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 (LC50) 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; genotoxicity; 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)

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)

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.,...
Blood parameters are therefore considered as pathophysiological 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 Cordier, 2000).\(^11\)

Effects of Malathion on \textit{Chama puncatus} 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 \textit{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 \textit{C. gariepinus} after long term exposure to diuron herbicide.

**Materials and Methods**

**Experimental Fish and Toxicant**

A total 250 of \textit{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 \textit{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 (LC50) 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 heparinized bottles for determination of hematocrit. Hemoglobin (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 Altingdag, (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

\[
\text{Cholesterol conc.} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times n
\]

The triglyceride concentration was determined colorimetrically using method of Tietz (1982)\(^18\) with formula

\[
\text{Triglyceride conc.} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times n
\]

**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 iccd 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:

\[
\text{MN}\% = \frac{\text{Number of cells containing micronuclei}}{\text{Number of cells containing micronuclei}} \times 100
\]

**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 60 hours exposure and shown in the table 2. There was a significant difference (p<0.05) in White blood cell, Haemoglobin, WBC and Neutrophyl with increase of concentrations compared with the control. Neutrophyl values ranged from 1.3±0.43 to 3.95±0.43, and decreased with increase in concentration when compared with the control. Red blood cell (RBC) ranged from 1.3±0.43 to 5.2±0.22, and increased with increase in concentration compared with the control. PCV (%), MCV (fl), and MCH (gm/100) respectively with increase of concentrations compared with the control Table 2.

There was a significant difference (p<0.05) in Packed cell volume (PCV), Haemoglobin, WBC and Neutrophyl with increase concentration of Diuron compared with the control. HB value ranged from 34.86±0.64 to 43.88±1.80, and the MCHC of the treatments increase with increase in concentration in MCHC result ranged from 34.86±0.64 to 43.88±1.80, and the MCH of the treatments increase with increase in concentration in MCH.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.00</th>
<th>0.50</th>
<th>0.75</th>
<th>1.0</th>
<th>1.25</th>
<th>1.50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (µS/cm)</td>
<td>231.00± 12.00*</td>
<td>250.00± 22.00*</td>
<td>252.50± 15.50*</td>
<td>282.00± 14.00*</td>
<td>428.50± 15.50*</td>
<td>403.50± 37.50*</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>112.35±15.95*</td>
<td>121.80±14.85*</td>
<td>123.20±7.70*</td>
<td>137.90±7.00*</td>
<td>210.00±7.00*</td>
<td>197.40±18.20*</td>
</tr>
<tr>
<td>Salinity ( g/kg)</td>
<td>0.05±0.00*</td>
<td>0.06±0.00*</td>
<td>0.06±0.00*</td>
<td>0.07±0.00*</td>
<td>0.10±0.00*</td>
<td>0.10±0.00*</td>
</tr>
<tr>
<td>pH ( mol / L )</td>
<td>7.81±0.09*</td>
<td>7.63±0.03*</td>
<td>7.72±0.03*</td>
<td>7.74±0.00*</td>
<td>7.84±0.04*</td>
<td>7.85±0.01*</td>
</tr>
<tr>
<td>Temperature(°C)</td>
<td>23.95±0.05*</td>
<td>23.95±0.05*</td>
<td>23.95±0.05*</td>
<td>23.95±0.05*</td>
<td>23.95±0.05*</td>
<td>23.95±0.05*</td>
</tr>
<tr>
<td>DO (mg/l)</td>
<td>5.65±0.05*</td>
<td>3.30±0.10*</td>
<td>3.05±0.05*</td>
<td>2.65±0.05*</td>
<td>2.15±0.05*</td>
<td>2.00±0.00*</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>PCV (%)</th>
<th>16.80±0.95ab</th>
<th>13.67±0.95ab</th>
<th>12.75±0.95ab</th>
<th>12.00±0.95ab</th>
<th>10.75±0.95ab</th>
<th>10.5±0.95ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB(g/100ml)</td>
<td>5.85±0.22</td>
<td>5.40±0.22</td>
<td>4.75±0.22</td>
<td>4.70±0.22</td>
<td>4.70±0.22</td>
<td>4.40±0.22</td>
</tr>
<tr>
<td>WBC(×10⁶/mm³)</td>
<td>4850±434.58</td>
<td>4600±434.58</td>
<td>3600±434.58</td>
<td>3050±434.58</td>
<td>2875±434.58</td>
<td>2075±434.58</td>
</tr>
<tr>
<td>RBC(×10⁶/mm³)</td>
<td>3.95±0.43</td>
<td>3.7±0.43</td>
<td>3.35±0.43</td>
<td>2.75±0.43</td>
<td>1.85±0.43</td>
<td>1.3±0.43</td>
</tr>
<tr>
<td>Neutrophyl (%)</td>
<td>4.60±0.61</td>
<td>5.2±0.61</td>
<td>5.12±0.61</td>
<td>4.67±0.61</td>
<td>7.21±0.61</td>
<td>8.55±0.61</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>23.60±0.54</td>
<td>24.0±0.54</td>
<td>25.50±0.54</td>
<td>26.9±0.54</td>
<td>24.10±0.54</td>
<td>26.18±0.54</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>69.86±0.28</td>
<td>68.34±0.28</td>
<td>69.20±0.28</td>
<td>68.34±0.28</td>
<td>68.10±0.28</td>
<td>69.20±0.28</td>
</tr>
<tr>
<td>MCV(fl)</td>
<td>42.78±2.27</td>
<td>37.15±1.77</td>
<td>38.33±2.11</td>
<td>44.18±3.40</td>
<td>60.19±8.93</td>
<td>88.06±21.95</td>
</tr>
<tr>
<td>MCHC(gm/100)</td>
<td>34.86±0.64</td>
<td>39.58±1.12</td>
<td>37.33±1.03</td>
<td>39.27±1.25</td>
<td>43.84±1.80</td>
<td>42.06±1.68</td>
</tr>
<tr>
<td>MCH(gm/100)</td>
<td>14.93±1.07</td>
<td>14.73±1.24</td>
<td>14.33±1.18</td>
<td>17.39±1.92</td>
<td>26.57±5.00</td>
<td>37.40±10.71</td>
</tr>
</tbody>
</table>

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.

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

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

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.

Discussion
Diuron, an herbicide used for specific crops and as an algaeicide 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 Hypessobrycon 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 Ukwapo 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 LC50 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 LC50 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 macrocyclic 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 betw

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 chromosomes 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 micronuclei

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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References


29. Güner U, Muranlı FD. Micronucleus test, nuclear abnormalities and accumulation of Cu and Cd on Gambusia affinis (Baird and Girard, 1853). Turkish Journal of Fisheries and Aquatic Science. 2011; 11615-622.


8. Tolga CN, Garanko N, Arkhipchuk VV. Induction of micronuclei and binuclei in blood, gill and liver cells of fishes subchronically exposed to cadmium chloride and copper sulphate. Food Chemical Toxicology. 2005; 43:569-574.


39. Banaee M. Physiological dysfunction in fish after insecticides exposure: Insecticides often undesired but still so Important, Edited by Stanislav Trdan, Published by InTech. 2013; 4:103-142.


