



Activities of pretilachlor and pyribenzoxim herbicide on the behaviour and blood profile of juvenile *Oreochromis niloticus* fish

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ABSTRACT

This work determined the effects of pretilachlor herbicide on behavioural and haematological parameters of juvenile *Oreochromis niloticus* at different concentrations. One hundred and eighty (180) apparently healthy fish were obtained and randomly distributed into 18 plastic tanks (75×45×45 cm) filled with 30,000 mL of unchlorinated water of six treatments (0.0, 0.10, 0.125, 0.15, 0.175 and 0.2 mL), in triplicate, of ten fish per tank for 96 h. 3 mL of blood was collected from each treatment after 96 h with a syringe and emptied into Ethylene Diamine Tetracetic Acid bottles. The 96-hour LC₅₀ of toxicity was 1.03 mL. The behavioural responses of *O. niloticus* to the treatments are loss of reflex, erratic swimming, and air gulping. The haematological parameters were significantly different ($p > 0.05$). There was decrease in Pack cell volume (22.50 ± 0.50 to 19.00 ± 2.00), Red blood cell (2.65 ± 0.55 to 1.75 ± 0.45), Haemoglobin (7.50 ± 0.17 to 6.33 ± 0.67), White blood cell (3.15 ± 0.95 to 1.05 ± 0.05) and Mean Cell Hemoglobin Concentration (33.18 ± 0.14 to 33.14 ± 0.20), while there was an increase in Mean Corpuscular Volume (89.14 ± 20.39 to 113.11 ± 17.66) and Mean Corpuscular Haemoglobin (29.71 ± 6.80 to 37.70 ± 5.89). Hence, the discharge of Pretilachlor herbicide in the aquatic environment should be restricted to reduce potential risk to fish.

HIGHLIGHTS

- Pretilachlor is an excellent herbicide for weed control in rice farms.
- Exposure of fish to herbicides affects their blood parameters.
- Haematological profile provides information on the health status of fish.
- The presence of the toxicant in the water alters the behaviour of the inhabitants.

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1. Introduction

Fish is an important resource for humans, especially as food. Commercial and subsistence fish farmers hunt for fish in the wild or culture them in different ponds and cages in the ocean. They are also caught by recreational fishers, kept as pets and raised by fish keepers and can also be exhibited in public aquaria (Helfman 2009). Fish is an important source of protein for the teeming population of Nigeria, and it provides 40% of the dietary intake of animal protein for the average Nigerian. Fish and fish products constitute more than 60% of the total protein intake in adults, especially in rural areas. The importance of fish in human nutrition includes a nutrient profile superior to all terrestrial meats (beef, parks and so on) being an excellent source of high-quality animal protein and highly digestible energy; a good source of sulphur and essential amino acids such as lysine, leucine, valine, and arginine (Helfman 2009).

Nile tilapia is one of the most cultured freshwater fish species in Nigeria. This species feeds on phytoplankton, periphyton, aquatic plants, invertebrates, benthic fauna, detritus, bacterial films (FAO 2012) and even other fish and fish eggs. Depending on

the food source, they feed either via suspension filtering or surface grazing (GISD 2012), trapping plankton in a plankton-rich bolus with mucus excreted from their gills. Nile tilapia, *O. niloticus*, was found to exhibit trophic plasticity depending on the environment and the species of fish they coexist (Bwanika et al., 2007).

Pretilachlor is a broad-spectrum systemic herbicide with the chemical name 2-chloro-2', 6'-diethyl-N-(2-propoxyethyl) acetanilide. It has excellent action against annual weeds, sedges, and broadleaf weeds in rice fields (Botelho et al., 2009). The mechanism of action of these groups of drugs is still not clearly understood, but they are known to act by inhibiting the biosynthesis of fatty acids, lipids, proteins, flavonoids, and so on. It is a chloroacetanilide herbicide belonging to the group of anilide herbicides, which also include alachlor, butachlor and metachlor. Chronic exposure of animals to these groups of herbicides has shown probable carcinogenic effects; however, acute toxicity from pretilachlor in humans has not been reported yet. Acute pretilachlor intoxication via ingestion can be mistaken for commonly encountered pesticides like organophosphates, resulting in fallacious management of the patient (Botelho et al., 2009).

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Blood is a transporter of constituents such as nutrients, hormones, minerals, immune components, microorganisms, water, gases, toxins, and waste products. The most important functions of blood are the supply of oxygen and nutrients (including glucose, amino acids, and fatty acids) to cell tissues and removal of wastes (carbon dioxide, urea, and lactic acid). Haematology provides useful information about fish welfare, health, immune system response, short-term and long-term effects of "suboptimal" farming conditions, water quality, potential disease outbreaks, and nutritional status. Therefore, the use of haematological techniques for toxicology research, environmental monitoring and assessment of fish health conditions is gaining importance (Shah and Altindag, 2004) due to their close association with their environment as physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish. Studies on fish blood will also reveal conditions within the fish long before there is an outward manifestation of diseases (Maheswaran et al., 2008). Blood parameters are used to study the extent of stress in fish exposed to different pollutants and toxicants such as heavy metals, pesticides, industrial influents such as chemicals, detergents and so on.

Fish, among others, are highly sensitive to the environmental contamination of water. Hence, pollutants such as pesticides may significantly affect certain physiological processes like biological and haematological parameters, alterations in genetic and protein levels, and changes in histological and, particularly, oxidative stress in fishes; thus, imparting serious impairment to the health status of fishes. According to Helfman (2009), fish is a major source of dietary protein. Thus, pollutants in fish can impact human health directly or indirectly. Modern food production has made the use of pesticides and herbicides an important aspect of agricultural practices, in which indiscriminate use can result in a negative impact on the aquatic ecosystems and the health of aquatic organisms. Therefore, it is imperative to investigate the effects of pollutants, particularly pretilachlor herbicide residue in fish, as they play a significant role in the food chain, and their contamination could impact the aquatic system and humans.

2. Methodology

The research took place in the Departmental Laboratory of Fisheries and Aquaculture at Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria, at latitude 7.4696° and longitude 5.7362°. A total of 250 healthy juveniles *O. niloticus* (Average length of 17.5 cm and weighing of 10.5-32.3 g) were collected from a reliable fish farm in Akure, Ondo state and acclimatised in the laboratory for 14 days. The fish were fed twice daily (7.00 am and 5.00 pm) with a commercial feed containing 40 % crude protein during the acclimatisation. Feeding was discontinued 24 h before the commencement of the experiment to reduce waste production in the test container. After acclimatisation, 10 randomly selected pieces of *O. niloticus* were distributed into each tank of six (6) treatments in triplicate. Each plastic tank of 75 cm × 45 cm × 45 cm, 50,000 mL capacity, was filled with 30,000 mL of unchlorinated water and the pretilachlor 300 g/L and pyribenzoxim 20 g/L EC were introduced with a 2 mL syringe.

2.1. Toxicity Test

A range-finding test was carried out to determine the effective concentrations of Pretilachlor to be used. Based on the results of the range finding test, a 96 h definitive test was carried out according to the static bioassay procedure described by Nimai et al. (2013). A batch of ten (10) juvenile *O. niloticus* was distributed into each set of 18 rectangular plastic tanks (75×45×45 cm) filled

with 30,000 mL of unchlorinated water. Six test solutions of Pretilachlor (0.00, 0.10, 0.125, 0.15, 0.175, 0.2 mL) previously determined from the range finding test were introduced directly into the tanks in a single dose. The test fish were not fed during the 96 h test. Changes in the behaviour of the fish to the toxicant and mortality in each tank were monitored and recorded every 15 min for the first hour, after which records were taken every hour for the next 4 h, once every 4 h for the next 24 h and once every 24 h for the rest 96 h. Dead fish were immediately removed to avoid a consequent Pollution of the test media.

2.2. Collection of Blood Samples

Blood samples of 3 mL from the fish body were collected after 96 h of exposure to the herbicide. This was done through the vertebral caudal blood vessel with the use of a 2 mL disposable hypodermic syringe and needle. The blood samples collected were emptied into a 5 ml sample bottle treated with anticoagulant, Ethylene Diamine Tetracetic Acid (EDTA). The analysis of the blood profile of the fish was carried out following the method described by Svobodova et al. (1991). Blood cell count (erythrocytes and leucocytes) was analysed in an improved Neubauer hemocytometer with the use of modified Yokoyama diluting fluid. Mean cell Haemoglobin concentration (MCHC), Mean corpuscular volume (MCV), basic erythrocyte indices, and mean corpuscular haemoglobin were calculated from the values of haemoglobin (Hb) and erythrocyte counts using a formula:

$$MCV = \frac{\text{Packed cell volume}}{\text{Red Blood Cells}} \times 10 \quad (1)$$

$$MCH = \frac{\text{Haemoglobin}}{\text{Red Blood Cells}} \times 10 \quad (2)$$

2.3. Calculation of Lethal Concentrations and Statistics

The results of the mortality rate during each of the specified exposure periods of exposure were used to calculate 96 h LC₅₀ (USEPA 2000).

2.4. Statistical Analysis

The results of the blood analysis were collated and statistically analysed using computerised probit and logit analysis (Litchfield and Wilcoxon, 1949). The median lethal concentration at a selected period of exposure and an associated 95% interval for the replicates of the toxicity test, haematological and water quality statistical analysis, was carried out with the use of SAS.

3. Results and Discussion

The LC₅₀ of Pretilachlor at various levels of concentration on *O. niloticus* juveniles is shown in Figure 1. The LC₅₀ represent the concentration at which 50 % of the population of the fish was killed when exposed to the toxicant. From the figure, the 96 h LC₅₀ is 103 ml with a maximum admissible concentration of 1.03 mL -10.3 mL when the figure obtained for LC₅₀ was multiplied by an application factor of 0.01 and 0.1.

The acute and chronic toxicity test shows how LC₅₀ determination is essential for routine bioassay experiments (Banerjee, 1993). The LC₅₀ value of Pretilachlor herbicide in this

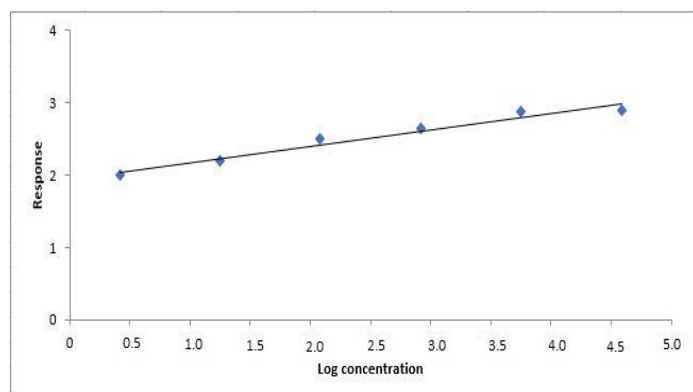


Figure 1: Log of concentration of Pretilachlor herbicide and its probit value on *Oreochromis niloticus* juveniles

study is 1.03 mL which is similar to previously reported LC_{50} values of butachlor to *O. niloticus* (Wang et al., 1992), *Heteropneustus fossilis* (Ateeq et al., 2005) and *Channa punctatus* (Tilak et al., 2007) which were 0.880 mgL⁻¹, 2.34 ppm and 247.46 ppb, respectively but of more toxic when compared to what was obtained when *O. niloticus* was exposed to endosulfan (10.20) (Werimo and Willen, 2010) and alachlor (381.9 µg/L) (Peebua et al., 2007).

The result of the haematological studies shows a significant decrease ($p \leq 0.05$) in the values of the blood parameters of *O. niloticus* after exposure to Pretilachlor herbicide for 96 h (Table 1). Pack cell volume decreased from 22.50 ± 0.50 to 19.00 ± 2.00 %, Red blood cell decreases from 2.65 ± 0.55 to 1.75 ± 0.45 ($\times 10^6/L$), Mean Cell Hemoglobin Concentration decreased from 33.18 ± 0.14 to 33.14 ± 0.20 (g/dL), Mean Corpuscular Volume decreased from 89.14 ± 20.39 to 73.71 ± 7.05 (FL) but increased with 113.11 ± 17.66 (FL) at T6, Mean Corpuscular Haemoglobin increased from 29.71 ± 6.80 to 37.70 ± 5.89 (pg/cell) Haemoglobin decreased from 7.50 ± 0.17 to 6.33 ± 0.67 (Hbg/dL), while White blood cell decreased from 3.15 ± 0.95 to 1.05 ± 0.05 ($\times 10^9/L$).

The haematological parameters of fish such as red blood cell count, hematocrit, haemoglobin (Hb) concentration, White blood cell, Mean corpuscle haemoglobin and Mean corpuscle volume are widely used as indicators of fish health status under toxicity (Adediji et al., 2002). These parameters can be biomarkers of sub-clinical health effects, and exposure to herbicides can cause a reduction in the level of the blood parameters (Adene et al., 2017).

In this study, a significant decrease was observed in pack cell volume (PCV), Mean cell Haemoglobin concentration (MCHC), Red blood cell (RBC), Haemoglobin (Hb) and White blood cell

(WBC), in the blood of *O. niloticus* juveniles exposed to Pretilachlor herbicide. The value of mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) decreased with an increase in concentration but was observed to increase in the highest concentration (0.2 mL). The decrease observed in the value of RBCs and Hb in the blood profile of *O. niloticus* juvenile exposed to Pretilachlor herbicide suggested poor efficiency of the fish blood to deliver enough oxygen to all tissues. This is similar to what was reported by Ghaffar et al. (2021) when *L. rohita* was exposed to glyphosate herbicide.

The decrease in the Haemoglobin (Hb) content observed in this study could be due to either its destruction or reduced synthesis or oxidative damage caused by reactive oxygen species (ROS) under the influence of herbicide (Mostakim et al., 2015). Also, stress caused by the toxicant could be responsible for the oxidation of Hb molecules leading to reduced oxygen and mortality.

The observed decrease in the Red Blood Cells (RBC) could be due to anaemia after exposure to the toxicant which could be attributed to malfunction of red blood cells and hemoglobin(Hb) synthesis and also an escalation of red blood cells damage in blood-forming sites. This observation aligns with Saravanan et al. (2011), who noted that red blood cell formation was inhibited and damaged when *O. niloticus* was exposed to Roundup. They reported that changes in MCV and MCH values might result from the release of undeveloped cells, similar to what was observed in Common carp fingerlings.

Furthermore, in this study, White blood cells (WBCs) of *O. niloticus* reduced after 96 h of exposure. The observed decrease may be attributed to disruption of WBCs development and discharge from the reservoirs of the tissue (Khan et al., 2016). White blood cell is a very important blood parameter that act as a defense mechanism to the fish and consist of granulocytes, monocyte, lymphocytes, and thrombocytes. The function of granulocytes and monocytes which are phagocytes is to remove debris from injured tissue and that of lymphocytes is to produce antibodies (Maheswaran, et al., 2008).

Table 2 shows the behavioural response of *O. niloticus* juvenile exposed to Pretilachlor rice herbicide. Normal behavioural responses were noticed in the control group (0.0 ml). Fish exposed to different concentrations (0.00, 0.10, 0.125, 0.15, 0.175 and 0.2 mL) of Pretilachlor displayed behavioural responses such as loss of reflex, air gulping, erratic swimming, but no sign of babel deformation. The behavioural response of *O. niloticus* to Pretilachlor herbicide was shown to be a sensitive indicator of physiological stress in fish under sub-lethal concentrations of pollutants (Maikai et al., 2008).

The results obtained in this study showed physiological malfunctions such as air gulping, loss of reflex, molting, fin

Table 1: The Haematological Parameters of *Oreochromis niloticus* Juvenile after Exposure to Pretilachlor for 96 h

Parameters (g)	T1 (0.0 ml)	T2 (0.10 ml)	T3 (0.125 ml)	T4 (0.15 ml)	T5 (0.175 ml)	T6 (0.2 ml)	P-value
PCV (%)	22.50 ± 0.50^a	21.50 ± 2.50^{ab}	20.50 ± 1.50^{ab}	15.50 ± 0.50^c	18.50 ± 2.50^{bc}	19.00 ± 2.00^b	0.006
RBC ($\times 10^6/L$)	2.65 ± 0.55^{ab}	3.55 ± 1.25^a	2.50 ± 0.50^{ab}	2.20 ± 0.30^b	2.50 ± 0.10^{ab}	1.75 ± 0.45^b	0.078
MCHC (g/dL)	33.18 ± 0.14^a	33.24 ± 0.09^a	33.23 ± 0.08^a	33.23 ± 0.11^a	33.23 ± 0.11^a	33.14 ± 0.20^a	0.887
MCV (FL)	89.14 ± 20.39^{ab}	66.30 ± 16.30^b	86.67 ± 23.34^{ab}	72.11 ± 12.11^b	73.71 ± 7.05^b	113.11 ± 17.66^a	0.056
MCH (pg/cell)	29.71 ± 6.80^{ab}	22.10 ± 5.44^b	28.89 ± 7.78^{ab}	24.04 ± 4.04^b	24.57 ± 2.35^b	37.70 ± 5.89^a	0.056
HB (Hbg/dL)	7.50 ± 0.17^a	7.17 ± 0.84^{ab}	6.83 ± 0.50^{ab}	5.17 ± 0.17^c	6.17 ± 0.84^{bc}	6.33 ± 0.67^b	0.006
WBC ($\times 10^9/L$)	3.15 ± 0.95^a	1.90 ± 0.20^b	1.90 ± 0.10^b	1.40 ± 0.30^{bc}	1.10 ± 0.10^{bc}	1.05 ± 0.05^c	0.000

Note: Means with the same row followed by the superscript alphabets are not significantly different ($p > 0.05$) from each other. PCV = Pack cell volume; RBC = Red blood cell; MCHC = Mean cell haemoglobin concentration; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; HB = Haemoglobin

deformation and erratic swimming which are well observed mostly in fish at the highest concentration of pretilachlor. The abnormal behavioural responses noticed was due to the toxic action of pretilachlor and its subsequent effect on the nervous system of *O. niloticus*. The observed malfunctions could certainly compromise

the organism's resistance to toxins and lead to constant mortality of the fish (Adewumi *et al.*, 2018). This study agrees with the report of Seveikova *et al.*, (2016) where common carp were exposed to copper. Similarly, Adene *et al.* (2017) reported a loss of reflex, erratic swimming and molting in *C. geriepinus* exposed to *Jatropha curcas* seed.

Conclusion and Recommendation

The findings of the study show that pretilachlor herbicide can cause huge damage to fish especially *O. niloticus* in ponds near rice farms and even low doses could modify the haematological profile of fish. The alterations in blood parameters may provide an early warning signal for the determination of the acute concentration of the herbicide in the field and its effects on an aquatic organism. However, damages can be stopped when there is perfect restriction of the use of this rice herbicide in the aquatic environment, to reduce its potential risk to fish in the ponds. Hence, it is suggested that all fish farms should regularly look out for areas not close to where such herbicides are used when locating their ponds and proper management should be made available during the applications of toxic chemicals to the crops planted and better still the fish should be harvested before the use of toxicants to prevent the mortality of fish and decrease in yield. In addition, it is advised that the use of pretilachlor herbicide be prohibited in paddy fish farms where fish are integrated with rice.

CRedit authorship contribution statement

ICA: Conceptualization, Methodology, Writing -original draft, Software, Data curation. **OJO:** Methodolog, Writing -review & editing. **OA:** Methodology, Writing -review & editing. **TOF:** Data curation, Writing -review & editing. **TAI:** Data curation, Writing -review & editing. **BMT:** Data curation, Writing -review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2: Behavioural response of *O. niloticus* exposed to Pretilachlor herbicide

Behaviour / Exposure	24 h of exposure (ml)						48 h of exposure (ml)						72 h of exposure (ml)						96 h of exposure (ml)					
	0	0.1	0.13	0.2	0.2	0.2	0	0.1	0.13	0.2	0.2	0.2	0	0.1	0.13	0.2	0.2	0.2	0	0.1	0.13	0.2	0.2	0.2
	0	0.1	0.13	0.2	0.2	0.2	0	0.1	0.13	0.2	0.2	0.2	0	0.1	0.13	0.2	0.2	0.2	0	0.1	0.13	0.2	0.2	0.2
Loss of reflex	-	+	-	-	+	+	-	+	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+
Air gulping	-	+	-	+	+	+	-	+	-	-	+	+	-	+	+	-	+	+	-	-	+	+	+	+
Erratic swimming	-	-	-	+	-	+	-	-	-	+	+	+	-	+	-	-	-	-	-	+	-	+	+	+
Fin deformation	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	-	+	+	+	+
Molting	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-

+ = reaction occurred; - = no reaction occurred

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