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Sublethal effect of diuron on DNA status, hematological and biochemical indices of fresh water catfish, *Clarias gariepinus* Juveniles

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Abstract
The toxicity of Diuron (N-(3, 4 dichlorophenyl)-N, N- dimethylurea), a herbicide was investigated on *Clarias gariepinus* popularly cultured fish in Nigeria. Hematological and biochemical indices status of DNA were examined in Diuron treated fish. Juvenile African catfish (*Clarias gariepinus*) (76.69±2.85g) were exposed to sub-lethal 0.00 ml/L, 0.5 ml/L, 0.75 ml/L, 1.00 ml/L, 1.25 ml/L and 1.50 ml/L of herbicide for 96 hours under static bioassay conditions. Another group of 2mg/l of cyclophosphamide was prepared. Water quality parameters were monitored during the bio-assay test. After 96 hours, blood was extracted from the fish and haematological parameters (Hb, PCV, WBC and RBC) were estimated. Biochemical indices of changes in creatinine, urea, cholesterol and triglyceride level were determined. The genotoxic effect was evaluated using micronucleus test. The median lethal concentration (LC_{50}) value was determined to be 1.29ml/L. There were significant differences ($p<0.05$) in the haematological, biochemical analysis as the parameters measured differed with concentrations of the toxicant. The induction of micronuclei was highest (15.67) with highest concentration of the toxicant in the peripheral blood. The study revealed that long exposure to diuron at sublethal concentration can cause mutagenic effects, alter blood chemistry and oxidative dysfunctions in *C. gariepinus.*

Keywords: diuron; sub-lethal toxicity; genotocity; haematological indices; biochemical indices; *Clarias gariepinus*; sub-lethal concentration

Introduction
Pollution in aquatic environment affect both flora and fauna which eventually result in disease, destruction of vital organs, reduces growth as well as reproduction performance and death of the aquatic organism when it is beyond the tolerant level of the organism (Beneet *et al.*,1995 and Rahaman *et al.*, 2002) [1, 2].

Herbicides have adverse effects on the environment and persist in the environment long after they have carried out their weed killing function. Their persistence in the soil is a public health concern because of the potential of leaching into ground water and contaminating surface water.

Fishes and other aquatic organisms are directly affected by pollution caused by herbicides consequently the effect on humans is indirect because they are the final consumer of these aquatic products. Usually, there is a wide spread exposure of humans to herbicides, especially during rainy season as a result of their use for land preparation by famers, and elimination of weeds and unwanted vegetation by households. Hence, the use of mortality or bioassay experiments is necessary to assess the environmental impact of toxic compounds.

Diuron (N-[3, 4-dichlorophenyl]-N, N-dimethylurea), A herbicide of the phenylurea family, is widely used to destroy unwanted weeds and as an antifouling agent in paints. Diuron is generally persistent in soil, water and groundwater. It is also slightly toxic to mammals and birds as well as moderately toxic to aquatic invertebrates depending on the concentration (Giacomazzi and Cochet, 2004) [3].

The fish haematology status is affected by the use of various herbicides whether selective or non-selective in which the effect may be acute or chronic. Changes in blood properties in response to environmental conditions are a response to environmental stress and can be considered as an important bio-indicator. Evaluation of the haematological profile usually gives information on the response of the fish to injury, size, weight and stress (Nussey *et al.*,

1995) [4]. Blood parameters are therefore considered as patho-physiological indicators of the whole body and are used in diagnosing the structural and functional status of fish exposed to various toxicants (Adhikari *et al.*, 2004; Maheswaran *et al.*, 2008) [5, 6].

The cytogenetic analysis, such as micronucleus induction, chromosomal aberrations and sister chromatid exchange offers reliable genetic assays in detecting genotoxic chemicals at sub-toxic levels. The micronucleus is a cytoplasmic chromatin mass with a small nucleus produced from a lagging chromosome in the anaphase stage as a result of some structural and/or numerical chromosomal aberrations in the cells during mitosis (Parveen and Shadab, 2012) [7]. This aberration is due to a genetic imbalance in the cells, possibly leading to carcinogenesis (Tolga Cavas *et al.*, 2005) [8].

There several biological test used in detection and identification of environmental pollutants (Güner and Muranlı, 2011) [9]. The use of micronuclei (MN) count has been reported by Bombail (2011) [10] an indices of chromosome aberration and non- functioning of spindle in mitosis. The benefits of this test involve its simplicity, reliability, and sensitivity.

Fish are considered good candidate for monitoring aquatic genotoxicity due to their ability to metabolise xenobiotics and accumulate pollutants (Grisolia and Corderio, 2000) [11]. Effects of Malathion on *Chana punctatus* has been reported by Parveen and Shadab (2011) [12] using the micronucleus test Mohamed *et al.* (2008) [13] analysed the cytogenetic damage by measuring the chromosomal aberration in the gill cells.

It is therefore necessary to know the effect of diuron herbicide on the juvenile of *Clarias gariepinus* bearing in mind that these juveniles grow to big size for human consumption.

This research therefore designed to examine the mutagenic and haematological effects as well as antioxidant stress biomarkers *C. gariepinus* after long term exposure to diuron herbicide.

Materials and Methods

Experimental Fish and Toxicant

A total 250 of *C. gariepinus* (mean weight, 76.69±2.85g) were collected between july and August 2017, acclimated for 72 hours in the Department of Fisheries and Aquaculture Research Farm of the Federal University of Technology, Akure. Diuron was obtained from Agro Shop in Akure Ondo State. The stock solution of Diuron was prepared by dissolving 150ml in 1 litre of distilled water from the different static toxicity test doses were calculated and prepared by appropriate dilution.

Experimental Procedure

Five concentrations (0.5, 0.75, 1.0, 1.25, 1.5 ml) of Diuron were prepared and tested on the *Clarias gariepinus* juveniles for the static bio-assay test together with another group containing 2mg/l of cyclophosphamide. A total of 14 glass tanks (0.7 x0.5x 0.45m^3) were used (5 treatment and 2 control groups) containing 10 litres of water in each was used for the dilution of each concentration. The exposure was continued for 96 hour during which physicochemical parameters of the test media were recorded. The physicochemical parameter measured and recorded during the experiment were temperature, total dissolved solid, conductivity and salinity using EXTECH Instrument EC 500 and the dissolved oxygen was measured using a Digital dissolved oxygen metre model 831E.

Mortality rate was also observed at 24, 48, 72 and 96 hours respectively. Confirmation of dead fish was done by gentle touch with a glass rod and by noticing no opercula movement and tail-fin beats. Dead fishes were promptly removed and mortality was recorded. The median lethal concentration (LC_{50}) at 96 h was computed using the probit analysis.

The blood samples from the life fish were collected after 96 hours for further study.

Haematological examination

Blood samples were collected from fishes by puncturing caudal vein utilizing ethylenediaminetetraacetate (EDTA) as anticoagulant.The blood, (2.5 ml) was emptied into heparinzed bottles for determination of hematocrit. Heamoglobin (Hb) fixation was estimated with Hb test unit utilizing the cyanmethemoglobin technique. Red blood cell count (RBC) and white blood cell count (WBC) count were checked under light microscope with an enhanced Neubauer haemocytometer using method of Shah and Altindag, (2005)[14]. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin fixation (MCHC) were computed in picograms = Hb/RBC x 10; and MCHC = (Hb in 100 mg blood/Hct) x 100.

Biochemical examination

The urea nitrogen was estimated according to Randox laboratories limited procedure (Weatherburn, 1967) [15]. The creatinine colorimetric assay was conducted according to Perone *et al.*, (1992) [16]. The total cholesterol concentration was determined using colorimetric method by Allain *et al.*, (1974) [17] with the standard formula

$$Cholesterol\ conc. = \frac{A_{serum}}{A_{standard}} \times n$$

The triglyceride concentration was determined colorimetrically using method of Tietz (1982) [18] with formula $Triglyceride\ conc. = \frac{A_{serum}}{A_{standard}} \times n$

Micronucleus assay

The method described by Ayllon and Garcia-Vazquez (2000) [19] for the piscine micronucleus test was used with little modification. Blood samples from five (5) selected fish from each of the sub-lethal concentration of diuron were smeared on clean, grease free iced glass slides. The treated slides were embedded in methanol for 12 min and later allow to air-dry at room temperature. The slides were again stained with 6% Geimsa in Sorenson buffer (pH 6.9) for 25 min. Graded alcohol was used for the dehydration of the slides followed by clearing in xylene solution. A mixture of distyrene, plasticizer and xylene were used in mounting the slides. From each of the slides, about 1000 erythrocyte cells were scored under a light microscope (Leica DM300). In identifying erythrocyte cell, each MN were subjected to the same color, plane of focus, clearly separated and smaller than one-third of the main nucleus as described by Nwani *et al.* (2011) [20].

The MN frequency was calculated thus:

MN% = $Number\ of\ cells\ containg\ micronuclei/$Number of cells containing micronuclei x 100

Data analysis

Values from the Haematological parameters, water quality

parameter and Biochemical indices were analysed by one factor analysis using test Statistical Package for Social Science (SPSS 23) and the difference in the mean was separated using the New Duncan Multiple Range Test (NDMRT). The mortality rate was determined using probit analysis. The frequency of micronuclei was presented using chart.

Results

The probit analysis of the log concentration against the probit percentage mortality of the fish exposed to diuron herbicide for 96 hours was presented in figure 1. In the control tank, the experimental fishes exhibited normal behavioural and swimming patterns compared with other treatments. There was a reduction in dissolved oxygen concentration, increases in conductivity and total dissolved solid level with increase in concentration and time Table 1.

Haematological Parameters

The haematological properties (PVC, HB, WBC, RBC, Neutrophyl, Monocytes Lymphocyte MCV MCHC and MCH) of the exposed fish at different concentrations were obtained after 96 hours exposure and shown in the table 2

There was a significant difference ($p<0.05$) in Packed cell volume (Haemtocrit) and was noticed to range from 10.5±0.95 to 16.80±0.95, which reduced with concentration of the toxicant. Haemoglobin exhibit significant different ($p<0.05$), its values ranged from 4.40±0.22 to 5.85±0.22 and decreased with increase in concentration of the Diuron.

There was a significant difference ($p<0.05$) in WBC with values ranged from 2075±434.58 to 4850±434.58, and WBC showed a decrease in Treatment 1(0.5ml/l) to Treatment 5 (1.50ml/l) with increase in concentration compared with the control. Red blood cell (RBC) ranged from 1.3±0.43 to 3.95±0.43, and decreased with increase in concentration when compared with the control. Neutrophyl values ranged from 4.60±0.61 to 8.55±0.61, the neutrophyl increased in Treatment 1, 2, 3, 4, 5(0.5ml/l, 0.75ml/l, 1.0ml/l, 1.25ml/l, 1.50ml/l) with increase in concentration compared with the control. Monocytes ranged from 23.60±0.54 to 26.92±0.54, which showed an increase in treatments with increase in concentration when compared with the control. Lymphocytes ranged from 68.10±0.28 to 69.86±0.28, and decreased in Treatments 1, 2, 3, 4, 5(0.5ml/l, 0.75ml/l, 1.0ml/l, 1.25ml/l, 1.50ml/l) respectively when compared with the control with increase in concentration. MCV ranges from 37.15±1.77 to 88.06±21.95, and decreased in Treatments 1 and 2 (0.5ml/l, 0.75ml/l), and increase Treatment 3, 4, 5(1.0ml/l, 1.25ml/l, 1.50ml/l) with increases in concentration when compared with the control.

MCH ranged from 14.33±1.18 to 37.40±10.71, the MCH value obtained showed a decrease in Treatment 1, 2(0.5ml, 0.75ml), and an increase in Treatment 3, 4, 5(1.0ml, 1.25ml, 1.50ml) respectively compared with the control with increase in concentration. MCHC result ranged from 34.86±0.64 to 43.88±1.80, the MCHC of the treatments increase with increase in concentration of Diuron compared with the control.

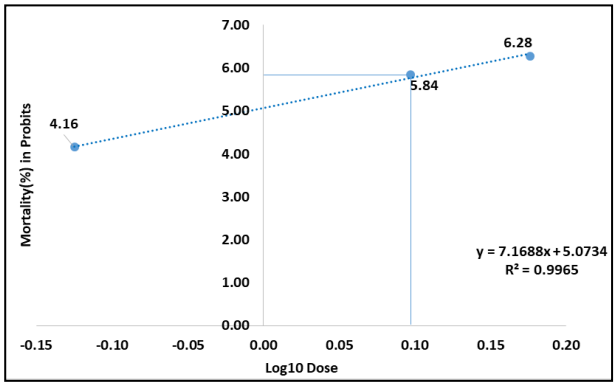

Fig 1: Lethal concentration (LC50) of Clarias gariepinus exposed to Diuron after 96 hou

Table 1: Physicochemical Parameter of water recorded 96 hour Bio-assay test

Parameter	0.00	0.50	0.75	1.0	1.25	1.50
Conductivity (µS/cm)	231.00 ± 12.00^{a}	250.00 ± 22.00^{a}	252.50 ± 15.50^{a}	282.00±14.00^{a}	428.50±15.50^{b}	403.50±37.50^{b}
TDS (mg/L)	112.35±5.95^{a}	121.80±14.85^{a}	123.20±7.70^{a}	137.90±7.00^{a}	210.00±7.00^{b}	197.40±18.20^{b}
Salinity (g/kg)	0.05±0.00^{a}	0.06±0.00^{a}	0.06±0.00^{a}	0.07±0.00^{a}	0.10±0.00^{b}	0.10±0.00^{b}
pH (mol / L)	7.81±0.09^{b}	7.63±0.03^{a}	7.72±0.03^{ab}	7.74±0.00^{ab}	7.84±0.04^{b}	7.85±0.01^{b}
Temperature (^{o}C)	23.95±0.05^{a}	23.95±0.05^{a}	23.90±0.00^{a}	23.95±0.05^{a}	23.95±0.05^{a}	23.95±0.05^{a}
DO (mg/l)	5.65±0.05^{e}	3.30±0.10^{d}	3.05±0.05^{c}	2.65±0.05^{b}	2.15±0.05^{a}	2.00±0.00^{a}

*TDS= Total dissolved solid *DO= Dissolved oxygen

The values are expressed as the mean ± standard error. Means in the same horizontal column followed by different superscript are significantly different ($p<0.05$).

Table 2: Haematological parameters of *C. gariepinus* exposed Diuron Concentrations

	Concentrations (ml/L)					
	0.00	**0.50**	**0.75**	**1.00**	**1.25**	**1.50**
PCV (%)	16.80±0.95^{b}	13.67±0.95^{ab}	12.75±0.95^{a}	12.00±0.95^{a}	10.75±0.95^{a}	10.5±0.95^{a}
HB(g/100ml)	5.85±0.22^{c}	5.40±0.22^{bc}	4.75±0.22^{ab}	4.70±0.22^{ab}	4.70±0.22^{ab}	4.40±0.22^{a}
WBC (×10^{3} /mm^{3})	4850±434.58^{c}	4600±434.58^{bc}	3600±434.58^{abc}	3050±434.58^{ab}	2875±434.58^{a}	2075±434.5^{a}
RBC (×10^{6} /mm^{3})	3.95±0.43^{c}	3.7±0.43^{c}	3.35±0.43^{bc}	2.75±0.43^{abc}	1.85±0.43^{ab}	1.3±0.43^{a}
Neutrophyl (%)	4.60±0.61^{a}	5.2±0.61^{ab}	5.12±0.61^{ab}	6.47±0.61^{abc}	7.21±0.61^{bc}	8.55±0.61^{c}
Monocytes (%)	23.60±0.54^{a}	24.20±0.54^{a}	25.50±0.54^{ab}	26.92±0.54^{b}	24.10±0.54^{a}	26.18±0.54^{b}
Lymphocyte (%)	69.86±0.28^{c}	68.34±0.28^{ab}	69.20±0.28^{bc}	68.33±0.28^{ab}	68.10±0.28^{a}	69.20±0.28^{bc}
MCV(fl)	42.78±2.27^{a}	37.15±1.77^{a}	38.33±2.11^{a}	44.18±3.49^{a}	60.19±8.93^{ab}	88.06±21.95^{a}
MCHC(gm/100)	34.86±0.64^{a}	39.58±1.12^{abc}	37.33±1.03^{ab}	39.27±1.25^{abc}	43.88±1.80^{c}	42.06±1.68^{bc}
MCH(gm/100)	14.93±1.07^{a}	14.73±1.12^{a}	14.33±1.18^{a}	17.39±1.92^{a}	26.57±5.00^{ab}	37.40±10.71^{b}

Values are mean ± standard error. Means in the same column followed by different superscripts are significantly different ($p<0.05$). PCV-Packed cell volume, HB- Haemoglobin, WBC-White blood cell, RBC-Red blood cell, MCV-Mean cell volume, MCH- Mean cell haemoglobin, MCHC-Mean cell haemoglobin concentration

Biochemical Examination

The result of biochemical parameters observed in this study is presented in table 3. There was a significant difference ($p<0.05$) in all the parameters measured. The result showed that the creatinine, urea, triglyceride and total cholesterol level increased with increase in the concentration of diuron.

Induction of Micronucleous

Figure 2 shows induced MN frequencies in red blood cell *C. gariepinus* exposed to Diuron for 96 hours. The highest value was recorded in concentration 1.50ml/l (15.67%) and least in 0.5ml/l (2.17ml/l) for 96 hours period. There was a reduction in MN induction frequency in the blood erythrocytes of the *Clarias gariepinus* and the induction of MN is observed to be concentration – dependent as the percentage of induction increased with exposure time.

Table 3: Biochemical Composition of *C. gariepinus* exposed to different concentration of Diron Concentration ml/l

Parameter	0	0.5	0.75	1.00	1.25	1.50
Creatinine	4.22±0.55^{a}	5.01±0.12^{a}	6.05±0.18^{b}	6.66±0.18^{b}	7.70±0.12^{c}	8.19±0.25^{c}
Urea	52.50±2.50^{a}	97.50±7.50^{b}	137.50±2.50^{c}	157.50±2.50^{d}	162.50±2.50^{d}	197.50±2.50^{e}
Triglyceride	54.36±2.18^{a}	81.54±3.27^{b}	100.02±4.35^{c}	121.77±4.35^{d}	139.15±4.35^{e}	161.99±5.44^{f}
Total Cholesterol	98.70±0.42^{a}	101.22±1.26^{a}	107.10±2.94^{ab}	113.40±2.52^{bc}	120.12±3.36^{c}	133.56±2.52^{d}
Lactate	0.56±0.47^{a}	1.45±0.47^{ab}	2.19±0.47^{abc}	2.77±0.47^{bc}	3.22±0.47^{c}	3.59±0.47^{c}

Values were derived in duplicate with significant difference observed across all the treatment ($p<0.05$).The values are expressed as the mean ± standard error. Means in the same horizontal column followed by different superscript are significantly different

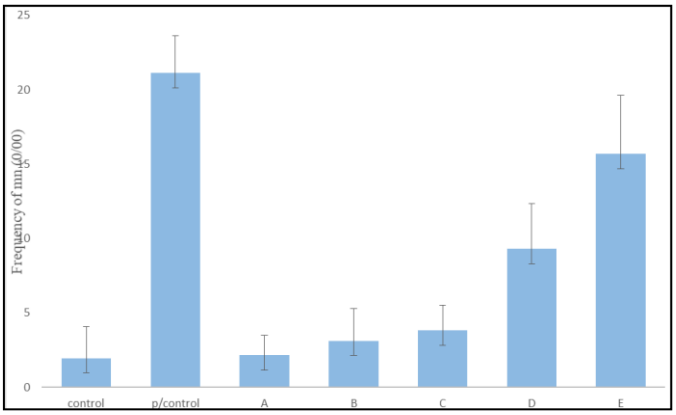

Fig 2: Frequencies (‰) of MNs, red blood cells from C. gariepiius exposed to the Diuron herbicide for 96 hours Cyclophosphamide (2 mg/L) was as positive control 0.o ml/l of diuron as negative control

Discussion

Diuron, an herbicide used for specific crops and as an algaecide in ornamental aquaria is toxic to fish and poses a threat to the health status of *Clarias gariepinus* juveniles. Increased concentration of Diuron could impair the biochemical indices of the fish. In terms of behavioral patterns, various behavioural response such as gulping air at the surface, abnormal movement was observed and the observations agreed with that of Lin and Liu,(1990) [21] who reported that clinical signs such as abnormal movement and high respiration rate in hybrid tilapia (*Oreochromis mossambicus*) induced by ammonia suggested neurological dysfunction and gill damage. Likewise, the deficiency of oxygen causes the hypoxic condition in fish which results in an increase in the breathing rate and to cope with the condition, the fishes gulp air by frequent surfacing. The test fish exhibited somersaulting activity, erratic swimming, swimming upside down and with time settled at the bottom of the tank and almost 95% died before the end of the exposure period. In the other concentrations the fish were seen to come to the water surface to gulp air, high rate fin and opercula movement. This is in agreement with the work of Omoniyi *et al.* (2002) [22]; Kulakkattolick and Kramer (1997) [23] who reported several abnormal behaviours such as incessant jumping and gulping of air, restlessness, surface to bottom movement, sudden quick movement and resting at the bottom also observed that the fish became inactive at higher concentrations with increasing time of exposure to toxicant which is a normal observation in acute and chronic toxicity test.

The physicochemical properties measured in the test solutions showed that water temperature and pH varied slightly between the treatments; there was also decrease in the dissolved oxygen level and increase in conductivity and total dissolved solids and this agrees with the findings of Yunis *et al.*, (2014) [24] who reported that fish mortality could not be attributed to pH; but instead were associated with the low levels of DO when the neotropical fish *Hyphessobrycon eques* was exposed to the aqueous extract of *Uncaria tomentosa* bark. The increase in the toxicant may be the cause of the depletion of oxygen which agrees with Ukwagu *et al.*, (2012) [25] who stated that the physicochemical parameters examined from the tank polluted with effluent showed a decline in Dissolved oxygen level and increase in total dissolved solid and conductivity level

According to Barkhordar (2013) [26] the 96 hrs LC_{50} tests are conducted to measure the susceptibility and survival potential of organisms to particular toxic substances. The result obtained from this investigation revealed that the 96-hour LC_{50} for the juvenile of *Clarias gariepinus* exposed to Diuron was 1.25ml/L.

The fish in the highest concentration 1.50ml/l showed 95% mortality while the fish in the lower concentrations of (0.5ml/l and 0.75ml/l) showed 0% mortality which showed that stress and eventual death of the fish is concentration dependent. The mortality rate was higher in the higher concentrations and this is in agreement with Fryer (1997) [27] who observed that the highest concentration of the toxicant resulted in the highest mortality rate which was described as normal, that in all toxicant thresholds is reached above which there is no drastic survival of animal. Below the threshold, animal is in a tolerance zone, above the tolerance zone is the zone of resistance.

In this study the result on the haematological parameters of *Clarias gariepinus* juveniles exposed to different concentrations of the toxicant showed a significant difference in the blood parameter when compared to the control. The result showed that there was a decrease in the PCV level of the test fish with an increase in the concentration; the fish subjected to the highest concentration (1.50ml/l) had the lowest PCV level although the decrease in packed cell volume

indicated that the fish might be suffering from anaemia. This corroborated the finding of (Oropesa *et al.,* 2009) [28] where fishes exposed to atrazine experienced decreases in the packed cell volume levels. The haemoglobin level decreased with increase in concentration, the fish subjected to the highest concentration (1.50ml/l) had the lowest haemoglobin level. The reduction in haemoglobin concentration in experimental fish might be due to the destruction of red blood cell as reported by (Svoboda *et al.,* 2001) [29] where he stated that exposure to heavy metals or pesticides leads to reduced haemoglobin content and haematocrit via disorders in haemopoietic and accelerated disintegration of erythrocyte cell membrane. The decrease in blood haemoglobin levels as seen in this research suggests a response of the juvenile fish to decrease in the blood oxygen carrying capacity. As haemoglobin level decreases, PCV level decreases as well. This is because haemoglobin will not decrease without a corresponding decrease in the PCV value which indicate a negative absorption of the toxicant in contrast with Olufayo (2009) [30]; Ayotunde *et al*, (2004) [31] who explained that exposure of *C. gariepinus* to sub-lethal concentrations of *Derris elliptica* caused a significant increase in PCV, haemoglobin, and erythrocyte of the fish treated with high concentration of *Derris elliptica*.

The decrease in blood haemoglobin and red blood cells may also be due to the presence of stressor (diuron) which causes haemodilution to occur as a result of impaired osmoregulation (Rottman *et al.,* 1992) [32]. There was reduction in the red blood cells with increase in concentration in comparison with the control group which suggested that at higher concentrations, there could have been destruction of red blood cells. Similar patterns were also observed for haemoglobin (Hb) values which suggested haemodilution which could lead to anaemia. The swelling of erythrocytes can be inferred from the increase in the MCV, which is an indicator of the size or state of the red blood cells which agrees with Larsson *et al.,* (1985) [33]. who attributed the increase in MCV to swelling of the red blood cells due to hypoxic conditions or impaired water balance (Osmotic stress) and this may be related to macrocytic anaemia in fishes exposed to metal pollution. Ayotunde *et al.,*(2004) [31]; Adekunle *et al.,* (2007) [34] stated that the haematological examination of the fingerlings showed an increase in RBC, WBC, PCV, Hb, MCH and MCHC values, while there was a reduction in the ESR, MCH and MCV values and lymphocytes percentage showed significant increase. In this study the white blood cell which serve as the immune response decreased significantly from the control group there was significant difference between treatments showing that the toxicant was beyond the immune level of the fish such that it caused destruction of the white blood cell. Mean corpuscular haemoglobin (MCH) measures the average amount of haemoglobin within a red cell show increase in values with concentrations increase. In accordance to this finding, (Ruperelia, 1990) [35] reported that MCHC levels increased in tilapia *Oreochromis mosambicus* when it was exposed to cadmium.

Creatinine is a nitrogenous waste product that is eliminated by the kidney when excretion is suppressed in renal inadequacy. In this research, the creatinine value of the fish exposed to diuron increased significantly with increase in toxicant concentration. Murray *et al.,* (1990) [36] and Lall *et al.,* (1997) [37] reported that elevated creatinine in serum might be induced by glomerular inefficiency and a rise in creatinine value is an indication of renal tubular damage. Thus, it could be inferred from this work that diuron is nephrotoxic. Triglyceride measured in this study showed a significant increase with increase in concentration. The increase in blood triglyceride and cholesterol may be due to dysfunction of liver and destruction of cell membranes can also lead to increased levels of cholesterol in plasma. Disorder in triglyceride uptake by adipose tissue may also increase triglycerides. John (2007) [38] explains cholesterol and glucose levels in blood of freshwater fish, *Mystus vittatus*, increased after exposure to metasystox and sevin. Increase of stress hormones such as cortisol in blood of fish exposed to various insecticides, which stimulates lipid breakdown in adipose tissue, were also found in Banaee (2013) [39]. Similar changes have also been reported by Ibrahim and El-Gamal (2003) [40], Lasram *et al*. (2009) [41] and Acker and Nogueira (2012) [42].

Genotoxic assays such as MN test had been reported by Mazzeo and Marin-Morales, (2015) [43] useful tools in evaluating the effects of pollutants in fish and other aquatic organisms (Ansari *et al.,* 2011) [44]. Elimination of amplified genetic materials from the cell (Fench, 2011) [45] non incorporated chromosomal breaks or losses during cell division cycle (Renu and Saxena, 2015) [46] have been reported to be the output of micronuclei

The present study revealed sub-lethal concentrations of Diuron in that it caused concentration-dependent increase of MN in the red blood cells of *C. gariepinus* as observed in 1.5ml/l after 96 hours. Piancini *et al*., (2015) [47] reported similar observation in *Rhamdia quelen* exposed to atrazine herbicide. Mahboob *et al*., 2013[48] give account of similar observation in *C. gariepinus* exposed to mercury chloride. The increase in MN frequency might be as a result of oxidative stress caused by the production of reactive oxygen specie (ROS). This oxidative stress is caused by the inability of the antioxidant system to eliminate the ROS (Dar *et al*., 2015[49]). The presence of ROS has been reported by Li *et al*., (2011) [50] that it could lead to the destruction of macromolecules, the implication of this is that long exposure of *C. gariepinus* to Diuron can cell damage leading to production micronulei

Recommendation

Diuron is of uttermost importance in the control of weed in agricultural farm land it is usually absorbed by the soil and gets into the water body either by runoff or ground water. It also has acute/chronic effect on fish. This study confirmed that exposure to pesticides can result in significant haematological and biochemical changes of fish. This study revealed not only toxicity on fish but also caused micronuclei induction. The indication that Diuron cause haematological alteration and can be mutagenic. Further research is encourage at molecular level to reveal more of the harmful effect of Diuron.

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