Toxicity of Pendimethalin (Herbicide) on Juveniles of *Oreochromis niloticus* (Linnaeus, 1758)

***Corresponding author:** bashirabdulmalik02@gmail.com, +2348171182597.

Abstract

The effect of the herbicide (pendimethalin) on juveniles of *O. niloticus* was investigated. The experiment had two phases i.e. acute and sub-lethal bioassays. The acute bioassay with varying concentrations of pendimethalin 5, 6, 7, 8, 9 mg/L and sub-lethal bioassay with concentrations 0, 0.46, 0.70 and 1.40 mg/L lasted for 96hours and 8weeks, respectively. Behavioural and hematological changes were studied in both phases. Fishes showed different behavioural changes which includes air gulping, discolouration, haemorrhage, restlessness, and vertical positioning in both acute and sub-lethal studies. 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 found to be 6.94 mg/L. It was concluded that Pendimethalin is moderately toxic to *O. niloticus* juveniles which led to the recommendation that the use of pendimethalin near water bodies should be restricted to avoid the risk associated with the use of the herbicide.

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

Introduction

Nile tilapia, *Oreochromis niloticus*, is one of the most common freshwater fishes used in toxicological studies (Figueiredo-Fernandes *et al*., 2006; Garcia-Santos *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 (Gadagbui *et al*., 1996). 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.,* 2018). 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.,* 2001). Pendimethalin is also good for cereal crops and ornamental plants (Inya *et al.,* 2019) 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 toxic (PBT) and a group C carcinogen "possible human carcinogen" by the United States Environmental Protection Agency (USEPA, 1997).

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 199 ug/L, 138 ug/L in rainbow trout, and 420 ug/L in channel catfish (Kidd and James, 1991).

Pesticides do not only deteriorate the life sustaining quality of a waterbody but also produce toxic effects on non-target organisms fish inclusive, 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 (Cerejeira *et al.*,2003; 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. 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 45 cm 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 30 x 30 x 45 cm 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 FAO (1977) and 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} (6.94 mg/L) was used to determine sub-lethal concentration range (Oladimeji and Ologunmeta, 1987; 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 pelletised commercial feed at 5% body weight for 8weeks.

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:

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

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

$$\text{MCHC (Mean Corpuscular Hemoglobin Conc.)} = \frac{Hb\ (\frac{g}{100}ml)}{Hct\ (\%)} \times 100 \qquad (\%)$$

Data Analyses

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.

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 Table 1. 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 the 5 mg/L concentration. The percentage mortality of 77% was recorded in the 9 mg/L concentration. The 96-hour median lethal concentration (LC_{50}) of pendimethaline for *O. niloticus* was found to be 6.94mg/L (Figure 1).

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). Mean corpuscular value (MCV) and mean corpuscular haemoglobin (MCH) did not vary significantly across groups. Packed Cell Volume, Hb and TRBC decreased with increase in concentration while TWBC increased with increase in concentration.

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 exposal. PCV, Hb and TRBC decreased with increase in concentration while TWBC increased with increase in concentration.

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 destress and has been collaborated by the reports of Baker *et al*. (2001), Auta and Ogueji (2007) 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 carbondioxide within the body of the fish (Inya *et al.,* 2019). The decrease in the TRBC in the highest concentrations of both acute (9 mg/L) and sublethal (1.40 mg/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). Modesto and Martinez (2010) and 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 hemato-poietic 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 buildup 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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References

Ademola MA, Jeremiah O, Jimoh A, Funmilayo O, Izah SC. (2019). Acute Toxicity of Paraquat Dichloride on Fingerlings of *Oreochromis niloticus*. *International Journal of Research Studies in Biosciences (IJRSB)*. 7(1): 29-36.

Aghoghovwia OA, Umoru OD, Izah SC. (2018). Physicochemical characteristics of nun river at Gbarantoru and Tombia Axis in Bayelsa State, Nigeria. *Bioscience Methods*. 9(1): 1-11.

Ahmed ENG, Moustafa GG. (2010). Evaluation the toxic effects of herbicide stomp 50% EC (Pendimethalin) on health of *Oreochromis niloticus*. *Zagazig Veterinary Journal*. 38(3): 8-17.

Ani LC, Nwamba HO, Ejilibe OC, Nnamonu EI. (2018). Evaluation of Lethality Effect and Morphometric Indices Alterations in *Clarias gariepinus* Exposed to Shapshooter. *Asian Journal of Biology*. 7(1): 1-8.

APHA. (2005). Standard methods for the examination of water and waste water, 21st ed. American Public Health Association, Washington, DC. 1368pp.

Attallah OA, Ali AM, Ibrahim AS, Sakr SF. (1997). Prevalence of pathologic changes associated with the fin – rot- indicating bacterial disease in freshwater fish. *Alex Journal of Veterinary Science*. 73(6): 629-644.

Auta J, Ogueji EO. (2007). Acute toxicity and behavioral effects of chloropyrifos-ethyl pesticide to juvenile of *Clarias gariepinus* Teugels. Proceedings of the 22nd Annual Conference of Fisheries Society of Nigeria (FISON), held at School of Nursing, Birnin Kebbi, 12th-16th November, 2007, 264-272.

Ayoola SO. (2008). Toxicity of glyphosate herbicide on Nile tilapia (*Oreochromis niloticus*) juvenile. *African Journal of Agricultural Research*. 3(12): 825-834.

Bacon BR, Adams PC, Kowdley KV, Powell LW, Tavill AS. (2011). Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology*. 54(1): 328-343.

Baker FJ, Silverton RE, Pallister CJ. (2001). Introduction to Medical Laboratory Technology. 7th Ed. Nigeria: Bounty Press Limited, Ibadan. 448pp.

Beitlich JH, Jenning BH, Lasse BA, Nelson RC, Ott JJ, Whitney JM, Wooly SK. (1995). Introduction to fish Health Management. B. A. Lasse 2nd edn. Onalaska, Wisconsin, USA.

Campbell TW. (1995). Avian Haematology and Cytology. Second edition, IOWA State University Press, Ames, IA, pp. 179-180.

Cerejeira MJ, Viana P, Batista S, Pereira T, Silva E, Valerio MJ. (2003). Pesticides in Portuguese surface and ground waters. *Water Research journal*. (37): 1055–1063.

CICAD OAS. (2005): The toxicology of selected substances used in the production and refining of Cocaine and Heroin: A tier- two Assessments. Washington DC, USA, CICAD, Organization of American States, Report Number OAS/ CICAD 2005-02.

Dacie JV, Lewis SM. (2001). Practical Haematology. 9th edition. Churchill Livingstone, London. 633pp.

El-Sayed YS, Samak DH, Abou-Ghanema IY, Soliman MK. (2013). Physiological and oxidative stress biomarkers in the freshwater monosex Nile tilapia, *Oreochromis niloticus* L., exposed to pendimethalin-based herbicide. *Environmental Toxicology*. 30(4): 430-438.

Engebretson J, Hall G, Hengel M, Shibamoto T. (2001). Analysis of pendimethalin residues in fruit, nuts, vegetables, grass, and mint by gas chromatography. *J. Agric. Food Chem.* 49: 2198–2206.

FAO. (1977). Manual of methods in aquatic environment research part 4: Bases for selecting biological test to evaluate marine pollution. FAO Technical Papers, (104), 31pp.

Farombi EO, Ajimoko YR, Adelowo OA. (2008). Effect of butachlor on antioxidant enzyme status and lipid peroxidation in fresh water African Catfish (*Clarias gariepinus*). *International Journal of Environmental Research and Public Health*. 5(5): 423-427.

Figueiredo-Fernandes A, Fontaínhas-Fernandes A, Peixoto F, Rocha E, Reis-Henriques MA. (2006). Effect of paraquat on oxidative stress enzymes in tilapia *Oreochromis niloticus* at two levels of temperature. *Pesticide Biochemistry and Physiology*. 85: 97-103.

Fink NE, Salibián A. (2005). Toxicological studies in adult amphibians: effects of lead. *Applied Herpetology*. 2(3): 311-333.

Flynn K, Spellman T. (2009). Environmental levels of atrazine decrease spatial aggregation in the freshwater mussel (*Elliptio complanata*). *Ecotoxicology and Environmental Safety*. (72): 1228–1233.

Fournier M, Cyr D, Blakley B, Boermans H, Brousseau P. (2000). Phagocytosis as a biomarker of immunotoxicity in wildlife species exposed to environmental xenobiotics. *American Zoologist*. 40: 412–420.

Gadagbui BKM, Addy M, Goksøyr A. (1996). Species characteristics of hepatic biotransformation enzymes in two tropical freshwater teleosts, tilapia (*Oreochromis niloticus*) and mudfish (*Clarias anguillaris*). *Comparative Biochemistry and Physiology*. 114C: 201-211.

Garcia-Santos S, Fontaínhas-Fernandes A, Wilson JM. (2006). Cadmium tolerance in the Nile tilapia (*Oreochromis niloticus*) following acute exposure: Assessment of some ionoregulatory parameters. *Environmental Toxicology*. 21(6): 33-46.

Hardersen S, Wratten SD. (1998). The effects of carbaryl exposure of the penultimate larval instars of *Xathocnemis zealandica* on emergence and fluctuating asymmetry. *Ecotoxicology*. 7(5): 297-304.

Inya OJ, Amuta E, Umeh E, Azua E, Odo E. (2019). Hematological Parameters and Serum Biochemistry of *Clarias gariepinus* Juveniles Exposed to Vestaline® (Pendimethalin) Herbicide. *American Journal of BioScience*. 7(1): 25-30.

Kidd H, James DR. (1991). The Agrochemicals Handbook.3RDEdn. Cambridge, UK: Royal Society of Chemistry Information Services, 1991 (As Updated). Pp.10–12.

Mekkawy IA, Mahmoud UM, Mohammed RH. (2013). Protective effects of tomato paste and vitamin -E on atrazine induced hematological and biochemical characteristics of *Clarias gariepinus* (Burchell, 1822). *Global Advanced Research Journal of Environmental Science and Technology*. 2(1): 11-21.

Modesto KA, Martinez CB. (2010). Effects of Roundup Transorb on fish: hematology, antioxidant defenses and acetylcholinesterase activity. *Chemosphere*. 81: 781–787.

Mohammed B. (1995). Haematological effcts of lindane on *O. niloticus*. M. Sc. Thesis, Federal University of Technology, Minna, Nigeria, 105pp.

Momoh AO, Oladunmoye MK, Adebolu TT. (2012). Haematological and histopathological effects of oil from castor seeds (Ricinus communis Linn.) on albino-rats. Journal of Pharmacognosy and Phytotheraphy, 4(4):40–43.

Oladimeji AA, Ologunmeta RT. (1987). Chronic toxicity of water borne lead to *Tilapia nilotica* (L). *Nigerian Journal of Applied Fish and Hydrobiology*. 2: 19-24.

Olusegun SA. (2001). Toxicity of Apron to juveniles of mudfish (*Clarias gariepinus*, Teugels). M.Sc. Thesis, Usman Danfodio University, Sokoto, Nigeria, 66pp.

Omitoyin BO, Ajani EK, Fajimi OE. (2006). Toxicity of Gramoxone (Paraquat) to juvenile African catfish, *Clarias gariepinus* (Buchell, 1822). *American Euroasian Journal of Agricultural and Environmental Sciences*. 1(1): 26–30.

Saleh YS, Marie MAS. (2016). Use of *Arius thalassinus* fish in a pollution bio-monitoring study, applying combined oxidative stress, hematology, biochemical and histopathological biomarkers: A baseline field study. *Marine Pollution Bulletin*. 106(1): 308-322.

Svobodova Z, Prada D, Palackova. (1991). Unified Methods of Haematological Examination of Fisheries Resources. Institute of Fish Culture and Hydrobiology, Vodany, Czechoslovakia. 31pp.

U.S. Environmental Protection Agency. (2011). Exposure Factors Handbook 2011 Edition (Final Report). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-09/052F.

U.S. EPA. (1997). Exposure Factors Handbook. U.S. Environmental Protection Agency, Washington, DC, EPA/600/P-95/002F a-c.

Velisek J, Stara A, Machova J, Svobodová Z. (2012). Effects long-term exposure to simazine in real concentrations on common carp (*Cyprinus carpio* L.). *Ecotoxicology Environmental Safety*. 76: 79-86.

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

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.	Number of fish Exposed	Mortality	% Mortality	Probit kill Values
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

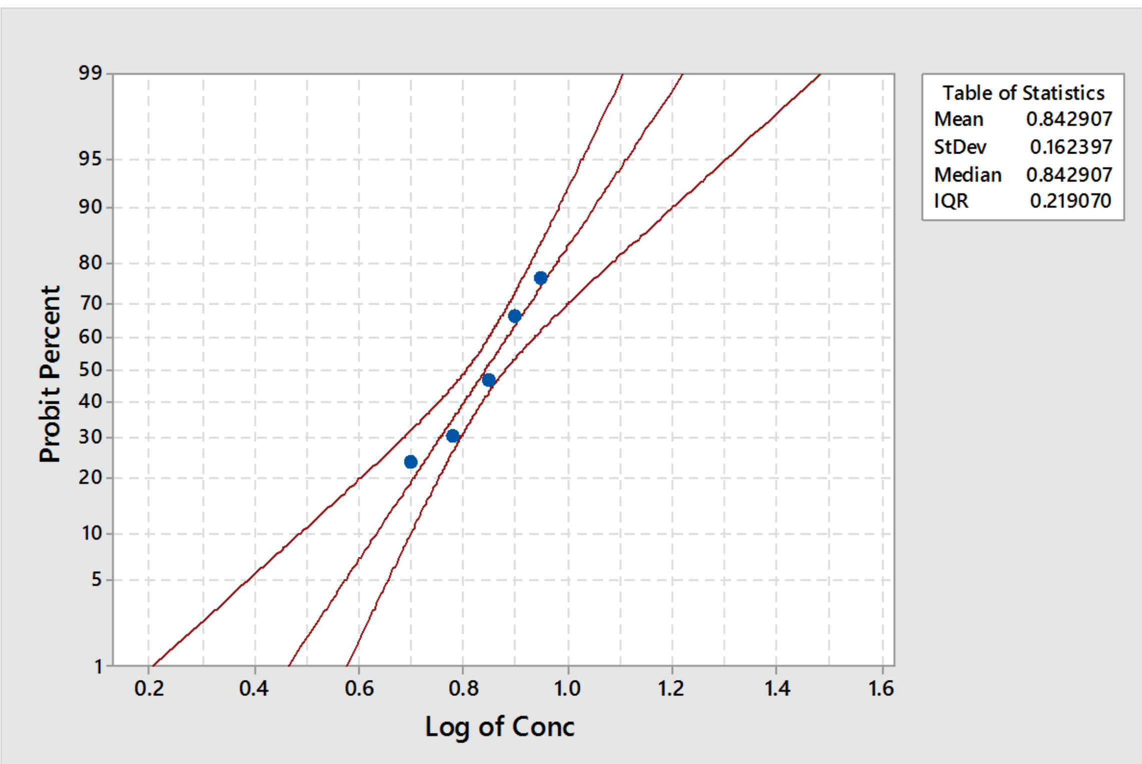

Figure1: LC_{50} graph for *O. niloticus* juveniles exposed to acute concentrations of pendimethalin

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

Parameters	Control	5 mg/L	6 mg/L	7 mg/L	8 mg/L	9 mg/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 along 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).

Table 3: 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).