Journal of Aquatic Pollution and Toxicology
ISSN: 2581-804X

Open Access Research Article

Hematological and Histological Studies to Determine the Toxicity of Profenofos on*Oreochromis niloticus* (Genetically Improved)

Dayananda NG*, Surendran SN

Department of Zoology, University of Jaffna, Jaffna, Sri Lanka

ABSTRACT

The extensive use of pesticides in modern agriculture can cause severe consequences because of their bio magnification and persistence. This research was designed to study the toxicity of profenofos on the genetically improved strain of farmed tilapia (*Oreochromis nilotic*). The LC_{50} value of Profenofos for fingerlings with 2 g ± 0.5 g average weight and 6 cm ± 0.5 cm average lengths was determined by probit analysis using MS excel 2013 software. Sub adults with 14.0 cm ± 0.5 cm average length and 71.0 g ± 0.5 g average weight were stocked and exposed to different concentrations of Profenofos ranging from 0 to 0.15 mg L^{-1} for four weeks and accessed the hematological parameters. The sub adults were exposed to different concentrations of Profenofos ranging from 0 to 0.26 mg L^{-1} for a week, and histological alterations of liver, kidney, and gills were examined. The 72 hours LC_{50} value for *Oreochromis niloticus* fingerlings was 0.26 mg L^{-1} at 30.1°C ± 1°C. One way ANOVA revealed that there was a significant decrease in erythrocyte (RBC) count ($P<0.05$) and Hematocrit (HCT) ($P<0.05$) for the groups exposed to profenofos concerning the exposure time, and there was a significant decrease in Hemoglobin (Hb) ($P<0.05$), Hematocrit (HCT) ($P<0.05$) and Mean Corpuscular Volume (MCV) ($P<0.05$) for the groups exposed to profenofos concerning the concentration of pesticide. Histological results revealed that profenofos causes histopathological alterations such as cytoplasmic vacillation, swelling hepatocytes with pyknotic nuclei, and severe infiltration of erythrocytes in the livers of exposed fish. Small vacuoles, psychotic nuclei, glomerular Shrinkage, renal epithelium degeneration, and infiltration of erythrocytes were noticed in the exposed kidneys. Degeneration of gill epithelium in secondary lamellae and infiltration of leucocytes were noticed in the gills of exposed fish. The study revealed that profenofos is toxic to the genetically improved strain of *Oreochromis niloticus* and should be considered when used in agricultural fields close to natural freshwater bodies.

Keywords: *Oreochromis niloticus*; Profenofos; Toxicity; Hematological; Histological

INTRODUCTION

The Nile Tilapia, *Oreochromis niloticus* is a widespread species used in tropical aquaculture. Natural populations of these fish occur in Africa, and the species *Oreochromis niloticus* has been introduced to almost every tropical country in the world. Pesticides and fertilizers play a major role in agriculture and improve food production worldwide. But these pesticides are intentionally produced to kill and control pests which can also be toxic to other animals, plants, and humans. Aquatic ecosystems that flow through the agricultural lands have a great chance of being contaminated by groundwater leaching and runoff of different chemicals, including pesticides and fertilizers. The most common, highly effective pesticides can

Received:	26-July-2022	**Manuscript No:**	IPJAPT-22-14025
Editor assigned:	28-July-2022	**PreQC No:**	IPJAPT-22-14025 (PQ)
Reviewed:	12-August-2022	**QC No:**	IPJAPT-22-14025
Revised:	10-October-2022	**Manuscript No:**	IPJAPT-22-14025 (R)
Published:	17-October-2022	**DOI:**	10.21767/2581-804X.6.7.61

Corresponding author Dayananda NG, Department of Zoology, University of Jaffna, Jaffna, Sri Lanka; E-mail: ngdayananda23@gmail.com

Citation Dayananda NG, Surendran SN (2022) Hematological and Histological Studies to Determine the Toxicity of Profenofos on Oreochromis Niloticus (Genetically Improved). J Aquat Pollut Toxicol. 6:61.

enter the aquatic environment and cause multiple changes in organisms like fish by altering their growth rate, behavioral pattern, nutritional value, etc. Alterations in the chemical composition of the natural aquatic environment affect the behavioral and physiological systems of the aquatic organisms, particularly those of the fish. The study of hematological parameters in fish is important for evaluating their physiological status, immune system, and possible pathological changes. The number and size of red blood cells, hematocrit, and hemoglobin concentrations indicate the oxygen carrying capacity of blood together with white blood cells. These parameters are also indicators of toxicity and could be potentially employed for environmental monitoring and toxicity studies in aquatic animals. The study of histopathological changes is important in evaluating the health of fish exposed to contaminants, both in the laboratory and in field studies. Histopathological biomarkers examine specific target organs, including the gills and liver, responsible for vital functions, such as respiration, excretion and accumulation, and biotransformation of xenobiotic in the fish. The present study aims at evaluating the toxicity of profenofos on hematological and histological parameters of the genetically improved strain of *Oreochromis niloticus* [1-4].

MATERIALS AND METHODS

Chemicals

The organophosphate insecticide profenofos, with the commercial name Hayleys profenophos (A.I. profenofos, 500 g/l), was used, manufactured by "Hayleys agriculture holdings limited, Colombo 10, Sri Lanka.

Experimental Animals

Clinically healthy fingerlings with (2 g ± 0.5 g) average weight and (6 cm ± 0.5 cm) average length were collected from the National Aquaculture Development Authority (NAQDA) Kilinochchi station, Sri Lanka. The Nile tilapia fingerlings (*O. niloticus*) of both sexes were maintained in the animal house laboratory at the zoology department, faculty of science, university of Jaffna. Fish were fed with commercial fish food (5% body weight) twice a day and maintained at approximately 30 °C ± 1°C with a 12 h:12 h light-dark cycle in tanks (240 L) for 2 weeks to acclimatize to laboratory conditions before experiments. The water of each tank was replaced daily during the acclimatization period. The grown sub adults total body length and bodyweight were (14 ± 0.5 cm) and (71 g ± 0.5 g), respectively. The underground well water used to culture the fish was continuously aerated (pH 7.2; dissolved oxygen 26.4% saturation; temperature 25.73°C photoperiod 12 h:12 h light: dark) [5].

Estimation of 72 h LC_{50}

The fingerlings were starved for 24 hours before the experiment and divided into four triplicated groups at a density of 4 fish per aquarium (27 liter capacity). The first group was kept in control. The second, third and fourth groups were exposed to 0.2 mg L^{-1} $^{-1}$, 0.3 mg L^{-1} and, 0.4 mg L^{-1} of profenofos concentrations, respectively. All these aquaria were kept for 72 hours under the same aeration, temperature, pH, and photoperiod conditions as during the acclimatization period. Mortality was assessed at 24,48, and 72 hours and the dead individuals were removed from the aquaria immediately. 72 hours LC_{50} (Concentration at which 50% mortality occurred after 72 hours) was determined using the Microsoft excel 2013 software [6].

Examination of Haematological Parameters

The sub adults were divided into four triplicated groups at a density of 3 fish per aquarium (27 liters capacity). The control group they were subjected to 0.05 mg L^{-1}, 10 mg L^{-1}, and 0.15 mg L^{-1} of profenofos. Two fish from each exposure were randomly selected at the end of each week at a fixed sampling time. Blood was sampled from the posterior caudal vein (vena caudal) using a 29 gauge × 0.5 attached to a 1 mL syringe. Ethylenediaminetetraacetic Acid (EDTA) was used as an anticoagulant at a concentration of 1.5 mg per 1 ml of blood. Blood (1 ml) from two replicates of the same concentration was pooled and transferred into heparinized tubes to determine blood parameters. Erythrocyte or Red Blood Cell (RBC) count, Leukocyte (WBC) count, Hemoglobin (Hb) Concentration, Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and Platelet (Plt) count are the indices used to evaluate the hematological profile. They were determined immediately on fresh blood using an auto hematology analyzer [7].

Statistical Analysis

Statistical analysis of the obtained data was done using the Microsoft excel 2013 computer software program, using analysis of variance (One way ANOVA).

Histological and Histopathological Examination

Fish were exposed to profenofos under two concentrations (0.26 mg L^{-1} (LC_{50}), 0.10 mg L^{-1}) with the control setup for 7 days. Two replicates for each concentration were used, and each replicates contained two fish (male: female=1:1). Randomly selected fish were sacrificed from each concentration at the end of the 7th day. The liver, kidney, and gills were fixed with a proper saline solution. The fixed organs were embedded in paraffin wax, sectioned at 7 µm in thickness, and then stained with hematoxylin and eosin.

RESULTS

72 hrs LC_{50} Value

The 72 hrs LC_{50} value of profenofos (Hayleys Profenophos) for the genetically improved strain of *Oreochromis niloticus* was 0.26 mg L^{-1} (at a 95% confidence interval).

Haematological Parameters

The hematological parameters during exposure to Profenofos for 4 weeks are presented in **Table 1**. "P" values of the hematological parameters concerning the concentration, exposure period, and control are presented in **Table 2**.

Table 1: Effects of different concentrations of profenofos on blood parameters of *Oreochromis niloticus.*

Parameter	WBC (cells*10^9/L)				RBC (cells*10^12/L)				Hb (g/dL)				HCT%			
Treatment (mg/L)	Ctrl	0.05	0.1	0.15	Ctrl	0.05	0.1	0.15	Ctrl	0.05	0.1	0.15	Ctrl	0.05	0.1	0.15
Week 1	34.2	58.3	46.3	41.7	2.48	2.37	2.16	2.1	8.6	7.8	7.9	6.7	40.3	38.7	36.4	32.1
Week 2	34.4	61.3	62.5	43.7	2.21	2.12	1.87	1.74	7.4	6.8	7.2	6.1	35.7	32.4	27.9	23.5
Week 3	34	34.8	53.9	41.9	1.92	1.91	1.22	1.12	7.4	6.8	7.2	6.1	31.3	28.4	17.8	14.6
Week 4	40.2	42.3	35.4	44.6	2.23	1.98	1.72	1.64	8.6	7.5	6.9	5.8	36.3	29.1	25	21.3
Parameter	MCV (fL)				MCH (pg)				MCHC (g/dL)				Plt (Cells*10^9/L)			
Treatment (mg/L)	Ctrl	0.05	0.1	0.15	Ctrl	0.05	0.1	0.15	Ctrl	0.05	0.1	0.15	Ctrl	0.05	0.1	0.15
Week 1	162	154	150	135	34.6	32.9	36.5	31.9	21.3	20.1	21.7	20.8	53	54	39	47
Week 2	162	153	150	135	33.4	32	38.5	35	20.7	20.9	25.8	25.9	56	47	48	68
Week 3	163	149	146	130	35.8	34	50.8	53.5	22	22.8	34.8	41	52	24	57	53
Week 4	163	147	146	130	38.5	32.8	37.8	35.3	23.6	25.7	27.6	27.2	50	23	56	47

Table 2: Significance levels between hematological parameters with exposure time, the concentration of profenofos and control test.

"P" values	Parameters	WBC	RBC	Hb	HCT	MCV	MCH	MCHC	Plt
	Concentration	0.13 NS	0.08 NS	0.00 S	0.04 S	1.2E-08 S	0.29 NS	0.21 NS	0.13 NS
	Weeks of exposure	0.60 NS	0.02 S	0.41 NS	0.04 S	0.96 NS	0.10 NS	0.10 NS	0.63 NS
	Control	0.13 NS	0.08 NS	0.02 S	0.04 S	1.2E-08 S	0.29 NS	0.21 NS	0.13 NS

S=Significant at 0.05 level, NS=Not Significant at 0.05 level

There was a significant decrease in RBC count (P<0.05) and HCT (P<0.05) for the groups exposed to profenofos over the exposure time, and there was a significant decrease in Hb (P<0.05), HCT (P<0.05) and MCV (P<0.05) for the groups exposed to profenofos over the concentration of pesticide. Compared to the control setup, it showed a significant decrease in Hb (P<0.05), HCT (P<0.05), and MCV (P<0.05). But there is no marked significant difference in WBC count and platelets (P>0.05).

Histopathological Changes

Liver histopathology: The liver of the control fish exhibits Hepatocytes (H) with homogenous cytoplasm and large central or sub central spherical nuclei (**Figure 1)**. The hepatic parenchyma of fish exposed to Profenofos at a concentration of 0.10 mg L^{-1} for 7 days showed an increase in cytoplasmic Vacillation (V) and infiltration of leucocytes (I). Some swelling hepatocytes with Pyknotic nuclei (P) were observed. The fish exposed to 0.26 mg L^{-1} for 7 days showed an increase in cytoplasmic vacuolation, severe infiltration of erythrocytes, and leucocytes (I) with swelling of pyknotic nuclei (N).

Kidney histopathology: The kidney of the control group of *Oreochromis niloticus* was reported to be renal corpuscles with well expand glomeruli and a system of renal tubules, showing capillaries in the Glomerulus (G). The kidney of the control group showed well organized blood vessels and hematopoietic tissues. The kidney of fish exposed to 0.10 mg L^{-1} showed glomerulus distortion (D). Some small vacuoles and pyknotic nuclei (I) were noticed. Glomerular shrinkage, renal epithelium degeneration (D), pyknotic nuclei (N), and infiltration of erythrocytes (I) were observed in the liver of fish exposed to profenofos concentration of 0.26 mg L^{-1} for 7 days.

Gill histopathology: Primary and secondary lamellae were observed in the gills of fish under the control setup. The fish exposed to a concentration of 0.26 mg L^{-1} for 7 days showed degeneration of gill epithelium in secondary lamellae and infiltration of leukocytes.

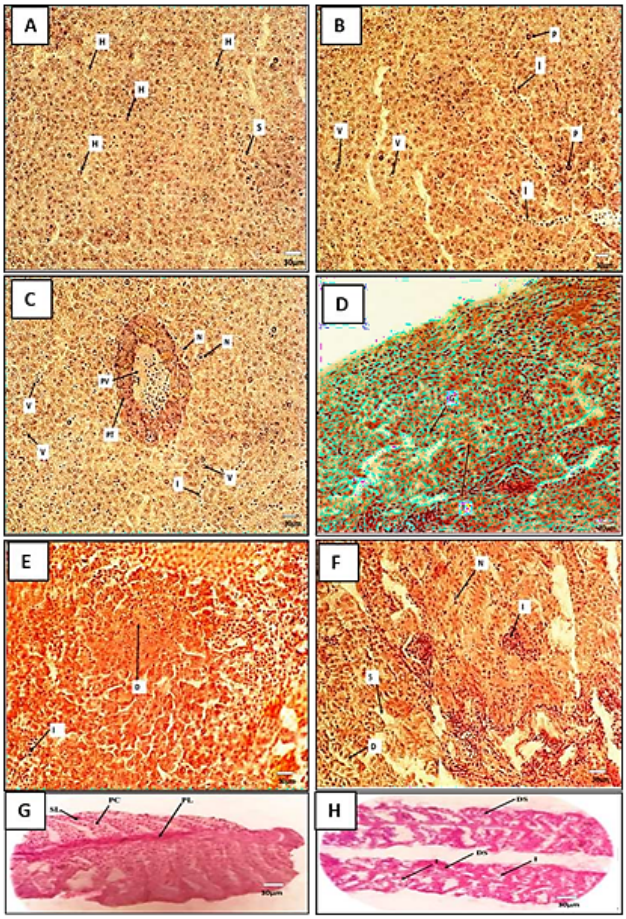

Figure 1: Light micrographs of a transverse section of *O.niloticus* liver (A,B,C), kidney (D,E,F), and gills (G,H). (A,D,G) control, (B,E) 0.10 mg L^{-1}, (C,F,H) 0.26 mg L^{-1}. Hepatocytes (H), Sinusoid (S), cytoplasmic Vacuolation (V), Infiltration of leucocytes/Erythrocytes (I), Pyknotic nuclei (P) swelling of hepatocytes with pyknotic Nuclei (N), Portal Vein (PV), Portal Tissue (PT), Glomerulus (G), Renal epithelium (T), Glomerulus distortion (D), Shrinkage of the glomerulus (S), Secondary lamella (SL), Pillar cells (PC), Primary lamella (PL), Degeneration of the epithelium of secondary lamellae (DS) hematoxylin and eosin x 400.

DISCUSSION

A reduction in the hematological values indicates anemia in the exposed fish due to erythropoiesis, chemosynthesis, osmoregulatory dysfunction, or erythrocyte destruction in hematopoietic organs. The decreased levels of red blood cell count and hemoglobin in the present study may be due to the disruptive action of the pesticide on erythropoietin tissue, which can affect the viability of cells. Red blood cell count can also be decreased due to the severely anemic state or haemolysing power of profenofos, particularly on the red blood cell membrane. The hemoglobin level can be decreased due to the rapid oxidation of hemoglobin to methemoglobin, or the release of oxygen radicals brought about by the toxic stress of profenofos, as xenobiotic are capable of undergoing redox cycling and can exert toxic effects *via* the generation of oxygen free radicals. These different leucocyte counts may

indicate the decreased non-specific immunity in fish. Profenofos can bind to the membranes of white blood cells in a similar manner where it binds with the cell membrane, and this binding may affect the function of white blood cells and may reduce the ability to engulf foreign substances. The changes in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) are more sensitive and can indicate the reversible changes in the homeostatic system of fish. The HCT value decreases when a fish loses appetite due to a disease or is poisoned by pesticides. The reduced HCT value indicates the anemia condition in fish. The most frequently encountered degenerative changes are hydropic degeneration, cloudy swelling, vacuolization, and focal necrosis. Pyknosis, Karyn orhexis, and karyolitic have been reported in cases of severe intoxication. The vacuolization of hepatocytes might indicate an imbalance between the synthesis rate of substances in the parenchymal cells and the rate of their release into the systemic circulation. The excretion of divalent ions is a major function of the renal tubular epithelium, and pollution with heavy metals or pesticides would highly likely affect these cells. The presence of hyaline droplets in renal tubules has been suggested to be an indicator of renal toxicity for various chemicals, including pesticides. Histopathological changes in gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm, and an increase in mucus secretion have been reported after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenols, and heavy metals. All these lesions may impair respiratory function. Filament cell proliferation and lamellar pavement cell hypertrophy reduce the inter lamellar space and cause a complete lamellar fusion reducing the total surface area for gas exchange. Otherwise, they increase the distance of the water blood barrier, which, together with epithelial lifting and the increase in mucus secretion, may drastically reduce the O_2 uptake and, if the damaging agent is not removed, can lead to the rupture of blood vessels with small hemorrhage focus.

CONCLUSION

The present study reveals that profenofos is moderately toxic to the genetically improved strain of *Oreochromis niloticus*. Significant hematological and histological alterations coupled with behavioral changes result from exposure to profenofos at low concentrations. These changes may be potentially disruptive to the survival of *Oreochromis niloticus* in their natural environments like lakes and reservoirs. So this aspect should be taken into consideration when using profenofos for controlling pests in agricultural fields close to natural freshwater bodies.

REFERENCES

1. Jenkins JA, Ourth DD (1993) Opsonic effect of the alternative complement pathway on channel catfish peripheral blood phagocytes. Vet Immunol Immunopathol. 39(4):447-459.
2. Jiraungkoorskul W, Upatham ES, Kruatrachue M, Sahaphong S, Vichasri Grams S, et al. (2002) Histopathological effects of Roundup, a glyphosate herbicide, on Nile tilapia (Oreochromis niloticus). Science Asia. 28(2):121-127.
3. Khan MZ, Law FC (2005) Adverse effects of pesticides and related chemicals on enzyme and hormone systems of fish, amphibians, and reptiles: a review. Proc Pakistan Acad Sci. 42(4):315-323.
4. Tavares-Dias M, Barcellos JFM (2017) Peripheral blood cells of the armored catfish *Hoplosternum littorale* Hancock, 1828: A morphological and cytochemical study. J Morphol Sci. 22(4):215-220.
5. Thompson S, Kruatrachue M, Upatham ES, Pokethitiyook P, Sahaphong S, et al. (2003) Histopathological alterations of white seabass, Lates calcarifer, in acute and subchronic cadmium exposure. Environ Pollu. 121(3):320.
6. Tripathi PK, Singh A (2002) Toxic effects of dimethoate and carbaryl pesticides on carbohydrate metabolism of freshwater snail Lymnaea acuminate. Bull Environ Contam Toxicol. 68(4):606-611.
7. Wester PW, Canton JH (1991) The usefulness of histopathology in aquatic toxicity studies. Comp Biochem Physiol C Comp Pharmacol Toxicol. 100(2):115-117.