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COMPARATIVE EVALUATION OF BIOCHEMICAL, OXIDATIVE STRESS AND HAEMATOLOGICAL INDICES OF *Clarias gariepinus*(BURCHELL, 1822) JUVENILES EXPOSED TO PROPANIL

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Abstract

Propanil is a selective contact herbicide. The present study was designed to evaluate and compare the sublethal toxicity of propanil pesticide and assess their effects on behavioural,mortality,biochemical and haematological changes in *Clarias gariepinus* juveniles.The 24,48,72 and 96hLC_{50} of propanil were 0.49,0.25,0.24 and 0.21mg/l. The safe levels estimated varied from 0.21x 10^{-1} to 0.29 x 10^{-5} mg/l in propanil. Behavioural changes, such as hyperactivity, gulping of air, skin discoloration, erratic swimming and mucus secretion were observed in fish exposed to both pesticides at 1,5,10 and 15 days. Therefore, the use of these pesticides in both terrestrial and aquatic ecosystems should be subject to strict control and monitoring to prevent potential ecotoxicological hazards to aquatic lives.

Introduction

Propanil (3, 4-dichloropropioanilide) is a selective contact herbicide used to control barnyard grass or a broad spectrum herbicide (Kellogg et al. 2000). According to Manson (2000), it is a selective broad spectrum herbicide used for the control of weeds in rice field. It decomposes within a short period after treatment which allows crops to be grown on the land in future seasons. According to Kellongg *et al* (2000), it hydrolyses in extremely acidic or basic condition. However, it is stable under normal temperature and pressure and may pose a slight fire hazard if exposed to heat or flame. It also poses a fire and explosion in the presence of strong oxidizers. Furthermore, the thermal decomposition of propanil will release toxic oxides of Nitrogen, carbon and corrosive fumes of chlorides African catfish (Clarias gariepinus) is one of the most important fish species currently being cultured both inside and outside its natural range of tropical and sub-tropical environment (Adewolu et al ,2008). It is of great commercial importance because it is the most common fresh water fish widely consumed in Nigeria and it also has the ability to grow fast and feed on a large variety of agricultural bye products, this fish species has a general characteristics of being hardy, tolerates adverse water quality conditions and can be raised in high densities, resulting in high yields (6-16t/ha/year) (Ansara-Ross *et.al*,2012). Found throughout Africa and Middle East, the African sharp tooth catfish live in fresh water lakes, rivers and swamps as well as human-made habitats, such as oxidation ponds or even urban sewage systems. Furthermore, the African sharp tooth catfish was introduced all over the world in the early 1980s for agricultural purposes and so is found in countries far

outside its natural habitat, such as Brazil, Vietnam, Indonesia and India (Adewole *et al* ,2008). *C.gariepinus* has an average adult length of 1-1.5m (3ft.3 in 4ft 11 in) (Annune *et al*, 1994). It reaches a maximum length of 1.7m (5ft 7in) and can weigh up to 60kg (130ib).Spawning in catfish mostly takes place at night in the shallow, inundated areas of the rivers, lakes and streams. These characteristics aid in the understanding and knowledge of the use of *C.gariepinus* as bio-indicator of environmental pollution and a good biomarker of aquatic pollution respectively. In addition to its important source of protein, it typically has rich contents of essential minerals, vitamins and unsaturated fatty acids (Agbohessi *et al* 2014).
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Research Methodology

Juveniles of African catfish, *C. gariepinus* (family: claridae, order: siluriformes) of average weight of 200.15$\pm$6.5 gariepinus and length of 20.8$\pm$1.5cm were purchased a from a reputable fish farm namely : Rogery Tourist Game village, idemili L.G.A of Anambra state and transported to Heldin's fisheries unit, old Airport Road, tinkers corner Emene, Enugu state. The juveniles were first acclimatized to laboratory conditions for a period of two weeks in a semi-static system in 1000 liters capacity tank before being transferred to ten litres tap water held in 90litres capacity aquarium bowls before commencement of the experiments. The fish were fed with 6mm coppens® fish feed for aquaculture (coppens international bv.5700 Am Helmond, Holland) according to the size and weight of the fish. Faecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. Dead fish were immediately removed with forceps to prevent deterioration of water quality. Feeding of the fish was terminated 24h before the commencement of the experiment as recommended by Ward and Parriah (1982) and Reish and Oshida (1987). Ethical clearance from the Ministry of Agriculture, department of fisheries management committee on Experimental Animal care (MANR/FD/2017/EC103) was obtained and strictly followed. The physicochemical properties of test water were analysed using standard methods (APHA, AWWA, WPCF 2005) were as follows: temperature 26.74$\pm$1.42^{0}C, Dissolved oxygen 7.82$\pm$0.14mgl^{-1}, P^{H}7.14$\pm$0.30. In the present study, commercial formulations of propanil (trade-named Arrosol, Bay 30130, Hubei

sanonda co., Ltd. Jingzhou, china containing $1000gl^{-1}$ propanil as an active ingredient) and endosulfan (trade-named Endosol, FMC 5462, Hubei sanonda co Ltd. Jingzlou, china containing $1000gl^{-1}$ endosulfan an active ingredient) were used as the stock solutions.

Range Finding test (mortality table)

Six different propanil concentrations (0.29, 0.43, 0.50, 0.71, 0.85, and 0.94mg/l) were selected for definitive exposure after a series of range findings. A set of 10 specimens of *C. gariepinus* juvenile were randomly exposed to each of the selected concentrations in 10L plastic aquaria and set in triplicates. Simultaneously, another set of 10 fish specimens was maintained as control in triplicate under the same conditions but contained only tap water. The test solution was renewed on alternate days in a flow through method to maintain the concentration of the chemicals. The behavioral responses in fish exposed to propanil and in control fish were observed and recorded. The mortality and survival values were recorded every 24h for a 96h exposure period.

Sub-lethal concentrations

Two different concentrations of propanil (0.05, $0.09mg^{-1}$) were made with three replicates and three control, having a total of 30 fish samples per concentration.
Haematological and biochemical analysis (Total protein and Glucose)
analyses were made for day 1,5,10 and 15 of the experimental period.

Biochemical Analysis of ALT,ALP,AST,Total Protein and Glucose Concentration

The second portion of the collected blood sample was dispensed into non-heparinised tubes. This was then centrifuged at 1,006xg for five minutes to obtain the serum. Serum glucose concentrations were estimated based on glucose oxidase method as described by Morgan and Iwana (1997). For serum total protein concentration were estimated based on Biuret method as described by Amer and Wolfson *et al* (1948) and Henry et al (1974).

TOTAL PROTEIN DETERMINATION

Principle: The (=C=O) and the (=N=H) of the peptide bonds of proteins react with cupric ions in moderately alkaline medium to form coloured chelate complex whose intensity is propotional to the concentration of proteins present in the sample.
Normal values: 6.2 to 8.0gm/dL.
Sample materials:serum and plasma
Reagents:Biuret reagent
Cupric sulphate 12mmol/L
Rochella salt 32mmol/L
Potassium iodide 6mmol/L
Sodium hydroxide 0.2N
Procedure:Wavelength:550nm(530 to 570nm)
Cuvette :1cm light path
Temperature:Room temperature
Measurement:Against reagent blank (RB)
Pipette into tubes
Allow to stand to stand at room temperature for at least 20minutes.Read the absorbance (A) of S and T against RB in calorimeter.
Calculation of protein: A (T)/A(S) X 6.0gm/Dl

Determination of Oxidative stress of *C. gariepinus*

Muscle samples were collected at day 1,5,10 and 15.The muscle samples collected were analysed for changes in catalase activity(K),Superoxide Dismutase (SOD) and lipid peroxidation,condition factor(CF) and Hepatosomatic indices (HSI) were also analysed.

Determination of Catalase Activity

Catalase activity was determined according to sinha(1972) 5g of potassium was dissolved in 100ml of distilled water, out of which 10ml was taken. The 10ml was added to 30ml of acetic acid out of which 2ml was put into as many test tubes as possible. Hydrogen peroxide was prepared 0.9ml of phosphate buffer at P^H of 7.4 was added into test tubes containing 0.1ml of sample each.2ml of potassium dichromate acetic acid solution was added after 0.2ml hydrogen peroxide(H_2O_2) and absorbence at 530nm for 15s,30s,60s,90s and 120s against the blank (H_2O)

Catalase activity (K) ={2.303/DTXLog A_1/A_1A_2}

Where A_1=initial absorbance.A_2=final absorbance (sinha, 1972).

Determination of Lipid peroxide (LPO)

Method:Wallen *et al*(1993) by estimation of thiobarbit uric acid reactive substances(TBARS)
Procedure: Add 0.5ml of sample, add 0.5ml of 10% TCA (Trichioroacetic acid, add 0.5ml TBA in the 10%TCA. Boil for 30 minutes and cool.
Prepare the blank as follows:Add 1ml of distilled water,0.5ml of TCA,0.5ml of TBA,boil for 30mins and cool.Add 1ml of distilled water and centrifuge for 30mins.Read the absorbance at 532nm and 600nm against the blank.
Calculation: LPO={532_600/0,066X 2X10}mg/100g

Determination of superoxide dismutase (SOD)

Superoxidase dismutase was determined according to Musa (1972)
Procedure: Prepare the blank as follows: Add 0.5ml of bicarbonate, then add 0.5ml of EDTA. Add 1ml of H_2O.Incubate for 5mins at room temperature.
Prepare the Test as follows: Add 0.5ml of bicarbonate buffer, then add 0.5ml of EDTA. Add 0.5ml of EDTA. Add 50ul of sample, add 1ml of distilled water and then incubate at room temperature for 3mins.Absorbance was read at 480nm for 3mins and records taken. (Misra, 1972).

Haematological Analysis

Blood samples were collected via caudal vein puncture as described by Kori-Siakpere *et al* (2005). Fish was held in a slanting position to collect the blood with the ventral part facing the researcher. Blood samples were collected with the EDTA Tube containing EDTA solution to prevent coagulation of blood. EDTA tubes for the various concentrations of propanil were dispensed into capillary tubes for haematological analysis.

White Blood Cell Count

380µl of tusk solution was added into the test tubes. Then, 20µl of the blood sample was added in each tube and mixed according to Russia and Sood (1992). After about 2 minutes, visualize under a low (10x) objective microscope and the cells are counted

Red Blood Cell Count

4mls of RBC fluid was added into the test tube and 20µl of blood sample was added in each tube and mixed. It was allowed to stand for 3 mins. and then visualized under a low (10x) objective microscope, cells were counted using counting chamber and cover slit and then converted to 10^6 as recommended by Russia and Sood (1992).

sPart Cell Volume Count (P.C.V)

Blood samples were collected using the capillary tube and sealed at one end with plasticine. Using the centrifuge, it was allowed to spin for 5 minutes after which the haematocrit reader was used to measure the blood plasma/serum percentage as described Hesser (1960).
Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values were estimated as follows (stockham and scott 2002).

$$\text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC}} \times 10$$

$$\text{MCH (pg)} = \frac{\text{Hb}}{\text{RBC}} \times 10$$

$$\text{MCHC (g/dl)} = \frac{\text{Hb}}{\text{PCV}} \times 100$$

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Statistical Analysis

The data obtained were analysed using the statistical package SPSS17.0 computer program (SPSS Inc. chicago, IL, USA).The data were subjected to one way analysis of variance and Duncan's multiple range tests to determine the significance difference at the 5% probability level.

RESULT

Fish exposed to different acute concentration of propanil displayed behavioural and physiological abnormalities such as air gulping,loss of equilibrium,erratic movement.Increase in exposure period resulted in exhaustion,weakness and general decrease in in fiah responses.Mortality during acute exposure in the treatment groups increased with increasing concentration and duration of exposure.It was found that at 0.05 and 0.09mg/l of propanil had lower toxicity.Furthermore,total protein concentration of endosulfan at 0.35mg/l showed to be 21 as compared to the control which showed to be 3 at 15 day period.Also,the result showed that there was a significant increase in the value of Alkaline phosphatase and Alanine transaminase as compared to the control.There was a significant decrease in the values of Superoxide Dismutase concentration at 0.05mg/l as compared to control.Also,Haematological parameters showed a significant increase in the value of packed Cell Volume,Red Blood Cell,White Blood Cell and Mean Cellular Volume.

CONCLUSION

This present study also observed that the higher the concentration of the toxicant, the higher the mortality rate. The analysis of data from the present study evidenced that propanil are toxic and had profound impact on behavior, biochemistry and haematology of *C. gariepinus* in sublethal concentrations. From this present results, it can be concluded that exposure of *C. gariepinus* to different doses of propanil and endosulfan for different duration times caused oxidative stress, morphological abnormalities in *C.gariepinus.*(Agbohessi et al,2014 andAbalaka,2013)

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