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TOXICITY OF PENDIMETHALIN (HERBICIDE) ON JUVENILES OF *Oreochromis niloticus* (LINNAEUS, 1758)

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ABSTRACT

Pendimethalin is a synthetic chemical primarily used as a pre- and postemergence herbicide against woody plants and broadleaf weeds. Pendimethalin contaminants surface water from aerial and groundwater application, runoff from rainfall and through irrigation. Aquatic animals are exposed to Pendimethalin via direct absorption through the skin, breathing through the gills and orally by drinking. The effect ofpendimethalinon *O. niloticus* was investigated following standard procedures. The experiment comprised of acute and sub-lethal bioassays. The acute bioassay with varying concentrations of pendimethalin 5, 6, 7, 8, 9mg/L and sub-lethal bioassay with concentrations 0, 0.46, 0.70 and 1.40mg/Llasted for 96hours and 8weeks, respectively. Behavioural and hematological changes were studied in both phases. Behavioural changes exhibited in both acute and sub-lethal studies include air gulping, discolouration, haemorrhage, restlessness, and vertical positioning. Packed Cell Volume, Haemoglobin, Total White Blood Cells, Total Red Blood Cells values in the acute and sub-lethal exposed groups showed similar response. PCV, HG and TRBC decreased with increase in concentration while TWBC increased with increase in concentration of toxicant. Mortality in acute and sub-lethal studies showed a dose dependent increase. The highest mortality was 23 (9mg/L) while the lowest was 7 (5mg/L) for the acute bioassay. For the sub-lethal bioassay, the highest survival was recorded in 0.5mg/L (24) and the lowest was in 1.40mg/L (18). From the acute bioassay the LC_{50} for 96hours was 6.94mg/L. It was concluded that Pendimethalin is moderately toxic to *O. niloticus* juveniles therefore its use near water bodies should be restricted.

Keywords: Toxicity, Haematology, Pendimethalin, Acute bioassay, Sub-lethal bioassay.

INTRODUCTION

Nile Tilapia, *Oreochromis niloticus*, is an edible fish and one of the most common freshwater fishes used intoxicological studies *(Figueiredo-Fernandes* et al. *2006*), because it presents a number of characteristics that makes it an appropriate model that can be used as indicator species in biomonitring programs. Tilapia, (*Oreochromis niloticus)* is a freshwater surface feeding fish. The fish has fast growth rate and can adapt to brackish and/or marine water (Ademola *et al.*2019).

Herbicides, also commonly known as weed killers, are chemical substances used to control unwanted plants (USEPA, 2011). The growing demand for increased food production to meet the ever-increasing global population has led to sophistication of agricultural technology including pesticides production. Nowadays, farmers rely much on pesticides (herbicides) for greater harvest to the extent of applying them excessively beyond or against manufacturers' recommendation not considering possible short- and long-term ecological effects (*Ani*et al. 2*018)*. Unfortunately, herbicides are indiscriminately used with little or no regulations in Nigeria, and they persist in the environment for a long time when released.Most soil pollutant (including pesticides) end up in nearby surface water including rivers, streams, creeks, creeklets, ponds, etc. Studies have suggested that water quality is rapidly declining due to human activities in the ecosystem especially in developing nations (Aghoghovwia *et al.* 2018).

Pendimethalin is a widely-used herbicide for the control of annual grasses and certain broadleaf weeds in commercial crops (*Engebretson* et al. 2*001). Pendimethalin is also good for cereal crops and ornamental plants (Inya* et al. 2*019) but at a low rate because it has little residual effects*. Direct overspray of a water body with a usual application rate of pendimethalin (2.4 kg/ha) can result in the concentrations severely toxic to algae, crustaceans, fish at a depth of 0.15 m (up to 1.6 mg/L) (*CICAD OAS, 2005*). Pendimethalin has been classified as persistent bioaccumulative toxicant (PBT) and a group C carcinogen "possible human carcinogen" by the United States Environmental Protection Agency (*USEPA, 1997*). People are exposed to this chemical through ingestion, inhalation, and dermalcontact withcontaminated water, including rain water. It isslightly toxic by the oral and eye routes and has been placedin Toxicity Category III; it is practically nontoxic by thedermal and inhalation routes and classified as Toxicity Category IV (Ramasahayam, 2014).

Aquatic animals are exposed to Pendimethalin via three ways, the first is dermally through direct absorption via the skin by swimming in the herbicide-contaminated water, the second way is breathing via direct uptake of the herbicides through the gills during the respiration process, the last way is orally via drinking the herbicides-contaminated water or feeding on herbicides-contaminated preys (Hardersen and Wratten, 1998). Pendimethalin is highly toxic to fish and aquatic invertebrates. The reported 96-hour LC_{50} for pendimethalin in bluegill sunfish is 199ug/L, 138ug/Lin rainbow trout, and 420ug/L in channel catfish (Kidd and James, 1991).

Pesticides do not only deteriorate the life sustaining quality of a water body but also produce toxic effects on non-target organisms such as fish. These pollutants, especially herbicides produce deleterious effects on aquatic flora and fauna by affecting various physiological, biochemical and cellular processes (Fournier et al. 2000). Over the last few decades the use of pesticides has dramatically increased in relation to increasing intensive agricultural practices. As a consequence of this massive use of pesticides in agriculture, pesticides have become significant ecological burden especially in aquatic ecosystems (Flynn and Spellman, 2009). The objective of this research was to assess the acute and sub-lethal toxicity of Pendimethalin on some hematological parameters of *O. niloticus* juveniles.

MATERIALS AND METHODS

Source of herbicide and fish

Herbicide that contains Pendimethalin (STOMP®) as active ingredient was purchased from Farmers Escort at Samaru market Zaria, Kaduna State.

500 Juveniles of *O. niloticus* with mixed sexes and average size (9.91g) were purchased from Garun Babba Integrated Farms Kano State and were transported in aerated polythene bags to the Fisheries laboratory, Department of Biology, Faculty of Life sciences, Ahmadu Bello University, Zaria, where the juveniles were acclimated for two weeks and fed at 5% body weight.

Range finding test

Range finding test was carried out to check for the concentrations of the herbicide used for the definitive tests following the recommendations of ATT Fish (2000). This was done by preparing stock solutions and placing six concentrations of the herbicide in separate tanks containing 20*l* of water each with 30 x 30 x 45cm dimensions. Mortality of fish was observed at 12, 24, 48,72, and 96 hours. The concentrations were graded using low ranges until about 80-90% mortality was recorded in the highest concentration and 20-30% for the lowest concentration.

Experimental set up

Exposure of fish to herbicide was carried out using glass aquarium tanks with 30x30x45cm dimensions. Six concentrations of the herbicide were prepared and labeled T_0, T_1, T_2, T_3, T_4 and T_5 as described by Beitlich *et al.* (1995). The fish were starved for 48 hours prior to the commencement of the experiment (Omitoyin et al. 2006). T_0 had zero concentration of pesticide and served as control. Ten fishes were selected randomly and stocked in each aquarium as described by Ayoola (2008). A static renewal method was used in the experiment, which was replicated three times.

Acute bioassay

Behavioural and physiochemical parameters

Fishes were observed for abnormal behaviors (hyperactivity and hypoactivity) at 12, 24, 48, 72, and 96 hours. Temperature and pH, were determined using Hanna instrument

(HI98129). Dissolved oxygen was determined according to the method described in APHA (2005) before and after introducing herbicide to fishes.

Mortality

Mortality of *O. niloticus* juveniles exposed to herbicide (Pendimethalin) was recorded at 1, 2, 4, 6, and 12 hours post exposure and then twice daily till termination as described by Olusegun (2001). Probit analysis was employed to determine the LC_{50} of the herbicide by using Microsoft Excel 2010.

Sub-lethal bioassay

These was based on the result of acute bioassay. Sub-lethal concentration of 1/5, 1/10, 1/15 of 96 hours LC_{50} (6.94mg/L) was used to determine sub-lethal concentration range (Mohammed, 1995). Twelve tanks were used with three replicates per treatment. Ten fishes per tank were exposed to 3 sub-lethal concentrations of Pendimethalin. During exposure, fresh solution was added every 48 hours to maintain the concentration level after the wastes were siphoned. Fish were fed twice daily with pelletized commercial feed at 5% body weight for 8 weeks.

Determination of haematological parameters

Haematocrit (PCV) was determined by the Wintrobe and Westergreen method as described by Svobodova *et al*. (1991). Percentage Haemoglobin (Hb) concentration was determined as described by Mohmoh *et al*. (2012) using Drabkin's solution and with the aid of a model XF-1C haemoglobinometer. The RBC count was determined using an improved Neubauer haemocytometer under ×40 objective and calculated (Dacie and Lewis, 2001). Total white blood cell count was determined as described using the standard two slide wedge technique to make blood films and the Giemsa's staining technique, counter stained with Leishmann's stain. Total leucocytes were calculated as formulated by Campbell (1995).

Erythrocyte indices which include Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) were calculated as follows:

$$\text{MCV (Mean Corpuscular Volume)} = \frac{\text{Hct (\%)}}{\text{RBC } (10^6/\text{mm}^3)} \times 10\ (\mu\text{m}^3)$$

$$\text{MCH (Mean Corpuscular Hemoglobin)} = \frac{\text{Hb } (\frac{\text{g}}{100}\text{ ml})}{\text{RBC } (10^6/\text{mm}^2)} \times 10\ (\text{pg cell}^{-2})$$

$$\text{MCHC (Mean Corpuscular Hemoglobin Conc.)} = \frac{\text{Hb } (\frac{\text{g}}{100}\text{ ml})}{\text{RBC } (10^6/\text{mm}^2)} \times 100\ (\text{g dL})$$

Data Analysis

The hematological parameters were analysed using One-way ANOVA at p? 0.05 level using SPSS. The comparison of means was carried out using Duncan's multiple range test. The mean lethal concentration (LC_{50}) for 96 hours was determined using Microsoft Excel. The LC_{50} graph was plotted using Minitab.

RESULTS

Behavioural changes were observed in the fishes within the first 12 hours of exposure. Fishes in the three (3) tanks with the highest concentration of pendimethalin (9.0, 8.0 and 7.0 mg/L) showed hyperactivity which was characterized by vertical positioning, fast opening of the operculum, agitated swimming, restlessness, and jumping (Plate I). These behaviours were later shown in fishes in lesser concentrations of the toxicant in a concentration dependent manner from 9-5 mg/L.

Plate I: Vertical positioning in *O. niloticus* juveniles exposed to pendimethalin

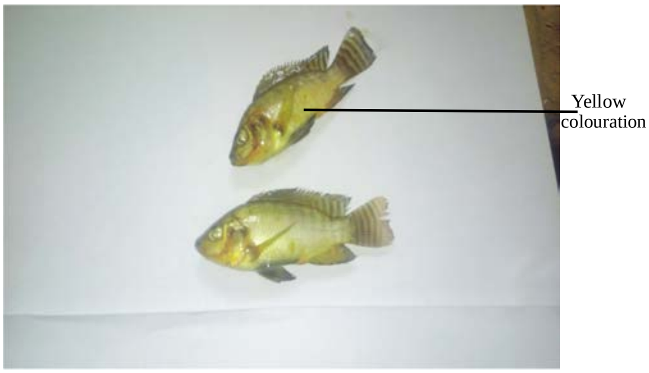

Plate II: Yellow colouration in *O. niloticus* juveniles exposed to pendimethalin

Plate III: Haemorrhage in *O. niloticus* juveniles exposed to pendimethalin

Some of the morphological changes observed in fishes of exposed group include mucus production and swollen abdomen which were more pronounced with increase in level of concentration.

At 48-72 hours post exposure, fishes of the exposed group began to show a decreased activity (hypoactive) which was characterized by slow swimming, gathering at the corners of the tanks, swimming to the surface to gulp air, impaired swimming, and loss of tail fins. Haemorrhage and yellow colorations were also observed at the anal fin region of some fishes and at the opercular region while the yellow colourations were on the body (Plate II and III).

The results of the acute toxicity bioassay showing the mean mortality, total mortality, percentage mortalities and probit kill values of *O. niloticus* are presented in Table1. Mortality was observed in all the treatment groups except the control. The highest mortality of 23 was recorded in the 9 mg/L concentration while the least mortality of 5 was recorded in the5 mg/L concentration. The percentage mortality of 77% was recorded in the 9mg/L concentration. The 96-hour median lethal concentration (LC_{50}) of pendimethaline for *O. niloticus* was found to be 6.94mg/L (Figure1).

Table 1: Mortality rates, percentage mortality and probit kill values of *O. niloticus* juveniles exposed to acute concentrations of pendimethalin

Conc. (mg/L)	Log of Conc	Numberof fish Exposed	Mortality	% Mortality	Probit killValues
00	00	30	0	0	0
5	0.70	30	7	23	4.27
6	0.78	30	9	30	4.48
7	0.85	30	14	47	4.92
8	0.90	30	20	67	5.43
9	0.95	30	23	77	5.73

Conc. = Concentration, %= percentage

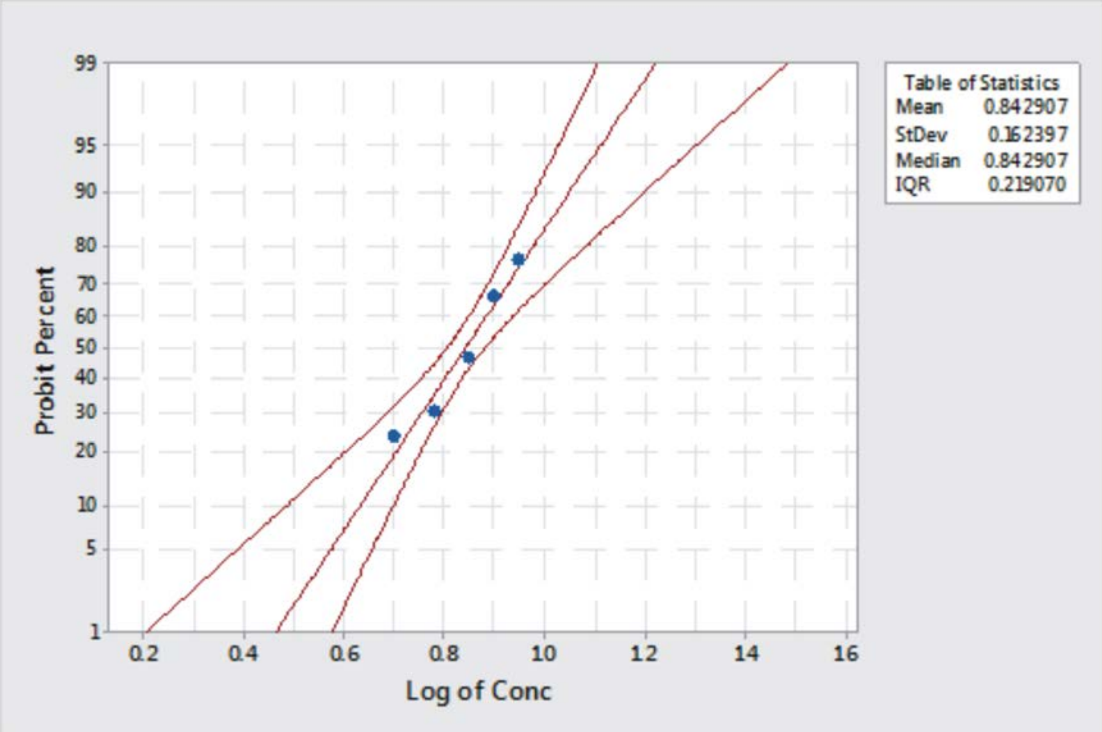

Figure1: LC_{50} graph for *O. niloticus* juveniles exposed to acute concentrations of pendimethalin

The results of the haematological parameters of *O. niloticus* exposed to acute bioassay concentrations of pendimethalin are presented in Table 2.*The control group had the highest* packed cell volume values (43.67 ± 0.88) while the lowest was recorded in 7mg/L concentration (38.00 ± 0.58). The highest value for haemoglobin and total red blood cells (TRBC) was recorded in the control group, 14.53 ± 0.29 and 7.30 ± 0.12, respectively, and the least was recorded in the highest concentration, 10.57 ± 0.30 and 5.30 ± 0.17, respectively. Total white blood cells (TWBC) recorded the highest value in the highest concentration 9mg/L (18.27 ± 0.09) while the least value was recorded in the control group (9.47 ± 0.07). There were significant differences (*P<0.05*) between PCV, Hb, TWBC and TRBC. The values of MCV, MCH and MCHC ranged from 58.88 -62.00 μm^3, 19.49 – 20.34pg $cell^{-1}$ and 32.78 – 33.37

Table 2: Haematology for acute bioassay of *O. niloticus* juveniles exposed to pendimethalin

Parameters	Control	5mg/L	6mg/L	7mg/L	8mg/L	9mg/L
PCV	43.67 ± 0.88^{a}	41.33 ± 0.88^{b}	39.67 ± 0.33^{bc}	38.00 ± 0.58^{c}	39.00 ± 0.58^{c}	31.67 ± 0.88^{c}
Hb	14.53 ± 0.29^{a}	13.67 ± 0.38^{b}	13.00 ± 0.00^{bc}	12.67 ± 0.20^{c}	12.90 ± 0.10^{bc}	10.57 ± 0.30^{d}
TWBC	9.47 ± 0.07^{e}	10.63 ± 0.26^{d}	11.00 ± 0.21^{d}	12.03 ± 0.15^{c}	16.00 ± 0.12^{b}	18.27 ± 0.09^{a}
TRBC	7.30 ± 0.12^{a}	7.00 ± 0.06^{ab}	6.40 ± 0.12^{c}	6.37 ± 0.15^{c}	6.63 ± 0.22^{bc}	5.30 ± 0.17^{d}
MCV	59.83 ± 0.33^{a}	59.07 ± 0.79^{a}	62.00 ± 0.71^{a}	59.73 ± 0.49^{a}	58.88 ± 1.38^{a}	59.80 ± 0.42^{a}
MCH	19.61 ± 0.09^{a}	19.53 ± 0.38^{a}	20.34 ± 0.38^{a}	19.85 ± 0.09^{a}	19.49 ± 0.51^{a}	19.95 ± 0.16^{a}
MCHC	33.29 ± 0.04^{a}	33.07 ± 0.29^{a}	32.78 ± 0.28^{a}	33.33 ± 0.05^{a}	33.08 ± 0.29^{a}	33.37 ± 0.04^{a}

Means with different superscripts across rows are significantly different (p? 0.05)

Note: Treatment (TRT), Pack Cell Volume (PCV), haemoglobin (HG), Total White Blood Cells (TWBC), Total Red Blood Cells (TRBC), Mean Corpuscle Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC).

The results of the haematological parameters of *O. niloticus* exposed to sub-lethal concentrations of pendimethalin are presented in Table 3. The control group had the highest PCV, Hb and TRBC, 52.67 ± 1.45, 17.5 ± 0.49 and 8.77 ± 0.20, respectively. Total White Blood Cell values in the control group had the lowest (9.20 ± 1.51) while the highest value was recorded in the highest concentration, 16.43 ± 2.13.The PCV, Hb, TWBC and TRBC values in the sub-lethal exposal responded similarly with values of the acute exposure. PCV, Hb and TRBC decreased with increase in concentration while TWBC increased with increase in concentration. The values of MCV, MCH and MCHC ranged from 53.37 - 60.03 μm^3, 17.70 – 19.97 pg $cell^{-1}$ and 33.17 – 33.27

Table3: Sub-lethal haematology of *O. niloticus* juveniles exposed to pendimethalin

TRT	PCV	HG	TWBC	TRBC	MCV	MCH	MCHC
0	52.67 ± 1.45 [a]	17.5 ± 0.49 [a]	9.20 ± 1.51 [a]	8.77 ± 0.20 [a]	60.03 ± 0.27 [a]	19.97 ± 0.09 [a]	33.23 ± 0.03 [a]
0.46	49.00 ± 1.53 [a]	16.30 ± 0.51 [a]	9.40 ± 2.47 [a]	8.30 ± 0.40 [a]	59.13 ± 1.22 [a]	19.67 ± 0.38 [a]	33.27 ± 0.03 [a]
0.70	39.00 ± 6.66 [a]	12.97 ± 2.22 [a]	10.23 ± 0.19 [a]	6.50 ± 1.07 [a]	59.87 ± 0.54 [a]	19.90 ± 0.15 [a]	33.23 ± 0.07 [a]
1.40	25.00 ± 4.04 [b]	8.30 ± 1.37 [b]	16.43 ± 2.13 [a]	4.13 ± 0.69 [b]	53.37 ± 7.48 [a]	17.70 ± 2.45 [a]	33.17 ± 0.09 [a]

Means with different superscripts along columns are significantly different (p? 0.05)

Note: Treatment (TRT), Pack Cell Volume (PCV), haemoglobin (HG), Total White Blood Cells (TWBC), Total Red Blood Cells (TRBC), Mean Corpuscle Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Concentration (MCHC).

DISCUSSION

A fundamental goal of ecotoxicology and hazard assessment is to determine the ecological effects of toxic chemicals on natural communities and ecosystems. The changes in behaviour observed in fish in this study are signs of distress and has been collaborated by the reports of Baker et al.(2001) and El-Sayed et al.(2013). The stressful and erratic behaviours of the *O. niloticus* also tend to indicate respiratory impairment probably due to the effect of the chemical on the gills. Fish breathe by movement of water, so the gills are usually the site of first contact of the internal organ. The observed behavioural changes and clinical toxicity signs in *O. niloticus* are in similar to the report of Ahmed and Moustafa, (2010), who reported that abnormal behavioural changes in the fish mainly manifested in their respiratory and nervous systems, and appeared immediately after exposure to a toxicant. The abnormal movements could have resulted from hyper contractions of the muscles due to cholinesterase inhibition at the highest pendimethalin concentration in addition respiratory manifestations may have resulted from excess mucus secretions forming a thick coating on the gill tissue (*Attallah* et al. 1997).

Hyperactivity of fish in the exposed group can be attributed to attempt to escape the test water (toxic environment). Hyperactivity in fish when introduced to an unfavorable environment has been suggested as primary and principal sign of nervous system failure due to pesticide poisoning which affects physiological and biochemical activities. Mekkawy *et al.* (2013) reported hyperactivity in *C. gariepinus* exposed to atrazine was characterized by rapid and erratic swimming or darting, partial loss of equilibrium, rapid pectoral fin and opercular movement, reduction in feeding activity, fin haemorrhage and loss of some parts.

Swollen abdomen observed in the exposed group may be attributed to necrotic damage to the gut and this shows that the toxicity of pendimethalin is not only on the outside or on the morphology of *O. niloticus*. Fast opening of the operculum in fish of exposed group may be due to impairment of respiration as a result of mucus secretion or attempt to counteract or offset the toxicant by breathing faster. This is attributed to increase in oxygen demand needed for increased metabolic activity as an attempt to metabolize the toxicant (pendimethalin). Colour change can be a means of adaption or a cryptic colouration to blend with the colour of the water which was from the herbicide during the sub-lethal bioassay. Most fishes have a mechanism of blending to colours from the environment they live as a means of protection.

Haemoglobin is the red pigment contained in the erythrocytes, it functions physiologically in the transport of dissolved gases, principally, dissolved oxygen and carbon dioxide within the body of the fish (Inya et al. 2019). The decrease in the TRBC in the highest concentrations of both acute (9mg/L) and sublethal (1.40mg/L) studies can be due to possible inhibition of erythrocyte production through destruction of the stem cells in the bone marrow which are progenitor cells (absolute anaemia). Similar reduction was observed in PCV values because volume of blood cells is a function of their numbers. However, white blood cell showed significant increase as the toxicant concentration increased, this might be due to an adaptation to fight toxic effects, increase in WBC counts recorded in this research may also suggest that the antigens (herbicide) stimulated the production of more WBC to improve the health status of the fishes (Baker et al. 2001; Inya et al. 2019). **Velisek et al. (2012)** reported that Pendimethalin herbicide may activate the immune system in fish by altering levels of total leukocytes, thus signaling an adaptive immune response. Fink and Salibian (2005) reported that increase in TWBC may reflect the proliferation of multi-potent hematopoietic cells as a consequence of chemical toxicity which was also observed in this study. The increase in TWBC indicates that the stress condition of the fish induced by pendimethalin caused hypoxia.

The result indicates synergistic action of Pendimethalin herbicide. This may also be due to haemo-concentration and polycythemia due to decrease in the amount of dissolved oxygen in water or may be due to Haemochromatosis in which too much iron in RBC and haemoglobin in the body causes haemochromatosis. Iron is important because it is part of haemoglobin, a molecule in the blood that transports oxygen from the lungs to all body tissues. Iron may build up in the organs and cause complications, including cirrhosis, or scarring of liver tissue, diabetes, irregular heart rhythms or weakening of the heart muscle, arthritis and erectile dysfunction. The complication most often associated with hemochromatosis is liver damage. Iron build-up in the liver causes cirrhosis, which increases the chance of developing liver cancer (Bacon et al. 2011). The calculated blood indices, MCV, MCH and MCHC have particular importance in describing anemia in most animals. The increase in MCV observed in this study may be attributed to the direct effect of catecholamines, cortisol, and glucose on adenylate cyclase activities in red blood cells, as a response to hypoxic stress (Saleh and Marie, 2016).

Mortality increased with concentration both in the acute and sub-lethal studies. It could be argued that mortality did not primarily result from haematological alterations alone, but also by causes made to other tissues as well (Forambi et al. 2008).

CONCLUSIONS

Pendimethalin is toxic to *O. Niloticus* and has a median lethal concentration of 6.94mg/L**.** *Oreochromis niloticus* exposed to acute concentrations of pendimethalin exhibited hyperactivity which was characterized by fast opening of the operculum, agitated swimming, restlessness, jumping and death which was concentration dependent. Haematological effects of pendimethalin in acute and sub-lethal bioassays showed acute anaemia with PCV, TRBC and Hb decreasing while WBC increased.

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