Effects of *Spondianthus preussii* Leaf Powder on Selected Haematological Parameters and Condition Factor of African Catfish - *Clarias gariepinus.*

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ABSTACT
The study investigated the alterations in haematological parameters and condition factor (K) of African catfish (Clarias gariepinus) exposed to Spondianthus preussii leaf powder during a long term experimental period in static renewable bioassay system. The fish with mean weight and length of 300 ±11.54 and 40.00 ±1.15 respectively were exposed to 1.00, 1.10 and 1.20g/l concentrations of Spondianthus preussii leaf powder for 7 - days. During the experimental period, some physicochemical parameters such as pH, temperature, total dissolved solids, dissolved oxygen, ammonia and nitrite were monitored daily. At the end of the experimental period, fish blood was collected to determine: Packed cell volume (%), Haemoglobin (g/dl), Red Blood Cells, White Blood Cell, Platelet, Mean Corpuscular Haemoglobin Concentration (gdl^{-}), Mean Corpuscular Haemoglobin (pg), Mean Corpuscular Volume (fl), Neutrophils (%), Leucocrit (%), Eosinophils (%), Monocytes (%) and Basophils (%). Fish weight (g) and length (cm) were also measured for K (Condition Factor). Total dissolved solids, ammonia and nitrite increased significantly ($p<0.05$) while pH and dissolved oxygen decreased ($p>0.05$) with increase in the concentration of plant leaf powder. Temperature was within range as control. All the haematological parameters dropped except WBC, Platelets, MCH, MCV and N which were significantly ($p<0.05$) raised with increase in plant powder concentration. K values generally dropped with increase in Spondianthus preussii leaf powder concentration when compared with control.

KEYWORDS: *Spondianthus preussii, Condition factor, bioassay, haematology.*

1. INTRODUCTION

Piscicides are often used to control competing species in fish production, especially in small water bodies/enclosures, eradicate fish to control parasites, and conserve or restore native species but their uses are not encouraged because of their toxicity to aquatic organisms and the degradation of the environment. The introduction of most chemicals into the aquatic environment occurs both directly and indirectly and the accumulative chronic effect of the toxicants on aquatic organisms is extremely hazardous to fish. There are many indigenous sources of botanical fish toxicants in Nigeria that are extremely toxic to a wide range of animals including fish. The introduction of some of these piscicides in the aquatic ecosystem will eventually lead to physiological stress in aquatic organisms and ultimately reduce production (Warren, 1977). Many plants contain chemicals which have traditionally been used to harvest fish in almost all parts of the world (Jennes, 1967). *Spondianthus preussii* is tropical swampy trees belonging to *Euphorbaceae*and commonly found in Africa. The leaf and stem bark contain extremely hazardous, very toxic acid as well as *saponins,flavonoid* and *Tanins*. All part of the plants is extremely poisonous and medicinal value of the plant is still unknown (Istvan, 2000 and Keay *et al.* 1964). Fish farmers in Nigeria have persistently and indiscriminately abused these natural plant piscicides by using much higher concentrations than necessary, causing mass mortality of fish in ponds, contaminating the freshwater bodies and affecting non target organisms.

Clarias gariepinus is a freshwater fish found in the tropical regions of West Africa (Nyamweya *et al.*, 2010. This species is widely distributed in Asia and Africa and inhabit most lakes, ponds and rivers where they feed mainly on plankton, insects' larvae, snails, crustaceans, worms and small fishes (Bruton, 1979). *Clarias* species is of the family *Clariidae* and apart from tilapia, *Clarias* is the most cultured fish species in Nigeria and are generally strong fish, they possess an accessory respiratory organ, comprising a paired shaped air chamber containing two arborescent structures located on the fourth bronchial arcs, that are supported by cartilage and covered by highly vascularised tissue which can absorb oxygen directly from the atmosphere. Since the air chamber communicates directly with the pharynx and the gill- chamber, this accessory air breathing organ enables them tolerate adverse aquatic conditions where other cultured fish species cannot survive. They grow faster with additional supplementary feed; the demand for the fish is very high due to its oily flesh (Nyamweya *et al.*, 2010) .

2. MATERIALS AND METHODS.

2.1 Location: This experiment was carried out at the University of Port Harcourt Demonstration fish Farm, Choba Port Harcourt Rivers State.

2.2 Sample Collection And Preparation: The fresh samples of the piscicide (*Spondianthus preussii*) was collected from the forest between Choba and Emouha of Rivers State, Nigeria and identified in Forestry Department, University of Port Harcourt. The sample was washed and oven- dried at 100 ^{O}C, ground into fine powder and stored in a container till it was ready for use.

2.3 Test Fish Species: The fish was obtained from Aqualife consult, a reputable farm in Rivers State. Care was taken to minimize stress during transportation of the fish.

2.4 Acclimation: This was done for a period of 7 days. During this period, the fish was fed once daily with an artificial feed at 3% body weight.

2.5 Experimental Design: Complete randomised design.

2.6 Physicochemical Parameters: Temperature, dissolved oxygen (DO), Ammonia, Nitrites, pH, Total dissolved solids were determined using standard methods.

2.7 Haematological Analysis: 5mls of blood sample was collected from each replicate in the treatment group using disposable syringes and preserved in Ethylene diaminetetra acetic acid (EDTA) bottles. In determining haematological parameters the methods of Blaxhal and Daisley (1973) were used or otherwise stated.

Estimation of Hb Concentration: The haemoglobin was estimated by cyanomethaemoglobin method recommended by International Committee for Standardization in Haematology (ICSH 1996 and 1997). In this method, the alkaline solution of ferricyanide converts haemoglobin ferrous (Fe^{2+}) iron to the ferric (Fe^{2+}) state to form Cyanomethaemoglobin. The colour developed was measured spectrophotometrically at 540 nm with the help of Systronics 106 Spectrophotorneter. Drabkins solution used for the quantitative. Spectrophotometric determination of haernoglobin concentration in whole blood at 540 nm, was prepared by mixing the following reagents in the proportion:

Sodium bicarbonate	1.0 gm
Potassium cyanide	0.05gm
Potassium ferricyanide	0.2gm
Distilled water	1000cc

Drabkin's Solution reacts with all forms of haemoglobin except sulfhemoglobin, a pigment that normally occurs in only minute concentrations in blood. The broad absorption peak of cyanmethemoglobin permits its measurement using both wide and narrow bandwidth instruments (530-550 nm).

Calculation:

$$\text{Hb}\ (\text{g}/100\text{ml}) = \frac{A_{540}\ \text{test sample} \times 15.06(\text{Std. Conc. as stamped on the vial} \times 0.25)}{A_{540}\ \text{standard}}$$

WHITE BLOOD CELL: A white cell count (TLC) estimates the total number of white cells in a cubic millimetre of blood. WBC diluting fluid or Turk fluid contains a weak acid to lyse the red blood cells and Gentian violet stain for staining the nucleus of white blood cells.

The Turks fluid with following composition was used for white blood cell:

Glacial acetic acid: 1.5ml

1% aqueous solution of Gentian violet: 1ml

Distilled water: l00ml

Neubauer'shemo cytorneter (Baker and Silverton, 1982) was used for counting of white blood cell. Using white cell pipette the blood was drawn upto 0.5 mark and the diluting fluid to 11

mark. Thus the dilution was 1:20.

Haematocrit or Packed Cell Volume: This was obtained by centrifuging blood (containing 5mg/mi EDTA) in a graduated tube until corpuscles were packed down to a constant volume. The volume of packed cell was then expressed as a percentage of the original volume of blood. With the aid of capillary pipette a Wintroheshaematocrit tube was filled to the 100 mark with the anticoagulated blood and centrifuged for 5-10 mm at 700RPM. As the original column of blood in the tube is 100mm long, the volume of packed cell is read directly as %age. The analysis was done according to England and Walford (1972).

Erythrocyte Indices: Wintrohe (1974) introduced calculation for determining the size, content and Haemoglohin concentration of red cell. These erythrocyte indices were found very useful in the morphological characterization of anaemia.

Mean Cell Volume (MCV): The MCV is the average volume of red cells and was calculated from the haematocrit (Hct .packed cell volume) and red cell count (TRBC).

$$\text{MCV} = \frac{\text{PCV}(\%)\times 10\,\text{cubic microns}}{\text{RBC}\,(\text{million/m})}$$

Mean Cell Haemoglobin (MCH):The MCH is the content (Weight) of the Hb of the average red cell. It was calculated from the Hb concentration and red cell count.

$$\text{MCH} = \frac{\text{H}b(\text{g/dl})\times 10\,\text{micrograms}}{\text{RBC}\,(\text{million/m})}$$

Mean Cell Haemoglobin Concentration (MCHC): The Mean cell haemoglobin concentration in g% for 100ml erythrocytes was calculated by following formula:

$$\text{MCHC} = \frac{\text{H}b(\text{g/dl})\times 100ml}{\text{PCV}(\%)}$$

Differential Leucocyte Count: A thin blood film was made by spreading a blood drop evenly on clean grease free slide using smooth edged spreader. Giemsas and Leishman's stains were employed for the staining of blood films. Using the 40 objective high-power lens, 100 leucocytes were counted in the blood smear. The percentage of each of the five basic leucocytes (Neutrophils, Eosinophils. Basophils, Lymphocytes, Monocytes) was calculated and DLC reported in percentage.

Fulton's Condition Factor: Values of Fulton's condition factor were calculated using the formula:

$$\text{Fulton condition factor (K)} = \frac{Weightof\ Fish}{l^3}\times 100$$ (Fang *et al.*, 2009).

2.8 STATISTICAL ANALYSIS: Result was subjected to statistical analysis with Duncan's multiple range f-test to test for significant difference ($P<0.05$ between various concentrations of the *Spondianthus preussii* and control).

3 RESULT

Marked behavioral changes like swift opercula movement, sudden jerky swimming body movements, loss of balance, respiratory distress, were literally observed on *C. gariepinus* adult as the concentration of *Spondianthus preussii* leaf powder increased. pH decreased with increase in toxicant concentration and differs significantly ($p > 0.05$) when compared with control (Table 1; Fig.1). Temperature, dissolved oxygen and nitrite did not differ significantly ($p > 0.05$) with increase in concentration – Table 1; Fig. 2, 4 and 6. Total dissolved solids (TDS) and ammonia (NH_3) differed significantly ($p < 0.05$) when compared with control - Table 1; Fig. 3 and 5. The exposure of C. *gariepinus* to *Spondianthus preussii* leaf powder caused the mean values of PCV, HB, MCHC, RBC and L to decrease with increase in concentration of the toxicant Their values peaked at treatment 0.00 mg/l (control) and the least at treatment 1.2 g/l. WBC, Platelets and N were raised with increase in concentration of the toxicant when compared with the control. The 3 blood parameters (White blood cells - WBC, Platelets and neutrophil - N) peaked at treatment 11.00 mg/l and least at 0.00 mg/l (control). The mean values of MCV, MCH, M and E, fluctuated while B was not found. MCV and MCH peaked at treatment 1.10g/ l and least at control. At 1.20g/l, M and E had the least values and highes at control - Table 3 and Figure 3

The highest condition factor (K) was at 1.10g/l and lowest at 1.20g/l. K, ranged from 0.35 to 0.47 as shown in Table 2.

Table 1. Physicochemical parameters

TREATMENTS	pH	TEMP	TDS	DO	NH_3	NITRITE
T_0 0.00g/l	8.5 ± 1.15^b	32.6 ± 1.27^c	15 ± 1.15^b	7.0 ± 0.011^a	0.18 ± 0.011^b	0.04 ± 0.011^a
T_1 1.00g/l	7.1 ± 1.27^c	31.4 ± 1.27^c	31 ± 1.15^b	**5.0** $\pm 0.011^a$	**1.5** $\pm 0.011^b$	**0.80** $\pm 0.011^a$
T_2 1.10g/l	7.0 ± 1.27^c	32.5 ± 1.27^c	44 ± 1.15^b	**4.9** $\pm 0.011^a$	**1.3** $\pm 0.011^b$	**1.0** $\pm 0.011^a$
T_3 1.20g/l	6.9 ± 0.011^a	31.6 ± 1.27^c	62 ± 12.7^d	**4.8** $\pm 0.011^a$	**1.26** $\pm 0.06^a$	**1.2** $\pm 0.011^a$

Mean±SE with same superscript across column values are not significantly different ($p>0.05$.)

Keys: TEMP. = Temperature (0^C); TDS = Total dissolved solid; DO = Dissolved oxygen;

NH_3 = Ammonia

Table 2. Condition factor (k) of *C. gariepinus*.

Conc.(mg/l)	MEAN WEIGHT	MEAN LENGTH	K

0.00g/l	300 ±11.54[a]	40.00 ±1.15[a]	0.47
1.00g/l	300 ±11.54[a]	41.16 ±1.01[a]	0.43
1.10g/l	300 ±11.54[a]	43.33 ±1.20[b]	0.37
1.20g/l	260 ±11.54[a]	42.16 ±1.48[c]	0.35

Mean±SE with same superscript across column values are not significantly different (p>0.05.)

Table 3. Haematological parameters of *C. gariepinus*

TREATMENTS	PCV	HB	RBC	WBC	PLT	MCHC
0.00g/l	45 ±1.15[a]	15.0 ±1.15[a]	6.2±1.27[b]	7.5±1.27[b]	153±14.52[c]	33 ±1.15[a]
1.00g/l	40 ±1.15[a]	13.3±1.27[b]	5.9±1.27[b]	9.3±1.27[b]	180±17.3[e]	32±1.7[a]
1.10g/l	34 ±1.15[a]	11.3 ±1.27[b]	4.5±1.27[b]	11.2±1.27[b]	225±12.7[d]	31 ±1.15[a]
1.20g/l	30 ±1.15[a]	10.0 ±1.21[b]	4.1±1.27[b]	12.5±1.27[b]	262±12.7[d]	30 ±11.5[c]

Mean±SE with same superscript across row values are not significantly different (p>0.05.)

Table 3. Haematological parameters of *C. gariepinus*

TREATMENTS	MCH	MCV	N	L	E	M	B
0.00g/l	24 ±1.15[a]	73 ±1.15[a]	25 ±1.15[a]	63 ±1.15[a]	5±1.15[a]	7±1.15[a]	-
1.00g/l	22±1.15[a]	68±12.7[d]	30±11.5[c]	62±12.7[d]	3±1.15[a]	5±1.15[a]	-
1.10g/l	25 ±1.15[a]	76 ±12.7[d]	35 ±12.7[d]	60 ±11.5[c]	2±1.15[a]	3±1.15[a]	-
1.20 g/l	24 ±12.7[d]	73 ±12.7[d]	38±1.12.7[d]	55 ±12.7[d]	2±1.15[a]	5±1.15[a]	-

Mean±SE with same superscript across row values are not significantly different (p>0.05.)

Key: PCV = Packed cell volume (%) HB = Haemoglobin (g/dl); RBC = Red Blood Cells; WBC = White Blood Cell; PLA = Platelet ; MCHC = Mean Corpuscular Haemoglobin Concentration (gdl-) MCH = Mean Corpuscular Haemoglobin (pg); MCV = Mean Corpuscular Volume (fl); N =Neutrophils (%); L = Leucocrit (%); E = Eosinophils (%); M= monocytes (%); B = Basophils (%).

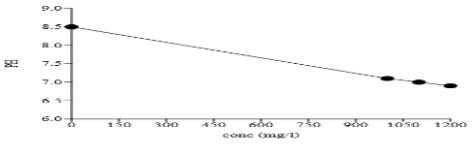

Fig. 1. pH

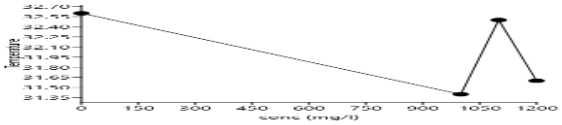

FIG 2. Temperature

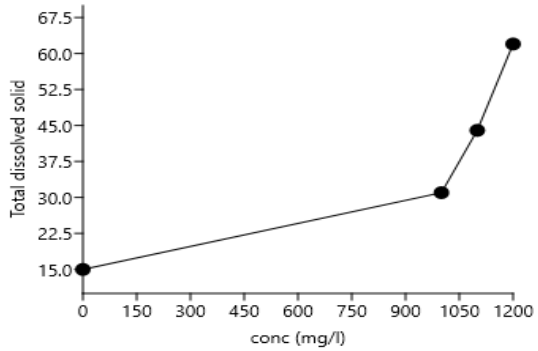

Fig. 4. Dissolved oxygen

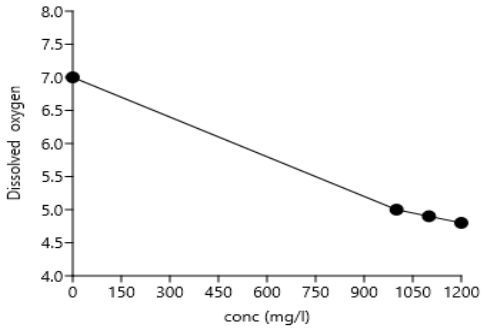

Fig. 3. Total dissolved solids

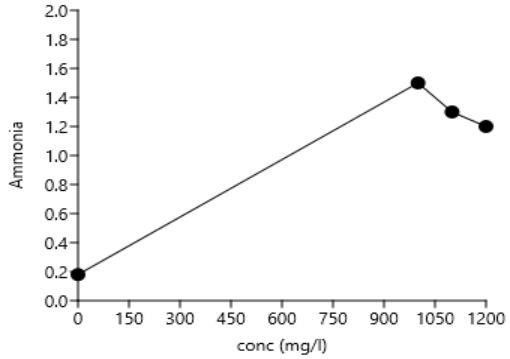

Fig. 6. Nitrite

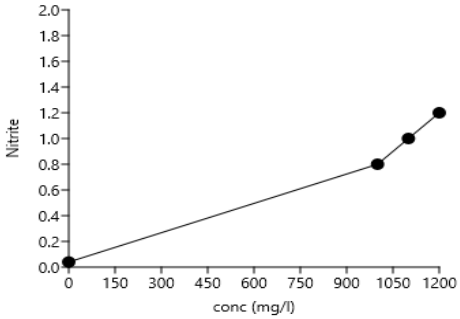

Fig. 5. Ammonia

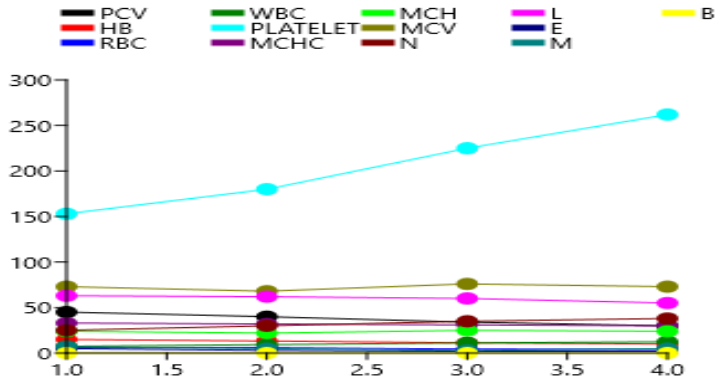

Fig.8. Haematological variables

4. DISCUSSION

The report by Muhammad *et al.*,(2010), Ajani and Ayoola (2010) and Ayuba and Ofojekwu (2002) which noted that fish exposed to varied concentrations of some toxic plants extracts exhibited marked behavioral changes like swift opercula movement, sudden jerky swimming body movements, loss of balance, respiratory distress, which demonstrated a sensitive indicator of physiological stress is in agreement with this work. The behavioral responses observed in this work are also in agreement with that of Nwani *et al.*, (2012) who also reported abnormal movements and high respiration rate in tilapia exposed to glyphosate herbicide. The findings on temperature, dissolved oxygen (DO), ammonia, nitrites, pH, total dissolved solids in this work were within acceptable range for fresh water fish culture as recommended by Boyd, (1979). This means that the results obtained in this study were not affected by physicochemical parameters of the culture tanks.

Stressors evoke non-specific responses in fish which enables the fish to cope with the disturbance and maintenance of its homeostatic state - Barton, (2002). If severe or long lasting, the reaction then becomes dangerous and threatens the fish health and comfort. Therefore, in the presence of stressors (contaminants/pollutants), blood parameters and blood chemistry can be employed as a standard laboratory test to determine diseased conditions and metabolic disorder in fish, Celik, 2004. In the present investigation, it has been discovered that low level sublethal contact of these compounds reduced hematological parameters. Omoregie *et al.*, (1910) reported that toxicants and pollutants have significant effects, which can result in several physiological dysfunctions in fish which induces changes in blood parameters.

Change of the blood cell distribution has been correlated with the changes in environmental conditions, De Wilde, 1967. The exposure of *C. gariepinus* adult to sublethal concentrations of *Spondianthus preussii* leaf powder caused a significant decrease in packed cell volume, haemoglobin, Red blood cell, MCHC, monocyte, leucocyte, erythrocyte and monocyte of the fish. The decrease in hemoglobin concentration is similar to those reported in *C. gariepinus* exposed to cassava effluents and tobacco (*Nicotiana tobaccu*) leaf extracts (Adeyemo, 2005). This pattern of response may be attributed to hemolysis which results in hemodilution, a means

of diluting the hemoconcentration of the leaf thus reducing the effect of the toxicants/pollutant in its system. This effect on *C. gariepinus* might have been achieved through failure or suppression of normal mechanisms promoting erythropoiesis and/or deficiency of some factors required for the maturation of the red cell. The causes of leucopenia observed in the present study are supposed to be according to the degeneration, depression, depletion and destruction of the blood forming materials by the active content of the leaf. The hemoglobin, hematocrit, red blood cells, MCHC, leucocytes and monocytes values depletion in the fish could be credited to the lysis of erythrocytes as reported by Kori-Siakpere and Ubogu, 2008. Thus, the significant reduction in these parameters is an indication of severe anemia. The slight fluctuations recorded in MCV and MCH of this work could be due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis.

Christensen *et al.*, 1978 reported that white blood cells in fish respond to various stressors including infections and chemical irritants. Thus increasing or decreasing numbers of white blood cells are a normal reaction on the exposure of toxicants, Kori-Siakpere and Ubogu (2008). In the present investigation the increase in WBC (leukocytosis) may have resulted from the excitation of defense mechanism of the fish to counter the effect of the toxicant. The increase observed in percentage neutrophils in this work is attributed to tissue damage. Finally, a slight but significant increase of platelets was recorded in this investigation.

The morphometric index which provides information on the physiological state of fish in their different habitats in relation to its welfare is generally referred to as Condition factor (K). It is based on the principle that fish with better growth rate are in a better condition. The condition factor recorded in this work ranged between 0.35 and 0.47 which is in agreement with that of Anyanwu *et al.* (2007) who reported 0.319 - 0.869 for *C. gariepinus*.

5. CONCLUSION

In conclusion, the alteration of all the haematological parameters and condition factor in this experiment shows that the use of *Spondianthus preussii* leaf powder for fishing in our streams and rivers by fishers is deleterious and should be highly discouraged. Relevant authorities saddled with the responsibility of protecting our water bodies should take note. There is also need for further research on the impact of this plant on other aquatic organisms that is of economic importance to man.

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