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Effect of Acute Nominal Concentrations of Imidacloprid on Some Haematological Parameters of *Oreochromis niloticus* in Static Exposure for 96 hours

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Abstract

This study investigated the acute toxicity of Imidacloprid on *Oreochromis niloticus*. Juveniles of *Oreochromis niloticus* of average weight of (10.0 ± 05 g) and length of (5.0 ± .3 cm standard length) were exposed to acute and sublethal concentrations of Imidacloprid. A total of eighten transparent plastic tanks of 40 litre capacity containing 30 litre of dechlorinated tap water was used during the experiment. The plastic tank was stock with ten juveniles *Oreochromis niloticus* in triplicate during the acute toxicity test. The LC_{50} values was 64.6 under static bioassay. The acute test was for 96 hr static bioassay using varying concentrations was 0.00, 33, 50, 66, 83, and 100 mg/L of Imidacloprid on *Oreochromis niloticus*. Behavioural and morphological symptoms observed during the acute study which preceded mortality included vertical standing, air gulping, skin lesion, abnormal swimming were observed. The acute and sublethal exposure of *Oreochromis niloticus* to imidacloprid elicited significant changes in some haermatological parameters. The acute concentrations of Imidacloprid affected the behavior and haematology. Application of this chemical in the environment should be below the LC_{50} value (64.6 mg/L) obtained during the research.

Keywords: Haematology; Static bioassay; Imidacloprid; *Oreochromis niloticus*

Introduction

Fish is the cheapest animal protein source in Nigeria because of its availability, palatability and health provision [1]. Also remarked that fish is a heavily traded food commodity in the country and it is becoming the fastest growing agricultural item in international markets. However, fish populations in water bodies are susceptible to environmental impacts caused by the introduction of exotic species, industrial wastes, oil spills, pesticides and other agents that directly affect the ecology and the survival of species. Pesticides contain poisonous substances (toxicants) that distort water quality and impose physiological stress on biotic community of the water body which is the home of fish.

Pesticide usage has become a necessary evil in developing countries and has increased several-fold where agriculture is anticipated to be the backbone of the economy. During the past few decades, agrochemicals have been widely used in most agricultural sectors for enhancing crop yield and improving the quality of the product [2]. Consequently, extensive application of pesticides poses potential risks to the biodiversity of freshwater aquatic environments because of their bioaccumulation and intrinsic toxicity [3].

Recently, evaluation of the potential adverse effects of pesticide stress on sensitive non-target aquatic organisms in aquatic ecosystems has been the subject of worldwide concern [4]. One of the major dilemmas in environmental risk assessment is that pesticide contamination is frequently detected as a mixture of different substances rather than individual chemicals in the aquatic environment. The worldwide usage of pesticides is about 2 million tonnes/year; about 45% of pesticides are used in Europe, USA 25% and the rest of the world consumes 25%. Toxicologists consider pesticides to have become a necessary evil and like many discoveries or developments, people have been quick to reap their benefits, but extra slow to comprehend and deal with their negative consequences. These effects may be sometimes irreversible, and harmful to humans and the environment. Given that their properties differ, toxicity to fishes can vary with each pesticide group, with insecticides typically the most toxic [5].

Thus, toxicity tests or studies are essential for determining sensitivity of animals to toxicants and also useful for evaluating the degree of damage to target organs and the consequent physiological, biochemical and behavioral disorders.

Materials and Methods

Juveniles of *Oreochromis niloticus* of mixed sexes and fairly uniform size of (5.0 ± 0.3 cm standard length) and (10.0 ± 05 g) were obtained from malgwis fish farm Kofare Yola south of

Adamawa state and transported using 50 litr jerrican to the laboratory department of fisheries, Modibbo Adama university of technology Yola. In the laborotory, the water from the farm was gradually replaced with dechlorinated water (bore hole water).

In the laboratory, the test organism (*Nile tilapia*) was kept for 14 days in plastic tanks to enable them acclimatize to the laboratory conditions. During this period, they were fed twice a day (9:00 am and 5:00 pm). With commercial fish feed (coppens) (2 mm) and the water was changed when polluted. Feeding stop 24 hours prior to the commencement of the experiment.

Pilot studies

Pilot studies, as range finding test were conducted to determine the actual concentrations of imidacloprid used for the definitive test. This was done by introducing one nominal concentration into one litre of water (stock solution) 5 mls were drown from the stock solution and introduced to 15 litres of water plastic tank in duplicate. Ten fishes were introduced in to each test tanks and no mortality were observed after 12,24,48,72 and 96 hours.

Then later stock solution was discarded. Two nominal concentrations were introduced into two transparent plastic tanks containing 30 litres of water in duplicate. Ten fish each were introduced in to each test tank and mortality of fish observed at 12,24,48,72 and 96 hours. When the fish died in all the test tanks, lower range of concentrations were prepared until when 80 to 90% of the fish died in the highest concentration test tank and 20 to 30% died in the lowest concentration test tank. The six nominal concentrations were then range between the highest and the lowest concentrations geometrically for proper experimentation.

Experimental design

The experiment was having six treatments and each treatment were triplicated including the control and the concentration of toxicant applied were determined after the pilot study.

Eighteen transparent plastic tanks of 40 l capacity containing 30 l dechlorinated tap water were used during the experiment.

Each treatment and its replicate were stocked with 10 freshwater fish (*Oreochromis niloticus*).

Acute toxicity studies

Acute 96 h static bioassays were conducted in the laboratory as described by Sprague 1973, APHA 1985, and OCED Guide 2010 [6,7]. To determine the toxicity of Imidacloprid to O*reochromis niloticus*. A total of one hundred and eighty (180) were used. Eighteen (18) transparent plastic tanks of 40 l capacity containing 30 l of dechlorinated tap water were used. Also, the physico-chemical parameters of the diluting water such as temperature, pH, dissolved oxygen, and alkalinity, were measured during the acute test as described by APHA. The Imidacloprid concentrations were measured and introduced into dechlorinated water in the plastic bowls. The mixtures were allowed to stand for 30 minutes before introducing test organisms *Oreochromis niloticus*. Thereafter the containers were stocked with 10 fish per plastic tank for the experimental run.

Haemotological study

Blood sampling: Blood sampled as described by Blaxhall and Daisely 1973. Blood were collected by severance of caudal peduncle from the caudal artery, the caudal region was cut 2 cm away and blood samples were collected using pipette and the blood taken was immediately took for laboratory analysis [8].

Total erythrocyte count: Hendricks solution were used for the erythrocyte count. Blood were drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool and adjusted the volume to exactly 0.5 marks.

The pipette was filled to 101 mark with the diluting fluid and shaken for 30 minutes to ensure thorough mixings the dilutes suspension of cells were thereafter draw in the Neubaueris chamber haemocytometer. The hemoglobin was placed on the microscope and cells within the boundaries of five small of the haemocytometer was counted using eyepiece microscope, the number of cells will be multiplied by X 10 and this was giving the total number of cells per cubic millimeter (mm) of blood.

Total leucocyte count: Leucocytes was counted by using Shaw's solutions A neutral red sodium chloride (0.9 g), distilled water (100 mls) and 13 crystal violet (12 mg), sodium citrate (3.8 g), distilled water (100 mls) The blood were drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A were drawn to shake the bulb of the pipette half way and then filled to 101 marks with solution B. A (few drops will be dispensed into the haernocytometer. The cells in the four large squares of the chamber was counted with 4 m Objective and X 40 eyepiece microscope. The number of cells were multiplied by 500 to obtain the total number of leucocytes per* cubic millimeter (mm) of blood.

Haematocrit (Packed cells volume): Determination of packed cells volume was carried out by micro-western method as described by Blaxhall and Daisely. The well mixed sampled blood from the severed caudal peduncle were drawn into micro-haematocrit tube (75 mm long, and 1.1-1.2 mm internal diameter). The tubes were then centrifuged for five minutes. This were taken with the aid of a micro-haematocrit reader and expressed as the volume of erythrocytes per 100 cm^3 [9].

Mean corpuseular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration

The absolute values Made up Corpuscular Haemoglobin Concentrations (MCHC), Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated from the results obtained for RBC, haernoglobin Dacie and Lewis.

Formular.

MCV (m^3) =Ht% × 10

RBC (Cells mm^3)

MCH (Pg cell)= ~ Hb (g/100 ml) × 10

RBC (Cells mm^3)

MCHC (g/100ml) = Hb (g/100 ml) × 10

RBC (Cells mm^3)

MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; Ht: Haemoglociit; Hb: Heamoglobin; RBC: Red Blood Cells; MCHC: Mean Corpuscular Haemoglobin Concentration.

Leucocyte differential count

Two drops of blood were placed on a slide, made into a thin smear with another slide and left dried.

The smear was fixed with absolute methanol, and then stained with giemsa's stain and 170 buffer distill water. It was allowed to stand for about 20-30 minutes after which the slide was rinsed again with buffer distilled water and allow to dried counting was made by used of microscope.

Statistical analysis

The experimental results were analyzed using one-way Analysis Of Variance (ANOVA) at α=0.05 level of significance. The differences among the treatment's means were determined by Duncan multiple range test.

Results

Result of the static bioassay on haematological parameters of fish exposed to acute nominal doses of Imidacloprid are presented in the Table 1 Red Blood Cell Counts (RBCC), White Blood Cell (WBCC), Haemoglobin (Hb), and Packed Cell Volume (PCV) of the exposed fish decreased with increase in concentrations except in concentration 33 mg/l where the WBC value were not significantly different when compare to the control. Fish exposed to acute nominal dose ≥ 4.3 mg/l had significantly lower ($p<0.05$) values of Red Blood Cell Caunts (RBCC) than the control fish. In addition, WBCC of the control fish were not significantly higher ($p<0.05$) than those exposed to ≤ 50 mg/L concentrations of the toxicant but not significant with those exposed to 33 mg/l concentration of the same toxicant. Haemoglobin values of fish exposed to various nominal concentrations were significantly lower ($p<0.05$) when compared to the control group [10].

Fish in the control group had MCH values significantly lower ($p<0.05$) than those exposed to ≥ 33 mg/l concentrations of Imidacloprid, but higher significantly than those exposed 33 mg/L concentrations of the toxicant. Mean Corpuscular Haemoglobin (MCHC) values of the control were significantly lower ($p<0.05$) than the fish exposed to 33, 83, and 100 mg/l concentrations, but not significant with fish exposed to 50 and 66 mg/l of the same toxicant. However, the values of MCHC of the exposed group differ significantly from the control group as shown in the Table 1 below.

Table 1: Shows the effect of acute nominal concentrations of Imidacloprid on some haematological parameters of *Oreochromis niloticus* in static exposure for 96 hours.

Conc. (mg/L)	RBC (cells mm^3)	WBC (cells 10^9)	PCV (%)	Hb (cells10^9)	MCV (m^3)	MCHC (g/100 m)
0	3.93 ± 0.21^a	10.33 ± 0.29^a	29.88 ± 0.41^a	10.33 ± 0.13^a	81.23 ± 0.90^a	19.17 ± 0.13^a
33	3.37 ± 0.39^b	10.07 ± 0.15^a	28.84 ± 0.38^b	7.53 ± 0.21^b	91.73 ± 0.87^b	20.77 ± 0.45^b
50	2.07 ± 0.05^c	9.57 ± 0.34^b	28.34 ± 0.30^c	6.09 ± 0.08^b	99.07 ± 1.60^c	22.00 ± 0.08^c
66	1.89 ± 0.17^d	9.27 ± 0.17^b	$27.67 \pm 0.26c$	5.20 ± 0.15^c	110.5 ± 0.85^s	21.37 ± 0.41^c
83	1.77 ± 0.05^d	9.27 ± 0.31^b	26.57 ± 0.26^c	4.67 ± 0.13^c	109.97 ± 9.99^e	22.53 ± 0.31^b
100	1.1 ± 0.08^d	8.33 ± 0.26^c	22.83 ± 0.41^c	3.7 ± 0.36^d	115.6 ± 12.04^e	23.53 ± 0.31^d
Note: Mean values along the columns with different superscript are statistically different at ($p<0.05$)						

Leucocyte differential counts of fish exposed to acute concentrations of Imidacloprid shown in Table 2. Neutrophil percentages were higher and dose dependent with the expoed fish compare to the control. Percentages of neutrophils of the control fish were decreased significantly ($p<0.05$) compared to the fish exposed to acute concentrations of Imidacloprid. While percentages of lymphocyte of the control fish were increased significantly ($p<0.05$) compared to fish exposed to acute concentrations of Imidacloprid. Lymphocytes of the exposed fish were significantly lower ($p<0.05$) compared to the control group. Basophils, eosinophils, and monocytes were not observed [11].

Table 2: Shows the leucocyte differencial counts of *Oreochromis niloticus* exposed to acute concentrations of Imidacloprid in static bioassay.

Conc. (mg/L)	Neutrophils (%)	Lympocytes (%)
0	14.17 ± 0.21^{a}	41.01 ± 0.09^{a}
33	15.4 ± 0. 37^{b}	40.27 ± 0.31^{a}
50	15.8 ± 0. 08^{c}	39.23 ± 0.34^{b}
66	16.5 ± 0. 15^{c}	38.4 ± 0.22^{b}
83	18.44 ± 0.21^{d}	37 ± 0.85^{c}
100	19.63 ± 0.77^{e}	36.63 ± 0.31^{c}
Note: Mean values along the columns with different superscript are statistically different at ($p<0.05$).		

Discussion

The use of this pesticide above the acute concentrations (64.6 mg/l) may be lethargic to aquatic organisms as such should be avoided, however the use of this insecticide at lethal concentration resulted to haemological alteration which lead to reduction in percentage of Haemoglobin (HB), White Blood Cell (WBC), Red Blood Cell (RBC), and Packed Cell Volume (PCV) as compared to the control ($P<0.05$), indicating the anaemic condition in the exposed fish and the anaemic effect could be attributred to destruction or inhibition in RBC production and as well these could be due to haemodilution resulting from impaired osmoregulation across the gill epithelium and also leading other types of anaemia example, poikilocythemic,and micocytic.Similar findings were reported after exposure of Clarias albopunctatus to acute and sublethal actellic concentrations by Mbenga et al.

Results of this research however revealed a significant and dose dependent increase of neutrophils in all populations of the exposed groups it suggested that the exposure of this chemical had a systemic effect on the bodys immune response. Neutrophils are the type of white blood cells that play a crucial role in the body defence agains infection,particularly bacterial infection. They are usually the cells to arrive at the site of infection or injury. In addition an increase in neutrophil is an indication of ongoing infection or inflammatory response similar was by Walter et al., 2022, Oluah et al. 2020.

Conclusion

The fishes subjected to toxicant exhibited toxicosis symptoms which include loss of equilibrium, agitated movement, air gulping, weakness and change in skin coloration. In addition to this a significant increased and decreased in some blood cells were also observed during the experiment.

Recommendation

- The results of these findings suggest the following recommendations.
- When this chemical most be used, sub lethal concentration should be encouraging in order to reduced it adverse effect on both the target and non-target organisms.
- Finally, the manufacturing industry should pay more attention in manufacturing less harmful substances.

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