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Haematological Responses in *Sarotherodon melanotheron* Exposed to Carbofuran in the Laboratory

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Abstract: *The haematological changes in Sarotherodon melanotheron exposed to sub lethal concentrations of carbofuran were investigated. One hundrerd and fifty adult sizes of S. melanotheron (mean length 12cm + 2.66 SD and mean weight 131.88g+11.77 SD) were exposed to different concentrations. (0.00,- control; 0.01, 0.02, 0.03 and 0.04 mg/L) of carbofuran for 15 days. The study assessed the effect of exposure to this chemical on some haenatological parameters which include Haemoglobin (Hb) , Red blood cell (RBC), packed cell volume (PCV), white blood cell (WBC), thrombocytes, neutrophils, lymptocytes, monocytes, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and oxygen carrying capacity (OCC). The results obtained indicated a significant (P<0.05) reduction in the values of Hb, RBC, PCV, thrombocytes, lymphocytes, MCHC, and OCC, with increasing concentrations of the contaminants. However, exposure of S. melanotheron to carbofuran, caused significant (P<0.05) elevation in the values of WBC, neutrophils, monocytes, MCV and MCH when compared to the control values. Exposure of this specie to sub lethal levels of this chemical distorted various mechanisms of its haematological variables, which may results in irregular metabolism of the fish. Consequently, there is need for proper disposal of domestic wastes containing pesticides into the aquatic environment.*

Keywords: *Sarotherodon melanotheron, Blood, Toxicants, Fish Aquatic environment*

1. Introduction

Various environmental pressures on aquatic ecosystems are increasing at faster rates as industrialization, urbanization and agricultural activities intensify all over the world. Myriads of kilograms of industrial chemicals find their way into fresh water bodies around the world annually including 140 billion kilograms of pesticides (Omer *et al*., 2012). 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 differs, toxicity to fishes can vary with each pesticide group, with insecticides typically the most toxic (Ezike, 2017). In different parts of Nigeria, agrochemicals that contain pesticides especially chlorinated ever-increasing use of numerous pesticides for pest control has caught much attention for extensive investigations on the mode of toxic actions of pesticides especially on aquatic fauna (Ayoola and Ajani, 2008). Among these pesticides are abamectin, carbofuran, chlorpyrifos, cypermethrin, deltamethrin, dichlorvos, dimethoate, fipronil, lambda-cyhalothrin and paraquat which are commonly deployed in different parts of the country. They are e characteristically employed as part of the integrated farming practice to protect crops and animals from insects, weeds and diseases (Fafioye *et al.,* 2001; Ezike, 2017).

In recent years, haematological parameters have become promising biomarkers in the assessment of chemical contaminant in teleost fish. Blood samples can regularly be obtained from test organisms, thus allowing the use of non- destructive approach in end product evaluation (Akinrotimi and Amachree, 2016). Characteristically, haematological parameters are non-specific in their responses towards chemical stressors. Nevertheless, they may provide important information in assessment studies, by providing an indication as to the general physiology and health status of the organism under investigation (Nte *et al.,* 2011). Several researcher have investigated the toxicity, uptake and tissues distribution and haematological changes of pollutants in fish (Gabriel et al., 2007; Akinrotimi et al., 2010a; Akani and Gabriel, 2015) and the use of hematological techniques in fisheries research is growing rapidly, as it is very important in toxicological research which result in monitoring and predicting health conditions of the fish (Gabriel et al., 2007b; Akinrotimi *et al.,* 2010b). Since fish are so intimately associated with the aqueous environment, the blood will reveal measurable physiological changes in the fish more rapidly than any physiological assessment parameters (Vani et al., 2011).Pollutants such as herbicides, pesticide and industrial effluent are known to alter the haematological indices of fish (Adhikari *et al.,* 2004; Akinrotimi *et al.,* 2009'; Ayanda *et al.,* 2017). According to Svobodova *et al.* (1996) study of haematological parameter are carried out on the fish to ascertain the normal range of blood parameter, find out the variation with age, sex, season, and determine the effects of disease condition on the fish. This study therefore aimed at assessing the haematological indices of black jaw Tilapia (*Sarotherodon melanotheron*) treated with various concentrations of carbofuran.

2. Materials and Methods

One hundrerd and fifty adult sizes of *S. melanotheron* (mean length 12cm + 2.66 SD and mean weight 131.88g+11.77 SD) were harvested from African regional Aquaculture Center, Buguma, Rivers State, Nigeria. They were transferred immediately in open, 50L tanks, half filled with water to the laboratory where they were acclimated to laboratory conditions for a period of seven day (Gabriel *et al*., 2006). Carbofuran is a systemic, broad spectrum N-methyl carbamate insecticide and nematicide. It was purchased off shelf in solid form, 1 g was weighed and dissolved in 1 L of water to obtain the stock solution (1 gL^{-1}). Various concentrations were measured into bioassay tanks for range finding and definitive tests. Appropriable graded concentrations were made by serial dilutions. The desired toxicant concentrations 0.00,- control; 0.01, 0.02, 0.03 and 0.04 mg/L). Each toxicant was replicated three times. The measured effluents were later introduced into 40L plastic tanks filled up to 30L mark. Fish (10 per tank) were introduced into the 15 tanks (3 tanks per each concentration and control). The fish were exposed to the chemical (for a period of 15 days. Water quality parameters in the experimental tanks during the study were evaluated: Water temperature was measured with mercury in glass thermometers, pH with pH meter (Model 3013, Jenway, China), and Salinity was determined with hand held refractometer (Atago products, Model H191, Japan). The values of dissolved oxygen, nitrite and ammonia were evaluated using the method described by APHA (1998).

Haematological analysis procedures described by Blaxhall and Daisly (1973) were used in the assessment of the various blood parameters or otherwise stated. Red blood cell (RBC) was evaluated using haemocytometer; while the packed cell volume (PCV) was determined using micro haematocrit tubes after centrifuging for five minutes. The hemoglobin content of the blood was assessed by cyanomethaemoglobin method. The white blood cell (WBC) was done using improved Neubauer counter. The values of thrombocytes were determined using the Rusia and Hood, 1992). The differential counts (neutrophils, lymphocytes, eosinophils and monocytes) were evaluated by dropping thoroughly mixed blood film on clean microscope slides and allowed to dry. The slides were then fixed in methanol and stained with leishman stain. The counting was done based on different cell types and recorded. The values of haematological indices was calculated using the method of Rusia and Sood (1992)

OCC = Hb x 1.34

$$\text{MCV} = \frac{HCT}{RBC} \times 10$$

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

$$\text{MCHC} = \frac{Hb}{PCV} \times 100$$

Source: *Miale (1982).*

Data obtained from the study were subjected to a one-way analysis of variance (ANOVA) test at 0.05% probability level, using statistical package for the social sciences (SPSS) version 17 .Differences among means where existed was done using Tukey test.

3. Results

The water quality parameters in the experimental tanks of *S.melanotheron* exposed to carbofuran indicated a concentration dependent change in the values of ammonia, dissolved oxygen and nitrites (Table 1). These values were distorted significantly ($P < 0.05$) when put side by side to the control values (Table 1). The haematological changes produced by the effects of carbofuran in *S.melanotheron,* showed a concentration dependent reduction in the values of Hb from 6.99 ± 0.92 in the control, to 3.37± 0.88 g/dl at 0.04mg/L concentration of the chemical. The values of RBC equally reduced from 5.92 ± 0.77 in the control, to 2.76 ±0.07 at 0.04mg/L concentration. In addition, similar trend was observed in PCV which reduced significantly ($P<0.05$) from 29.81 ± 1.99% to 20.99 ± 2.88% at 0.04mg/L of the chemical. Likewise significant reductions ($P<0.05$) comparable to the control values were similarly recorded in leucocrit, thrombocytes, and lymphocytes. On the contrary, concentration dependent elevations were recorded in WBC, neutrophils and monocytes of the exposed fish. (Table 2). The results of the red blood cell indices and oxygen carrying capacity were shown in Table 3. Consistent reductions relative to the concentrations of the chemical were recorded in the values of MCHC, and OCC, however, no definite trend was observed in the values of MCH and MCB across all the concentrations.

Table1. *Physico-chemical parameters of water in Experimental tanks (Meant ± SD)*

Parameters	Concentrations of Carbofuran (mg/L)				
	0.00	**0.01**	**0.02**	**0.03**	**0.04**
Temperature (^{0}C)	29.22±2.65^a	28.08±4.22^a	28.77±1.88^a	28.54±3.05^a	28.99±1.88^a
pH	6.44±1.66^a	6.71±1.07^a	6.81±1.44^a	6.77±1.99^a	6.61±1.991^a
Ammonia (mg/l)	0.06±0.01^a	0.18±0.02^a	0.19±0.02^{ab}	0.19±0.02^{ab}	0.27±0.10^{ab}
DO (mg/l)	6.99±0.02^a	6.31±0.12^a	5.44±0.88^{ab}	4.11±0.12^b	3.66±0.09^b
Nitrite (mg/l)	0.01±0.01^a	0.06±0.01^b	0.07±0.01^b	0.08±0.01^c	0.09±0.01^c
Salinity (ppt)	12.88±1.62^a	12.14±1.88^a	12.24±1.21^a	12.96±1.55^a	12.47±1.77^a

Means within the row with different superscripts are significantly different (p<0.05)

Table2. *Haematological Parameters of S.melanotheron Exposed to Different Concentrations of Carbofuran (mean ± SD)*

Parameters	**0.00**	**0.01**	**0.02**	**0.03**	**0.04**
Hb (g/dl)	6.99±0.92^c	6.12±0.77^c	5.01±0.88^b	4.44±0.11^b	3.37±0.88^b
RBC (cellsx10^6)	5.99±0.33^c	4.51±0.51^b	4.22±0.66^b	3.57±0.55^b	2.76±0.07^a
PCV (%)	29.81±1.99^c	27.65±0.88^c	24.42±1.81^b	22.02±1.57^b	20.99±2.88^b
WBC (Cellsx10^9)	18.88±1.77^a	19.99±1.77^a	22.77±4.99^b	27.99±1.89^b	32.99±5.07^c
Leucorit (%)	9.88±1.69^c	8.02±0.73^c	7.11±0.77^b	6.55±0.51^b	3.51±0.72^a
Thrombocytes (%)	175.99±9.82^c	161.63±9.55^a	156.82±8.55^a	94.61±9.07^b	85.62±2.83^a
Neutrophils (%)	14.00±2.09^a	18.12±1.12^b	26.11±1.19^b	33.82±1.21^b	38.22±2.88^c
Lymphocytes (%)	78.26±9.88^c	70.96±1.03^c	60.95±1.17^b	50.21±1.89^b	46.89±1.51^b
Monocytes (%)	7.74±1.78^a	10.92±1.99^a	13.97±1.99^a	15.07±1.77^b	15.69±3.77^b

Means within the row with different superscripts are significantly different (p<0.05)

Table3. *Red Blood Cell Indices of S.melanotheron Exposed to Different Concentrations of Carbofuran (mean ± SD)*

	Concentrations of Cabofuran (mg/L)				
Blood Indices	**0.00**	**0.01**	**0.02**	**0.03**	**0.04**
MCHC (g/dl)	21.26±7.99^b	21.73±9.88^b	20.22±7.82^b	18.62±1.21^b	16.91±5.88^b
MCH (pg)	12.61±3.34^a	13.17±3.88^a	12.54±2.86^a	13.55±1.89^b	16.67±1.77^b
MCV (fl)	59.16±8.99^a	60.63±7.01^b	62.04±8.75^b	64.23±9.88^b	98.57±10.99^c
OCC (g/d/)	8.48±1.88^a	8.05±1.77^a	6.74±1.83^a	6.39±1.78^a	4.56±1.88^a

Means within the same row with different superscripts are significantly different (p < 0.05).

Key: *MCHC – mean corpuscular Hoemoglobia concentration; MCH – mean corpuscular Haemoglobin; mean corpuscular volume, OCC – Oxygen carrying capacity.*

4. Discussion

Haematological parameters of juveniles of *S. melanotheron* exposed to sub lethal concentration of carbofuran revealed a significant difference in all of the parameters across the concentrations. The significant decrease observed due to pesticide exposures in the levels of Hb and RBC recorded in this study differs with findings of some previous investigations using similar pesticides, indicating some level of inconsistencies in haematological responses. For instance, a significant decrease in RBC and Hb level after exposure to pesticides and other toxicants were reported by several investigators (Akinrotimi *et al.,* 2013;Akinrotimi *et al*., 2018a; Akinrotimi *et al.,* 2018b). These changes observed in this study can be attributed to direct responses of structural damage to RBC membrane resulting in haemolysis and also the subsequent need to quickly produce replacement blood cells to minimize risk of anaemia. According to Campana et al. (1999), the RBC elevation may be due to blood cell reserve combined with cell shrinkage as a result of osmotic alterations of blood by the actions of the pesticides. In disagreement with results of the present study, Riaz-ul-Haq et al. (2018) have previously observed an increase in HCT (or PCV) of freshwater fish Channa punctatus exposed to endosulphan pesticide.

The values of, PCV followed the same trend of reduction observed in the exposed S. *melanotheron.* PCV is an important tool for evaluating the amount of plasma and blood corpuscles in the blood. Values of PCV in the exposed *S.melanotheron* in this study are an indicative anaemia and haemodilution consequent of gill damage in the exposed fish (Akinrotimi *et al*., 2007).. The toxic effect of the chemical has been reported by Chindah (2004), to cause reduction in blood parameters and weight loss. The reduction in the blood parameters was as a result of destruction of the cells of the fish by the surfactant and traces amount of heavy metals found in pesticides.

The haematological results obtained in this study showed that the exposure of *S.melanotheron* to sub-lethal concentrations of carbofuran in the laboratory caused a significant increase in WBC count of the fish. An increase in WBC count can be correlated with an increase in antibody production, which helps in survival and recovery of fish exposed to sublethal concentration of pesticides (Lahr *et al.,* 2000). This response was also observed in the same fish species exposed to chlorpyrifos and DDforce (Adewumi et al., 2018), and in Cyprinus carpio after acute exposure to phenithrotion and dichlorvos (Svobodova, 1996). This may be due to the release of WBC from spleen in the blood stream to combat the toxicant. As suggested by Nwani *et al.* (2015), several chemical compounds including insecticides generate antibodies owing to their interference with the immune system

Moreover, the reduction recorded in the leucocrit, which represents the leucocytes and thrombocytes packed value expressed as a percentage of the blood, is an indication internally disturbed stressful condition of the fish (Gabriel *et al.,* 2011a). Elevated levels of some differential counts namely neutrophils and monocytes were observed in the exposed fish. This indicated a state of pathological stress induced by contaminants, as Gabriel and Ugbomeh, 2016) noted that exposure of fish to some toxicants may results in the increase of some differential counts which leads to stressful condition and ultimately results in their dysfunction in the blood stream. In this study, the values of MCV, MCH and MCHC decreased in *S. melanotheron* treated with detergents with concomitant decline in oxygen carrying capacity of the fish. The significant decrease in the MCH and MCHC level of fishes exposed to sub-lethal concentrations of carbofuran in this study is a good indicator of RBC swelling in the fish which has been related to hypoxia. In a study carried out by Akinrotimi and Amachree (2016), in *Tilapia guineensis* showed significant decrease in RBC, and HCT after exposure to conditions of hypoxia. Given that RBC is important in oxygen transport to cells, increased RBC counts would ensure that cells continue to receive enough oxygen for metabolism. Previous studies involving catfishes have reported that under extreme hypoxia, reduced activity is reflection of the differences in blood respiratoty functions of different species (Akinrotimi *et al.,* 2012).

5. Conclusion

Exposure of *S.melanotheron* to sublethal levels of carbofuran in the laboratory distorted some element of its haematological parameters which may lead to abnormal metabolism and ultimately results in the death of the fish. There is therefore the need to inform the public on proper application of these chemicals and prevent them from gaining access to aquatic environment. In view of the toxicity of pesticides to fishes, farm lands should be sited far away from natural water bodies so as to minimize the risk of intoxication of fishes by pesticides through surface runoffs and atmospheric deposits.

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