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Effect of Diuron Herbicide (N- 3, 4-Dichlorophenyl -N, N-Dimethylurea) to Serum Biochemistry of *Clarias gariepinus*

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Abstract

The effect of Diuron herbicide on post Juvenile African catfish (*Clarias gariepinus*) using biochemical indicators was investigated and this could provide vital information concerning the health status of aquatic organism in *clarias gariepin*were investigated. The fish were exposed to different concentrations of Diuron of 0.5mg/L, 0.75mg/L, 1.0mg/L, 1,25mg/L, and 1.5mg/L for 96 hours. Different behavioural changes were observed. Also, blood samples were collected after 96 hours of exposure to check for the effect of different concentration of Diuron herbicide on the fish. The blood samples were analysed for serum enzymes (Alanine Aminotransferase ALT, Alkaline Phosphate ALP, Aspartate Aminotransferase AST and Lactate Dehydrogenase LDH). Also, total protein and glucose in the fish blood were monitored. Blood ion level examination was carried out to assess changes in sodium (Na^+), potassium (K^+) and chloride (Cl^-) and the alterations in the serum enzymes, metabolites and ion level were determined. It was observed that there were significant differences ($p<0.05$) in the serum enzymes analysis, biochemical examination and ion level examination and these parameters revealed differences with varying concentrations of Diuron herbicide.

Keywords:
Diuron, Herbicide, Clarias gariepinus, serum enzymes, biochemical indices

Introduction

The use of herbicides has gained worldwide use due to increase in population that required adequate food production to meet the demand of the teaming population, their usage could contaminate water resulting to bioaccumulation in tissue of some aquatic organisms. Report from Ladipo *et al.* (2011) confirmed that herbicides can store in aquatic ecosystem resulting in adverse effect on zooplankton community, the primary source of food for young fish. Akinsorotan, (2014) and Ladipo *et al*., (2011) explained that in the course of applying pesticides into the environment (terrestrial or aquatic), there is the possibility of non – target organisms been affected owning to the toxic nature of such pesticides. The effects of pesticides has been reported by Napit (2013) to cause disease conditions, behavioural abnormalities, physiological malformation, histological, hematological and biochemical changes, cancer and gene mutations in fishes especially in their early stages.

Diuron is a broad-spectrum herbicide used for weed, grass, and bush control on highway. It stops photosynthesis in plants and also inhibits seed germination. In fish, sodium and potassium are predominant electrolytes, with the predominance of sodium in the serum and in other fluids and potassium in extracellular fluids. The main function of proteins and electrolytes, mainly the sodium and potassium, is to regulate the acid-basic balance and thereby maintain an ionic adequacy on the tissue functions (Tavares-Dias, 2004; Tavares-Dias *et al.,* 2008). Many aquatic organisms are known to accumulate toxic solutes from their habitat without any obvious damage to themselves and therefore act as toxicant amplifiers, making the toxicants available to predators at dangerously high levels. Several

cases of the adverse effects of environmental pollution on fish and its consumers have been reported (Yuan *et al.,* 2004; Dix, 1981; Karthikeyan *et al.,* 2006; Iweala and Okeke, 2005). More commonly, aquatic organisms are subjected to long-term stresses from exposure to sub-lethal concentrations. However, in the long run, these sub-lethal concentrations may also prove to have negative effect on animals as do lethal concentrations (Yuan *et al.,* 2004).

The assessments of blood biochemical parameters are important to evaluate the health of many vertebrates, including fish (Cnaani *et al.,* 2004; Tavares-Dias *et al.,* 2008). Since blood biochemical parameters in fish may vary with ambient and other factors (Tavares-Dias *et al.,* 2008), they have been used by fish biologists for a variety of purposes, such as detecting cellular damage caused by toxicant exposure, infection by pathogenic agents, and traumatic handling.

Serum biochemistry data is of immense importance in monitoring the health status of aquatic organisms, especially in fisheries management programmes. Serum contains many chemical substances which include protein, enzymes, lipids, hormones etc. Testing for these various substances provides information on tissues and organs in the body of the fish as well as their metabolic state. Biochemicals are the assessable body contents for checking the toxicity of any chemicals (Singh *et al.,* 2010) and the results of such biochemical parameters results in serious outcome in the form of various diseases in fishes/ animals and also reveals underlying physiological conditions of the organs/ tissues of organisms (Obomanu *et al*., 2009).

Clarias species is one of the most widely cultured fish in Nigeria and acceptable among consumers probably due to their high economic and nutritive value. *Clarias gariepinus* can survive in poorly oxygenated waters; hence it has been widely used for ecotoxicological studies (Ogundiran *et al.,* 2010). This present study assessed the effects of different concentrations of Diuron on the serum biochemistry of *Clarias gariepinus*.

Materials and Methods

Chemical material and preparation

The test chemical (Diuron – (3-3,4-dichlorophenyl)-1,1-dimethylurea 40 .7% active ingredients) was purchased from Agro Shop at Arakale market, Akure Ondo State. The stock solution of Diuron was prepared by dissolving 150ml in 1 litre of distilled water. It was from this solution that the different static toxicity test doses were calculated and prepared by appropriate dilution.

Experimental animal

Clarias gariepinus juveniles (n = 250; mean weight76.69 ± 2.85g; mean standard length of 11.58 ± 0.50cm) were purchased from a private fish hatchery in Akure, Nigeria and conveyed to Federal University of Technology Akure, Teaching and Research Farm in a 50-litre plastic container and acclimated to laboratory condition for 14 days.

Fish were randomly divided into five plastic tanks (100 litre) supplied with oxygenated water maintaining constant dissolved oxygen of 4.5 ± 0.5 mg/L, temperature at 24 ± 2 °C, pH at 7.88 ± 0.2, and natural photoperiod. During the acclimation, fish were fed commercial diet on a maintenance ration of fish feed starter diets per day.

Experimental design

The *Clarias gariepinus* used for this study were randomly divided into six groups of ten fishes each, representing one control group and five other treatments. Experimental test was carried out using twelve plastic tanks of 16 litres capacity filled with 10 litres of water each. The concentrations of toxicant (0.5,

0.75, 1.0, 1.25, and 1.5 ml/L) were introduced into the plastic tanks with water, a control of (0.0ml/L) in duplicate.

Physico-chemical properties of the test media

The physico-chemical properties such as conductivity, temperature, pH, turbidity and dissolved oxygen were monitored in each aquaria for the period of the trial using EXTECH Instrument EC 500 and the dissolved oxygen was measured using a Digital dissolved oxygen metre model 831E.

Metabolites analysis assay of total protein level

This was carried out using the manufacturer protocol of Randox Total Protein Kit. One miliiter (1ml) of reagent R1(Sodium hydroxide (100 mmol/l), sodium-potassium tartrate (16 mmol/l), Potassium iodide (15 mmol/l) and copper II sulphate (6 mmol/l)) was added to 0.02 ml of the test sample, the mixture was incubated at 25^0C and the absorbance was then measured against the reagent blank at a wavelength of 546 nm.

Total Protein Concentration = $((Abs\ Sample/\ Abs\ Standard)\ \times\ standard\ concentration)$

Determination of Serum Glucose

The serum glucose was determined with Qomatest Kit® which relies on the principle that glucose oxidase (GOD) catalyses the oxidation of glucose into gluconic acid to form hydrogen peroxide which was detected by a chromogenic oxygen acceptor, phenol- ampyrone, in the presence of peroxidose (POD). The red quinone formed is proportional to the amount of glucose present in the serum sample.

The serum glucose concentration was determined from the calculation.

$$\frac{A\ of\ unknown}{A\ of\ standard} \times 100 = mg/100ml$$

Serum Enzymes Analysis

Determination of alkaline phosphate (ALP)

The quantitative in vitro ALP in serum was determined with the use of RANDOX® kit EC 3.1.3.1. The initial reading on 405nm absorbance was taken and then a timer was started simultaneously, the absorbance was read again at 1, 2, and 3 min. To calculate the ALP activity, the following formula was used:

$$U/I\ =\ 2760\ X\ absorbance/min.$$

Determination of Alanine Amino Transaminase (ALT)

The RANDOX® kit was used for the determination of the alanine amino transaminase (ALT). The absorbance of the ALT in serum was read at 546nm. The results were read on the calibrated graph respectively.

Determination of Aspartate Aminotranferase (AST)

The RANDOX® kit was used for the determination of the aspartate aminotranferase (AST/SGOT). AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The absorbance of AST in serum was read at 546nm.

Determination of Lactase Dehydrogenase (LDH)

Lactase dehydrogenase activity was measured using the change in optical density (OD) at 340nm for five minutes. The supernatant was used directly as an LDH source in the kinetic study. The oxidation of NADH at 340nm was done in a circulating thermobath at 25^{0}C. The reaction mixture was contained in a total volume of 1ml, 50mM Imidazol, 1mM KCN buffer pH 7.4 at temperature of 25^{0}C , 0.13 mM of NADH and different concentrations of pyruvate for LDH saturation plots.

Data Analysis

A significant difference in biochemical characteristics and physicochemical properties of test media of fish exposed to different concentrations of diuron was examined using one-factor analysis (ANOVA) after examining the data for normality using Kolmogorov-Smirnov test. The significant difference in means were compared by New Duncan's Multiple Range test (NDMRT) at $P < 0.05$. Statistical analyses were performed using SPSS 21 (IBM) software (Chicago, IL, USA). Data were presented as mean ± SD.

Result

Results from this study showed that there was no significant difference in the temperature at the end of the experiment while some significant differences were observed in DO, pH, Conductivity, Salinity and TDS. (Table 1).

Table 1: Effects of diuron herbicide on the physico-chemical parameters of water after the 96 hour exposure

Parameters	T0	T1	T2	T3	T4	T5
Temperature (^{o}C)	24.05 ± 0.05^a	24.20 ± 0.00^a	24.20 ± 0.00^a	24.20 ± 0.00^a	24.20 ± 0.00^a	24.20 ± 0.00^a
Dissolved Oxygen (mg/l)	4.51 ± 0.01^a	3.43 ± 0.03^b	3.15 ± 0.15^c	3.10 ± 0.20^c	3.10 ± 0.20^c	2.35 ± 0.15^d
pH	7.87 ± 0.03^a	7.55 ± 0.22^c	7.70 ± 0.00^b	7.76 ± 0.04^b	7.74 ± 0.04^b	7.86 ± 0.01^a
TDS (mg/l)	114.80 ± 4.90^d	114.10 ± 1.40^d	129.15 ± 5.95^c	148.05 ± 5.25^b	146.30 ± 28.70^b	174.65 ± 3.15^a

Means within the same column were not significantly different (P>0.05)

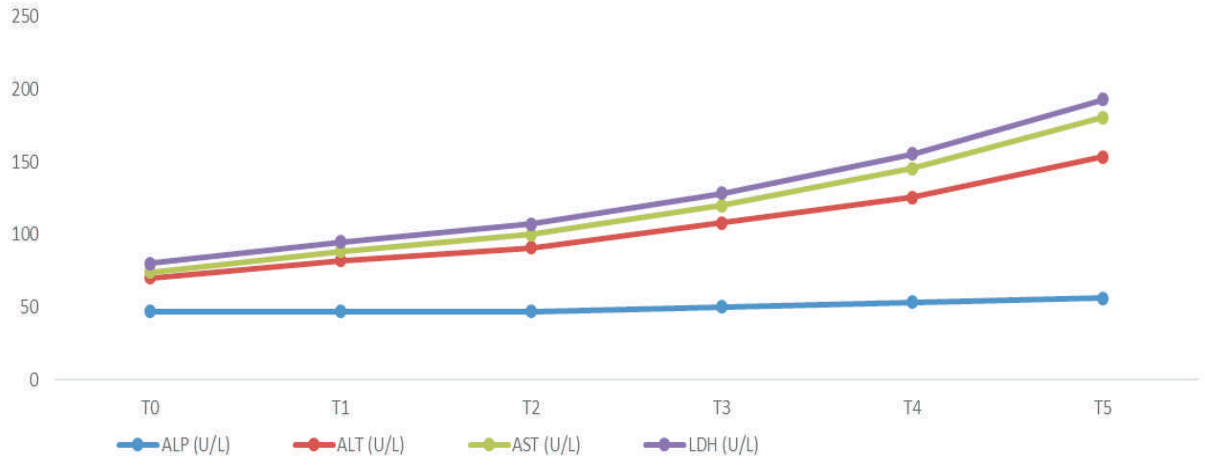

Figure 1: Serum enzymes concentration in *Clarias gariepinus* exposed to diuron for 96 hours

Serum Enzymes Examination

There were differences in the serum enzymes of *Clarias gariepinus* juveniles exposed to different concentration of Diuron herbicide after a period of 96 hours as shown in Figure 1. For all the enzymes measured, the values increased with increase in concentrations of the toxicant. Alkaline phosphate (ALP) value was noticed to range from 47.00 ± 0.00 to 56.00 ± 0.00. Alanine amino transferase (ALT) values ranged between 35.00 ± 1.00 and 97.50 ± 2.50. The values for Aspartase aminotransferase (AST) were between 6.50 ± 1.50 and 27.00 ± 1.00, while the values of Lactase Dehydrogenase (LDH) ranged from 6.43 ± 0.24 to 12.39 ± 0.35.

Metabolite Level Concentration

The metabolite level of *Clarias gariepinus* exposed to different concentrations of Diuron herbicide after 96hours showed significant difference across the various concentrations of the toxicant. The total protein value ranged from 25.27 ± 1.14 to 17.58 ± 0.48. The obtained value decreased in concentration from treatment 1 to treatment 5 as compared with the control. The Glucose ranged from 9.45 ± 0.09 to 28.3 ± 0.22. Glucose level showed an increase in values from treatment 1 to treatment 5 as compared with the control. The Cortisol values ranged from 20.67 ± 1.31 to 53.71 ± 0.62 and there is increase in cortisol values from treatment 1 to treatment 2 as compared to control as shown in Table 3.

Table 2: Ion level concentration in *Clarias gariepinus* exposed to diuron for 96 hours

Parameters	T0	T1	T2	T3	T4	T5
Sodium (mmol/L)	133.50 ± 9.50^{d}	121.00 ± 16.00^{c}	120.50 ± 2.50^{c}	114.50 ± 4.50^{b}	103.50 ± 0.50^{a}	101.00 ± 1.00^{a}
Chloride (mmol/L)	205.79 ± 14.59^{d}	186.98 ± 24.57^{c}	186.02 ± 3.26^{c}	176.81 ± 6.34^{b}	159.53 ± 0.96^{a}	156.46 ± 1.34^{a}

Means within the same column are not significantly different (P>0.05)

Table 3: Metabolite level concentration in *Clarias gariepinus* exposed to Diuron for 96 hours

Parameters	T0	T1	T2	T3	T4	T5
Total Protein (g/L)	32.49 ± 0.19^{d}	25.27 ± 1.14^{c}	21.66 ± 1.33^{b}	21.28 ± 0.76^{b}	18.91 ± 0.09^{ab}	17.58 ± 0.48^{a}
Glucose (mmol/L)	4.31 ± 0.03^{a}	9.45 ± 0.09^{b}	16.31 ± 0.47^{c}	23.77 ± 0.86^{d}	25.59 ± 0.41^{e}	28.33 ± 0.22^{f}

Discussion

Musa and Omoregie (1999) reported that fish are intimately associated with their environment and physiological changes in fish is a function of physical and chemical changes in the environment. Studies have shown that when water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (Van, 1986). Thus, water quality is one of the major factors, responsible for variations in fish biochemical parameters, due to their close association with their environment and are sensitive to slight fluctuation that may occur within their surrounding water (Popoola *et al*., 2018). Although, the variation were noticed in some water parameters, but the values recorded are still with the tolerance range for *C. gariepinus.*

The ALP, ALT AST and LDH are considered as physiological stress parameters and can be used as a standard while measuring effect of Diuron on aquatic organisms, as it serves as indicator for toxicity effect of pollutant on fish (Conn *et al.* 2009). In the present study ALP, ALT AST and LDH were analysed as blood indicators of pollutant stress in fish.. Administration of Diuron at different concentrations resulted in increased levels of blood parameters (ALP, ALT AST and LDH). The values recorded for all the enzymes increased across the treatment (p<0.05). The significantly higher ALP, ALT

AST and LDH activities in the C.*gariepinus* exposed to increasing Diuron level across the treatment when related with control groups could be linked with leakage of aminotransferase (ALT) enzymes from injured liver cells. These results were similar to the observation of Konstantinova and Russanov, (1999) who studied paraquat induced oxidative stress in rat liver.

Electrolytes are ions in solution which acquire the capacity to conduct electricity and their balance in the body of organism is essential for the normal function of cells and organs. According to Gabriel*et al.* (2009), the basic function of electrolytes in the body lies in the control of fluid distribution, intracellular and extracellular acido-basic equilibrium which culminates in the proper maintenance of osmotic pressure of body fluids and normal neuro-muscular irritability. The study found that there is variation in the sodium, potassium and chloride content in the blood serum, of the *C. gariepinus* under varying concentrations of the toxicant (Diuron). The electrolytes (sodium and chloride) increased with toxicant concentrations in the blood suggesting that the fishes responded to the stress. This suggested that little variation in the chemical composition of the aquatic ecosystem might alter the electrolytes of *Clarias gariepinus*. Chloride ion as the major extracellular fluid in the body, combines with sodium to form NaCl (sodium chloride), which then helps in the osmotic balance of the organism (Adeoye, 2007). The levels of chloride significantly decreased ($P<0.05$) during prolonged toxic exposure periods of *Clarias gariepinus* to Diuron herbicide. Decrease in Cl- ion has been found to be associated with handling stress and kidney disease (Tomasso *et al.,* 1980). Potassium ion (K+) is essential for normal cell function and body regulation. The perceived increase in potassium (K+) ion in this study might be a result of the interference of diuron with normal function of the gill and liver as reported by Gabriel *et al.,* (2009). An increase or decrease in potassium (K+) ion normally results in irregular heartbeats (arrhytamias) which can be fatal in extreme conditions (Gabriel *et al.,* 2009). Sodium and potassium had been reported to be essential for the activity of many enzymes in animal system, also in transportation of ATP in several metabolic processes. Na+ and K+ATPases are present in the membranes of cells and are in charge of active transport of Na+ and K+ across cell membrane (Rajanna *et al.,* 1981). The imbalance electrolytes in fish has also been reported to cause lateral line imbalance and hormonal disorder through affective endocrine organs as a result of pesticides attack (Tella, 2005; Gabriel *et al.,* 2009).

However, this study provided evidence that glucose and protein levels were altered in fish induced with Diuron. The meaning of this is that, endocrine-disrupting effects were connected with hyperglycemia and liver impairment. The increase in blood glucose in this study significantly increased while the protein decreased with increase in the toxicant concentrations and this agrees with the findings of Yousafzai, *et al.,* (2011) who reported that high glucose concentrations in the blood indicate that the fish is in stress and is intensively using energy reserves. The increase in the glucose levels reported in this research indicated that the *Clarias gariepinus* juvenile became hyperglycemic in the sub-lethal exposure of 1.5mL/L concentration of the herbicide. According Omoregie *et al.* (1990) this incomplete metabolism of blood sugar could be as a result of impaired osmoregulation.

Total protein is an important constituent of cells and tissues as it plays a vital role in the physiology of living organisms. There was a decrease in total protein indicating increased mobilization of protein reserves to adapt to stress conditions. The concentration of the fish blood protein serum is an index of the general health condition of the fish. Das *et al.* (2004) have reported that increased energy demand might increase protein consumption, a process where protein is converted into energy, and therefore the protein serum will be reduced.

This study revealed the significant effects of Diuron on the blood biochemical parameters of C. *gariepinus* on exposure to varying concentrations of Diuron leading to blood biochemical alterations and homeostasis disruption. Therefore, the environmental guideline on the use of pesticides such as Diuron should be followed, because the presence of this herbicide in small amounts in fish tissue, can lead to their bioaccumulation in organisms at higher levels of the food chain.

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