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Sublethal Toxicity and Histological Effect of *Balanites aegyptiaca* Bark (Aqueous extract) on Juvenile of *Clarias gariepinus* (Burchell, 1822)

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Abstract
The sublethal concentration of the *Balanites aegyptiaca* extract on juvenile of *Clarias gariepinus* was assessed after exposing them for 112 days at various concentration of 14mg/L, 7mg/L, 5mg/L. and 3mg/L. The effect of the sublethal concentration on liver, gills, and gonad of fish was investigated. Like-wise the resultant effect of the extract on haematocrit percentage of the fish, growth rate and nutrient utilization of the fish was also assessed. The histological study shows that the liver was vacuolated hepatocytes with frequent necrosis, the bile canaliculi were dilated. The gills were pale and congested and the gill filaments was hyperplastic and edematous with vacuolated epithelial covering of the gill rakers. The highest feed utilization was recorded in the tank with lowest sublethal concentration of 3mg/L. While the lowest feed utilization was recorded in the tank with concentration of 14mg/L.

Keywords: sublethal, histological, *Balanites aegyptiaca*, *Clarias gariepinus*

Introduction
In Nigeria however, the national fish production has been on the decrease from 80's to 90's (Bouari, 1995). This decrease was due to low aquaculture production and over exploitation of the resource by the use of unorthodox fishing methods. The most commonly used unorthodox method is the application of *Balanites aegyptiaca* (Del., 1813) (date palm desert) powder along the River Niger, which is the unique permanent river that traverses the country with a length of over 550 km. It has toxic properties, which has been found to be useful in the control of intermediate host of some diseases that are waterborne, for example, snail, etc. (Maydell, 1991).

A sub-lethal effect of a substance will not cause death but may cause stress, Many times there are sub-lethal effects and are not immediately noticed, as the organism so affected does not die. There are, however, consequences of the impending impairment of the health of the organism, its behavior, physiology (metabolism), and life cycle. For example, some toxic plants interfere with biochemical activities at the cellular level in many organisms. While not directly leading to death, in many cases this 20 impairment can lead to poor health and subject the animals to infections and other equally debilitating illnesses.

Omoregie *et al*. (1994) observed that sub-lethal concentrations of toxicants in the aquatic environment did not result in mortality of the aquatic organisms, however they had significant effects, which resulted in several physiological dysfunctions. Results from various investigations revealed that fish exposed to sub-lethal concentrations of toxicants grew significantly less and had poorer food conversion compared to their counterparts, which were not exposed to the toxicant. Oluwole and Bolarinwa (1995) [9]. noted growth reduction and disruptive properties in animals exposed to sub-lethal concentrations of some plants (*Jatropha curcas* and *Azadirachta indica*) extracts.

The toxicity of the plant extract on fish and some other aquatic organisms has been reported by many authors (Agbon *et al*., 2002). The effect of bark extract of *Azadirachta indica* (A. Juss, 1830) (Neem) on *Tilapia zillii* were investigated by Omoregie & Okpanachi (1997) [8]. Agbon *et al*. (2002) studied the effect of Tobacco leaves (*Nicotiana tabacum*, L. 1753) on O. Niloticus. The toxicity of *B. aegyptiaca* and *Kigelia africana* (Lam.) Benth., 1849, on O. niloticus was investigated by Ufodike & Omoregie (1994). The need therefore arises to investigate the effects of the bark of *B. aegyptiaca* alone, on *Clarias gariepinus*, with a view of investigating the effect *B. aegyptiaca* bark extract on the histology. *Clarias gariepinus* is one of the most popular culture fish species in many tropical countries (Yi, 1999) [11]. such as Nigeria (Ibrahim, 2002). The objective of this study is to evaluate the sublethal toxicity effect of *Balanites aegyptiaca* on the histology of *Clarias gariepinus*.

Materials and Methods
Experimental Area
Adamawa State is located within the climate of Northern Guinea Savannah zone and lies between latitude 8^0 and 11^0 N and Longitude 11.5^0 and 13^0 E and climate is tropical with two distinct seasons; dry and wet seasons. The experiment took place in Fisheries Department Laboratory of Modibbo Adama University of technology which is located in Yola.

Plant Sample Collection
The fresh samples of the plant was collected from Sangere,

Area of Adamawa state and were taken fresh to Department of Fisheries laboratory for further analysis and study.

Extraction procedure of *Balanite aegyptica*

The bark of *Balanites aegyptiaca* was obtained from Sangere, and was taken to Modibbo Adama University of Technology Yola and dried in shade for three days. The dried bark was then crush into powdered form using pestle and mortal and sieved using a 0.1mm sieved. 200g of the fine powder was soaked in 1litre distilled water and was allowed to stand in this condition for two days with occasional mixing. At the end of 48h, the sampled was filtered using whatman no.1 filter paper. The filtrate was concentrated by drying in an oven at 40^0C

Fish Sample Collection

Juvenile of *Clarias gariepinus* with average weight and length of 3.1g and 6.2cm respectively, was obtained from Fisheries unit of Devine farm, and transported to the Fisheries Laboratory, Modibbo Adama University of Technology, Yola in plastic, container containing cool water, in the early hour of the day, covered with screened Nylon net to prevent the fish from jumping out of the bucket and allow easy percolation of atmospheric oxygen. The fish was acclimatized in glass tanks for at least five (5) days prior to the commencement of the study. For sub-acute toxicity test (16weeks) fish was randomly divided into group of 10 fish each. One group was serve as control while the remaining group was expose to different Balanite concentration. At the end of the 16weeks period blood samples was taken before sacrificing the fish. Liver was collected, its fresh weight was recorded. Small slice of liver was use for histology.

Stocking of the fish

The fish was distributed at random in duplicates into 10 glass tanks (10 fish/tank and a total number of 100 catfish Juvenile was used.

Water quality

The pH of the solution was measured with the pH meter, temperature with mercury-in glass thermometer dissolved oxygen with a digital $D0_2/C0_2$ meter and total alkalinity was measured by titration, 0.01N of hydrochloric acid against 25m1 of test solution while two drops of methyl orange indicator was added with a continuous shaking until the colour changes from blue to pale pink. The exposure was lasted for 96h. Water temperature, pH dissolved oxygen and total alkalinity was determined every six hours daily of the experiment.

Experimental set up and management.

Ten tanks with dimension 30cm x 30cm x 45cm were set up. The bioassay was conducted in duplicate. A total of 100 juveniles of *C. gariepinus*, each were sorted randomly into the concrete tanks at 10 fish per tank as already described. Experimental tanks were cleaned every two days before feeding by siphoning dirt, unconsumed feed and faeces with flexible hose. The water level of 60L in the tanks were then maintained by adding fresh water from the reservoir and appropriate volumes of the toxicant added into each tank, except the control tanks while Feeding of fish was carried out thrice daily at a feeding rate of 5% body weight. Daily ration was split to three and fed thrice (8:00am, 1:00pm and 6:00pm) per day. Quantity of daily ration was adjusted weekly based on fish weight increment.

Histological examination

Gonad and liver specimens was collected from the fish and preserved in 10% formalin and processed for histological examination using standard histological techniques as described by Drury and Wallington (l967) [3]. Small slices of liver and gonad (approximately 3mm) was fixed in Bouins fluid which was change after 24hour for 70% alcohol. All sample was ultimately embedded in paraffin wax block from which 5µm section was taken and subsequently stained with heamoxylin and eosin, the stained sections was examined with the aid of a light microscope. The liver sections was classified as stage1 to 3 according to the stage of spermatogenesis. Ovaries was classified 1 to 6 dependent upon the stage of cogenesis. The histological score for the gonads was based upon method recommended by Drury and Wallington (1967) [3].

Data analysis

All data generated was subjected to ANOVA and correlation, to ascertain the significance level at $p < 0.05$ using SPSS 10.0 window 2007 package using Duncan methods (Duncan, 1955) [4].

Results

The results of haematological parameters of *C. gariepinus* exposed to various concentrations of *Balanites aegyptiaca* for 16 weeks are summarized in Table 1. Exposure of *C. gariepinus* to *B. aegyptiaca* for 16weeks showed that Packed cell volumes (PCV) ranges between 19.5±0.10 and 36.1±0.10. The highest PCV (%) of 36.1% was recorded in the control group and the lowest PCV was recorded in the treatment, T_4 with the value of 19.5%. Haemoglobin had a range of 6.5±0.1 and 12.03±0.2g/100mL respectively. The highest haemoglobin value was recorded in the control (0.00g/L) (12.03±0.2g/100mL) while the lowest haemoglobin was 6.5±0.1g/100mL in treatment, T_4. Red Blood Cells (RBC) was the highest in control ($3.2±0.3 \times 10^6$ mm^3) and lowest in T_4 ($1.1±0.0510^6$ mm^3). Control group recorded the lowest Neutrophils of 16.2±0.3% while T_4 recorded the highest neutrophils of 20.42±0.1%. Lymphocytes values ranges from 39.5±0.01% to 43.12±0.4% while T4 and control treatment having the lowest and highest value respectively. White blood cell (WBC) had a slight range of 11.1±0.1 and $12.3±2.1 \times 10^4$ mm^3. T4 recorded the highest while the control had the lowest white blood cell value. These haematological parameters showed a decreasing trend with increasing concentrations of *B. aegyptiaca*.

Table 1: Some Haematological Parameter of *Clarias gariepinus* Exposed to various Concentration for 16 weeks

Blood Parameters	Control	T1	T2	T3	T4
MCV (μm^3)	112.1±1.2[a]	114.3±0.1[a]	115.1±0.3[b]	118.0±0.2[c]	121.3±0.2[c]
MCHC (%)	21.3±1.7[a]	23.3±0.02[b]	24.1±0.01[b]	23.1±0.05[b]	24.7±0.1[b]
PCV (%)	36.1±0.1[a]	28.4±0.01[b]	24.02±0.00[b]	22.1±0.1[bc]	19.5±0.1[c]
Hb (g100m1$^+$	12.03±0.2[a]	9.5±0.10[b]	8.07±0.01[b]	7.37±0.1[b]	6.5±0.1[c]
RBC ($X10^6mm^s$)	3.20±0.3[a]	2.10±0.1[a]	2.0±0.05[a]	1.21±0.1[b]	1.1±0.05[b]
WBC ($X10^4mm^3$)	12.3±2.1[a]	12.1±0.3[a]	11.9±0.04[a]	11.4±0.2[b]	11.1±0.1[b]
Lymphocyte (%)	43.12±0.4[a]	42±0.3[a]	41.1±0.02[a]	40.3±0.5[a]	39.5±0.01[b]
Neutrophils (%)	16.2±0.3[a]	17.3±0.2[b]	17.82±0.1[b]	18.39±0.5[b]	20.42±0.1[bc]

NB: Values with different superscript along row are statistically different to each other at $p<0.05$
key: T_1 = 0.014g/L. T_2 = 0.007g/L. T_3 = 0.005g/L. T_4 = 0.003g/L.

Table 2 shows the parameters observed and recorded in the growth performance and nutrient utilization of *Clarias gariepinus* exposed to different concentration of *B. aegyptiaca*. The mean weight gain across the treatments were significantly different ($p<0.05$). Treatment (T_0) had the highest mean weight gain of 818.43±2.55g and lowest mean weight gain was recorded in Treatment (T_3) which is 366.47±1.63g. Mean feed intake was significant across the treatment, where Treatment (T_0) had the highest value of 843.12±3.11g and the lowest mean feed intake was 812.12±2.94g in Treatment (T_1). The relative growth rate (RGR) observed in the Table 7 showed that Treatment (T_0) had the highest relative growth rate of 1439.3% followed by Treatment (T_4) (1145.3%), T_3 (619.77%), and T_1 (592.79%) respectively. Similarly, T_0 had the highest specific growth rate (SGR) value of 1.06±028%/day while the lowest SGR value was recorded for T_1 (0.75±0.03%/day). The feed conversion rate (FCR) was significant across the treatment; the highest FCR of 2.30±0.21 was shown in T_1; however T_0 showed the lowest FCR value of 1.03±0.11.

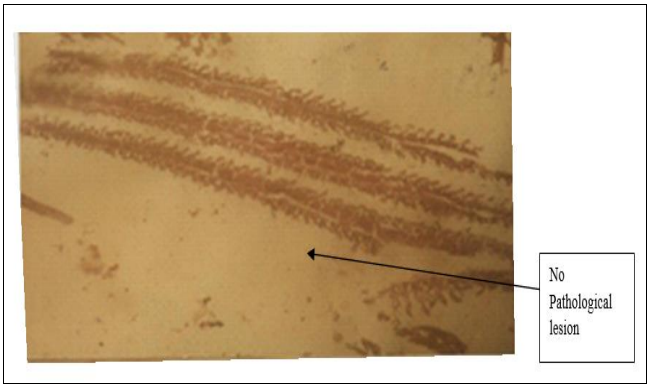

Fig 1: Histology of Gill of *Clarias gariepinus* Exposed to Control Solution

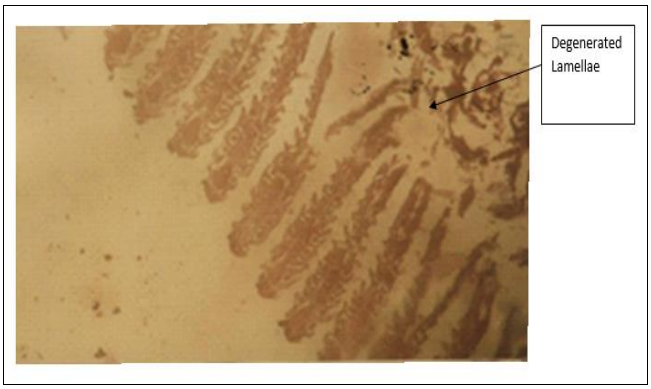

Fig 2: Photograph of Gill from *Clarias gariepinus* Exposed to 0.003g/L of *Balanites aegyptiaca*

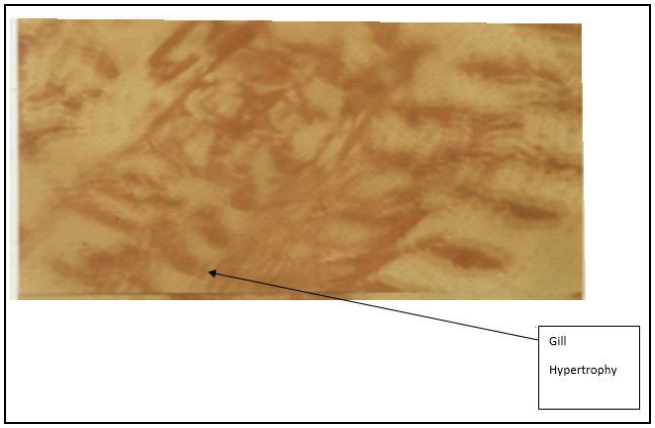

Fig 3: Photograph of Gill from *Clarias gariepinus* Exposed to 0.005g/L of *Balanites aegyptiaca*

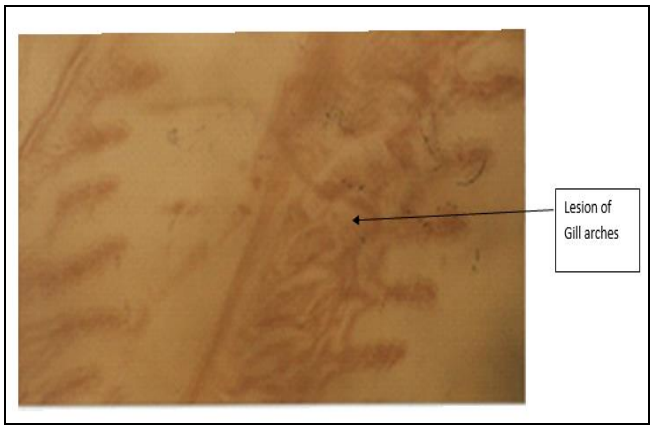

Fig 4: Photograph of a Gill from *Clarias gariepinus* Exposed to 0.007g/L of *Balanites aegyptiaca*

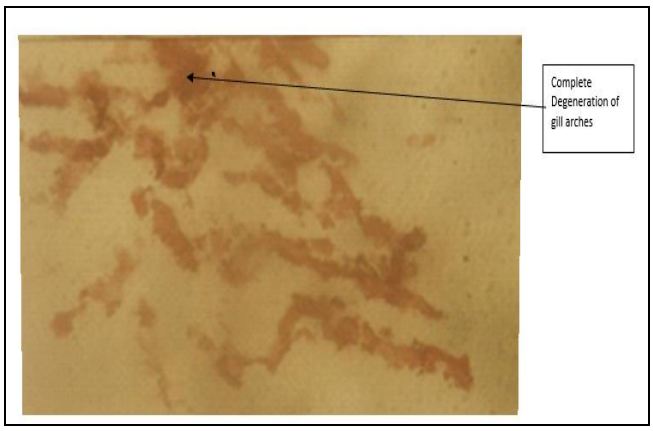

Fig 5: Photograph of Gill of *Clarias gariepinus Exposed to 0.014g/L of Balanites aegyptiaca*

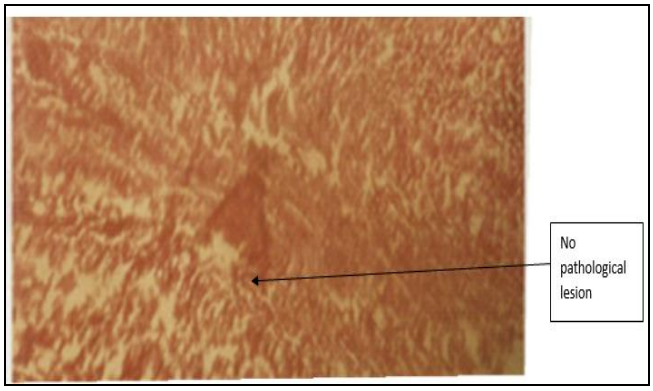

Fig 6: Photograph of Liver of *Clarias gariepinus* of Control Tank

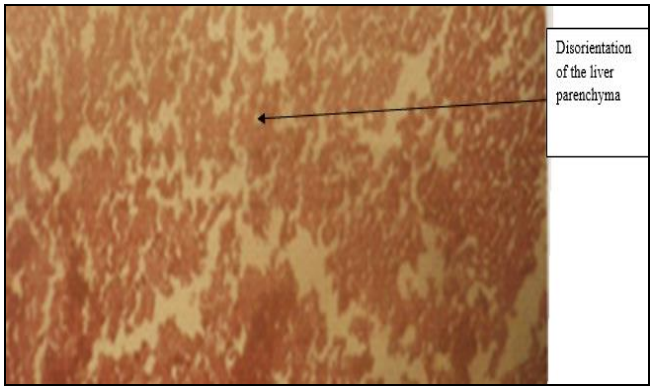

Fig 8: Liver from *Clarias gariepinus* exposed to 0.005g/L of *Balanites aegyptiaca*.

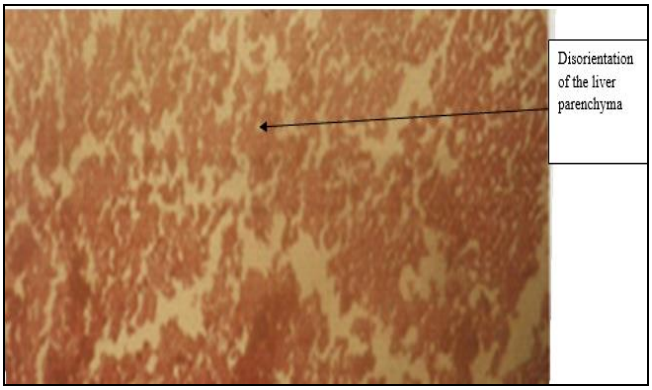

Fig 7: Photograph of Liver from *Clarias gariepinus* Exposed to 0.003g/L of *Balanites aegyptiaca*

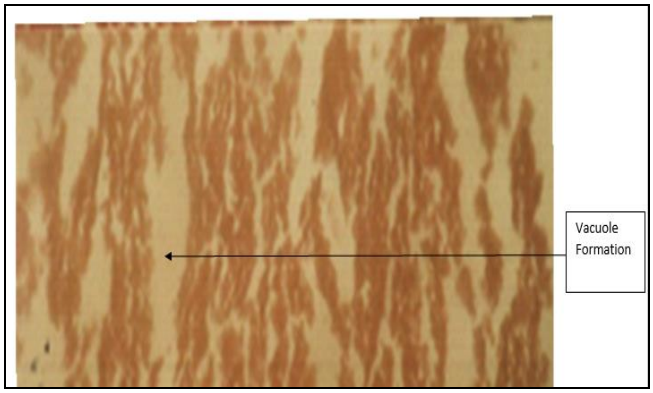

Fig 9: Photograph of Liver from *Clarias gariepinus* Exposed to 0.007g/L of *Balanites aegyptiaca*

Fig 10: Photograph of Liver from *Clarias gariepinus* Exposed to 0.014g/L of *Balanites aegyptiaca*.

Table 2: Growth Parameters, Feed Utilization of *C. gariepinus* Exposed to Different Concentration of *B. aegyptiaca*

Parameters	T_0 ($^1/_5$ LC_{50})	T_1 ($^1/_{10}$ LC_{50})	T_2 ($^1/_{15}$ LC_{50})	T_3 ($^1/_{15}$ LC_{50})	T_4 ($^1/_{20}$ LC_{50})
Initial mean weight (g/fish)	56.87	59.21	60.22	59.13	58.23
Final mean weight (g/fish)	875.3	410.2	430.32	425.6	725.1
Mean weight gain (g/fish)	818.43±2.55[a]	350.99±1.59[b]	370.1±1.66[c]	366.47±1.63[c]	666.9±2.04[d]
RGR (%)	1439.12	592.79	614.5	6.1977	1145.3
SGR (%/day)	1.06±0.28[a]	0.75±0.03[b]	0.76±0.04[b]	0.77±0.06[b]	0.98±0.85[a]
Mean feed intake (g)	843.12±3.11[a]	812.12±2.94[b]	814.4±2.90[b]	821.3±2.99[c]	832.6±3.00[d]
FCR	1.03±0.11[a]	2.3±0.21[b]	2.2±0.01[b]	2.24±0.30[b]	1.2±0.09[a]
Condition factor (k)	4.45	3.85	6.69	2.72	2.97

NB: Values with different superscript along row are statistically different to each other at $p<0.05$

Discussion

The results of erythrocytes count (RBCs), haemoglobin content (Hb) and haematocrit value (PCV) obtained from the fish exposed to sub lethal dose of *Balanites aegyptiaca* shows significant reduction in RBCs, Hb and (PCV). The reduction of these parameters in *Clarias gariepinus* at sub lethal levels of the extract agrees with the reports of (Wintrobe, 1978) that this might be due to the destruction of mature RBCs and the inhibition of erythrocyte production, hence leading to reduction of heme synthesis. Also, the decrease in RBCs count may be attributed to hematopathology or acute haemolytic crisis that results in

severe anemia in most vertebrates including fish species (Khangarot and Tripathi, 1991) [5]. It may also be the decrease in the RBCs which can be attributed to reduction of growth and other food utilization parameters resulting in severe anaemia (James and Sampath, 1999). The calculated blood indices MCV, MCH and MCHC have a particular importance in anaemia diagnosis in most animals. The perturbations in these blood indices increase MCV, decrease of MCH and MCHC may be attributed to a defense against plants toxicity through the stimulation of erythropoiesis or may be related to the decrease in RBCs, Hb and PCV.

Histological changes in the gill, liver, tissues were observed in all treatments and pathologic lesions were similar for all treatments, although the intensity of cell damage increased with increasing concentration and time of exposure. studied the histology on the liver and gill of *Clarias gariepinus* exposed for 112days to different concentrations of *Balanites aegyptiaca* with concentration of 0.003g/L, 0.005g/L,.0.007g/L and 0.014g/L. The gills were pale and congested. The liver showed vacuolated hepatocytes with frequent necrosis, evidenced by pyknosis. The bile canaliculi were dilated. The epithelial covering of the gill filaments was hyperplastic and edematous with vacuolated epithelial covering of the gill rakers. The lamellar blood spaces showed telangiectasis. Wade *et al*. (2002) [7] studied the toxicity of cassava (*Manihot esculenta*) effluent on *O. niloticus*. He histological examination of the gill, liver and kidney tissues indicated damage ranging from oedema and telangiectasia of the gill lamellae and gill hyperplasia to vacuolation of the liver cells and necrosis.

The result for growth parameter (SGR, FCR, MFI) and nutrient utilization shows protein intake (PI) of fish in the control tank with the highest value of 49,406, Followed by the one with least concentration of 0.003g/L and a corresponding value 46375.8. while the one with lowest PI value is that which has higher concentration 0.014g/L the Specific growth rate (SGR) was highest in control tank (1.06) and lowest in treatment tank with concentration 0.014g/L. The best feed conversion ratio (FCR) is recorded in the control tank, which is an indication of an optimum level of utilization of the diet by the *C. gariepinus* juveniles. which is in agreement with Adikwu (2003) [1] who stated that the lower the FCR, the better the feed utilization by the fish. In this study, the lowest FCR value is an indication of better feed utilization by the fish and this account for better growth performance of *C. gariepinus*. also the increase in weight gain indicate that the fish were able to utilize the feeds given to them during the period of sub-lethal exposure.

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