

## Haematological effect of acute concentration of cypermethrin on juveniles of *clarias gariepinus*

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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, 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. 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. cypermethrin is therefore toxic to juveniles of *Clarias gariepinus*.

**Keywords:** cypermethrin, synthetic pyrethroids, nominal concentration and cypavest.

### I. INTRODUCTION

Toxicology is the study of poisons and as such is concerned with their chemical nature, their interaction with biological systems and of particular relevance in this day and age, with then safety evaluation or toxicity testing of potentially poisonous materials. Poisons or toxicants are chemicals which have harmful or adverse effects on living organisms. A chemical may be poisonous under one set of condition but in other circumstances, it may be harmless or even essential but when administered or accidentally consumed in large doses it may be distinctly hazardous (David, 1983).

Haematological parameters and physiological profiles can be useful indicators of the physiological disturbances in animals and so can be crucial in providing vital information on the general well-being of fish (Barton and Iwama 1991; Wendelaar Bonga 1997; Tavares-Dias and Moraes 2004, 2007). Stoskopf (1993) considered evaluation of blood cells, blood biochemistry as useful for the diagnosis of diseases and assessing the physiological status of fish. Blood cell indices (RBC, WBC and DLC counts) are good indicators of systemic response to external stimulus and any changes are therefore reflected in their morphology and distribution in the blood.

Therefore, detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. Early diagnosis is also possible when evaluating haematological data, particularly blood parameters (Folmar, 1993; Golovina, 1996; Luskova, 1997). Furthermore, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Lebedeva *et al.*, 1998; Vosylienė, 1999a; 1999b). Previous haematological study of nutritional effects (Rehulka, 2000), infectious diseases (Rehulka, 2002a) and pollutants (Rehulka, 2002b) brought knowledge that erythrocytes are the major and reliable indicators of various sources of stress (Rainza-Paiva *et al.*, 2000; O'neal and Weirich, 2001).

The African catfish *Clarias gariepinus* is an ecologically important and commercially valued fish for the Nigerian fishing industry (Ita, 1980). These mud fish are frequently and widely cultured in ponds and they also occur freely in Nigerian's Natural Freshwater. According to Musa and Omoregie, (1999) fish are intimately associated with the aqueous environment, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish.

### II. MATERIALS AND METHODS

The experiment was carried out at the toxicology section of the of Water Resources, Aquaculture and Fisheries Technology Department, School of Agriculture and Agricultural Technology of the Federal university,

Minna, Niger State and Federal college of Freshwater Fisheries Technology, Baga Maiduguri, Borno State. 180 healthy juveniles of *Clarias gariepinus* of the same cohort with average weight ( $16.62 \pm 4.36$ )g, standard length ( $12.64 \pm 1.03$ ) cm and total length ( $14.97 \pm 8.94$ ) 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.

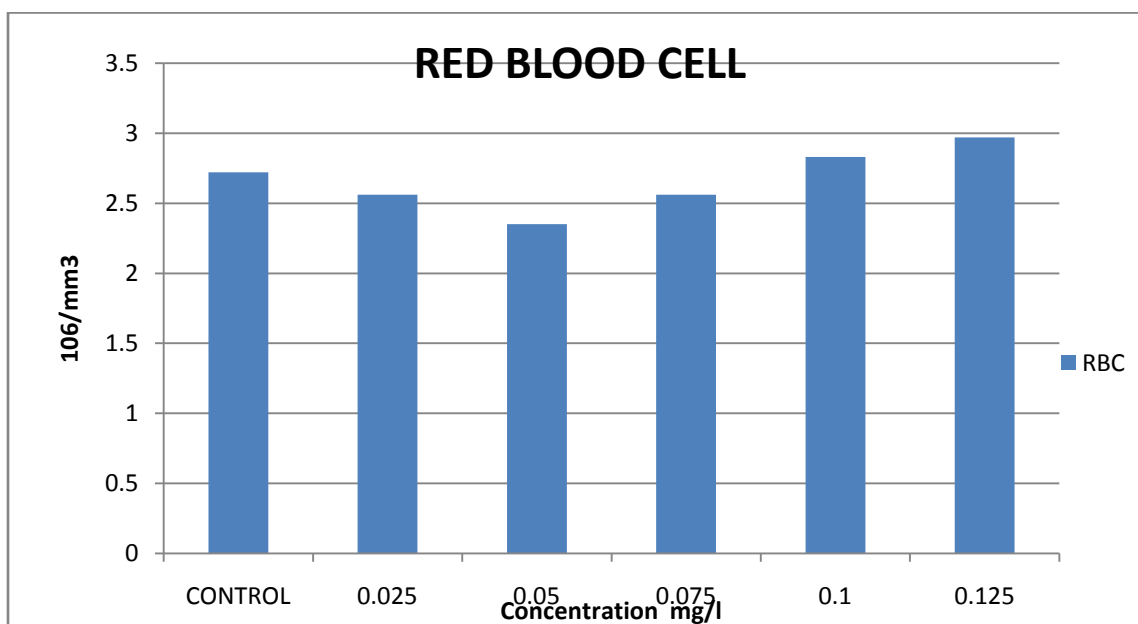
A preliminary range finding test was carried out based on the concentration of the active ingredient in the test chemical. The range finding was done using the following concentrations; 0.1mg/l, 10mg/l and 100mg/l of Cypermethrin for 24hrs in triplicates. The concentration was done using a serial dilution 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 experiment was carried out using a static non-renewal bioassay for 96 hours. Mortality and general behavior of fish were noted 24 hourly.

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 were presented as Mean  $\pm$  Standard Deviation. Analysis of variance was used to test the variation between the means (Mead and Curnow, 1983).

### III. RESULT

The red blood cell (RBC) Table 1 and Fig.1 of the sampled fish exposed to cypermethrin were observed to decrease ( $P > 0.001$ ) with increase in concentration of cypermethrin at 0.025mg/l, 0.050mg/l and 0.075mg/l with mean values of ( $2.56 \pm 0.07$ ), ( $2.35 \pm 0.10$ ) and ( $2.56 \pm 0.09$ )  $10^6/\text{mm}^3$  respectively. However, the red blood cell of fish in 0.100mg/l and 0.125mg/l shows a significant ( $P < 0.05$ ,  $P < 0.001$ ) increase with ( $2.83 \pm 0.06$ ) and ( $2.97 \pm 0.08$ )  $10^6/\text{mm}^3$  as mean values. The control had ( $2.72 \pm 0.07$ )  $10^6/\text{mm}^3$  of red blood cell.



**Fig. 1 Mean Red Blood Cells of *Clarias gariepinus* juveniles exposed to different concentration of Cypermethrin for 96hrs**

**Packed Cell Volume (PCV)**

Packed cell volume (PCV) (Table 4.2 & Fig 4.2) of the fish showed no significant ( P> 0.05) difference in the blood of fish exposed to 0.025mg/l, 0.05mg/l and 0.075mg/l. their mean values were (26.20 ± 1.30), (34.80 ± 1.48) and (35.80 ± 1.11) 10<sup>6</sup>/mm<sup>3</sup>. A marked significant (P< 0.001) increase in PCV was noticed in 0.100mg/l and 0.125mg/l with (45.80 ±1.14) and (45.80 ± 1.11) %, as compared to the control (35.40 ± 0.89).

**Table 1: Changes in Haematological Parameter of *Clarias gariiepinus* juveniles exposed to various concentration of cypermethrin in water**

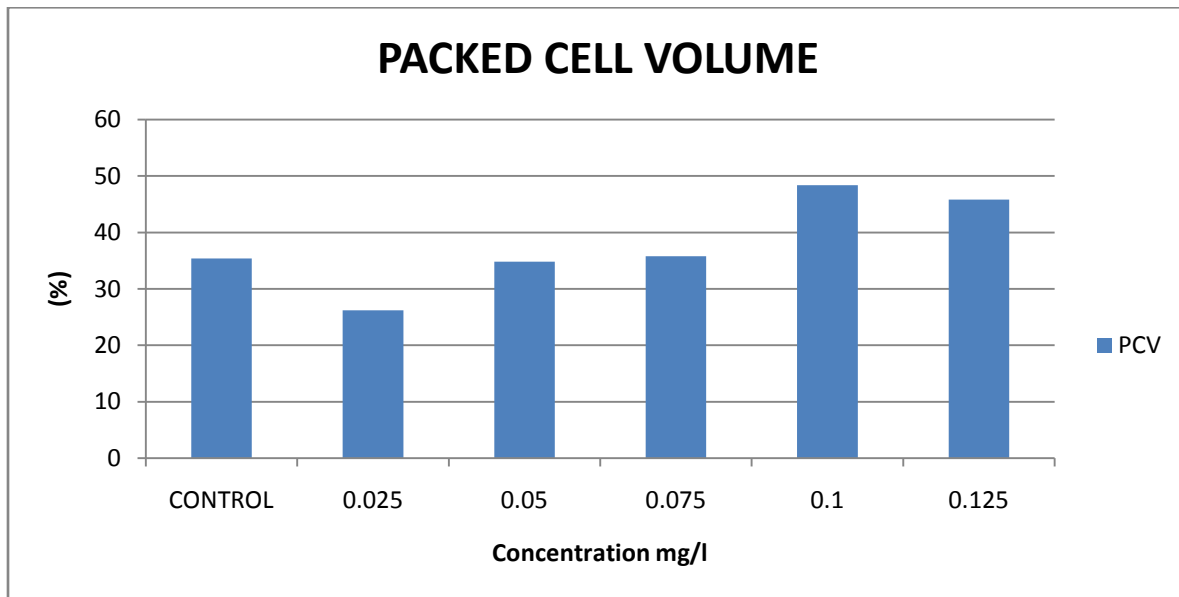
CONC. (mgL <sup>-1</sup> )	RBC (10 <sup>6</sup> /mm <sup>3</sup> )	PCV (%)	Hb (g/dl)	WBC (10 <sup>3</sup> /mm <sup>3</sup> )	MCV (fl)	MCH (Pg)	MCHC (%)	HETR (%)	MONO (%)	EOSI (%)	BASO (%)	LYMP (%)
0.025	2.56±0.07*	26.20±1.30	8.26±0.36	3.64±0.26*	10.24±0.54***	32.30±2.04	31.57±1.71***	32.00±1.58	8.00±0.71**	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

Mean ±SD, n =5.

\*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.



**Fig 2: Packed Cell Volume of *Clarias gariiepinus* juveniles exposed to different concentration of Cypermethrin for 96hrs**

**Haemoglobin Concentration (Hb)**

The exposure of fish to different concentration of cypermethrin shows that there was no significant (P> 0.05) in the haemoglobin concentration (Hb) (Table 4.2 & Fig 4.3) in the blood of fish at 0.025mg/l and 0.075mg/l with corresponding (8.26 ±0.36) and (8.70 ± 0.49) g/dl as their mean values respectively. The treatment with 0.05mg/l, 0.100mg/l and 0.125mg/l shows significant (P< 0.05) increase in haemoglobin concentration. The mean values were (9.50 ± 0.30), (9.26 ± 0.40) and (11.62 ± 0.43) g/dl respectively. The control had (8.62 ± 0.41) g/dl.

### White Blood Cell (WBC)

The results on white blood cell (WBC) are presented in (Table 4.2& Fig 4.4).It shows that there was no significant difference ( $P > 0.05$ ) in the WBC of fish exposed at 0.05mg/l and 0.075mg/l value compared with the control ( $4.20 \pm 0.49$ )  $10^6/\text{mm}^3$ . Treatment with 0.025mg/l concentration of cypermethrin showed significant ( $P < 0.05$ ) decrease in WBC ( $3.64 \pm 0.26$ )  $10^6/\text{mm}^3$ . Marked significant ( $P < 0.001$ ) increase in the WBC count was evidential in treatments with 0.100mg/l and 0.125mg/l of cypermethrin. The mean values are ( $4.88 \pm 0.18$ ) and ( $4.96 \pm 0.17$ )  $10^6/\text{mm}^3$  respectively.

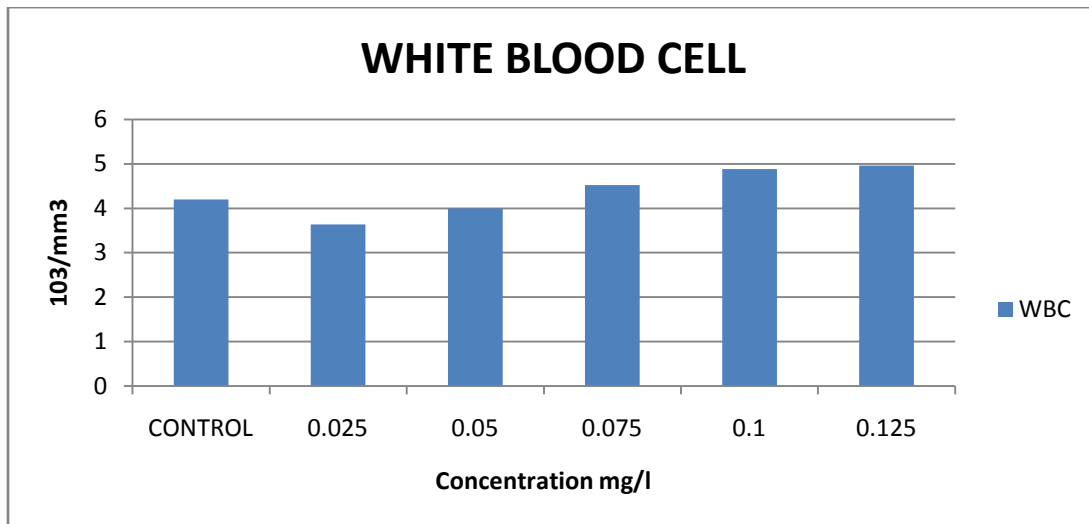


Fig 3 : White Blood Cells of *Clarias gariepinus* juveniles exposed to different Concentration of Cypermethrin for 96hrs.

### Mean Corpuscular Volume (MCV)

The result of mean corpuscular volume (MCV) presented on (Table 4.2 & Fig 4.5) shows that there was highly significant ( $P < 0.001$ ) increase in the in the blood of fish exposed at 0.100mg/l and 0.125mg/l with ( $17.10 \pm 0.43$ ) and ( $15.42 \pm 0.32$ ) Fl as mean values respectively. MCV of fish exposed at 0.05mg/l showed a moderately significant ( $P < 0.01$ ) increase ( $14.85 \pm 1.2$ ) Fl as compared with the control ( $13.02 \pm 0.39$ ) Fl. The result also show a highly significant ( $P < 0.001$ ) decrease in MCV of fish blood at the lowest concentration (0.025mg/l) with ( $13.02 \pm 0.39$ ) Fl as the mean value.

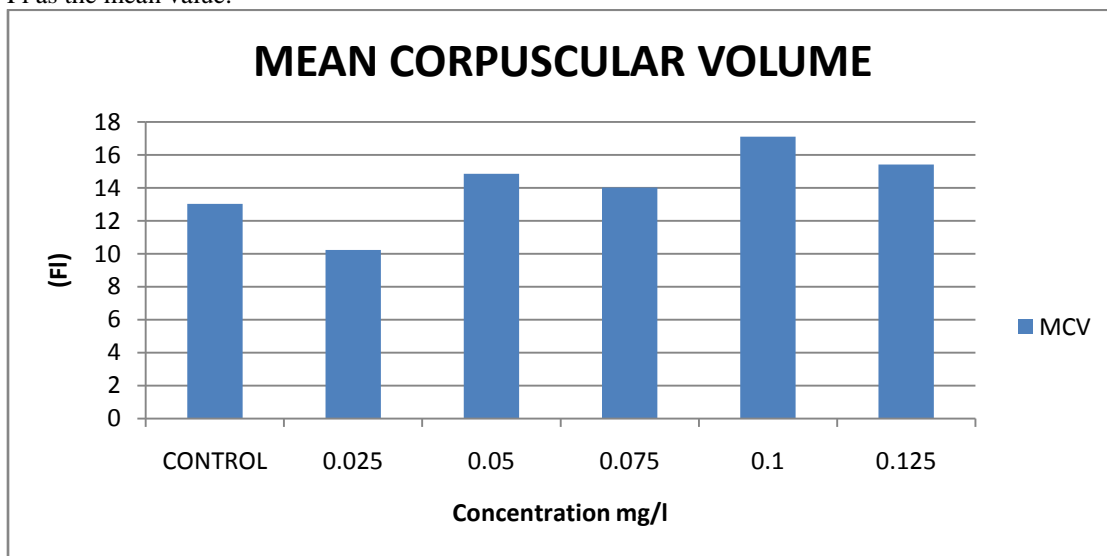


Fig 4: Mean Corpuscular Volume of *Clarias gariepinus* juveniles exposed to

different concentration of Cypermethrin for 96hrs

Mean Corpuscular Haemoglobin Count (MCHC)

Mean corpuscular haemoglobin count (MCHC) (Table 1 & Fig 5) values at 0.075mg/l and 0.125mg/l are not significantly ( $P>0.05$ ) different compared with the control. Mean value ( $19.15 \pm 1.19$ )% obtained for 0.100mg/l showed highly significant ( $P< 0.001$ ) decrease as compared to the control ( $24.63 \pm 1.17$ ) %. Mean values for 0.025mg/l and 0.05mg/l concentration showed significant ( $P<0.001<0.05$ ) increase in MCHC with ( $31.27 \pm 1.71$ ) and ( $27.34 \pm 1.42$ )% as the mean value respectively. The control had ( $24.63 \pm 1.17$ ) %.

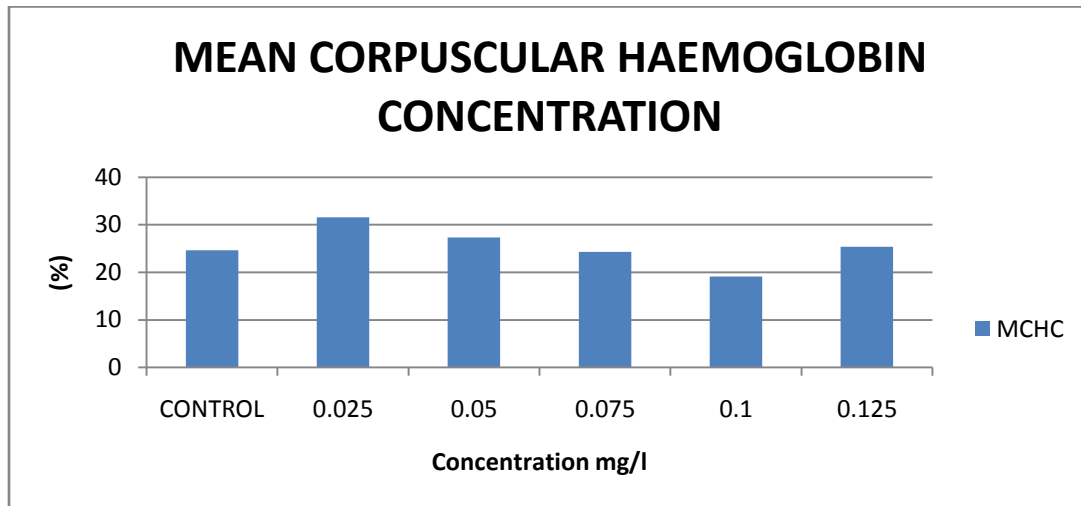


Fig 5: Mean Corpuscular Haemoglobin Concentration of *Clarias gariepinus* juveniles exposed to different concentration of Cypermethrin for 96hrs

Heterophils

The result on (Table 1 & Fig. 6) also shows that the heterophils values obtained in 0.025mg/l of Cypermethrin exposed fish showed no significant ( $P>0.05$ ) difference compared to the value for the control. Values obtained for heterophils at 0.075mg/l, 0.100mg/l and 0.125mg/l showed highly significant ( $P<0.001$ ) increase as compared to the control ( $31.20 \pm 1.30$ ) %. The mean values are ( $35.00 \pm 1.58$ ), ( $35.60 \pm 0.89$ ) and ( $36.60 \pm 1.52$ ) % respectively.. At 0.05mg/l concentration, a significant ( $P<0.05$ ) increase was noted in heterophils with ( $34.00 \pm 1.41$ ) % as the mean value.

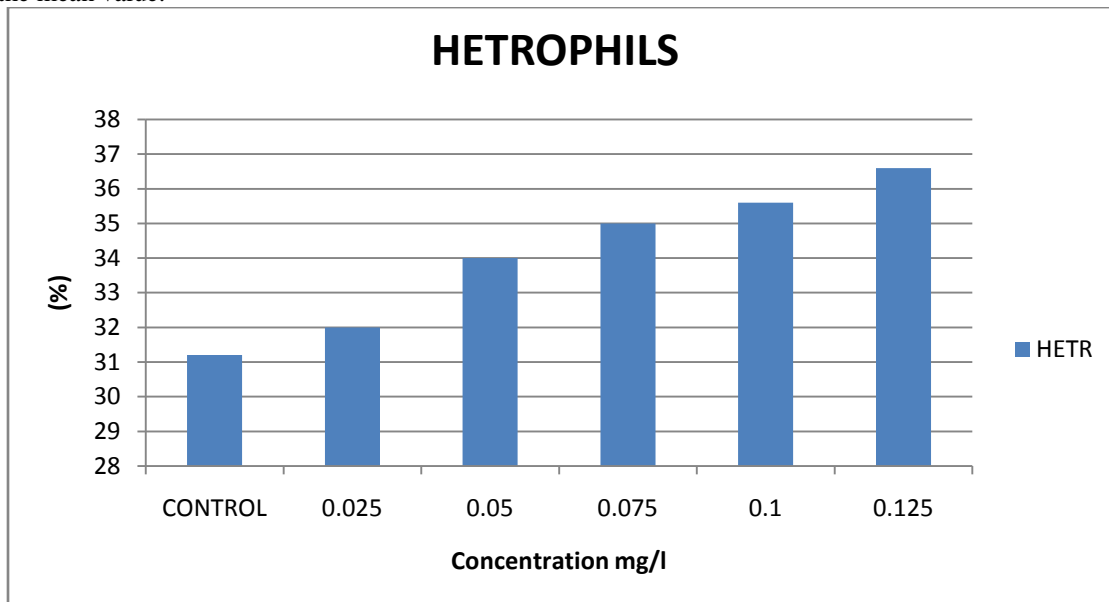
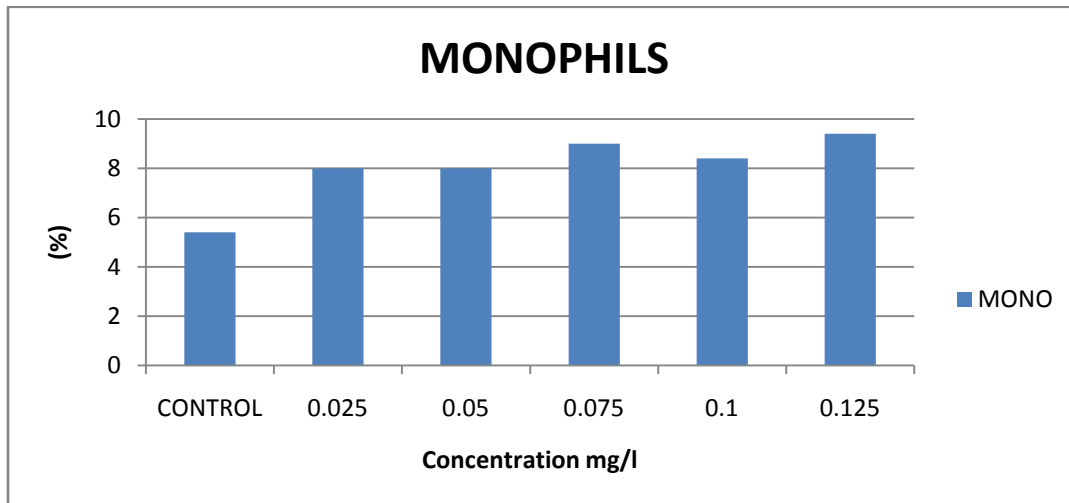


Fig 6: Mean Heterophilsof *Clarias gariepinus* juveniles exposed to different

concentration of Cypermethrin for 96hrs

**Monophils**

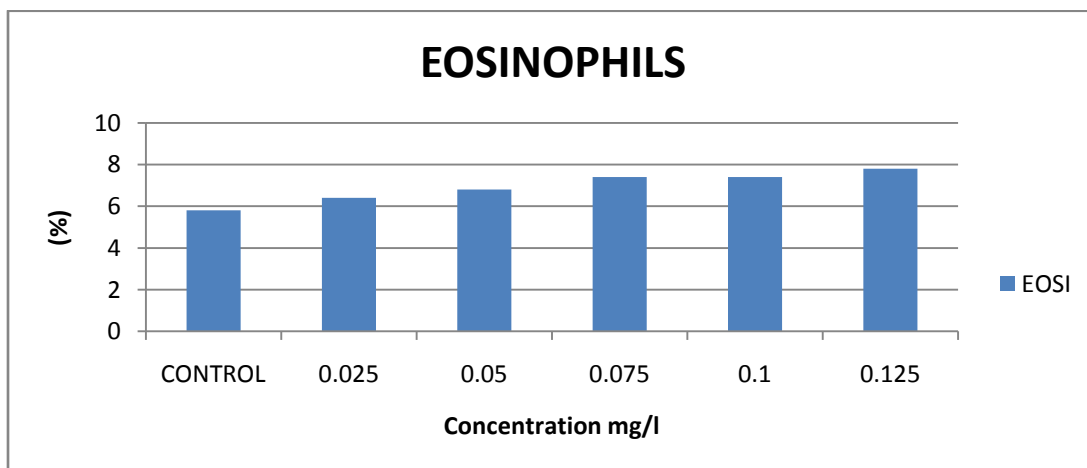
Monophils value obtained (Table 1 & Fig. 7) shows that there was highly significant ( $P < 0.001$ ) increase in all treatment concentration as compared to the control. The mean values are  $(8.00 \pm 0.71)\%$ ,  $(8.00 \pm 1.00)\%$ ,  $(9.00 \pm 0.71)\%$ ,  $(8.40 \pm 0.50)\%$  and  $(9.40 \pm 0.55)\%$  at 0.025mg/l, 0.05mg/l, 0.075mg/l and 0.125mg/l respectively. The control had  $(5.40 \pm 0.55)\%$ .



**Fig7: Monophils of *Clarias gariepinus* juveniles exposed to different concentration of Cypermethrin for 96hrs**

**Eosinophils**

Eosinophils values obtained (Table 1 & Fig. 8) showed that at 0.025mg/l, 0.05mg/l and 0.100mg/l concentration of cypermethrin exposed fish showed no significant ( $P > 0.05$ ) difference compared to the control group. However, there was significant ( $P < 0.05$ ) increase in eosinophil values at the highest concentration (0.125mg/l) with  $(7.80 \pm 0.84)\%$  as the mean value. The control had  $(5.84 \pm 0.84)\%$ .



**Fig 8: Mean Eosinophils of *Clarias gariepinus* juveniles exposed to different concentration of Cypermethrin for 96hrs**

**Basophils**

Table 1 and Fig. 9 also showed that there was no significant difference ( $P > 0.05$ ) in the values obtained for basophils at 0.025mg/l, 0.05mg/l, 0.075mg/l and 0.100mg/l concentration of cypermethrin compared with the control. At 0.125mg/l concentration, there was moderately significant ( $P < 0.01$ ) increase in basophils level. The mean value is  $(1.60 \pm 0.55)\%$  as compared to the control  $(0.00 \pm 0.00)\%$ .

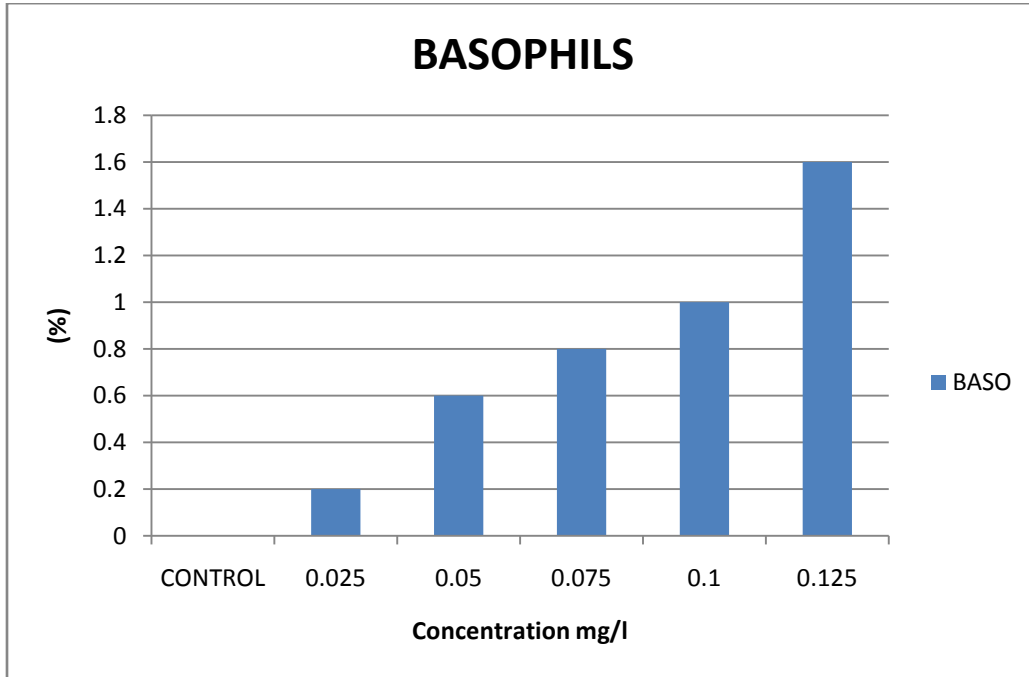


Fig 9: Mean Basophils of *Clarias gariepinus* juveniles exposed to different Concentration of Cypermethrin for 96hrs

#### Lymphocyte

The result on (Table 4.2& Fig. 4.12) also showed that there was highly significant ( $P<0.001$ ) decrease in the lymphocyte in the blood of exposed fish at 0.05mg/l, 0.075mg/l, 0.100mg/l and 0.125mg/l with ( $50.40\pm 2.51$ ), ( $47.80\pm 0.84$ ), ( $47.60\pm 1.95$ ) and ( $44.60\pm 1.52$ )% respectively. However, a moderately significant ( $P<0.01$ ) decrease in lymphocyte was obtained at 0.025mg/l with mean value of ( $53.40\pm 2.61$ ) % as compared to the control ( $57.60\pm 1.34$ ) %.

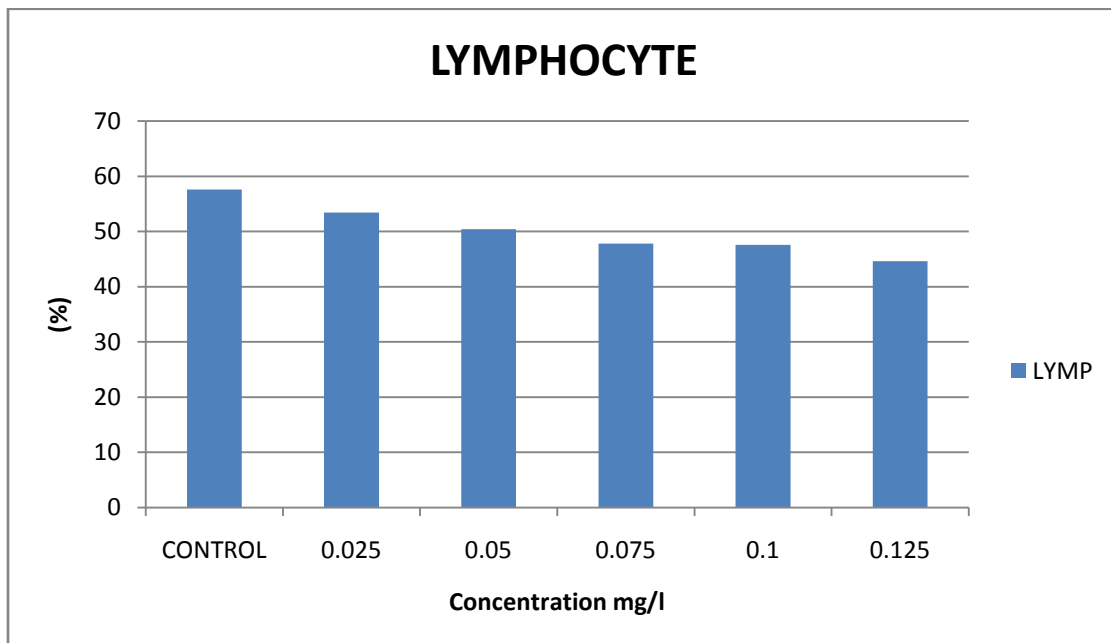


Fig 10: Mean Lymphocyte of *Clarias gariepinus* juveniles exposed to different concentration of Cypermethrin for 96hrs

#### IV. DISCUSSION

The results obtained on the haematology parameters of juveniles of *Clarias gariepinus* exposed to acute concentration of cypermethrin over a 96hr period showed a significant variation in the blood parameter compared to the control group of fish. Haematology parameters are important in the health status of any organism (Baker *et al.*, 2001). In fishes, they are used for clinical diagnosis of fish physiology which is determined by the effect of the internal and external physical environment (Adeyemo, 2005). 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 Aderojuet *al.*, (2010). When they expose fish to pollutant under laboratory condition. The significant reduction in this parameter could be an indication of severe anaemia caused by destruction of erythrocyte (Omoniyiet *al.*, 2002). Adhikariet *al.*, (2004), reported similar trend of result. Their experiment showed that exposure of *Labeorohita* to sub-lethal levels of cypermethrin and carbofuran resulted in significant ( $P < 0.05$ ) decrease in erythrocyte count (RBC)

Packed cell volume (PCV) and haematology 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* (19.0, 22.5g/ml). PCV could be used as a tool for detecting haemolysis or anaemic conditions. 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). Similarly, Ada *et al.*, 2011 observed significant difference in WBC of *Oreochromis niloticus* exposed to higher concentration of Butachlor an herbicide thus resulting to a leukemia condition.

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. Adeyemoet *al.*, (2008) reported similar trend. The authors exposed *Clarias gariepinus* adult to acute toxicity of lead nitrate and observed a significant increase in the erythrocyte value. 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 Suggest that 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)

#### V. CONCLUSION

The result of the findings indicated that the physiology of the fish was disturbed by the Cypermethrin acute exposure. The toxicant caused haematological disturbances which could lead to impairment of fish ability to combat diseases, reduce its chances for survival for growth. Also adequate data build-up on the haematological parameter of our local fish species might be useful in the selection of healthy stock for breeding purposes, in the possible early diagnosis of a disease before it becomes an epidemic.

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