



## SUBLETHAL EFFECT OF SNIPER 1000EC ON BIOCHEMICAL PARAMETERS OF *Clarias gariepinus* (BURCHELL, 1882) UNDER LABORATORY CONDITIONS

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### ABSTRACT

The study was carried out to ascertain the effect of sub-lethal concentrations of 2, 3-dichlorovinyl dimethyl phosphate (Sniper 1000EC) on biochemical parameters of *Clarias gariepinus* (Burchell, 1822) under laboratory conditions. Experimental fish were exposed to test water separately diluted with sub-lethal concentrations of sniper 1000EC (0, 0.27, 0.31, 0.41 and 0.55 mg/L). Water quality parameters and physiological parameters were monitored according to standard procedures. The monitored water quality parameters such as temperature, free carbon (iv) oxide, pH and dissolved oxygen significantly decreased while total alkalinity and conductivity increased significantly in the exposed media compared to the control test. A 28 day exposure to sub-lethal concentrations of the toxicant resulted in changes in biochemical parameters of the fish on the exposure days (1, 14 and 28). Biochemical parameters such as total protein, albumin, globulin and lactate dehydrogenase (LDH) decreased significantly ( $p < 0.05$ ) with increasing concentrations of the toxicant. Aspartate aminotransferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP) increased significantly ( $p < 0.05$ ) with increasing concentrations of the toxicant. This study showed that sniper 1000EC has adverse effect on activities of liver enzymes and decreased protein concentrations, as indicator of possible liver damage. It is recommended that the use of Sniper 1000EC by local fishermen be banned to save aquatic ecosystem.

**Keywords:** Sniper 1000EC; *Clarias gariepinus*; chronic toxicity and biochemical parameters

### INTRODUCTION

The Africa catfishes of the genus *Clarias* are a highly esteemed group of fishes in tropical Africa and they command high market value. Their hardy nature and possession of accessory air-breathing organs enable them tolerate adverse aquatic conditions (Reed *et al.*, 1967). Nonetheless, *Clarias gariepinus* are very delicate and sensitive to aquatic pollutants. The African catfish (*Clarias gariepinus*) is an important food fish in Nigeria, which is also good for research work (Abubakar, 2012). *Clarias gariepinus* is not only the most predominant fish species raised in aquaculture in Nigeria, but has also served as an experimental model of aquatic vertebrate for two decades (Cavaco *et al.*, 2001).

The assessment of serum levels of biochemical factor could be used not only for the diagnosis of disease, but also to obtain information which could be of use for taking preventive measures during aquaculture. It would be very useful to measure the levels of this parameter at different times to ascertain the possible beneficial effects of treatment with toxicant and immunostimulants, as well as to detect the occurrence of any potential negative effect of stressors involved in aquacultural management (Swain *et al.*, 2007). Many studies have shown that biochemical changes occur in fishes that are exposed to environmental contaminants (Luskova *et al.*, 2002). These biochemical changes elicited mainly in the blood and organs under toxicant exposure are among the most important indices of the status of the internal environment of the fish under chemical exposure (Edsall, 1999). Therefore, the changes in the levels of metabolites in the organs and biochemical processes of the organisms, resulting from the effects of various pollutants, make it possible to assess the effects of these chemicals in the organisms (Chang *et al.*, 2005). The enzymes of common interest are the transaminases, alanine transaminase (ALT) and aspartate transaminase (AST); and phosphatase: alkaline phosphatase (ALP) and acid phosphatase (ACP) (Begum, 2004). Enzyme is used as a potential biomarker for a variety of different organisms due to its highly sensitivity, less variability, high conserveness among species and often easier to measure as stress indices (Vijayavel and Balasubramania, 2006; Sanjib *et al.*, 2009). Numerous biochemical indices of stress have proposed to assess the health of non-target organisms exposed to toxic chemicals in aquatic ecosystem (Nimmi, 1990). However, it has been reported that apart from nerve tissue, tissues like blood, liver and gills also contribute information in detection of toxic symptoms caused by certain groups of pesticides (Venkataramana, *et al.*, 2006).

Widespread application of various pesticides has aggravated the problem of pollution to aquatic environment. Due to these synthetic chemicals, environment has failed to keep its healthy characteristics. The insecticides of proven economic potentialities could not do well in the ecosystem when viewed on extra fronts since these revenue poisons, in a residual form or as a whole, get into the aquatic ecosystem. They cause a series of problems to aquatic organisms (Mastan and Ramayya, 2010).

Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate), a brand of dichlorvos, is contact acting and fumigant insecticide (Idi-Ogede *et al.*, 2016). Like all organophosphates, it kills insects and other target organisms because of its toxicity to the nervous system. This is achieved by inhibition of enzyme acetylcholinesterase (AChE) that breaks down acetylcholine at the receptor site for partial uptake into the nerve terminal. Without functioning AChE, accumulation of acetylcholine results in depolarizing block of muscle membrane, producing rapid twitching of involuntary muscles, convulsions, paralysis and early death. Indiscriminate use of Sniper 1000EC is common among local fishermen from Northern parts of Niger state.

Despite the indiscriminate use of Sniper 1000EC by local fishermen, there is a paucity of information on its toxicity. The aim of this study was to evaluate the effect of sublethal concentrations of sniper 1000EC on biochemical parameters of *Clarias gariepinus* (Burchell, 1822) under laboratory conditions.

## MATERIALS AND METHODS

### Study Area

The study was conducted in Minna which lies between Latitude 9° 36' 50" North and Longitude 6° 32' 24" East with estimated population of about 304, 113 (National Population Commission, 2007). It is the capital of Niger State; Minna is connected to neighboring cities by road.

The climate of Minna lies within a region described as tropical climate. It has a tropical dry and wet climate. The region is characterized by double rainfall maxima. The town has a mean annual precipitation of 130mm. the rainy season commences most of the time in April and lasts till October, with fluctuation in amount of rainfall received per year. The highest mean monthly rainfall is September with almost 300mm. Temperature is uniformly high throughout the year reaching the peaks of 40°C (February/March) and 30°C (November/ December).

The vegetation of Minna consists of Open Savanna. The Fadamas of the larger rivers support savanna with occasional streams covered with dense riparian woodlands or forestry area (Source: Ministry of Agriculture Minna).

### Procurement of Test Fish

Juveniles of *Clarias gariepinus* (mean body weight  $19.47 \pm 1.05$ ; mean standard length,  $20.00 \pm 0.45$ cm) were obtained from Abdullahi fish farm, Chanchaga, Minna and brought to the laboratory. The fishes were kept in the glass aquaria to observe any visible pathological symptoms. Before introducing into the aquarium, fishes were treated with 0.1% KMnO<sub>4</sub> solution to obviate any dermal infection.

### Acclimation of Test Fishes

Fishes were acclimatized to laboratory conditions for a period of two weeks. No mortality was recorded during acclimation period. The fishes were fed with pelleted feed containing 35 % crude protein at 5% body weight per day. Daily ration was divided into three portions and fed thrice per day. After acclimatization, fishes were kept in different concentrations of sniper 1000EC in different aquaria. The test solutions were renewed fortnightly.

### Sources of Sniper 1000EC and its Exposure

Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate) was purchased from Minna central market. Renewal toxic test method (APHA, 1992) was used. Fishes were exposed to sub-lethal concentrations for 28 days. Control fish were also maintained under identical conditions without the toxicant.

### Experimental Design

The experimental design was a complete randomized design. A total of one hundred and fifty (150) juvenile of *Clarias gariepinus* were randomly distributed into the tanks at a

stocking rate of 10 fish per tank. The fifteen (15) tanks were assigned to 5 treatments (control inclusive). In order to determine the LC<sub>50</sub>, the *C. gariepinus* were exposed to four different concentrations (5mg/L, 10mg/L, 15mg/L and 20mg/L) of sniper 1000EC for 96hr. The mortalities recorded were 1 mortality in 5 mg/L concentration of sniper 1000EC, 6 mortalities in 10mg/L concentration of sniper 1000EC, 9 mortalities in 15mg/L concentration of sniper 1000EC and 10 mortalities in 20mg/L concentration of sniper 1000EC respectively. LC<sub>50</sub> value obtained using EPA Probit Analysis programme version 1.5 was 8.21mg/l and one fifteen (1/15), one twenty (1/20), one twenty five (1/25) and one thirty (1/30) were taken as sublethal using the method of Idi-Ogede *et al.* (2016) to produce 0, 0.27, 0.31, 0.41 and 0.55mg/L respectively.

### Sampling Techniques

Blood samples were collected from both the control and experimental fish at intervals of 1, 14 and 28 days. The fish were stunned with a gentle knock on the head. The stunned fish was placed in a trough and blood was taken by caudal venous puncture using 23GX 11/4 (0.6 x 32 mm) syringe. Serum was obtained by centrifugation using Hawkley centrifuge for 10 minutes at 3,000rpm. The serum was transferred into anticoagulant free test-tube and stored at 2°C until analyses. Total protein concentration was carried out using Biuret method. 5.0ml of Biuret reagent was pipette into tubes labeled blank, standard, test, and control. 0.1ml of distilled water, standard, sample and control were pipette into their respective tubes, mixed and incubated for 30 minutes at 25°C. The absorbances were measured against the reagent blank at wavelength of 546nm. The concentration of total protein was calculated by dividing the absorbance of sample against absorbance of standard multiplied by concentration of standard (Henry *et al.*, 1974). Bromocresol green (BCG) method by Doumas *et al.*, (1971) was used for albumin estimation. 3ml of Bromocresol green reagent was pipette into tubes labeled blank, standard, sample and control. 0.1ml of distilled water, standard, sample and control were pipette into their respective tubes, mixed and incubated at 25°C for 5 minutes. The absorbance was measured at 578nm against the reagent blank. The concentration of Albumin was determined by dividing by the standard. Serum globulin was determined using Bromocresol green method. It was determined by taking the differences between total protein and albumin.

Determination of AST and ALT was done by monitoring the concentrations of Pyruvate hydrazone formed with 2, 4 dinitrophenylhydrazine. 0.5ml of buffer solution was dispensed into test tubes labelled blank, sample, control blank and control respectively for AST and ALT. 0.1ml of sample and control was dispensed into their respective test tubes. All the tubes were incubated at 37°C for 30 minutes. 0.5ml of 2, 4 dinitrophenylhydrazine was dispensed into all test tubes. 0.1ml of sample and control was dispensed into their respective blank test tube. The content of each test tube was mixed and allowed to stand for 20 minutes at 25°C, 5ml of 0.4N solution hydroxide was added to each tube, mixed and read at 550nm against the respective blank prepared. The activity of the unknown was extrapolated from the calibration curve already prepared (Reitman, and Frankel, 1957).

The activity of ALP and ACP were estimated by the method of Englehardt (1970) and Richterich (1962) respectively. Alkaline phosphatase activity was measured spectrophotometrically at 405nm. The reaction principle followed:

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p-nitrophenylphosphate + H<sub>2</sub>O → ALP Phosphate + p-nitrophenol.

Sample absorbance was read against air.

Acid phosphatase activity was measured spectrophotometrically at 405nm. The reaction principle followed:

p-nitrophenylphosphate + H<sub>2</sub>O → ACP Phosphate + p-nitrophenol.

Sample absorbance was read against reagent blank.

Determination of lactate dehydrogenase (LDH) was carried out following the reaction:

Pyruvate + NADH  $\xrightleftharpoons{\hspace{1cm}}$  Lactate + NAD

The reaction velocity was determined by a decrease in absorbance at 340nm resulting from the oxidation of NADH. One unit causes the oxidation of one micromole of NADH per minute at 25° C and Ph 7.3 under specified conditions. The spectrophotometer was set at 340nm and 25° C. The following reagents were pipette into spectrophotometer cuvette: 2.8 ml of 0.2M, HCl. 0.1ml, Mm NADH and 0.1ml of Sodium pyruvate. The curvette content was incubated in the spectrophotometer for 4-5 minutes to achieve temperature equilibration and establish a blank rate. Another 0.1ml of appropriately diluted enzyme was added and spectrophotometer reading recorded

$\Delta A_{340}/\text{min}$  from initial portion.

### Statistical Analysis

All the data generated were managed with Microsoft office Excel 2003. Data were analyzed with one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 16.0 for window. Statistical significance of difference among means was compared using Turkey (HSD) test at 95%.

## RESULTS

### Water Quality Parameters

The mean values of the water quality parameters of the different sublethal concentrations of sniper 1000EC and control to which the test fish (*C. gariepinus*) were exposed over the 28 days exposure period is presented in Table 1. The value of temperature and free carbon (iv) oxide, pH and dissolved oxygen were found to significantly (p<0.05) decreased as the concentrations of sniper 1000EC increased. However, the values of total alkalinity and conductivity in the exposed media were found to be significantly (p<0.05) increased as the concentrations of sniper 1000EC increased, compared to the control test.

Table 1: Mean values of water quality parameters of different sublethal concentrations of sniper 1000EC and control to the test fish during the 28 days exposure period

CSNIPER (mg/L)	Water Quality parameters					
	Temp (°C)	FCO (mg/L)	TAL (mg/L)	pH	DO (mg/L)	Cond µs/cm)
0.00	30.53 (0.33)	1.75 (0.11)	0.25 (0.02)	7.16 (0.06)	6.67 (0.00)	148.43 (0.35)
0.27	29.59 (0.60)	1.50 (0.13)	0.34 (0.03)	6.58 (0.02)	6.47 (0.08)	151.72 (0.38)
0.33	28.62 (0.34)	1.45 (0.17)	0.44 (0.06)	6.24 (0.02)	5.20 (0.06)	156.78 (0.90)
0.41	28.74 (0.34)	1.25 (0.15)	0.55 (0.09)	6.09 (0.02)	4.21 (0.08)	160.20 (1.23)
0.55	28.76 (0.33)	0.77 (0.12)	0.67 (0.09)	6.05 (0.02)	3.11 (0.08)	167.42 (1.23)

Mean value obtained from the sampling with replicate: SE-Standard error; CSNIPER-Concentration of sniper 1000EC; Temp-Temperature, FCO-Free Carbon iv Oxide; TAL- Total Alkalinity; DO-Dissolved Oxygen; Cond-Conductivity,  $p < 0.05$

### Effects of Sublethal Concentrations of Sniper 1000EC on Biochemical Parameters

Blood disorder attributable to sublethal concentrations of sniper 1000EC were observed in *Clarias gariepinus*, 28 days after exposure. Exposure of *Clarias gariepinus* to sublethal concentrations of sniper 1000EC for 28 days produced blood alteration with a significant dose- dependent ( $P < 0.05$ ) decrease in blood protein (plasma protein, albumin and globulin). The values for their exposed groups were significantly lower (Hypoproteinaemia, hypoalbuminaemia and hypogammaglobulinaemia) than their control groups. The values for the exposed group of blood enzymes (AST, ALT, ALP and ACP) increased ( $p < 0.05$ ) significantly compared with their control. LDH of the exposed groups decreased ( $P < 0.05$ ) significantly compared with their controls (Table 2).

Table 2: Biochemical parameters of *C. gariepinus* exposed to sublethal concentrations of sniper 1000EC (Mean  $\pm$  SD)

Parameters	Concentration(mg/L)				
	Control	0.27	0.33	0.41	0.55
Total protein (mg dL <sup>-1</sup> )	60.4 $\pm$ 0.96 <sup>a</sup>	56.0 $\pm$ 0.77 <sup>b</sup>	51.9 $\pm$ 0.36 <sup>c</sup>	48.0 $\pm$ 0.62 <sup>d</sup>	44.8 $\pm$ 0.51 <sup>e</sup>
Albumin(mgdL <sup>-1</sup> )	35.5 $\pm$ 0.72 <sup>a</sup>	27.6 $\pm$ 0.34 <sup>b</sup>	25.0 $\pm$ 0.27 <sup>c</sup>	23.6 $\pm$ 0.19 <sup>d</sup>	22.1 $\pm$ 0.19 <sup>d</sup>
Globulin(mgdL <sup>-1</sup> )	23.4 $\pm$ 0.23 <sup>a</sup>	18.1 $\pm$ 0.24 <sup>b</sup>	17.1 $\pm$ 0.32 <sup>c</sup>	16.3 $\pm$ 0.19 <sup>c</sup>	15.7 $\pm$ 0.25 <sup>d</sup>
AST(iµL <sup>-1</sup> )	5.6 $\pm$ 0.24 <sup>a</sup>	6.2 $\pm$ 0.15 <sup>b</sup>	7.4 $\pm$ 0.17 <sup>c</sup>	7.7 $\pm$ 0.13 <sup>c</sup>	8.8 $\pm$ 0.26 <sup>d</sup>
ALT(iµL <sup>-1</sup> )	4.3 $\pm$ 0.22 <sup>a</sup>	5.6 $\pm$ 0.15 <sup>b</sup>	6.5 $\pm$ 0.25 <sup>c</sup>	7.0 $\pm$ 0.15 <sup>c</sup>	7.5 $\pm$ 0.14 <sup>c</sup>
ALP (iµL <sup>-1</sup> )	42.9 $\pm$ 0.20 <sup>a</sup>	45.0 $\pm$ 0.22 <sup>b</sup>	53.3 $\pm$ 0.17 <sup>c</sup>	54.7 $\pm$ 0.28 <sup>d</sup>	57.1 $\pm$ 0.28 <sup>e</sup>
ACP(iµL <sup>-1</sup> )	3.4 $\pm$ 0.17 <sup>a</sup>	3.9 $\pm$ 0.22 <sup>a</sup>	4.1 $\pm$ 0.15 <sup>b</sup>	4.7 $\pm$ 0.18 <sup>b</sup>	4.9 $\pm$ 0.15 <sup>b</sup>
LDH(iµL-1)	104.7 $\pm$ 0.18 <sup>a</sup>	89.7 $\pm$ 0.22 <sup>b</sup>	81.4 $\pm$ 0.32 <sup>c</sup>	80.0 $\pm$ 0.17 <sup>c</sup>	77.0 $\pm$ 0.27 <sup>d</sup>

Means of parameters with the same superscripts along the rows are not significantly different at  $p < 0.05$ .

AST – Aspartate amino transferase; ALP – Alanine phosphatase; ALP- Alkaline phosphatase; ACP- Acid phosphatase; LDH – Lactate dehydrogenase.

**Biochemical Parameters in *Clarias gariepinus* at Various Exposure Days**

Biochemical parameters of *Clarias gariepinus* on exposure days (1, 14 and 28) also showed reduction in blood protein (plasma protein, albumin and globulin), reflecting hypoproteinaemia, hypoalbuminaemia and hypogammaglobulinaemia. Blood enzymes (AST, ALT, ALP and ACP) in the exposed groups were however, increased significantly ( $p < 0.05$ ) at the exposure days while LDH in the exposed groups declined significantly ( $p < 0.05$ ) at different exposure days compared with their controls (Table 3).

Table 3: Biochemical parameters of *C. gariepinus* at the various duration of exposure to sniper 1000EC (Mean  $\pm$  SD).

Parameters	Duration of exposure (Days)		
	1	14	28
Total protein (mg dL <sup>-1</sup> )	54.6 $\pm$ 1.20 <sup>a</sup>	51.0 $\pm$ 0.33 <sup>b</sup>	53.1 $\pm$ 0.40 <sup>c</sup>
Albumin (mgdL <sup>-1</sup> )	28.1 $\pm$ 0.37 <sup>a</sup>	25.4 $\pm$ 0.34 <sup>b</sup>	25.6 $\pm$ 0.31 <sup>b</sup>
Globulin(mgdL <sup>-1</sup> )	19.6 $\pm$ 0.31 <sup>a</sup>	17.6 $\pm$ 0.23 <sup>b</sup>	17.1 $\pm$ 0.20 <sup>b</sup>
AST( $\mu$ L <sup>-1</sup> )	7.3 $\pm$ 0.25 <sup>a</sup>	7.1 $\pm$ 0.16 <sup>a</sup>	7.0 $\pm$ 0.15 <sup>a</sup>
ALT ( $\mu$ L <sup>-1</sup> )	5.9 $\pm$ 0.18 <sup>a</sup>	6.5 $\pm$ 0.16 <sup>b</sup>	6.2 $\pm$ 0.21 <sup>b</sup>
ALP( $\mu$ L <sup>-1</sup> )	51.0 $\pm$ 0.23 <sup>a</sup>	50.1 $\pm$ 0.18 <sup>b</sup>	50.1 $\pm$ 0.22 <sup>b</sup>
ACP( $\mu$ L <sup>-1</sup> )	3.8 $\pm$ 0.19 <sup>a</sup>	4.0 $\pm$ 0.12 <sup>b</sup>	4.8 $\pm$ 0.22 <sup>b</sup>
LDH( $\mu$ L <sup>-1</sup> )	88.0 $\pm$ 0.21 <sup>a</sup>	86.4 $\pm$ 0.22 <sup>b</sup>	84.8 $\pm$ 0.27 <sup>c</sup>

Means of parameters with the same superscript along the rows are not significantly different at  $p > 0.05$ .

AST – Aspartate amino transferase; ALP – Alanine phosphatase; ALP- Alkaline phosphatase; ACP- Acid phosphatase; LDH – Lactate dehydrogenase.

**DISCUSSION**

Water quality parameters such as temperatures, dissolved oxygen, free carbon (iv) oxide, pH, alkalinity and conductivity are important parameters that affect the fish health, growth and reproduction (Camus *et al.*, 1998). However, Richards (1977) reported that the main cause of mortality in aquarium fish is the adequate maintenance of water environment. In this study, the monitored parameters were observed to be significantly different from the control test throughout the 28- days exposure period which shows that sublethal concentration of sniper 1000EC has effect on water chemistry. The variation in the reported result of monitored water quality parameters may be associated to the exposure period and the concentration of sniper 1000EC. Noga (1996) recommended the pH for fresh water fish to be 6.5 to 8.5, the value for pH in the highest concentration of sniper 1000EC was found to be lower than the recommended value. Thus, the significant decrease in pH value as the concentration of sniper 1000EC increased shows that the toxicant resulted in acidic condition. This was reported in the findings of Omoniyi *et al.*, (2002) who reported acidic condition in water of *Clarias gariepinus* exposed to tobacco leaf dust. The acidic condition of the water had resulted in the decrease in the level of dissolved oxygen, free carbon (iv) oxide and temperature with a corresponding increase in the values of total alkalinity and conductivity. Omoniyi *et al.*, (2002) reported decrease in temperature, dissolved oxygen with increase in the values of total alkalinity and conductivity.

The measurement of biochemical changes in blood and tissues of fish exposed to pollutants has been widely used to predict effects of chronic exposure (Christensen, 1975). Such biochemical indices include changes in tissue enzyme activity (Nemesok and Hughes,

1988). In this study, a significant change in biochemical parameters was taken to indicate an early sign of toxicity showing that the aquatic concentration of the pollutants was unsafe for the survival of organisms. Exposure of *C. gariepinus* to sublethal concentrations of sniper 1000EC resulted in blood dyscrasia with reduction in total plasma protein (hypoproteinaemia), plasma albumin (hypoalbuminaemia) and plasma globulin (hypogamma-globulinaemia). Proteins can be expected to be involved in the compensatory mechanism of stressed organisms (Ramalingam and Ramalingam, 1982), in the present study, when the fish were exposed to sniper 1000EC, the protein content were found to have decreased. Krishnamohan *et al.*, (1985) and Chandravathy and Reddy (1994) have suggested that decline in the protein content may be due to reduced protein synthesis, increased proteolysis and also due to utilization for metabolic processes. The concentration of total protein in blood plasma was used as a basic index for the health status of fish (Swain *et al.*, 2007). The plasma protein consists of albumin and globulin (Ganong, 2000). Plasma albumin concentration was decreased in the presence of hepatic cirrhosis, liver abscess, gastrointestinal diseases, nephritic syndrome and chronic renal failure (Idi-Ogede *et al.*, 2016). Hyper bilirubinaemia could further decrease the albumin binding capacity of acidic drugs (Baggot, 2001).

The protein concentrations of the treated groups were lower than the control groups. This was not surprising since some pesticides are known to interfere with protein synthesis (Suzuki, 1977). Raj and Sathyaesan (1987) reported a decrease in protein content of fish exposed to safe dose of a mercurial fungicide. Bittencourt *et al.* (2003) and Chen *et al.* (2003) reported decrease in serum protein in blood chemistry of healthy, nephrocalcinosis affected and ozone-treated *O. niloticus* in a recirculating system. The same results were reported by Mourad *et al.*, (1999) on *Tilapia zilli* exposed to organochlorine lindane (10 and 20  $\mu\text{gL}^{-1}$ ). Saganuwan (2006b) reported hypoproteinaemia in Nigerian montrel dog exposed to ceftriaxone. The result might be attributed to reduced protein in fish body due to decreased metabolic activity (Abo-Hegab *et al.*, 1999). The decrease in protein content suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. The fall in protein level during exposure may be due to increased catabolism and decreased anabolism of proteins. Decrease in protein content under toxicity stress has already being reported (James *et al.*, 1979; Natarajan, 1983; Khare and Singh, 2002). AST, ALT and ALP are the most sensitive biomarkers employed in the diagnosis of hepatic damage because they are cytoplasmic in nature and are released into the circulation (blood) after cellular damage (Leelanvinonthan and Amali, 2005) and Mayne (2002). The results show a significant increase in the concentrations of Aspartate amino transferase (AST) and alanine amino transferase (ALT) ( $P < 0.05$ ) as well as that of alkaline and acid phosphatase ( $p < 0.05$ ). This increase was in agreement with the result of Christensen, *et al* (1972) who reported an increase in the activity of ALT in adult brook trout exposed to a mixture of salts of heavy metals. Similarly, the activities of acid and alkaline phosphatases increased in *C. gariepinus*. Aminotransferases are important as they convert amino acids into ketoacids and incorporate them into TCA cycle. Both AST and ALT levels increased in the exposed fish suggesting the conversion of amino acids released by the proteolysis into ketoacids for energy production. The increase in activities of Aspartate amino transferase (AST) and Alanine amino transferase (ALT) agrees with the findings of Nemskok (1988) and Wilard (1989) who reported a similar change of activity in fish following exposure to copper sulphate solution. Similarly, elevated levels of lysosomal hydrolytic enzymes acid and alkaline phosphatase are indicative of degeneration of hepatocytes and rupture of the lysosomes. Elevated ACP and



ALP suggests an increase in lysosomal mobilization and cell necrosis due to toxicant toxicity (Celik *et al.*, 2005). Satry and Sharma (1980) recorded elevated activities of acid and alkaline phosphatase and other enzymes in the blood of fish exposed to HgCl<sub>2</sub> and suggested that these changes were due to hepatic damage or dysfunction. The increase in alkaline and acid phosphatases may affect bone mineralization. Jee *et al.* (2005) reported increase in serum aspartate aminotransferase in Korean rock fish (*Sebastes schlegeli*) exposed to cypermethrin. The increase in serum aspartate aminotransferase was attributed to the process of either deamination or transamination due to the effect of the toxicant. The increase in aspartate and alanine aminotransferase in the experimental fish revealed that the toxicant has an effect on the parenchymatous tissue and skeletal musculature which probably might disturb the permeability and integrity of cell organells as supported by Adamu and Iloba (2008). Yakubu *et al* (2005) reported significant increase (p<0.05) in serum aspartate aminotransferase in rats exposed to *Khaya senegalensi* during 18-days exposure period. This is also reported by Murray *et al.* (2000), but in contradictory to Saganuwan (2006a) who reported decrease in alkaline and acid phosphatases and affirmed their contribution to bone mineralization especially in young animals between 6-8 months old. Lactate dehydrogenase activity decreased in all the exposed fish. Decrease in LDH suggests a decrease in the incorporation of lactate into TCA cycle. Lactate dehydrogenase catalyses the conversion of pyruvic acid to lactic acid in aerobic condition: thus, acts as an indicator of hepatobiliary disease. The activity of lactate dehydrogenase in the test fish species exposed to various concentrations of the toxicant revealed significant decrease in lactate dehydrogenase with increase in concentrations. Rashatwar and Ilyas (1983) reported significant decrease in lactate dehydrogenase activity in fresh water fish *Nemachelius denisoni* exposed to sublethal concentrations of Basalin. The decreased activity of LDH (Lactate dehydrogenase) could possibly indicate early hepatic damage (Saganuwan, 2006b). Sastry and Sharma (1979) stated that the decrease in the activity of the enzyme may be due to either enzyme inhibition or decreased synthesis of the enzyme. The reduced lactate dehydrogenase might have occurred due to the stress- induced increase in the rate of glycolysis. This is in agreement with Idi-Ogede *et al.*, (2016) who reported decrease in lactate dehydrogenase of *Oreochromis niloticus* exposed to sublethal concentrations of sniper 1000EC under laboratory conditions. Increase in protease activity, decrease in protein level and increase in amino acid levels suggest degradation of proteins. Increased levels of AST and ALT activities indicate the conversion of liberated amino acids into keto acids for energy production. Decrease in LDH activity suggests the organism's adaptation to avoid the toxicant toxicity (Satyaparameshwar *et al.*, 2006).

## CONCLUSION

This study revealed that sniper 1000EC at sublethal concentration levels had mild effects on some basic function of the serum and liver of *Clarias gariepinus*. Therefore, the determined enzymatic activities can be suitably used to determine the effect of the toxicant on the physiology of fish under sublethal condition prior to sudden death of the fish. By this context, the toxicant has to be taken into more consideration as an environmental contaminant.

The use of sniper 1000EC by fishermen should be banned to save aquatic ecosystem and more studies recommended for further evaluation of this toxicant.

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