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HISTOPATHOLOGICAL AND HAEMATOLOGICAL EFFECT OF ACUTE TOXICITY OF CYPERMETHRIN ON *Clarias gariepinus* JUVENILE.

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ABSTRACT

The experiment was conducted to evaluate the acute toxic of cypermethrin, a synthetic pyrethroids on juveniles of *Clarias gariepinus*. The effect was assessed based on the comparism results of haematology and histopathological tissues examinations of control and experimental group exposed to five nominal concentration of cypavest, 10EC Pesticide Preparation (active substance 100mg/l) of cypermethrin in a static non- renewal bioassay for 96hours. The 96hrs LC_{50} value of the exposed juveniles was found to be 0.062 mg/l. Fish exhibited progressive loss of balance, respiratory distress, erratic movement and death. Examination of haematology significantly showed higher value ($P < 0.001$; 0.01) of white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), packed cell volume (PCV), monophilis and heterophilis; a significant reduction ($P < 0.001$, 0.01) in Red Blood Cell (RBC) and Lymphocyte was obtained as compared to the control. Severe necrosis, haemorrhages vacuolation, congestion and focal interstitial haemorrhages were the histopathological changes in the tissues of gill and liver, thus concluding that cypermethrin is toxic to juveniles of *Clarias gariepinus*.

Key word: Cypermethrin, cypavest, synthetic pyrethroid, static-non renewal and bioassay

INTRODUCTION

Water is undoubtedly the most precious natural resource that exists on our planet. It comprises over 70% of the earth surface (Terry, 1996).Water pollution is any chemical, physical or biological changes in the quality of water that has a harmful effect on any living thing that drinks, uses or lives in (Lenntech, 1998). However, there is overwhelming evidence that agricultural use of pesticides has a major impact on water quality and leads to serious environmental consequences.Although pesticide use is low to nothing in traditional and subsistence farming in Asia and Africa, environmental public health and water quality impacts of inappropriate and excessive use of pesticide are widely due to economic factor.Pesticides are one group of toxic compounds linked to human use that have a profound effect on aquatic life and water quality The amount of pesticides that migrates from the intended application area is influenced by particular chemical properties; its propensity for building to soil, its vapour pressure, its water solubility and resistance to being broken down over time, (Kellogg *et al.,* 2000). Factors in the soil such as its texture, its ability to retain water and the amount of organic matter contained in it also affect the amount of pesticides that will leave the area.(Kellogg et al, 2000). Some pesticides contribute to global warming and depletion of the ozone layer (Reynolds, 1997). Pesticides impacts on aquatic system are often studied using a hydrology transport to study movement and fate of chemical in rivers and streams.The use of pesticides has increased considerably to reduce the damage cause by pests to standing crop. Among these pesticides, the synthetic pyrethroids are commonly used because of their rapid biodegradability and non-persistence nature. These compounds, which frequently enter the aquatic ecosystem through agricultural run-off and spraying operations adversely affect non-target animal such as fish (Murphy, 1996 and Singh *et al,* 2003). Pyrethroids are used preferably over organochlorine, organophosphorous and carbonate due to their high effectiveness, low toxicity to birds and mammals and easy biodegradability (Kale *et. al*, 1999).Therefore, the objective of this paper is to examine the effect of acute concentration of Cypermethrin on histopathology and haematology of Claris gariepinus juvenile in a static system

MATERIALS AND METHODS

One hundred and eighty healthy juveniles of *Clarias gariepinus* of the same cohort with average weight (16.62±4.36)g, standard length (12.64±1.03) cm and total length (14.97±8.94) cm were sourced from the hatchery unit of the Federal college of Freshwater Fisheries Technology, Baga Maiduguri, Borno State. They were acclimatized for seven (7) days during which they were fed 5% of their body weight with commercial Coppens (2mm). Feeding was stopped 24hrs prior to the commencement of the toxicity test experiment.A preliminary range finding test was carried out based on the concentration of the active ingredient in the test chemical. The range finding was done using the following concentrations; 0.1mg/l,10mg/l and100mg/l of Cypermethrin for 24hrs in triplicates. The concentration was done using a serial dilution formula $C_1V_1 = C_2V_2$.The result obtained from the range finding test provided a guide for the definitive test. Following this, the definitive test was carried out using; 0.025mg/l, 0.050mg/l, 0.075mg/l, 0.100mg/l, 0.125mg/l and 0.000mg/l of Cypermethrin. The result obtained was used to determine the median lethal concentration (Lc_{50}) using Probit analysis.A total of eighteen (18) glass aquaria were used for the definitive toxicity test. Ten juveniles of *Clarias gariepinus* were introduced into each aquarium with 20 litres of water with; 0.025mg/l, 0.050mg/l, 0.075mg/l, 0.100mg/l, 0.125mg/l and 0.000mg/l concentration of

Cypermethrin at the same time. Each of the toxicant concentration was replicated three times each.The histology of the gills and liver of *Clarias gariepinus* was carried out after 96hours exposure period to the various concentration of the toxicant (Cypermethrin).Organs were collected and fixed in 10% formal saline for one week. They were then processed for routine paraffin histological sectioning. The tissues were dehydrated through graded concentration of ethanol (70%, 90%, absolute ethanol and cleared in xylene. The tissues were pre-impregnated in xylene paraffin wax in the oven and embedded in pure paraffin wax. The organs were sectioned at 7μm thickness and tissues were stained with Haematoxylin and Eosin (H&E) for light microscopic examinations (Drury *et al.* 1975).Blood samples were collected from the caudal vein of each fish per treatment into a heparine bottles and transported in ice packed to the laboratory unit of Biological department, Ahmadu Bello university, Zaria for the analysis of red blood cells count (RBC), white blood cell counts (WBC), packed cell volume (PCV), haemoglobin concentration (Hb), differential leucocytes count (DLC) and mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were determined by calculation.

RESULTS AND DISCUSSION.

During exposure, the test fish exhibited the following behavioral patterns before death occurred; restlessness, respiratory distress, loss of balance, gasping for air, vertical movement, excessive accumulation of mucus and death. The reaction to the toxicant was more pronounced in the aquaria containing the highest concentrations of the toxicant. The stressful behaviour exhibited by the fish may be attributed to the effects of the toxicant on the gill. This was clearly noted from the result of histopathological examination of the gill. The and liver observed increase in erratic swimming, instability and subsequent immobilization before death which is directly proportional to the concentration of the toxicant could be attributed to the respiratory impairment of the gills filaments by the insecticide.Several authors have reported work on similar patterns of abnormal behavioural responses in fish exposed to toxicant (Wade, *et al.,* 2002, Oti, 2002) The result obtained from this research work showed that the 96 hour LC_{50} was determined to be 0.062mg/l thus giving an indication that cypermethrin a synthetic pyrethriod is highly toxic to *Clarias gariepinus*juveniles.This result was in line with the findings of Ayoola and Ajani(2008). These authors worked on the toxicity of Cypermethrin on juveniles of *Clarias gariepinus* but were much lower than the findings of Aguigwo (2002) who studied the toxicity of cymbush pesticides on growth and survival of *Clarias gariepinus*. The result obtained for water quality parameter were observed to be to be within the tolerable limits for toxicity test and were not adversely affected by toxicant. The histopathology observation on the gill and liver showed Varied morphological changes occurred in the gill and liver tissue of fish in the aquaria treated with cypermethrin pesticide.Light microscopic studies showed that the morphologic changes were more evidence in the liver of exposed fish as changes were not observed in the control fish. The liver of the exposed fish compared to the control showed varying degree of degeneration of cell, hypertrophy of hepatocytes, Fatty degeneration, vascular channel congestion and vascuolization of cell cytoplasm; these alterations were dose-dependent

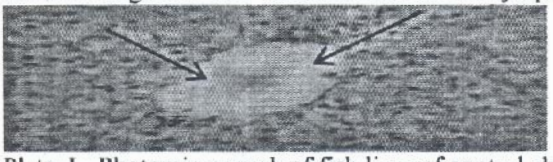

Plate. I. Photomicrograph of fish liver of control showing normal hepatocytes (arrows), radiating away from central vein (CV) and sinusoids (arrow head) H&E x400

Plate II. Photomicrograph of fish liver treated with 0.025mg/L of Cypermethrin showing wide spread sinusoidal haemorrhages (S) and mild areas of coagulative necrosis (CN) H&E x400.

Plate:III Photomicrograph of fish liver treated with 0.025mg/L of Cypermethrin showing coagulative necrosis (CN) and vacuolation (arrows) H&E x400.

Plate : IV .Photomicrograph of fish liver treated with 0.050mg/L of Cypermethrin showing enlarged vascular channel (arrow), congestion (CG) and cloudy swelling hepatocytes (CW) H&E x400.

Plate :V Photomicrograph of fish liver treated with 0.75mg/L of Cypermethrin showing wide spread fatty tissues H&E x400.

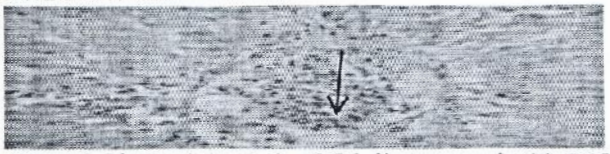

Plate.IV. Photomicrograph of fish liver treated with 0.10mg/L of Cypermetrin showing enlarged vascular channel (arrow) and cloudy swelling (CW) H&E x400

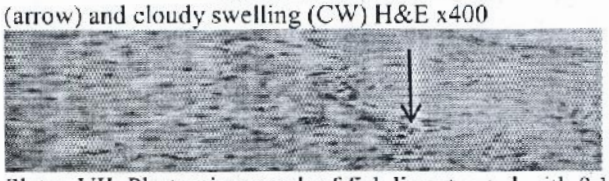

Plate:. VII Photomicrograph of fish liver treated with 0.125mg/L of Cypermethrin showing thick fibrous connective tissue (FB), inflammatory cells (arrows) and vacuolation (arrows) H&E x400.

Plate:VIII. Photomicrograph of control fish gills showing normal gills H&E x400

Plate :IX Photomicrograph of fish gills treated with 0.025mg/L of Cypermethrin showing gill necrosis (GN), congestion (CG), cartilage (CT) and interstitial haemorrhage (arrows) H&E x40.

Plate: X. Photomicrograph of fish gills treated with 0.050mg/L of Cypermethrin showing interstitial haemorrhage (arrows) H&E x400

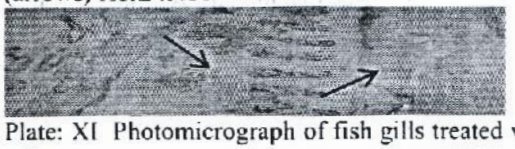

Plate: XI Photomicrograph of fish gills treated with 0.075mg/L of Cypermethrin showing severe gill necrosis H&E x40

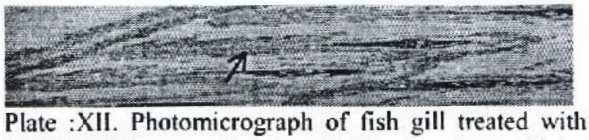

Plate :XII. Photomicrograph of fish gill treated with 0.100mg/L of Cypermethrin showing necrosis of the gill filament (thin arrows), interstitial haemorrhage (thick arrows) cartilage (C) H&E x400.

Haematology parameters are important in the health status of any organism (Baker *et al.*, 2001).The result obtained in this study showed a decrease in Red Blood Cell (RBC) of fishes exposed to increased concentration of cypermethrin similar reduction had been reported by Adeyemo, (2005) and Aderoju *et al.*, (2010).The significant reduction in this parameter could be an indication of severe anaemia caused by destruction of erythrocyte (Omoniyi *et al.*, 2002).Packed cell volume (PCV) and haemoglobin concentration (Hb) showed significant ($P< 0.05$) increase at increasing concentration of toxicant. The result evidentially showed that increase in PCV lead to corresponding increase in the Hb content as well. The result obtained in this present study is been corroborated by the findings of Olufayo, (2009).The author reported that exposed of *Clarias gariepinus*to sub-lethal concentration of *Derris elliptica* caused a significant increase in PCV, haemoglobin and erythrocyte of the fish, packed cell volume increased with high concentration of *Derris elliptica*.The white blood cell count increased significantly ($P< 0.01$) at higher concentration of the toxicant. This can be correlated with an increase in anti-body production which helps in survival and recovery of fish exposed to pesticide (Joshi et al., 2000).Result obtained for the erythrocytes values (MCV, MCH and MCHC) showed that there were significant increase ($P< 0.001$, $P< 0.01$) as compared to the control group of fish. Adeyemo *et al.*, (2008) reported similar trend.These alterations were attributed to direct or feedback responses of structural damage to RBC membrane resulting in haemolysis and impairment of haemoglobin synthesis.However,in the white blood cell count, a sharp increase was observed in the percentage of heterophils, monophils, basophils and eosinophils.The increase in WBC counts recorded in this research, the antigens (pollutant) and this augmented the production of more WBC to improve the health status of the fishes which however, agreed with the reports of Adeyemo (2005) .

Table 1: Changes in Haematological Parameter of *Clarias gariepinus juveniles* exposed to various concentration of cypermethrin in water Mean ±SD, n =5.

CONC. (mgL^{-1})	RBC ($10^6/mm^3$)	PCV (%)	Hb (g/dl)	WBC ($10^3/mm^3$)	MCV (Fl)	MCH (Pg)	MCHC (%)	HETR (%)	MONO (%)	EOSI (%)	BASO (%)	LYMP (%)
0.025	2.56±0.07*	26.20±1.30	8.26±0.36*	3.64±0.26	10.24±0.54***	32.30±2.04	31.57±1.71***	32.00±1.58	8.00±0.71***	6.40±0.89	0.20±0.45	53.40±2.61**
0.050	2.35±0.10***	34.80±1.48	9.50±0.30*	4.00±0.20	14.85±1.21**	40.49±2.40***	27.34±1.42*	34.00±1.41*	8.000±1.00***	6.80±0.84	0.60±0.55	50.40±2.51***
0.075	2.56±0.09**	35.80±1.11	8.70±0.49	4.52±0.34	14.01±0.89*	34.01±2.07	24.33±1.79	35.00±1.58**	9.00±0.71***	7.40±1.14	0.80±0.8	47.80±0.84***
0.100	2.83±0.06*	48.40±1.14***	9.26±0.40	4.88±0.18**	17.10±0.43***	32.74±1.86	19.15±1.19***	35.60±0.89***	8.40±0.55***	7.40±0.89	1.00±0.71	47.60±1.95***
0.125	2.97±0.08***	45.80±1.11***	11.62±0.43***	4.96±0.17**	15.42±0.32***	39.15±2.02***	25.38±1.17	36.60±1.52***	9.40±0.55***	7.80±0.84*	1.60±0.55**	44.60±1.52***
control	2.72±0.07	35.40±0.89	8.62±0.41	4.20±0.49	13.02±0.39	31.69±1.14	24.63±1.17	31.20±1.30	5.40±0.55	5.80±0.84	0.00±0.00	57.60±1.34

*P<0.05 Significance increase or decrease compared to control
**P<0.01 moderately Significance increase compared to control
***P<0.001 Highly Significance compared to control.

Table 2: The mean water quality parameters obtained during exposure of *Clarias gariepinus* Juveniles to Cypermethrin for 96hrs.

Concentration (mg/l)	Physico- chemical parameters			
	Hardness (U/L)	pH	Temp. (°C)	Dissolved Oxygen (mg/l)
0.00	$99.13^{b} \pm 0.74$	$7.03^{a} \pm 0.04$	$26.13^{a} \pm 0.29$	$6.40^{a}\pm$ 0.05
0.025	$91.77^{c} \pm 0.47$	$6.87^{b} \pm 0.13$	$26.10^{a} \pm 0.46$	$6.10^{b} \pm$ 0.08
0.05	$94.27^{c} \pm 0.76$	$6.60^{c} \pm 0.21$	$26.03^{a} \pm 0.54$	$6.08^{bc}\pm$ 0.20
0.075	$101.3^{ab} \pm 4.28$	6.50^{c} +0.02	$26.70^{a} \pm 0.82$	$5.92^{cd}\pm 0.07$
0.1	$102.67^{ab} \pm 2.05$	$6.33^{d} \pm 0.01$	$26.30^{a} \pm 0.31$	$5.83^{d} \pm 0.15$
0.125	$104.00^{a} \pm 2.45$	$6.17^{e} \pm 0.04$	$26.40^{a} \pm 0.22$	$5.61^{e} \pm 0.05$

* Mean values followed by the same superscript on the same column are not different significantly ($P<0.05$)

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