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Effect of Diazinon on Organosomatic Indices and Behavioural Responses of *Clarias gariepinus* (a Common Niger Delta Wetland Fish)

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ABSTRACT

Lethal and sublthal concentrations of toxicants affect on fish behaviour, reproduction, growth and general physiological processes in exposed organism. The purpose of this research was to determine the organosomatic indices and behavioural responses of *Clarias gariepinus* exposed to diazinon. Adult fish (mean length, 35.24±2.80cm) were acclimated to laboratory conditions for 7 days and then exposed to varying sublethal concentrations of diazinon (1.00, 2.50, 5.00, 7.50 and 10.0mg/l) in a semi-static bioassay for 30 days. Organosomatic index was determined in the liver, spleen, kidney and heart. Fish behaviour was also monitored for several hours on and before exposure to diazinon. The final condition factor values were not statistically significant (P>0.05). All organosomatic indices tested were significant, unveiling the effect of diazinon on the probe organism organs. Behavioural aberrations were also observed in experimental group/treatment that received the highest dose of the toxicant. Based on the results, diazinon used for agricultural are anthropogenic purposes could have a devastating effect on *Clarias gariepinus* (a common Niger Delta wet land fish).

INTRODUCTION

In spite of the immerse contribution of pesticides to agriculture and household uses, they have been reported to have negative ecological consequences on the environment (Far et al., 2012). The food chain/web is majorly affected. Pesticides applied in agricultural field or careless management of used pesticide containers find their way into the aquatic environment, either through water run-off and or as aerosols carried by wind (Inyang et al., 2016a-f, 2017).

Pesticides are classified according to their function including herbicides, insecticides, fungicides, acaricides, fumigants etc. They are also classified based on their chemical composition. Irrespective of their mode of classification, they affects target organisms, and in some cases non-target organisms as well.

Organophosphate insecticides are widely used to control a variety of agricultural pests as well as ectoparasites in fish. The organophosphorus based insecticides are derived from the phosphoric or phosphorothioic acid (Díaz-Resendiz et al., 2015). Organophosphorus pesticides are group of highly toxic compounds and they are readily available commercially for domestic, agricultural and industrial purposes (Aardema, 2008; Kapka-Skrzypczak et al., 2011). They account for about 50% of all insecticides applied worldwide (Yang and Deng, 2007). Organophosphorus insecticides are toxic because of their inhibition of the enzyme acetylcholinesterase (Al-Ghanim, 2014; Muranli et al., 2015). These inhibition processes of the enzyme may results in the accumulation of acetylcholine in nerve tissue and effector organs, with the main site of action being the peripheral nervous system (PNS) (National Research Council, 1982; Hodgson et al., 2004).

Diazinon (an organophosphrate insecticide) is one of the most frequently used pesticides in the world (Muranli et al., 2015). According to Al-Ghanim (2014), diazinon is the most generally used pesticide in agricultural field and environmental health. In Nigeria, it is sold in an open market and it is well embraced by farmers. Diazinon is mostly used for the control flies, lice, insect pests (of ornamental plants and food crops, nematodes and soil insects in lawns and croplands (Vajargah et al., 2013), and other insect pest such as cuttworms, wireworms and maggots in soil and ectoparasites on sheep (Virtues and Clayton, 1997). Additionally diazinon uses in urban areas include dormant sprays on fruits trees, professional landscape and pest control services (Baily et al., 2000). Diazinon may be found in formulations with a variety of other pesticides such as pyrethroids lindane and disulpoton. The mechanism of toxic effect of diazinon is the same as those of other organophosphate substances (Leudke and Bartley, 2006).

Lethal and sublethal concentrations of toxicants usually have adverse effect on fish behaviour, haematology, histopathology, growth, reproduction, feeding and general physiological developments of exposed organisms (Butler, 1971). This research work examines the suitability of somatic indices of *Clarias gariepinus* as yet effective biomarkers of diazinon induced stress in fish.

MATERIALS AND METHODS

Fish samples for this study were purchased from a private fish farm at Abuloma road, Port Harcourt, Rivers State of Nigeria. The fish samples were transported to the Department of Fisheries and Aquatic environment, Rivers State University of Science and Technology, Port Harcourt, Nigeria, were the assays were conducted. Fifty-eight adult *Clarias gariepinus* (mean weight 275±53.12g, mean length, 35.24±2.80cm) were acclimatized individually in rectangular aquaria for one week during which they were fed once a day (9.00-11.00 hours) with 35% crude protein diet at 1% biomass.

Sublethal concentrations of diazinon for the assay (1.0, 2.5, 5.0, and 10.0mg/l) were determined based on the range finding test (Inyang, 2008). These were prepared by transferring 0.02, 0.013, 0.25, 0.37 and 0.5ml, respectively of the original concentration of diazinon and making it up to 30L with borehole water in the aquaria, 30L of the diluent water was used as control. Four replications of each treatment level (concentrated) and control were set up by introducing fishes individually into each aquarium. Daily renewal bioassay was employed during the experiment which lasted for 30 days. The physico-chemical properties of the water used for the fish bioassay was carried out using standard methods (APHA, 1998); The result of the water quality were; temperature 26ºC, pH 6.620 – 6.37, dissolved oxygen 5.38 – 7.21 mg/l, alkalinity 15.25 – 17.09mg/l, conductivity 99.50 – 136.12 µs/cm and turbidity 0.042 – 0.50NTU.

After 30 days of exposure, fishes were weighed before sacrifice for collection of target organs (heart, liver, kidney, and spleen) for organosomatic index text (OIT). The OIT was determined by weighing each organ on digital weighing balance. Organosomatic index for all the organs was calculated using the expression:

$$\frac{\text{organ weight }(g)}{\text{total body weight}} \times 100 \text{ (Simeon, 2007)}$$

While the condition factor (k) of each specimen was calculated using foulton's formular:

$$K = \frac{100W}{L3} \times 100$$

W = weight of fish
L = Length of fish

Fishes were closely observed for abnormal or adverse behavioural change outside the normal range of variability, during and after exposure to diazinon.

Data analysis

The data were subjected to analysis of variance (ANOVA). Where variation exist, Duncan multiple range test (DMRT) were used determine the source of the observed difference between treatments at P=0.05 (Wahua, 1999).

RESULTS AND DISCUSSION

Condition factor

The initial condition factor of the control and experimental group values were not statistically significant (P>.05). Albeit a slight progressive rise in values were observed as the concentration increased. The final condition values were not significantly different (P>0.05). Values fluctuate as the concentration increased. A slight increase in values at 1.00mg/l and 2.50mg/l were recorded and then a reduction in values at 5.00, 7.50 and 10.0mg/l (Table 1). A sudden drop in values is clear indication of effect of diazinon on general physiology of the probe organism.

Organosomatic index

Our data unveiled a statistically significant values in all somatic indices tested. A slight deviation in values was recorded in the cardiosomatic (Heart) and Spleenosomatic indices. Renosomatic (kidney) and spleenosomatic (Spleen) indices values reduced as the concentration of the toxicant increases. These fluctuation in values showed the effect of diazinon on these organs. The results were similar to those of *Clarias gariepinus* exposed to monoclotophos insecticide (an organophosphorus insecticide) for 30 days (Simeon, 2007).

Table 1: Condition factor of *Clarias gariepinus* exposed to diazinon for 30 days (mean ±SD)

Conc of diazinon (mg/l	Initial Condition (K1)	% of Control	Final Condition (K2)	% of Control
0.00	0.68 ± 0.14^{a}	100	0.69 ± 0.12^{a}	100
1.00	0.88 ± 0.14^{a}	129	0.78 ± 0.15^{a}	113
2.50	0.72 ± 0.15^{a}	106	0.72 ± 0.15^{a}	104
5.00	0.76 ± 0.12^{a}	112	0.68 ± 0.69^{a}	99
7.50	0.67 ± 0.08^{a}	99	0.59 ± 0.06^{a}	86
10.0	0.70 ± 0.07^{a}	103	0.63 ± 0.07^{a}	91

Means with the same superscript in the row are not significantly different (p<0.05).

Table 2: Condition factor of *Clarias gariepinus* exposed to diazinon for 30 days (mean ±SD)

Conc of diazinon (mg/l	Renasomatic (kidney)	Cardiosomatic (Heart	Hepetosomatic (Liver	Spleenosomatic (Spleen)
0.00	0.01 ± 0.02^{b}	0.18 ± 0.03^{a}	1.29 ± 0.32^{ab}	0.23 ± 0.03^{b}
1.00	0.8 ± 0.03^{a}	0.15 ± 0.02^{ab}	1.28 ± 0.45^{ab}	0.9 ± 0.03^{b}
2.50	0.09 ± 0.02^{a}	0.14 ± 0.02^{ab}	1.27 ± 0.35^{ab}	0.11 ± 0.04^{b}
5.00	0.08 ± 0.02^{a}	0.13 ± 0.03^{ab}	1.25 ± 0.39^{ab}	0.07 ± 0.01^{b}
7.50	0.10 ± 0.01^{a}	0.16 ± 0.03^{a}	1.45 ± 0.35^{a}	0.08 ± 0.04^{b}
10.0	0.09 ± 0.01^{a}	0.14 ± 0.05^{ab}	1.44 ± 0.23^{a}	0.07 ± 0.03^{b}

Means with the same superscript in the row are not significantly different (p<0.05).

Pesticides are metabolized in the liver through cytochrome P450 to hepatotoxic intermediates (Das and Gupta, 2013). Several field and laboratory studies have recorded an increase in hepatosomatic indices in fishes exposed to pesticides (Krurutap and Duran, 2001; Roche et al., 2000. Liver values increased slightly at the last two concentrations of diazinon (7.50 and 10.00mg/l). Liver enlargement suggests increased detoxification processes.

Spleen filters foreign substances from blood cells, release blood to sections in need, while kidney plays the major role in excretion of nitrogenous waste, control of water and electrolyte balance in the fish. A significant decline in size of these organs as recorded in this present study could impair these functions resulting in a more toxic internal environment which is detrimental to the fish.

Behavioural observation

There was no behavioural changes in the control and the first group that received the least dose (1.00mg/l) of the toxicant. The rest of the doses caused changes in behavioural patterns. Slight restlessness was observed in the group/treatment that received the highest dose of the toxicant. Intermediately the fish turned on the flank and swam in half circles and weakening of jerks were observed. Also, sudden quick movement, settling at the bottom and excessive mucus secretion on the skin were observed. Additionally, fishes showed unusual movement as a result muscle spasm and lethargy. Similarly findings have been reported by several researchers in a number of fish species including *Clarias gariepinus, Heterobrancus bidorsalis,* Common Carp (*Cyprinus carpio L) and Branchydario rerio* (Omeregie and Okpanachi 1992; Svobodova et al., 2001; Avoajeh and Oti, 2002; Auta et al., 2004). These abnormal behavioural responses exhibited by *Clarias gariepinus* in this present study suggest that they suffered respiratory impairment due to the effect of the toxicant precisely on gills and general metabolism.

CONCLUSION

Our data and physical observation unveiled the effect of diazinon on *Claria gariepinus*. We also observed that the concentration of diazinon that caused negative effect (specially, the behavioural responses) was much lower than anticipated, hence anywhere this insecticide run-off is present; it is assumed that organism in that microenvironment will suffer negative effects from exposure to diazinon. Further research of the impact of diazinon on developing aquatic organisms should be done for better understanding of the effect of diazinon in aquatic ecosystem.

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