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Sub-lethal Concentration of Paraquat on Growth performance and haematological parameters of *Clarias gariepinus* (Burchell, 1822) Juveniles.

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Abstract

Juveniles of Clarias gariepinus mean weight 32.73 ± 0.00g were obtained from Makurdi, Benue state and were exposed to different sub-lethal concentrations of paraquat to produce 0, 0.5, 1.0, 1.5, 2.0, and 2.5mg/L respectively for 8 weeks under laboratory conditions with continuous aeration. Growth and hematological parameters were determine under 60 day's and water quality parameters were monitored. Results showed that parameters such as temperature, Dissolved oxygen and Potential hydrogen fluctuated slightly except for alkalinity; Total dissolved solid and Electrical conductivity that varied significantly across treatments. The growth indices such as SGR decreased initially but increased from 2.0 to 2.5mg^{L}, FCE decreased steadily as concentration increased, Mean Initial Weight (MIW) were statistically the same, MFW decreased steadily as concentration is increased, Survival rate (SR) decreased with increased concentration, FCR increased with increase in concentration, PER decreased with increase in concentration. Results of the haematological parameters of Clarias gariepinus exposed to sub-lethal concentrations (0.5-2.5mg^{l}) showed that paraquat was toxic as evidenced by decrease in RBC, HGB, HCT, MCV with increasing concentration of toxicant, MCH and MCHC fluctuated with increasing concentration of toxicant while WBC increased with increasing concentration of toxicant which were all significantly different from control and treatment exposed at ($p<0.05$). Precautions are observed in the use of paraquat for controlling weeds on the farm before it washes into the streams or river where fishes and other aquatic organisms live.

***Keywords**: Clarias gariepinus, sub-lethal, growth performance, heamatology & toxicants.*

Introduction

Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals as reported by Olaifa *et al.*, (2003). The application of environmental toxicology studies on non-mammalian vertebrates is rapidly expanding, and for the evaluation of the effects of noxious compounds as reported by Ayoola, (2008). The toxicity of a chemical is totally dependent on the concentration of the chemical in organisms or even the concentration at the target receptor in the organism as observed by Ayoola, (2008). Their role in degradation of the aquatic ecosystem cannot be ignored as reported by Omitoyin *et al.*, (2006). They could therefore be accumulated in the tissues of these non-target organisms, thereby influencing their ability to adapt to the environment. Herbicides could hamper reproduction, food conversion efficiency, growth and cause mortality of inhabitants as observed by Ateeq *et al.*, (2002).

The use of haematological techniques in fish culture is growing and is important for toxicological research; environmental monitoring and fish health. Often, physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish due to their close association with the environment. Shah and Altindag (2004) noted that studies on fish blood gives the possibility that fish blood will reveal conditions within the fish long before there is an outward manifestation of diseases. *Clarias gariepinus* is a common tropical freshwater fish and is widely used in aquaculture in Africa; hence it is choice for this study. Blood parameters are considered patho-physiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants as observed by Adhikari and Sarkar, (2004), and Maheswaran *et al*., (2008). These parameters provide an integrated measure of the health status of organism which overtime manifest changes in weight (growth).

Due to the dearth of information on the sub-lethal effects of paraquat on the growth performance and haematological parameters of *C. gariepinus* in the tropics, the study evaluated the growth performance and haematological parameters changes of *C. gariepinus* exposed to sub-lethal toxicity of paraquat for 8 weeks.

Materials and Methods.

General bioassay.

Juveniles of the African catfish, *Clarias gariepinus* of the same brood stock were brought from fish farm in Makurdi, Nigeria and brought to Fisheries and Aquaculture laboratory, University of Agriculture, Makurdi, Nigeria. The fish (mean weight of 32.73 $\pm$ 0.00g) were subjected to sub-lethal concentration of 0, 0.5, 1.0, 1.5, 2.0, and 2.5mg/L paraquat respectively in plastic containers measuring 70L with dechlorinated well aerated University of Agriculture, Makurdi water from River Benue. During the study period, the experimental fish were fed 3% of their body weight weekly to observe growth with commercial feeds, Coppens. During this period, (10) fish weighing 32.73 $\pm$ 0.00g were randomly selected and stocked with triplicates following methods by Ayoola, (2008) for each concentration and exposed to sub-lethal concentrations; The exposure period lasted for 8 weeks during which each plastic container were well aerated. Physico-chemical parameters of test solution were determined also following the methods of APHA (1985) and measured with Hanna II check meter.

Growth determination.

Growth was monitored by weekly weighing; Measurements of growth were taken for 60 days.

This is because the experiment was designed to last for 60 days so as to observe, the long term sub-lethal effects of Paraquat on the growth parameters.

The fish in each container were weighed individually with Metler Toledo weighing balance at the start of the experiment and weekly. Thereafter the mean weight was computed for each treatment for each weighing period. The amount of feed given was adjusted to new weights.

Haematological techniques.

At the end of the 8 weeks, blood samples were taken by randomly selecting fish from the various treatments and injecting a 2mm needle and syringe through the dorsal aorta puncture and placed in ethylene-diamine-tetra-acetic-acid (EDTA) treated bottles to prevent coagulation and analyzed at federal medical center for the following: haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC) using an automated haemoglobin analyser (Cobus U 411) model, while Mean Corpuscular Haemoglobin Concentration(MCHC) Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were determined by calculations.

$$MCV = \frac{Hematocrit\ (\%)}{RBC\ count\ (\frac{million}{mm^3}\ blood)} \times 10$$

$$MCH = \frac{Hemoglobin\ (g/100ml)}{RBC\ number\ in\ Millions} \times 10$$

$$MCHC = \frac{Hemoglobin\ (g/100\ ml)}{Hematocrit\ (\%)} \times 100$$

The following haematological indices were calculated from the equations given above by Svobodova (2001).

Statistical analysis

Water quality parameters were analysed for sub-lethal test concentrations in Table 1. Analyses of differences if there exist in growth and nutritional response of *Clarias gariepinus* exposed to sub-lethal concentrations of paraquat in table 2. Means in the same column of treatments followed by different superscripts differ significantly ($p<0.05$). Means in the haematological parameters were analyzed using analysis of variance (ANOVA) at 0.05% level by Genstat at Discovery edition 13 as shown in table 3.

Results and Discussion

The physiochemical parameters of the test solution fluctuated slightly during the bioassays but were not thought to have affected fish mortality since they were within tolerance range as suggested by Mackereth, (1963) which agrees with the findings of Adigun, (2005).

In Table 1, the observation in the growth data showed an increase in that of the control group and decrease significantly ($p<0.05$) in exposed group. The reduction in growth is the effect of pollutants in the environment which would result in physiological dysfunction in fish since, water is essential for fish stability as reported by Ayuba and Ofojekwo, (2007), who reported that the extracts from the leaf of *Dattura inoxia* at sub-lethal concentration on fingerlings of *Clarias gariepinus,* impaired the growth between the control and experimental groups. According to Zaghlour, (1997), the decrease in muscle of total protein in fish affected with herbicide could be attributed to the reduction in food consumption or decrease in gross food conversion and the decrease in insulin level, detected by the higher plasma glucose level. Growth is an evident feature of fish feeding normally, the exposure of Thiobencarb herbicide on *Oreochromis niloticus* for 8 weeks (chronic experiment on body weight and length gain) revealed that, fish showed a reduction in body weight gain compared to the control group during the entire experimental period according to Hossam *et al.,* (2007). Therefore, this becomes more realistic when it is known that in nature stressed fish will more readily reject feed.

Also showing in table 2, the growth indices such as SGR decreased initially but increased from 2.0 to 2.5mg/L, FCE decreased steadily as concentration increased, MIW were statistically the same, MFW decreased steadily as concentration is increased, SR decreased with increased concentration, FCR increased with increase in concentration, PER decreased with increase in concentration. Exposure to toxicant without causing death to organisms can still cause harm according to Stephan, (1982) and survival of estuarine and marine organisms in relatively low concentrations of toxicant on the first day, does not necessarily indicate that they are resistant to the toxicant pollution as reported by Mironov, (1972). Decreased growth rate found in this study is similar reduction in growth was also observed by Toussain, *et al.,* (2001) and Onusiriuka (2002) when they exposed Japanese Medaka fish and *Clarias gariepinus* to sub-lethal concentrations of chloroform and formalin respectively, better growths were reported in control groups of the fish than those exposed to toxicants just as it was observed in this study. This might be due to the fact that they were able to utilize the feeds or that the feeds were palatable.

Table 1: Mean Physico-chemical parameters of the test water obtained during the exposure of *Clarias gariepinus* to sub-lethal concentrations of paraquat.

Concentration of paraquat (mg/L)	DO(mg/L)	Temperature °C	pH	Alkalinity (mg/L)	Total dissolved solid (mg/L)	Electrical conductivity (mg/L)
0.00	5.64±0.01[a]	25.64±0.22[a]	6.68±0.01[a]	47.66±0.05[f]	63.40±0.09[f]	127.00±0.15[e]
0.50	5.56±0.02[b]	25.80±0.13[a]	6.64±0.01[b]	49.69±0.12[e]	74.83±0.61[e]	130.33±0.38[e]
1.00	5.51±0.02[b]	25.81±0.12[a]	6.60±0.01[c]	50.85±0.16[d]	79.36±0.20[d]	143.25±0.97[d]
1.50	5.54±0.02[b]	25.79±0.10[a]	6.59±0.01[c]	52.90±0.11[c]	83.09±0.63[c]	156.11±1.61[c]
2.00	5.39±0.02[c]	25.82±0.09[a]	6.55±0.01[d]	53.39±0.19[b]	84.91±0.63[b]	164.33±0.51[b]
2.50	5.33±0.03[d]	25.83±0.09[a]	6.52±0.01[d]	53.91±0.16[a]	89.53±0.28[a]	179.43±2.87[a]

Means in the same column followed by different superscript differ significantly (P<0.05)

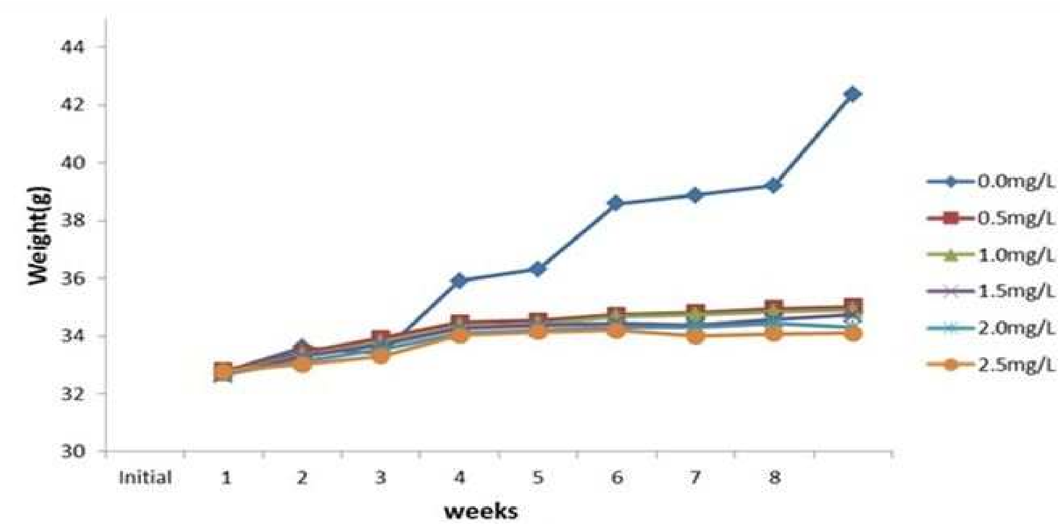

Fig 1: Mean weight gain of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of Paraquat for 8 weeks.

Table 2: Mean growth indices of *Clarias gariepinus* juveniles exposed to sub-lethal concentrations of Paraquat for 8 weeks.

Concentrations of paraquat (mg/L)	MIW (g)	MFW (g)	MWG (g)	SGR (g)	FCR (%)	FCE (%)	SR	PER
0.00	32.75 ± 0.00[a]	42.37 ± 0.21[a]	9.62 ± 0.21[a]	0.33 ±0.04[a]	1.47 ± 0.03[c]	68.01 ± 1.53[a]	100.00 ± 0.00[a]	0.270±0.01[a]
0.50	32.80 ± 0.00[a]	35.02 ± 0.01[b]	2.22 ± 0.01[b]	0.23 ±0.01[c]	5.89 ± 0.02[bc]	16.97 ± 0.07[b]	100.00 ± 0.00[a]	0.063±0.00[b]
1.00	32.72 ± 0.00[a]	34.91 ± 0.00[b]	2.19 ± 0.00[b]	0.20 ±0.01[c]	5.95 ± 0.00[bc]	16.82 ± 0.01[b]	100.00 ± 0.00[a]	0.062±0.00[b]
1.50	32.65 ± 0.00[a]	34.73 ±0.01[b]	2.08 ± 0.01[b]	0.21 ±0.02[c]	6.24 ± 0.03[abc]	16.03 ± 0.08[bc]	100.00 ± 0.00[a]	0.059±0.00[c]
2.00	32.78 ± 0.00[a]	34.30 ± 0.20[b]	1.52 ± 0.20[c]	0.28 ±0.03[b]	8.81 ± 1.32[ab]	11.82 ± 1.54[cd]	93.33 ± 3.33[b]	0.044±0.01[d]
2.50	32.70 ± 0.00[a]	34.10 ±0.35[b]	1.40 ± 0.35[c]	0.29 ±0.06[c]	11.07±3.70[a]	10.86 ± 2.73[d]	93.33 ± 3.33[b]	0.040±0.00[d]

Where; MIW=mean initial weight, MFW=mean final weight, MWG=mean weight gain, SGR=specific growth ratio, FCR=food conversion ratio, FCE=food conversion efficiency, SR=survival rate, PER=protein efficiency ratio.

Results in table 3, on the haematological parameters of *Clarias gariepinus* exposed to sub-lethal concentrations (0.5-2.5mg/l) showed that Paraquat was toxic as evidenced by decrease in RBC, HGB, HCT, and MCV with increasing concentration of toxicant. Also, fluctuations in MCH and MCHC with increasing concentration of toxicant, and WBC with increasing concentration of toxicant, were significantly different from the control ($p<0.05$). These findings are in line according to reports by Annune *et al.,* (1994) and Ayuba and Ofojekwu (2007). A reduction in the values of HGB and TEC as the concentrations of Paraquat increase is an indication of severe anemia caused by the herbicide on the exposed fish. The anemic response could be as result of destruction/ inhibition of erythrocyte production and as the result of the destruction of intestinal cells by the toxicant according to Samprath *et al.,* (1993) and Patnaik and Patra, (2000). Also, the decrease in RBC and MCV values with increase in Paraquat concentration in this report suggest that anemic effect could be attributed to the destruction of the erythrocytes or inhibition of erythrocytes production, similar trends RBC in fishes exposed to various toxicants have been observed by (Ayuba and Ofoejekwo, (2007) and Kori-Siakpere, (2007).

Fluctuations in MCH and MCHC values found in this study were found to be in line according to Annune *et al.,* (1993), which he found that there was increase in MCH, MCHC and a decrease RBC and WBC were observed when *Oreochromis niloticus* were exposed to zinc. But different with the findings of Kori-Siakpere *et al.,*(2007) which he reported that, potassium permanganate can adversely affect haematology of fish at 19.25 and 13.60mg/L for *Clarias gariepinus* exposed to sub-lethal level showed a decrease in MCH, and MCHC were reported to be significantly different ($P<0.05$) between the control and sub-lethal concentrations. The fluctuations in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in the present study, indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish showing anemic condition this is similar with the reports observed by Bhagwart and Bhikajee, (2002) and Ayuba and Ofojekwu, (2007) observed similar fluctuations. According to Dzenda *et al.,* (2004), MCHC is a good indicator of red blood cell swelling hence its reduction as concentrations of Paraquat increases.

The increase in WBC with increased concentration of the Paraquat in this study is likely due to heighten immune mechanism of the experimental fish species, and there will be stimulation to fight against the effect of the toxicant, this is in line with reports by Kori-Siakpere, (2007), Ayuba and Ofoejekwo, (2007). Increase in white blood cell in this study is also similar according to Ayoola (2007) and Ayotunde *et al.,* (2010). In this study, the mean cell volume did not change significantly in the exposed group compared to the control. It could therefore be assumed that the increase in packed cell volume was due to increase in cell population. Sub-lethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms but they have significant effects which can result in several physiological changes in the fish according to Olufayo *et al.,* (2007).

It is therefore recommended that precautions be observed in the use of paraquat for controlling weeds on the farm before it washes into the streams or river where fishes and other aquatic organisms live.

Table 3: Mean effects of Sub-lethal concentrations of Paraquat on haematological parameters of *Clarias gariepinus*

Conc. of paraquat (mg/L)	WBC(10^{3L})	RBC(10^{6L})	HBG(g^{dL})	HCT (%)	MCV(fL)	MCH(g^{dL})	MCHC(g^{dL})
0.00	1.69 ± 6.11^c	1.93 ± 2.00^a	10.00 ± 0.12^a	32.73 ± 0.47^a	173.33 ±9.78^a	47.10 ± 1.00^a	30.03 ± 0.17^a
0.50	1.59 ± 3.18^d	1.89 ± 1.00^a	9.10 ± 0.10^a	30.23 ± 0.17^a	156.87 ± 2.43^a	47.20 ± 0.39^a	31.47 ± 0.15^a
1.00	1.67 ± 3.51^c	1.90 ± 8.51^a	8.97 ± 0.13^a	28.33 ± 0.27^a	155.81 ± 6.59^a	52.77 ± 2.00^a	30.53 ± 0.74^a
1.50	1.74 ± 2.52^b	1.51 ± 2.71^a	8.20 ± 0.03^a	24.43 ± 0.65^a	150.57 ± 0.84^a	48.28 ± 0.47^a	33.60 ± 2.84^a
2.00	1.74 ± 1.53^b	1.71 ± 5.55^a	7.50 ± 0.65^a	24.01 ± 2.36^a	145.90 ± 1.60^a	48.43 ± 1.50^a	33.73 ± 0.56^a
2.50	1.85 ± 5.49^a	1.55 ± 1.58^a	7.21 ± 1.31^a	22.53 ± 5.54^a	145.57 ± 5.68^a	48.43 ± 1.15^a	31.23 ± 0.80^a

Means in the same column followed by different superscript differ significantly (P<0.05)
Where; WBC=white blood cell, RBC=red blood cell, HGB=haemoglobin, HCT=haematocrit, MCV=Mean corpuscular volume, MCH=mean corpuscular haemoglobin, MCHC=mean corpuscular haemoglobin concentration.

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