DOI: https://doi.org/10.67390/gjids.2016.8wrsg2u0

Gashua Journal of Irrigation and Desertification Studies

Available online at www.gjidsfugashua.org.ng

Volume 2, Issue 2

EFFECTS OF SNIPER 1000EC ON BODY WEIGHT, LIVER AND KIDNEY HISTOLOGYOF *OREOCHROMIS NILOTICUS* UNDER LABORATORY CONDITIONS

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Abstract

Juveniles of *Oreochromis niloticus* (mean body weight 7.05± 1.02cm) were subjected to sniper 1000EC at 5 sublethal treatment levels of 0.00, 0.12, 0.15, 0.19 and 0.25mg/L. There was no significant difference between water quality parameters of the exposed and control groups. A 21 day exposure to sublethal concentrations of the toxicant led to a significant decrease ($p<0.05$) in (specific growth) weight of the exposed fish in comparison with their controls. The effects on liver and kidney were observed majorly at 0.19 and 0.25mg/L of the exposed fish. Liver tissues revealed steatosis, haemosiderosis and vacuolations. Kidney tissues revealed tubular nephrosis and hyperplasia. It is concluded that abnormalities in body weight and visceral organs of the exposed fish were consequences of exposure to the toxicant. It is recommended that the use of Sniper 1000EC by local fishermen be banned to save the aquatic environment from destruction.

Keywords: Sniper 1000EC, *Oreochromis niloticus,* weight, liver, kidney and histology

INTRODUCTION

Weight measurements provide information on the stock composition, mortality and growth (Moutopoulos and Stergiou, 2000). Weight data is a useful tool for estimating growth rates (Froese, 2006). Weight information allows estimation of biomass (Froese and Pauly, 1998). Weight data is useful for fisheries research because they are very useful for stock assessment (Moutopoulos and Stergiou, 2000).

Histological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory (Thophon *et a*l., 2003) and field studies (Tech *et al*., 1997). The alterations found in the organs are easier to identify than functional ones (Fanta *et al*., 2003), and serve as signs of damage to animal health (Hinton and Lauren, 1990). Studies have been conducted on histological changes in the liver and kidney of fish exposed to various toxicants which have been reported to cause pathological alteration in the exposed C. *gariepinus* (Auta, 2001). The liver of fish can be considered as a target organ to pollutants; alterations in its structure can be significant in the evaluation of fish health (Kolbasi *et a*l., 2009).

Sniper 1000EC (2, 3-dichlorovinyl dimethyl phosphate) is a contact acting and fumigant insecticide which has deleterious effects on visceral tissues (Abubakar, 2013). It contains carcinogenic, mutagenic, growth retardative and immunosuppressive compounds (Halliwell, 1993a).

Oreochromis niloticus is the best species for culture-among the tilapia family-with squat shape (Arrignton, 1998). They are disease resistant, highly prolific; feed on wild variety of foods, tolerant of poor water quality with low dissolved oxygen level (Fagbenro, 2002). Tilapia is one of the fisheries resources that suffers from environmental effects and needs to be protected because world production of tilapia exceeds two million tons per year far exceeding the harvest of Atlantic salmon and secondary only to carp as a culture food fish (FAO, 2005).

The aim of the present study was to evaluate the effect of sublethal toxicity of sniper 1000EC on weight, liver and kidney histology of *Oreochromis niloticus* under laboratory conditions.

MATERIALS AND METHODS

EXPERIMENTAL FISH AND TEST CHEMICAL

Juveniles of *Oreochromis niloticus* (mean body weight 7.05± 1.02cm) were purchased from a reputable fish farm in Minna, Niger State. The samples were transported to the laboratory in plastic container of 100L capacity filled with water to two-third volume between 0700 hours and 0900 hours. They were held in large water baths of 160L capacity and acclimated for 14days to laboratory conditions. The top of water bath was covered with netted material to prevent jumping out of the fish. A slit was made at middle of the net to allow for feeding fish and cleaning of the bath. Feeding commenced two days after the arrival and stopped twenty-four hours before the commencement of the experiment. During acclimation, fish were fed twice daily (0800 and 1600 hours) with formulated feed (35% crude protein) at 3% body weight. The fishes were accepted as well as adapted to laboratory conditions when less than 5% death was recorded for the 14 days. The water in the bath was changed daily and uneaten food and faecal matters were siphoned out. Dead fish were also removed to minimize contamination of water.

Test chemical (2, 3-dichlorovinyl dimethyl phosphate) with the trade name Sniper 1000EC was obtained from Minna central market and was used for the study. The test concentrations were prepared with reference to the Manual of Method in Aquatic Environment Research.

WATER QUALITY PARAMETERS

Dechlorinated municipal tap water was used. It was allowed to stand for 72 hours during which it was aerated. The water quality parameters determined were temperature, dissolved oxygen, pH, water hardness and total alkalinity which could adversely affect survival and growth of fish in tanks. Temperature and dissolved oxygen were monitored on daily basis (09:00 and 14:00 hours) using a thermometer and oxygen meter (Cole Parma model 5946; Sigma Chemical, Berlin, Germany). The values of pH were measured daily using an Orion digital pH meter (Model 210; Sigma Chemical, Lisbon, Portugal). Water hardness were monitored daily using a German hardness scale (degrees of hardness - ^{0}dH) while total alkalinity were monitored on weekly basis using standard method of APHA

(1987). These were done to ensure proper recordings and calculations of the parameters.

EXPERIMENTAL DESIGN

The experimental design was a complete randomized design. A total of one hundred and fifty (150) juvenile of *Oreochromis niloticus* 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_{50}, the *O. niloticus* were exposed to four different concentrations of sniper 1000EC for 96hr. LC_{50} value obtained using EPA Probit Analysis programme version 1.5 was 3.81mg/l and one fifteen (1/15), one twenty (1/20), one twenty fifth (1/25) and one thirty (1/30) were taken as subletha using the method of Abubakar (2013) to produce 0.00, 0.12, 0.15, 0.19and 0.25mg/L respectively.

Feeding was ad libitum 3 times daily. The test solutions were renewed weekly. The average weight of the fish were measured weekly using a 3- digital sensitive weighing balance Meter pm 2500 Delta range ®. The experiment lasted for 21 days (3weeks). Growth in weight was based on specific growth rates which were calculated using standard formulae:

G**R**= $\frac{Wt - WO}{}$ X 100 ----- Haghighi, 2009:WO

$SG_R = \ln \frac{Wt-WO}{}$ ----- Akintola et *al.*, 2010: WO

Where:

Wt = Final body weght at time t (weeks);

WO = Initial body weight at time 0 (weeks);

GR = Growth rate;

SG_R = Specific growth rate;

T_1 = Initial time;

T_2 = Final time:

HISTOLOGICAL PROCEDURES

Fish specimens from both control and experimental groups were excised at the end of the experiment. They were rinsed in physiological saline and fixed in 4% paraformaldehyde and were sequentially embedded in paraffin wax blocks. Tissue sections of 5µm thick were cut, and stained slides were cleared with haematoxilin-eosin (H-E) and masson's trichrome (Suzuki and Suzuki, 1998) for conventional morphological evaluation. The slides were examined under light microscope (BX50; Olympus, Tokyo). The images were obtained by digital camera system (Pixceral Co., Osaka, Japan) attached to the microscope. Photomicrographs were then downloaded into a computer. Photomicrographs of control groups were compared with those of exposed groups under the guidance of a pathologist.

STATISTICAL ANALYSIS.

Data were analyzed with one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS), version 16.0 for window. Statistical significance of difference among means was compared for significant differences using Duncan's New Multiple Range tests at 95% probability.

RESULTS AND DISCUSSION

EFFECTS OF SUBLETHAL CONCENTRATIONS ON WATER QUALITY PARAMETERS

The water quality variables at various sublethal treatments for the exposed and control groups did not differ

significantly ($p>0.05$) as shown in Table 1.

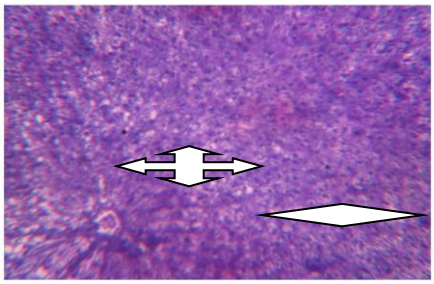
EFFECT ON GROWTH RATES

Differences in the Mean change in weight among the exposed fish at various sublethal concentrations were significant ($p<0.05$). Mean change in weight ranged from 16.4gm in the control to 13.6gm in the highest concentration (Table2).

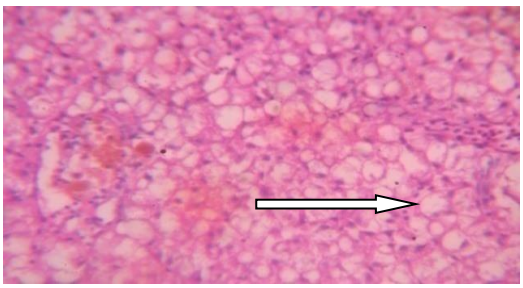
The toxicant induced depressive effect on growth rates and specific growth rates on the exposed fish. Table 3.

HISTOLOGY OF THE LIVERS

The liver of the control contained mild to moderate steatosis and haemosiderosis (plate 1A). The most common pathological changes in the livers of exposed fish (0.19and 0.25) were vacuolations (plate 1**B**).



1A



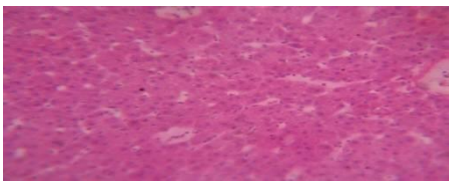
1B

Plate 1A: Photomicrograph of a section of liver from a control *Oreochromis niloticus* showing mild to moderate steatosis ⇔ and Haemosiderosis ◇

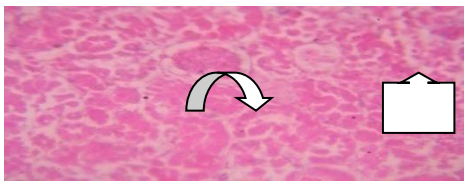
1B vacuolations ⇒

HISTOLOGY OF THE KIDNEYS

No alterations were observed in the kidney of the control (plate 2A). The most common pathological changes in the kidneys of exposed fish (0.19and 0.25) were tubular nephrosis and hyperplasia of epithelial cells (plate 2**B**).

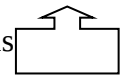


2A



2B

Plate 2A: Photomicrograph of a section of kidney from a control *Oreochromis niloticus* showing no visible lesion and 2B showing tubular nephrosis  and hyperplasia

Of epithelial cells 

The water quality parameters (Temperature, P_H, dissolved oxygen, alkalinity and hardness) were within the optimal range for fish which indicated that the water quality parameters did not have influence on the toxicity of the pesticide to the test fish. This study showed that sniper 1000EC has depressive effect on the weight of *Oreochromis niloticus* at various concentration levels.

Abubakar and Abdulsalami(2013) reported decreased in growth rate of *Clarias gariepinus* subjected to sublethal concentrations of Sniper 1000EC under laboratory conditions. A similar reduction in growth was also observed by Onusiriuka (2002) when he exposed Japanese Medaka fish and *Clarias gariepinus* to sub-lethal concentrations of chloroform and formalin respectively, better growths were reported in control groups of certain fish than those exposed to toxicants as 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. This observation was in agreement with the reports of Omoregie and Okpanach (1995) in *Tilapia zilli*. Omoregie *et al* (1998) in *Oreochromis niloticus* ; Omoregie and Onwogu (2000) in *Aphyosemion gardneri*. Most of the authors often attributed the decline in growth rates to the impairment of feeding by fish in the toxicant polluted area as observed in this study. Several workers have reported similar findings (Shanmugavel *et al.*, 1988 and Toussain, *et al.,* 2001). This might also be due to the presence of a dominant aggressive fish that caused an increased activity for others and consequently, a reduction in their growth rates. The dose- dependent effects of sniper 1000EC on the growth rates of the fish species suggested that high concentration of the toxicant inhibited the feeding rate of the fish or make the feed unpalatable for them. Pal and Konar (1987) similarly, reported reduced growth rate on *Oreochromis mossambicus* subjected to sublethal concentrations of organophosphorus insecticides. Other pollutants have also been reported to decrease fish growth and survival as recorded in Petroleum effluent by Omoregie *et al.* (1997), Paraquat by Babatunde (1997) and Tannery by Adakole (2005). Decrease in weight of the treated fish may be attributed to the stress they experience while adjusting to attain a tolerance level with the toxicant (Abubakar and Abdulsalami, 2013). Though, no mortality was reported but the toxicant has decline effects on the weight of the exposed fish as well as alterations on their livers and kidneys. The observed hepatomegaly and fat deposition obtained in this study following progressive increase in duration of treatment of sniper to suspicion of some degree of toxicity. The steatosis and haemosiderosis were similar to those reported for fish exposed to various chemicals under laboratory conditions (Brand *et al*., 2001; Fafioye *et a*l., 2004 and Aniladev*i et al.,* 2008). Fatty degeneration of the liver (haemosiderosis/steatosis) of the exposed

fish might be due to metabolic disorders commonly associated with dietary deficiency in response to xenobiotic (Myers *et al*., 1987). These changes are usually reported in organisms exposed to toxicants (Ogbulie and Okpkwasili, 1999). It might have resulted from steps leading to fatty acid entry to lipoprotein exit (Mitchell and Cotran, 2004). Similar changes have been reported in the liver of *Astyanax sp* exposed to WSFs of crude oil (Akaishi *et al*., 2004). *Perca fluviatilis* and gold fish exposed to oil seed (Nero *et al*., 2006). Several studies have reported that chronic accumulation of some toxicant in fish livers causes hepatic-cirrhosis and eventually death (Varanka *et al.*, 2001). Pathological changes in kidneys of exposed fish were tubular nephrosis and hyperplasia. As in higher vertebrates, the kidneys of fish perform an important function related to electrolyte and water balance and maintenance of a stable internal environment (Supap *et al*., 2009). Teh *et al*. (1997) observed alterations at the level of tubular epithelium and glomeruli in *O. niloticus* exposed to a toxicant. The kidney is a major site for toxic effect due to a wide variety of environmental pollutants (Hook, 1980). Winkaler *et al.* (2001) found anomalies such as hyperplasia, hypertrophy and dilation of epithelial cells in Neotropical fish, *Astyanax altiparanae* collected in Cambe stream. Similar alterations were found in fishes exposed to organic contaminants (Veiga *et al*., 2002) and mixed environmental contaminants (Pacheco and Santos, 2002).

CONCLUSION

The abnormalities in fish body weight, liver and kidney histology of the exposed fish species were associated with the effects of sublethal toxicity of sniper 1000EC. Therefore, the toxicant should be considered as an environmental contaminant.

RECOMMENDATION

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

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Table 1. Physico-chemical parameters of chronic test solutions for *Oreochromis niloticus* (mean ±SD)

Concs	**P^{H}**	**Temperature (^{O}C)**	**Dissolved oxygen (mgL^{-1})**	**Hardness (mgL^{-1})**	**Alkalinity (mgL^{-1})**
0.00	6.64±1.70	28.6±1.52	7.50±0.30	39.1±2.81	33.7±2.13
0.12	6.67±0.51	28.8±2.09	7.63±0.28	40.2±3.26	33.3±1.54
0.15	6.56±0.42	28.6±1.51	7.65±0.29	40.4±2.22	33.0±2.37
0.19	6.59±0.49	28.5±2.04	7.68±0.27	40.2±3.42	34.1±2.14
0.25	6.62±0.45	28.6±1.61	7.67±0.22	39.4±3.86	34.3±1.56

Values of parameters along the same column are not significantly different at (p>0.05).

Table 2. Weekly Mean change in weight (gm) at different concentration levels of sniper1000EC in *Oreochromis niloticus*.

Concentration (mg/L-1)	Mean change in weight (gm)		
	WK1	WK2	WK3
0.00mg	16.4[a]	16.6[a]	16.6[a]
0.12mg	16.2[a]	15.9[a]	15.8[a]
0.15mg	15.8[a]	15.3[a]	15.2[a]
0.19mg	14.7[a]	14.5[a]	14.3[a]
0.25mg	14.0[a]	13.8[a]	13.7[a]

Means with the same superscript along column are not significantly ($p<0.05$) different

Table 3. Mean initial weight, Mean final weight, Growth rate and Specific growth rate in *Oreochromis niloticus*:

Concentration (mg/L-1)	Mean Initial weight (gm)	Mean Final weight (gm)	Growth rate (gm)	Specific growth Rate (gm)
0.00mg	13.0[a]	16.7[a]	28.5[a]	1.2[a]
0.12mg	13.4[a]	16.2[a]	21.0[b]	0.9[a]
0.15mg	12.9[a]	15.3[b]	18.6[c]	0.8[a]
0.19mg	13.2[a]	15.0[b]	13.6[d]	0.6[a]
0.25mg	13.5[a]	15.3[b]	13.3[d]	0.6[a]

Means with the same superscript along column are not significantly ($p<0.05$) different