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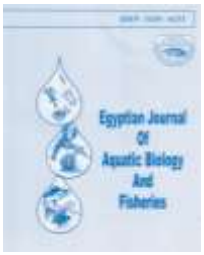

Haematology and Biochemical Responses in *Oreochromis niloticus* exposed to sub-acute Doses of Aronil (Propanil) in a Flow through Bioassay

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ABSTRACT

Effects of sub-acute exposure of pesticide aronil (propanil) on haematology and serum biochemistry of *Oreochromis niloticus* juvenile was investigated in the present study. Sub-acute doses (0.21 mg/l, 0.43 mg/l and 0.83 mg/l) were derived from 1/20, 1/10 and 1/5 of the 96h LC_{50} (4.17 mg/l) of aronil, in which fish specimens were exposed for eight weeks. Control group was exposed to dechlorinated tap water.

Haematology and serum biochemistry of fish were examined at week 2, 6 and 8 of aronil exposure. Significant and dose dependent decreases in red blood cell, haemoglobin and haematocrit were observed in aronil exposed fish compared to the control. White blood cell, mean cell haemoglobin and mean cell haemoglobin concentration of the control were significantly lower compared to the exposed fish groups. Neutrophil and lymphocyte percentages were elevated significantly in 0.83 mg/l exposed fish when compared to other exposed fish and the control at the eight week. Basophils, eosinophils and monocytes were not observed in aronil exposed fish. Significant and dose dependent increases in glucose, protein, aspartate aminotransferase, alanine aminotransferase and triglyceride were observed in aronil treated fish with reference to the control while insignificant changes in cholesterol level was observed in the exposed fish compared to the control. Aronil pesticide induced stress and had toxic effect on *O. niloticus.* Use of pesticide aronil should be regulated especially in farm lands along the coast and riversides to avoid the influx of pesticides into the aquatic ecosystems.

INTRODUCTION

Aronil (propanil) is a known common pesticide widely used in agriculture andagro-forestry tocontrol pests. It is mainly used in rice farms to control weeds. However, due to increased agricultural activities in both urban and rural areas, the use of this pesticide has increased tremendously (Nwani *et al.*, 2015).

This has direct consequence both to humans (Hill, 1989) and its immediate environment especially the aquatic ecosystem (Firat*et al.,* 2011) which is at the receiving end (surface runoff from agricultural lands discharges into streams and rivers).

Presence of propanil in aquatic ecosystem and fish although in trace amount has been reported (Call *et al.,* 1983). According to Ensibi *et al.* (2013), presence of toxic substances in aquatic ecosystem can affectfish growth ultimately by reducing food accessibility, or by altering their metabolism. Toxicsubstances even at minute concentrations in aquatic environment can cause fish kill (Dinis-Oliveira *et al.,* 2008). Toxicity of propanil to different fish species are reported by several researchers (Call *et al.,* 1983; Oyibo *et al.,* 2014; Mallum *et al.,* 2015). Sancho *et al.* (2009) observed lethary and erratic movement in European eel (*Anguilla Anguilla*) after two day exposure to propanil. Reduced reproduction rate, declined feeding rate and poor growth was reported in propanil exposed *Daphnia magna* (Villarroel *et al.,* 2003). Mallum *et al.* (2015) reported hyperactivity and mortality in propanil exposed *Oreochromis niloticus*.

Blood and biochemical parameters are essential biomarkers for assessing the health status of animals with regards to environmental pollution (Adhikari *et al.,* 2004; Iheanacho *et al.,* 2017). Changes in blood parameters reflect pathophysiological conditions in animals most especially in pollution studies (Firat*et al.,* 2015). Alterationsin the biochemical parametersindicate changes in the metabolic rates in organisms, as a consequence of exposure to toxicants (Luskova *et al*. 2002).

Fish are good bioindicators of aquatic pollution as toxic substances bioaccumulate in them for a long time (Ogueji *et al*. 2017). Nile tilapia *O. niloticus* is one of important commercial fish species widely distributedaround the globe with economic importance for aquaculture (Iheanacho *et al.,* 2018). Nile tilapia is an excellentcandidate for toxicological studies, due to its uniquefeatures, such as high resistance todiseases, high prolific nature, high growth rates, ability to reproduce easilyin captivity and hightolerance to wide range of ecological conditions (Firat *et al.,* 2011). For this reason, *O. niloticus* juvenile was used in this study, as model for evaluating the toxic effect of aronil (Propanil) pesticide using blood parameters as biomarkers for assessment.

MATERIALS AND METHODS

Experimental Fish and Chemical

Nile tilapia (*Oreochromis niloticus*) Juveniles were procured from Regina fish farm in Abakaliki and transferred to the wet laboratory of the Department of Fisheries and Aquaculture, Alex Ekwueme Federal University, in 50 Litre plastic container filled with water. On arrival, fish specimens were carefully transferred to plastic pond (500 litres) containing fresh water, thus aeration pumps were provided to enable constant supply of dissolved oxygen for the two weeks acclimation period. Fish were fed with commercial feed twice daily (9.00hr and 18.00 hr) at 3% body weight. Feeding stopped 24 hours prior to the commencement of the experiment. Pesticide aronil (propanil) was purchased from Jude agrochemicals Limited, at Abakpa market Abakaliki Ebonyi State and dissolved in distilled water (80mg/L) to formulate a stock solution that was used in the experiment. Various sub-acute concentrations of aronil were prepared from the stock solution,using distilled water. Stock solution was prepared fresh each time theconcentrations were renewed.

Determination of sub-acute concentrations and Experimental design

The experiment lasted for eight weeks and was done under natural photoperiod (12:12 light-dark cycle). The experiment was conducted in an intermittent flow through bioasay. The system set-up consists of delivery and test tanks as prescribed by EPA (1996) and OECD (1992) guidelines for toxicity tests with fish.Flow rate was controlled by the gate valves to allow continuous flow of the nominal concentrations by the toxicants from the delivery tanks into the test tanks at 4L/hour. The concentrations of aronil used were 0.83, 0.42 and0.21 mg L^{-1}derived from 1/5, 1/10 and 1/20mg L^{-1} of 4.17 mg/L being the 96 h $LC_{50}$of propanil to *O. niloticus*.

Total of 120 juveniles of *Oreochromis niloticus* (24±0.52g and 9.1±0.68cm) were randomly assigned to four (4) sub-acute doses of aronil as follows; 0.83 mg/l, 0.42 mg/l, 0.21 mg/l and 0.00 mg/l (contains dechlorinated tap water as the control). Each treatment (test medium) was triplicated in completely randomised design (CRD), hence making it a total of twelve (12) aquarium tanks (35 cm × 35 cm × 40.5 cm) used for the experiment. Each replicate contained ten fish. The fish were fed twice daily at 3% total body weight at 9.00 and 18.00 h with commercial fish feed (4mm Coppens, Netherland), having 40% crude protein. Water quality was monitored while the remnantsof the unconsumed feed and the excreta were also siphoned. Water quality parameters such as temperature, pH, and dissolved oxygen were monitored following the procedure of APHA (1985), and the means for the respective parameters are reported as follows; 29.15±0.12°C, 6.89-7.05 and 5.78±0.14mg/L^{-1}. Fish specimens from each treatment were sampled at weeks 2, 6 and 8 to ascertain the toxic effect of aronil on the fish.

Haematology and Serum biochemistry analysis

Fish specimens from each replicate were sampled for blood collection.Blood was collected by severance of caudal peduncle from the caudal artery.The caudal region of fish was cut 2cm away and blood then collected into different plastic tubes (heparinized and non-heparinized) for haematology and serum biochemistry analysis respectively.Blood samples were transferred immediately to the haematology/Biochemistry unit of Federal Teaching Hospital (FETHA1) Abakaliki, Ebonyi State Nigeria. Haematological analysis was done using automated haematology analyzer (Abacus 360, India). Haematological parameters examined in this study include; Red blood cell (RBC), White blood cell (WBC), Haemoglobin (Hb) content and Haematocrit (HcT), red cell indices (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)) and leukocyte differentials (neutrophils, lymphocytes, monocytes, basophils and Eosinophils).

For biochemical analysis, blood was placed in micro centrifuge tubes, and centrifuged at 1500 rpm for 15 minutes. Serum was then isolated by pipetting and stored at 4^0C prior to the determination of biochemical parameters. Total protein (TP), glucose (GT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), cholesterol andtriglycerides (TG) levels were measured with an automatic biochemical analyzer (Olympus AU 400 biochemical analyzer, Tokyo Japan). Analysis was done following the manufacturer's instructions.

Statistical Analysis

Haematology and biochemical data obtained from experiment were analyzed using statistical SPSS 22.0 (SPSSInc. Chicago, Illinois, USA).Differences in the test concentrations and control were subjected to one-way analysis of variance (ANOVA),followed by Tukey'smultiple range tests to determine the level of significance at 5% probability level.

RESULTS

Haematology

Results of haematological parameters of *O. niloticus* exposed to sub-acute concentrations of aronil in flow through bioassay are presented in Table 1. Fish exposed to ≥ 0.42mg/L concentrations at week 2 recorded significant ($p < 0.05$) reduction in RBC and HcT compared to the control. However, insignificant changes in the same parameters were observed in 0.21 mg/L aronil exposed fish compared to the control. WBC of the exposed fish had a significant ($p < 0.05$) and dose dependent increase compared with the control fish. Furthermore, haemoglobin content (Hb) of the aronil exposed fish were lower significantly ($p < 0.05$), when compared to the control. The decrease in haemoglobin was noticed in 0.83 mg/l exposed fish and adjudged to be dose dependent. MCV, MCH and MCHC values of the control fish were not significant with those exposed to sublethal concentrations of aronil (Table 1).

RBC and Hb values of the exposed fish at week 6 showed a significant decrease ($p < 0.05$) with concentrations ≥0.42mg/L against the control (Table 1), but were not significant with those exposed to 0.21mg/l concentration of the toxicant.

Table 1: Effects of sub-acute concentrations of aronil on some haematological parameters of *Oreochromisniloticus* in flow through bioassay.

	Conc. (mg/l	RBC ($x10^{12}$L)	WBC ($x10^{9}$L)	Haemoglobin (g/100ml)	HcT (%)	MCV ($x10^{6}$Pg cell)	MCH ($x10^{6}$pg cell)	MCHC (g/100mg)
2 weeks	0.00	2.33± 6.01^{a}	45.33± 3.33^{d}	12.00± 2.65^{a}	28.67± 0.67^{a}	1.50± 0.01^{a}	0.63± 01.5^{a}	41.47± 8.24^{a}
	0.22	1.93± 4.37^{a}	54.33± 6.01^{c}	9.67± 1.20^{ab}	28.00± 5.30^{a}	1.47± 0.23^{a}	0.53± 0.08^{a}	38.57± 11.40^{a}
	0.42	1.73± 6.01^{b}	61.00± 5.77^{b}	7.67± 1.20^{ab}	20.67± 1.20^{ab}	1.23± 0.10^{a}	0.67± 0.07^{a}	37.90± 7.79^{a}
	0.83	1.63± 4.37^{b}	66.33± 4.91^{a}	5.67± 1.20^{b}	16.67± 2.91^{b}	1.03± 0.20^{a}	0.37± 0.07^{a}	38.73± 14.49^{a}
6 weeks	0.00	3.43± 6.01^{a}	57.27± 12.02^{c}	11.33± 0.88^{a}	31.00± 1.00^{a}	0.90± 0.06^{b}	0.33± 0.03^{b}	36.63± 3.33^{c}
	0.21	3.30± 17.64^{a}	57.60± 11.55^{c}	9.67± 1.20^{a}	29.00± 2.65^{a}	0.90± 0.12^{b}	0.33± 0.06^{b}	33.13± 1.08^{c}
	0.42	1.83± 4.37^{b}	58.31± 7.69^{b}	8.00± 2.08^{b}	24.00± 3.21^{a}	1.33± 0.15^{a}	0.93± 0.09^{a}	71.10± 1.31^{b}
	0.83	1.78 ± 6.01^{b}	77.52± 18.90^{a}	7.67± 0.67^{b}	16.00± 1.00^{b}	0.90± 0.00^{b}	0.90± 0.06^{a}	98.87± 8.68^{a}
8 weeks	0.00	3.39± 6.57^{a}	57.69± 45.70^{b}	12.00± 0.58^{a}	31.33± 1.45^{a}	0.93± 0.03^{b}	0.33± 0.03^{c}	35.50± 1.33^{b}
	0.21	3.23± 8.82^{a}	57.65± 8.82^{b}	10.67± 1.20^{ab}	30.67± 1.76^{a}	0.93± 0.09^{b}	0.37± 0.03^{c}	37.97± 1.25^{b}
	0.42	1.74± 8.82^{b}	57.96± 38.28^{b}	9.67± 1.20^{b}	28.67± 1.76^{a}	1.67± 0.18^{a}	0.57± 0.07^{b}	33.57± 2.62
	0.83	1.63± 6.01^{b}	77.51± 8.82^{a}	7.00± 1.15^{c}	16.00± 1.15^{b}	1.00± 0.12^{b}	0.87± 0.03^{a}	89.50± 13.78^{a}

Means with the same superscript along columns are not significantly different ($P<0.05$) (Mean values±SE), n=3.

Red blood cell =RBC, White blood cell = WBC, Haematocrit = HcT, Mean cell volume = MCV, Mean cell haemoglobin = MCH, Mean cell haemoglobinconcentration=MCH

However, WBC, MCH, and MCHC values recorded with the control fish were significantly lower ($p<0.05$) than those exposed to 0.42 and 0.83mg/L sub-acute concentrations of toxicant, but not significant with those exposed to 0.21mg/L concentrations of the same toxicant (Table 1).

Fish exposed to 0.83mg/l aronil recorded significant lower ($p<0.05$) values of HcT compared to the control group. But HcT values of those exposed to ≤0.42mg/L concentrations were not significant compared to the control. MCV values recorded with the control fish were not significant with fish exposed to 0.21 and 0.83mg/L concentrations, but significant with fish exposed to 0.42mg/L concentration of the toxicant.

At week 8 exposure period, RBC values of the control fish recorded were significantly higher ($p < 0.05$) than those exposed to ≥ 0.42mg/L concentrations of aronil, but not significant with those exposed to 0.21mg/L concentration (Table 1). WBC, Hb and MCHC values of the control fish were significantly lower ($p <0.05$) than fish exposed to 0.83mg/L of aronil, but were not significant with those exposed to 0.21 and 0.42mg/L concentrations of the toxicant. Control fish recorded significant ($p <0.05$) higher HcT values than those exposed to 0.83mg/L, but insignificant with fish groups exposed to 0.21 and 0.42mg/L of propanil. MCV values of the control fish were significantly lower ($p <0.05$) compared to fish exposed to 0.42mg/L concentrations, but were insignificant with fish groups exposed to 0.21 and 0.83mg/L concentrations of the same toxicant. Fish in the control group recorded significant ($p<0.05$) lower levels of MCH compared to fish group exposed to ≥0.42mg/L sub-acute concentrations of propanil, but were not significant with those exposed to 0.21mg/L concentrations of the toxicant.

Leucocyte differential count

Leucocyte differential count percentages of *O.niloticus*exposed to sub-acute concentrations of aronil pesticide at week 2, 6 and 8 are presented in Table 2.

Table 2: Effects of sub-acute doses of aronil on leucocyte differential count of *Oreochromis niloticus* in a flow through bioassay.

	Conc. (mg/l)	Neutrophils (%)	Lymphocytes (%)	Basophils (%)	Eosinophils (%)	Monocytes (%)
2 weeks	0.00	32.33 ± 1.20^{a}	59.33 ± 13.78^{a}	0.00	0.00	0.00
	0.21	36.67 ± 7.69^{a}	79.00 ± 10.60^{a}	0.00	0.00	0.00
	0.42	32.00 ± 3.06^{a}	74.67 ± 6.49^{a}	0.00	0.00	0.00
	0.83	34.67 ± 5.24^{a}	64.67 ± 12.88^{a}	0.00	0.00	0.00
6 weeks	0.00	27.33± 1.20^{a}	54.33± 13.78^{a}	0.00	0.00	0.00
	0.21	31.67± 7.69^{a}	74.00± 10.60^{a}	0.00	0.00	0.00
	0.42	27.00± 3.06^{a}	69.67± 6.49^{a}	0.00	0.00	0.00
	0.83	29.67± 5.24^{a}	59.67± 12.88^{a}	0.00	0.00	0.00
8 weeks	0.00	36.33 ± 1.76^{bc}	64.33 ± 12.45^{b}	0.00	0.00	0.00
	0.21	31.33 ± 0.88^{c}	69.00 ± 2.08^{b}	0.00	0.00	0.00
	0.42	39.67 ± 0.88^{b}	85.33 ± 4.63^{ab}	0.00	0.00	0.00
	0.83	46.00 ± 2.65^{a}	95.33 ± 6.74^{a}	0.00	0.00	0.00

Means with the same superscript along columns are not significantly different ($P<0.05$) (Mean values±SE), n=3

At week 2, Neutrophil and lymphocyte percentages in exposed fish were higher numerically but statistically not significant ($p>0.05$) compared with the control fish. Basophils, eosinophils, monocytes were clumpsy and not observed in both control and exposed fish.

Neutrophils and lymphocytes values of *O. niloticus* recorded in the control fish at week 6 were not significant compared with propanil exposed groups (Table 2). Basophils, eosinophils and monocytes were not found to be zero at the time of examination.

At week 8, significant and dose dependent increases in neutrophil and lymphocytes percentages were observed in 0.83 mg/l exposed fish with reference to

the control (Table 2). No traces of basophils, eosinophils and monocytes observed (Table 2).

Biochemical response

Oreochromis niloticus of the control at week 2 recorded significantly ($p < 0.05$) lower values for serum total glucose (TG), total protein (TP) and triglycerides than those fish exposed to sub-acute doses of aronil (Table 3). Similarly, serum total AST and ALT of the control fish were lower significantly ($p<0.05$) than the exposed fish groups (Table 3). Cholesterol values of the exposed fish were not significantly different ($p>0.05$) compared to the control group. The control group had lower values for TG, TP, AST and ALT, and were significantly different ($p < 0.05$) from fish exposed to $\geq$0.42mg/L concentrations of aronil, but insignificant ($p>0.05$) when compared to 0.21mg/L aronil exposed fish at week 6(Table 3). Triglyceride of the control were significantly ($p < 0.05$) lower than those exposed to 0.83mg/L, but significantly higher ($p < 0.05$) than those to 0.42mg/L and were not significant with fish group exposed to 0.21mg/L concentration of aronil. Cholesterol values of the exposed groups were not significant ($p>0.05$) compared to the control fish.

Table 3: Effects of sub-acute doses by aronil on some biochemical parameters of *Oreochromisniloticus* at week 6 in flow through bioassay.

	Conc. (mg/l)	Glucose (mg/dl)	Protein (g/dl)	AST (IU/L)	ALT (IU/L)	Triglyceride (Mgl/dl)	Cholesterol (Mg/dl)
2 weeks	0.00	4.60 ± 0.42^{b}	41.33 ± 6.01^{c}	10.33 ± 3.84^{d}	19.33 ± 6.57^{c}	0.63 ± 0.03^{b}	3.00 ± 0.58^{a}
	0.21	7.33 ± 8.88^{a}	57.33 ± 5.46^{b}	20.33 ± 6.01^{c}	18.33 ± 4.37^{b}	0.80 ± 0.12^{ab}	3.60 ± 0.42^{a}
	0.42	6.97 ± 0.58^{a}	86.33 ± 4.91^{a}	38.67 ± 3.71^{b}	24.33 ± 5.46^{a}	0.83 ± 0.03^{a}	3.53 ± 0.33^{a}
	0.83	8.96 ± 0.32^{a}	89.33 ± 4.37^{a}	41.33 ± 5.46^{a}	24.33 ± 4.37^{a}	0.93 ± 0.03^{a}	2.43 ± 0.33^{a}
6 weeks	0.00	2.97 ± 0.22^{c}	30.67 ± 1.76^{b}	30.33 ± 6.84^{c}	19.67 ± 7.69^{c}	1.07 ± 0.12^{ab}	3.33 ± 0.38^{a}
	0.21	2.87 ± 0.17^{c}	33.33 ± 2.036^{b}	29.00 ± 2.89^{c}	19.67 ± 6.67^{c}	1.27 ± 0.07^{ab}	3.13 ± 0.49^{a}
	0.42	4.07 ± 0.33^{b}	36.67 ± 3.48^{a}	41.67 ± 9.28^{b}	27.67± 10.37^{b}	0.87 ± 0.12^{ab}	2.33 ± 0.33^{a}
	0.83	7.37 ± 0.27^{a}	48.67 ± 4.63^{a}	61.67 ± 10.91^{a}	38.40 ± 10.69^{a}	1.47 ± 0.18^{a}	2.43 ± 0.38^{a}
8 weeks	0.00	2.97 ± 0.21^{c}	31.86± 1.23^{c}	33.35± 5.14^{c}	18.27 ± 3.39^{c}	1.03± 0.16^{b}	3.85± 0.21^{a}
	0.21	3.78± 0.12^{b}	33.39± 2.04^{b}	37.04± 2.19^{b}	22.47 ± 2.37^{b}	1.19± 0.15^{ab}	3.57± 0.26^{a}
	0.42	4.17 ± 0.43^{b}	35.47 ± 2.58^{ab}	39.67 ± 4.21^{b}	24.57 ± 4.87^{b}	1.95± 0.54^{ab}	2.13 ± 0.34^{a}
	0.83	6.27 ± 0.77^{a}	45.67 ± 3.43^{a}	58.87 ± 6.71^{a}	37.32± 2.17^{a}	2.37 ± 0.04^{a}	2.12± 0.18^{a}

Means with the same superscript along columns are not significantly different ($P<0.05$) (Mean values±SE), n=3. Aspartate aminotransferase = AST, Alanine aminotransferase = ALT.

At week 8 exposure period, TG, TP, AST and ALT values of the control fish significantly decreased ($p < 0.05$) compared to aronil exposed fish (Table 3), However, triglyceride values of the control fish decreased significantly ($p<0.05$) from 0.83 mg/L propanil exposed fish, but insignificant ($p>0.05$) when compared to $\leq$ 0.42 mg/L propanil exposed fish group. Highest value for cholesterol was seen in the

control group, but insignificant ($p>0.05$) when compared to aronil exposed fish groups.

DISCUSSION

The findings of the present study revealed that aronil was moderately toxic to exposed fish compared to the control. Considering the 96 hr LC_{50} (4.17 mg/L), aronil pesticide was found to be moderately toxic to the exposed fish. The findings of this study validates the report of the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) (1997), who indicated that the 96 hr LC_{50} ratings between 1-10 mg/L of biocides are moderately toxic to living resources. Significant reduction in RBC, Hb and HcT values of aronil exposed fish suggest inhibition in haematopoisis and erythrogenesis caused by aronil.Similar findings were reported in fenthion exposed fish (Muralidharm, 2013; Jaya and Ajay, 2014). Banaee*et al*. (2011) also state significant and dose dependent decreases in PCV, RBC and Hb counts in dianion exposed fish (*Oncorhynchus mykiss*). Mallum *et al*. (2015) report significant reduction in RBC, Hb and PCV levels in *O. niloticus* exposed to lethal doses of aronil. Significant decrease in PCV value were reported inmalathion exposed fish (*C. gariepinus*) (Ahmad, 2012). Significant and dose dependent increase in WBC values of the exposed fish suggest the occurrence leucocytosis in defence to the invasion of aronil toxicant. Similar findings have been reported in *C. gariepinus* exposed to fenthion (Nwani*et al.,* 2016) and in paraquat exposed fish (Nwani*et al.,* 2015).Saravannan *et al.* (2011) reported increased WBC counts in *Cyprinus carpio* exposed to lindane. Insignificant changes in MCV, MCH and MCHC in exposed fish and the control at week 2 may suggest normocytic anaemia while significant and dose dependent increases in the same parameters observed at week 6 and 8 indicate macrocytic anaemia caused by aronil. The result of this study differed from the report of Nwani *et al.* (2015) who reported significant reduction in MCV, MCH and MCHC in *C. gariepinus* exposed to sub-lethal concentrations of pesticide paraquat.

Leucocytes are important biomarkers of stress thus give information on health condition of animals with regards to their immune status (Jeney, 2017).Aronil did not affect neutrophils and lymphocyte cells in exposed fish at week 2 and 6 duration exposure.Furthermore, Progressive and dose dependent increases observed in neutrophil count in 0.83 mg/l exposed fish at week 8 and suggests immune defense mechanism against stress elicited due to prolonged exposure to pesticide aronil. Nwani*et al.* (2016) reported insignicant changes in neutrophil and lymphocyte percentages in *C. gariepinus* exposed to sub-acute exposure to fethion for 7 days but observed significant increases after the 14^{th} day fethion exposure. Eosinophils, monocytes and basophils were not seenin fish, thus indicate decreased concentrationof these cells in fish. Romao *et al.* (2006) also confirmed theabsence of eosinophils, basophils and granulocytes in *Hoplias malabaricus* captured from the wild. According to Ranzani- Paiva (1995), preservation of basophils are not easy, hence could be the reason for its scarcity in fish.

Biochemistry

Aronil pesticide elicited glucose and protein levels hence, elevated activities of *O. niloticus.* Progressive increases in glucose and protein levels in aronil exposed fish with reference to the control suggest secondary response to stress (Cicik and Engin, 2005; Sepici-Dincel *et al.,* 2009), and disruption in carbohydrate metabolism following the elevation of glucose activity in liver or glucose synthesis from

superfluous hepatic tissue proteins (Firat*et al.*, 2011). Induced hyperglycemia have been recorded in cypermethrin exposed *O. niloticus* (Firat *et al*., 2011), *S. schlegeli* (Jee*et al.*, 2005) and paraquat exposed *C. gariepinus* (Nwani*et al.*, 2015). The findings of the present study (elevation in protein levels) collaborate the reports of Monteiro *et al.* (2005) and Jee *et al*. (2005), who accentuate that pesticides can either increase or decrease the levels of glucose, protein cholesterol depending on the type of toxicant, fish species and duration of exposure.

Serum enzymes (ALT, AST, ALT) are important biomarkers for evaluating liver condition and function in animals (Iheanacho *et al*., 2017). Alternations in the activities of these enzymes indicate damage of hepatic cells and tissues which is tantamount to degenerative changes and dysfunction of the liver causing the release of serum enzymes into the blood serum (Firat *et al*,. 2011). Increases in ALT and AST activities in aronil exposed fish throughout the duration exposure suggest necrotic damage of liver caused by invasion of aronil pesticide. John (2007) reported increased ALT and AST in *Mystus vittatus* exposed to pesticide metasystox. Elevation in ALT, AST and ALP activities in *O. niloticus* were attributed to the prolonged exposure to cypermethrin (Firat*et al.*, 2011), *Rhamelia quelen* (Borge *et al*., 2007), *Labeo rohita* (Das and Muklerjee, 2003). Elevated levels of triglyceride observed in 0.83 mg/l exposed fish may suggest inhibition in the storage of glycogen induced by aronil incursion, causing the release of triglyceride into the blood system. Higher levels of triglyceride has been reported in *S. schlegeli* exposed to pesticide cypermethrin (Jee *et al.*, 2005). Insignificant changes in chlosterol levels in *O. niloticus* indicate that aronil had no adverse effect in chlosterol content in fish.

CONCLUSION

Reduction in some haematological parameters (RBC, Hb, HcT) tells more about the immune-suppressive potentials of aronil pesticide. There is no doubt that aronil elicited stress in the exposed fish as seen in progressive increases in glucose and protein. Liver necrosis was evident in exposed fish, affirming the toxic effect of aronil pesticide. Therefore, the use of pesticides should be regulated especially in farm lands along the coast and riversides to avoid the influx of pesticides into the aquatic ecosystems.

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