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TOXICITY AND SOME BIOCHEMICAL RESPONSES OF AFRICAN CATFISH *CLARIAS GARIEPINUS* EXPOSED TO BENTAZON HERBICIDE.

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ABSTRACT

Toxicity and some biochemical responses of African catfish *Clarias gariepinus* were investigated using static bioassay for a period of 96 hours intervals at different concentrations (3.84, 4.08, 4.32, 4.56 and 4.80 mg/L). The results from the study revealed that 96 hours LC_{50} of the exposed fishes was 3.89 mg/L, This study shows that the mortality of fish occurred steadily with increase in duration of exposure and concentrations of the bentazon toxicant. At the end of the experiment, blood samples from treatments collected for some biochemical variables show a significant ($P<0.05$) increase in the total protein level, triglycerides level and activities of glutamic pyruvic acid transaminase (GPT) and glutamic ozaloacetic acid transaminase (GOT) levels, with a noticeable decrease in both glucose and cholesterol levels compared to the control. This study shows that acute concentrations of bentazon herbicide is harmful to *Clarias gariepinus* which is incapable of adapting to changes caused in the water quality by the added toxicant.

Keywords: Bentazon, toxicity, biochemical, *Clarias gariepinus*

INTRODUCTION

Fish has been in the diet of man from time immemorial. The increased and continued use of fish arises from the fact that fish provides not only all essential amino-acids required by man; it supplements these with polyunsaturated fatty acids not found in other sources of protein, mineral salts and some vitamins (Kent, 1984). About, 80-90 million people in the world depend on fish for their daily source of protein and as a source of income (Nikolsky, 1983). Nigeria's domestic fish production hardly meet the local demand because of the over-increasing population of mankind, as a result there has been yearly increase in fish importation in order to meet the demand (FDF, 2000). For example, the fish supply in Nigeria is mainly from the capture sector which contributes about 85% of total domestic production and Nigeria imports about 700,000 tonnes per annum and annual deficit of about one and half million tonnes still exist (FAO, 2002).

In spite of the inability to meet the local demand for fish, certain constraints limit the local fish production. One of which is aquatic pollution. Water bodies are the ultimate recipients of industrial effluents, indiscriminate use of agro-chemicals for crops and animal production, post harvest technology and public health activities. These pollutants enter the aquatic environment directly, by point source dumping or indirectly by rain; water run-off and ground water leaching, these pollutants have been known to have toxic effects which may be poisonous to fish and other aquatic organisms.

Bentazon is an herbicide widely used in agriculture, residential landscaping and recreational areas for the control of post emergence broad-leafed weeds and sedges. It is often mixed with phenoxyacetic acid and other herbicides for the purpose of other weeds control. In the USA, it is used to control cock lebur, morning glory, night shade and shepherd's purse to control Canada thistle and yellow nutsedges.

Clarias gariepiunus is the common ecologically important and commercially valued cultured fish in Nigeria; it is an omnivore fresh water fish, and popular delicacy throughout Africa, because of its hardness and fast growth (Okeke, 2004). The realization of the polluting and potential public health effects of herbicides resulted in a number of studies on the toxicity of various pesticides on fish (Zikic *et al.*, 2001; Zang *et al.*, 2003; Simonato *et al.*, 2006 and Ayoola, 2008). Amongst these, reports on toxicological study of bentazon on *C. gariepinus* are insufficient particularly in Nigeria where it is mostly use to control weeds. This study aimed to access the effects of bentazon on *Clarias gariepinus*.

MATERIALS AND METHODS

Experimental Animals

Juvenile *(Clarias gariepinus)* (24.56±9.86 g; 16.06±2.33 cm; Mean±SD) were obtained from a private farm in Zaria, transported to the Department of Biological Sciences Ahmadu Bello University Zaria. Fish were held in the laboratory for acclimatization for two weeks in dechlorinated municipal water during which they were fed commercial pellets at 2.5% of their body weight (twice daily). During the acclimation period, about 50% of the water in the water bath was renewed every two days. Throughout the acclimation period and subsequent periods of bentazon exposures, fish were held under a photoperiod of 12 hours of light and 12 hours of darkness. The fishes were maintained at 26.2±0.7^{0}C, pH 5.86±1.52 and dissolved oxygen 6.5±3.6 mgl^{-1} throughout the acclimation period.

Experimental Design

The toxicity test design was conducted based on guidelines provided by Altinok *et al.* (2006). The fish were determined to be free of external parasites prior to the exposure (AFS-FHS, 2003). After acclimation, fish from the bath of the acclimatization were randomly transferred into glass aquaria containing 25 litres of water. Duplicate random group of 10 fish each were subjected to experimentation for 96hours in water containing different concentrations of bentazon.

Duplicate aquaria were designated for each concentration of bentazon (3.84, 4.08, 4.32, 4.56 and 4.80 mg/L) dispensed into the test tanks and the last tanks without the

toxicant to serve as the control (0.00 mg/L). The mixture was allowed to stand for 5minutes to be evenly distributed via diffusion before introduction of the test organisms.

At the end of the experimental period blood samples were collected from the fish by a puncture behind the anal fin using 2 G hypodermic syringe and needle. Samples thus collected were stored in EDTA embedded bottles. The blood samples collected were immediately centrifuged at 1500 rpm for 10minutes. Serum was then removed and stored at 4^0C prior to determination of biochemical analysis. The enzymes activities of glutamic pyruvic acid transaminase (GPT) and glutamic ozaloacetic acid transaminase (GOT) were analyzed using the method of Reitmans and Frankel, (1957) blood glucose were analyzed using the method of Trinder, (1969) blood Cholestesterol were analyzed using the procedure of Pearson *et al.* (1953), blood triglycerides were determined using the method of Rice (1970) and the biuret method was used to analyzed the value of total protein, respectively.

Statistical Analysis

The percentage mortality in each concentration was determined. The LC_{50} after 96hours was also determined and probit graph was drawn using Microsoft excel package. Analysis of variance (ANOVA) and Duncan multiple range tests were used to test for differences between levels of treatments and to separate means respectively. Test of significance were at 95% ($P<0.05$) probability (Duncan, 1955).

RESULTS

No mortality was recorded at 12 hours at concentration 4.32 mg/L of bentazon but at 24, 48, 72 and 96 hours exposures, mortality were observed at all concentrations. The highest recorded mean mortality of 9 occurred at the highest concentration of 4.80 mg/L. Table 1, showed the mean percentage mortality and the percentage kill probit values. The 96 hours LC_{50} of bentazon to *Clarias gariepinus* was determined to be 3.89 mg/L as represented in Figure 1.

The mean biochemical analysis of *Clarias gariepinus* exposed to bentazon is represented in Table 3. A marked reduction glucose level of the exposed fish compared to the control was observed which were seen to increase with increase in toxicant concentration. Cholesterol level at higher concentrations of toxicant 4.32, 4.56 and 4.80 mg/L reduced with increase concentrations and were seen to be lower than the control value. Triglycerides levels of the exposed fish were significantly higher than the control groups.

Total protein, glutamic pyruvic acid transaminase (GPT) and glutamic ozaloacetic acid transaminase (GOT) levels of the exposed fish were higher than the control group. Also the three values were seen to be concentration dependent but not significantly different ($P<0.05$) with the control group.

Table 1. Mortality of *C. gariepinus* juveniles exposed to concentrations of bentazon for 96hours.

Concentration (mg/L) Exposure	Tsbr0.00	Tsbr3.84	Tsbr4.08	Tsbr4.32	Tsbr4.56	Tsbr4.80
12	- -	- -	- -	- -	1 -	2 2
24	- -	- -	1 2	2 1	1 3	2 1
48	- -	1 2	2 1	1 2	- 1	1 2
72	- -	2 2	2 -	2 3	2 2	2 1
96	- -	1 -	1 1	1 -	2 2	2 2
Mean Mortality	0/10	5/10	5/10	6/10	7/10	9/10

Tsbr = Toxicity and Some Biochemical Responses

Table 2: Mean, percentage mortality and probit values of *C. gariepinus* exposed to acute concentrations of bentazon.

Conc(mg/L)	Log_{10} of Conc.	Mean Mortality	% Mean	% Probit Kill
Tsbr0.00	0.000	0/10	0%	0
Tsbr 3.84	0.584	510	50%	5.00
Tsbr 4.08	0.611	5/10	50%	5.00
Tsbr 4.32	0.634	6/1	60%	5.25
Tsbr 4.56	0.659	7/10	70%	5.52
Tsbr 4.80	0.681	9/10	90%	6.28

Tsbr = Toxicity and Some Biochemical Responses

Table 3: Levels of biochemical analysis (X ± SD) in the serum of *C. gariepinus* exposed to bentazon concentrations.

Parameters Bentazon Conc. (Mg/L)	Glucose ($Mg100^{-1}$)	Cholesterol ($Mg100^{-1}$)	Triglycerides ($Mg100^{-1}$)	Total Protein ($Mg100^{-1}$)	GPT (μL^{-1})	GOT (μL^{-1})
Tsbr 0.00	60.03±0.77a	233.69±2.21a	86.54±3.62a	3.0±0.1a	60.6±1.15a	36.3±1.52ab
Tsbr 3.84	54.86±3.01b	237.54±5.78a	140.90±1.53b	3.0±0.26a	66.6+2.08b	43.6±1.52ab
Tsbr 4.08	43.9±2.01b	233.79±5.39a	135.18±5.22b	3.46±0.42b	67.69±1.73b	51.6±1.52c
Tsbr 4.32	38.6±0.81c	226.2±2.12b	132.26±2.94c	3.56±0.10b	71.6±0.57bc	55.0±1.0c
Tsbr 4.56	35.6±1.52c	230.5±1.53b	124.3±1.87d	3.73±0.15ab	71.3±1.52bc	60.3±1.52b
Tsbr 4.80	30.4±0.86c	217.58±0.96c	124.18±5.27d	3.78±0.15ab	73.0±1.0b	63.6±1.52a

Means with the same letters along the column are not significantly different ($P<0.05$).

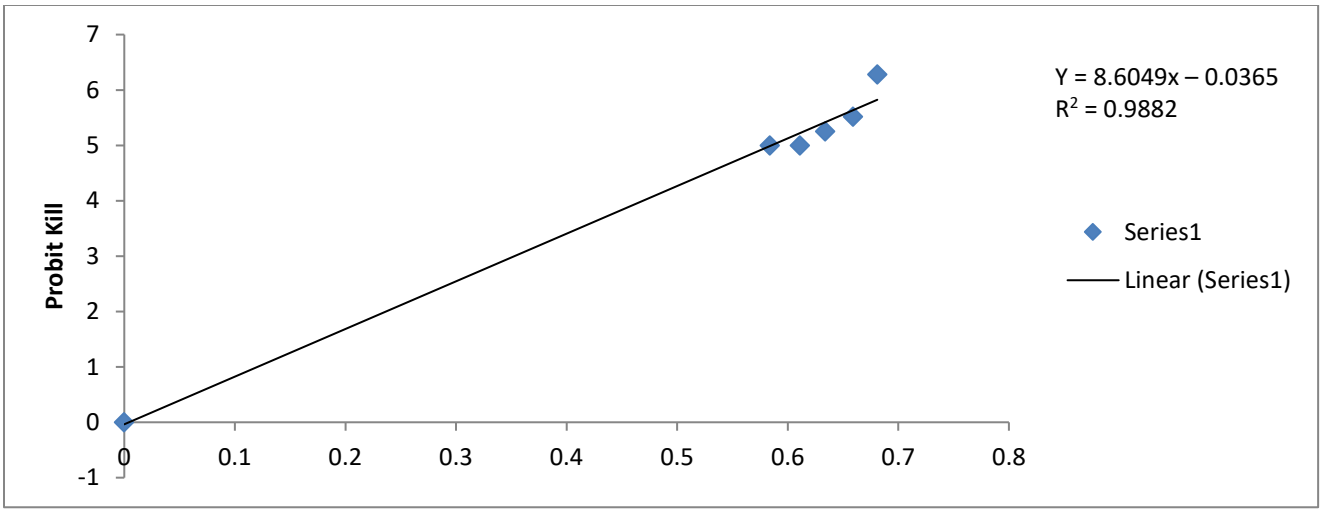

Fig. 1. Determination of the LC_{50} of Bentazon to *C. gariepinus* at 96 hrs.

DISCUSSION

In the present study the LC_{50} value of bentazon to *Clarias gariepinus* juveniles exposed for 96hours was 3.89 mg/L. The 96 hours LC_{50} value of bentazon in rain bow trout is 510 mg/L for wettable powder in bluegill sunfish, the 96hours LC_{50} for technical bentazon was 616ppm, and in rainbow trout it was 190ppm as reported by Hartley *et al;* (1983). In the present study the mortality of fish occurred steadily with the increase in exposure and concentration of the bentazon toxicant. Ayotunde *et al.* (2011) observed that the mortality rates in the study had a clear relationship between the dose, mortality and exposure period. The concentration of the toxicant is directly proportional to the mortality rate. The fish in the present study died due to certain physiological and biochemical changes. Oh *et al*. (1991) attributed that specific sensitivity of fish to bentazon may be associated with different rate of absorption, acetyl cholinesterase inhibition and detoxification. Neibor and Richardson (1980) also reported that, the level of toxicity of any pesticide depends on the bioaccumulation of the toxicant, the different chemistry of the compound forming the pesticide and the reaction of the organism receiving the toxicant.

The biochemical responses of non-target organisms exposed to different herbicides have been documented (Coppage and Mathews, 1974; Corbett, 1974). Exposure of fish to chemical pollutants has led to many molecular and biochemical changes in fish which precede cellular and systematic dysfunctions, so that, if appropriate parameters are monitored, early warnings signs of distress may be detected (Palmer, 1976).

The present study, demonstrate that the fish *Clarias gariepinus* exposed to Bentazon herbicide showed a significant decrease in glucose level and activities of GPT and GOT enzymes. Blood triglyceride and total protein were markedly elevated from the treatment group. Significant changes in the level of blood cholesterol were also noted. These activities were seen to be dose-dependent. Wedemer and Mc Leay (1981) reported the decreased levels of blood glucose and activities of GPT and GOT are caused by disorder in carbohydrate metabolism appearing in the conditions of physical and chemical stress. A number of stressors stimulate the adrenal tissue, resulting in increased level of circulating glycocorticoids (Partap *et al.*, 1976; Witters *et al.*, 1991). Forlin *et al*. (1986) reported that the apparent glucose depletion in rainbow trout hepatocytes following exposure to cadmium may reflect acceleration rate of glycolysis which will deplet the liver glycogen storage. The decrease in liver glycogen is due to glycogeneolysis and normally the decrease of tissue glycogen content runs parallel to an decrease of glucose in serum (Gluth *et al.*, 1985; James *et al.*, 1992; Hontela *et al*., 1996). Similarly, Kozaric *et al.* (1992) reported that the liver glycogen content of carp, *Cyprinus carpio* reduced significantly, but the activities of hexokinase and glucose phosphate isomers were elevated. They suggested an intensive glycogenolysis in cadmium intoxicated carp. However, the reduction of blood glucose is a response to the increased rate of glycogenolysis or gluconeogenesis.

The significant elevations in the activities of blood GPT and GOT clearly shows that the blood enzymes were highly increased in the fish treated with bentazon. Shakori *et al.* (1990) reported that the increased of blood enzymatic activity was either due to leakage of these enzymes from hepatic cells and thus raising levels in blood or increase in synthesis and induction of these enzymes. Campbell (1984); Tietz (1987) reported that these enzymes liberates to the blood streams when the hepatic parenchyma cells are damaged. The significant rise in blood triglycerides level in *C. gariepinus* exposed to bentazon herbicide may be due to hypothyrodism induced by the herbicide and or liver dysfunction, because the liver is the principal centre of lipid metabolism Tietz (1987). Forlin *et al*. (1986) and Thophon *et al.* (2003) found structural and ultra structural damage in the liver of rainbow trout and White Sea bass following cadmium exposure. The observed increase in the activities of GPT and GOT suggest that the observed proteolysis intended to increase the role of protein in the energy production during bentazon stress hence the possible reason for total protein elevation.

Despite the benefits derived from pesticides, the result of this study clearly revealed that herbicides can potentially harm aquatic life and our health. Fish are usually harvested from these polluted aquatic environments for human

consumption, since most of the world's population depends upon aquatic animals for food. The agricultural community should therefore be constantly reminded of safe level of usage of these pesticides because of potential adverse effects of pesticides and other farm chemicals to aquatic lives and humans..

REFERENCES

AFS-FHS (American Fisheries Society-Fish Health Section). (2003). Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens, fifth ed. Fish Health Section, American Fisheries Society, Bethesda, MD, USA: pp. 140-190.

Altinok, I. and Capkin, E. (2006). Histopathology of rainbow trout exposed to sublethal concentrations of methiocarb or endosulfan. *Toxicologic Pathology*, 35: 405-410.

Arasta, T.,. Bais, V. S. and Thakur, P. (1996). Effect of Nuvan on some biological parameters of Indian catfish, *Mystus vittatus. J. Environ Biology*, 17: 167-169.

Ayotunde, E. O., Fagbenro, O. A. and Adebayo, O. T. (2011). Toxicty of aqueous extract of Moringa oleifera seed powder to Nile tilapia *Oreochromis niloticus*, finger lings. *International Research Journal of Agricultural Science and Soil Science*, 1(4): 142-150.

Ayoola, S. O. (2008). Toxicity of Glyphosate herbicides on Nile tilapia (*Oreochromis niloticus*) juvenile. *Afri. J. Agri. Res.* 3(12): 825-834.

Bruton, M. N. (1979). The breeding biology and early development of *Clarias gariepinus* (pisces, Clariidea) in lake sibaya, South Africa, with a review of breeding of species of subgenus *Clarias*. *Trans, Zool. SOC*, 35: 1-45.

Cambell, E. J., Dickinson C. J., Spater, J. D., Edwards C. W. and Sikora K. (1984). Clinical Physiology Bulter and Tanner Ltd. London.

Clay, D. (1979). Population biology, growth and feeding of Africa catfish (*Clarias gariepinus*) with special reference to juvenile and their important to fish culture. *Arch, Hydrobiol.* 87: 453-482.

Coppage, D. L. and Mathews, E. (1974). Short term effects of organ phosphorus pesticides on cholinesterase of estuarine fishes and pink shrimps. *Bull. Environ Contam. Toxicol.* 11:P.483.

Corbett, J. R. (1974). The Biochemical Mode of action of pesticides. Academic Press, New York. 80 p.

Duncan, D. B. (1955). Multiple range and multiple F. test. *Biomet.* 11: 1-10.

FAO. (2002). Food and Agriculture publication, Year Report 2002. FAO Rome. pp.1-6.

Forlin, L., Haux, C. Karlsson-Norrgem L., Runn, P. and Larsson, A. (1986). Biotransformation enzymes activities and histopathology in rainbow trout Salmo gaidnernri, treated with cadmium, *Aquat, Toxicol.*, 8: 51-64.

Gluth, G. and Hanke, W. (1985). A comprison of physiological changes in cerp, *Cyprinus Carpio.* Induced by several pollutants at sublethal concentrations. I. The dependency on exposure time. *Ecotoxcol. Saf.* 9: 179-188.

Hartley, D. and Kidd, H. (Eds.) (1983). The agrochemicals handbook. Nottingham England; Royal Society of Chemistry, pp. 64-69.

Hontela, A., Daniel C. and Ricard A. C. (1996). Effects of acute and sub-acute exposure to cadmium on the internal and thyroid function in rainbow trout, *Oncorhyncus mykiss. Aquat. Toxicol*. 35: 171-182.

James, R., Sampth, K. and Alagurathinam, S. (1992). Effects of lead on respiratory enzymes activity, glycogen and blood sugar levels of the teleost *Orechromis mossambicus* (Peter) during accumulation and depuration *Asian Fish Sci*. 9: 87-100.

Kent, G. (1984). National Fisheries policies and Alleviation of Malnutrition in the Philippines and Thailand. FAO Fish Cir. No. 77.

Kozaric, Z. Novak, R. and Srebocan, E., (1992). The effects of sublethal concentrations of cadmium on the glycogen content and activities of some enzymes of glycose metabolism in the liver of carp (*Cyprinus carpio) Verterinarshi Archiv* 62: 335-340.

Neibor, E. and Richardson, D. H. (1980). Replacement of non descript term heavy metal by a Biological and chemical classification of metal ions. *Environmental Pollution Series* 3(1): 3-26.

Oh, H. S., Lee, S. K., Kim, Y. H. and Roh, J. K. (1991). Mechanism of selective toxicit of diazinon to killifish *Orzias latipes* and loach *misgurnus auguillicaudatus. Aquatic Toxicology and Risk Assessment.* 14: 343-353.

Okeke P. U. (2004). Toxicity of Diazinon to Juvenile of the African catfish, Clarias gariepinus (Teugels) Unpublished M.Sc. Thesis. Ahmadu Bello University, Zaria, Nigeria.

Palmer, E. D. (1976) Measurement and significance of sub clinical effects of chemicals *Clin. Toxicol.* 9 P. 723.

Partap, H. P. and Bonga S. E. W. (1990). Effect of waterborne cadmium on plasma cortisol and glucose in the cichlid fish *Orochromis mosambicus. Comp. Biochem. Physicol* 95: 313-317.

Pearson, S.S., Stern, and Megavack T. H., (1953). A rapid procedure for the determination of Serum cholesterol. *Journal. Clin. Endocrinol*, 25:8-13.

Reitman, S. and Frankel, S. (1957). Calorimetric method for the determination of serum glutamic oxalaoacetic and glutamic-piruvic transaminases. *Amer J Clin Pathol.* 28: 65-63.

Rice, E. (1970). Triglycerides (Neutral Fat) in serum Stand. *Math. Clin. Chem.* Vol. 6, Academic Press. New York., 215 p.

Shakori, A. R., Alam, J., Aziz, F. and Ali, S. S. (1990). Biochemical effects pf bifenthrin (talstar) administered orally for one month on the blood and liver or rabbit. *Proc. Congr. Zool.*, 10: 61-81.

Stalin, I. S., Kruba, S, and Das, S. S. M. (2008) A comparative study on the toxicity of a synthetic pyrethroid, Delta methrin and a Neem based pesticide, Azadirachtin to poecilia reticulate

peters 1859 (*Cyrpinodontiformes: Poecilidae*) *Turkish Journal of Fisheries and Aquatic Sciences*, 8: 1-5.

Simonato, J. D., Alibanati, A. C. L. and Martinez, C. B. R. (2006). Effect of the water soluble fraction of diesel fuel oil on some functional parameters of the neotropical fresh water fish, *Prochilodus Lineatus Valenciennes*. *Bull. Environ Contam. Toxicol*, 76: 505-511.

Thophon, S., Kruatrachue, M. Upatham, E .S., Pokethitiyook, P., Sahaphong, S. and Jarikhuan S. (2003). Histopathological alterations of white seabass, Lates calcarifer, in acute and subchronic cadmium exposure. *Environ. Pollut.* 121: 307-320.

Tietz, N. W. (1987). Fundamentals of Clinical Chemistry. Saunder W.B. Co. pp. 42-51.

Trinder, P. (1969). Determination of blood glucose using 4-antiophenazone, *Journal Clinic. Patho* 22: 246.

Wedemer, G. A. and Mcleay, D. J. (1981). Methods for Determining the Tolerance of Fish to Environmental Stressors. In Stress and Fish. Edited By Pikering A. D. *Academic Press, London* pp. 274-275.

Witters, H. E., VanPuymbroek S. and Vanderborght, O. L. J. (1991). Adrenegic response to physiological disturbances in rainbow trout, *Oncorhynchus mykiss,* exposed to aluminium at acid pH Can. *Journal Fish Aquat. Sci.* 48: 414-420.

Zang, J. F., Shen, H. and Xu, Y. F. (2003). Effects of long term exposure of low-level diesel oil on the antioxidant defence system of fish. *Environ. Conatin Toxicol*, 71: 234-239.

Zikic, R. V., Stajin, A. S., Pavlovic, S. Z., Ogunjanovic, B. I. and Saicic, Z. S. (2001). Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminase of gold fish. *(Carassius auratus)* exposed to cadmium. *Physiol Res*. 50: 105-111.