Haematological Reaction Of Clarias Gariepinus (Burchell 1822) Juveniles Exposed To Tetrapleura Tetraptera Leaf Powder

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Citation

Abstract
Acute toxicity of Tetrapleura tetraptera leaf powder on Clarias gariepinus juveniles (46.68±0.62g) was conducted using static bioassay tests over a period of 96 hour. The range finding test was conducted to determine the lethal concentration of the botanical on C. gariepinus juveniles and was found to induce varying behavioral response in the fish. The 96 h median lethal concentration, LC50 of 1.60 g L⁻¹ was determined graphically. Percentage mortality of the test organisms followed a regular pattern, increasing with decreasing concentration. Prior to death, fish exhibited marked behavioural changes like hyperventilation, erratic swimming (vertical/spiral uncoordinated swimming movement), irregular operculum and tail frequencies, loss of reflex and settling at the bottom. Haematological analysis carried out after experiment showed significant haematological variations, the Pack Cell Volume (PCV), White Blood Cell (WBC), Red Blood Cell (RBC), Platelet and Lymphocyte decreases as concentration of Tetrapleura tetraptera leaf dust increases. The Dissolved oxygen (DO2), pH and temperature values of the water were within tolerable limits for fish culture.

INTRODUCTION
The use of ichthyotoxic plants for capturing fish is a common practice worldwide (Ayotunde et al., 2011). Fisher folks in Africa extensively use plants and plant products for capturing fish (Neuwinger, 2004; Fafioye et al., 2004). Indiscriminate use of these natural biocides in Nigeria water bodies is now increasing at an alarming rate (Fafioye et al., 2004). Fish farmers and fisher folks haphazardly use various kinds and parts of these plants due to their narcotic, pesticidal and molluscidal properties in other to stupefy fish for easy catch also to clean up the aquatic ecosystems off some pests (Ologe and Sogbesan 2007). Studies have been conducted on the response of fish to some plant toxicants (Jegede and Olanrewaju 2012; Olufayo, 2009; Fafioye et al 2004; Wade et al., 2002; Ayuba and Ofaje, 2002; Ufodike and Omorogie, 1994; Omorogie and Okpanachi, 1992; Omorogie and Ufodike, 1994); but the piscicidal effect of Tetrapleura tetraptera leaf powder on fish has not been given much attention.

Tetrapleura tetraptera is a species of flowering plant in the pea family native to Western Africa, (Steentoft 1988). It is a single stemmed, robust, perennial tree of about 30m. It has a grey-brown, smooth-rough bark with glabrous branchlets. The flower is yellow or pink and racemes is white, while the fruit is dark brown, with four winged pods 25 x 6.5cm. It is generally found in the lowland forest of tropical Africa. The fruit consists of a fleshy pulp with small, brownish-black seeds. The fruit possesses a pungent aromatic odour, which is attributed to its insect repellent property (Aladesanmi 2007). It is used as spices and aroma (exotic tropical scents) and fish poisoning. Tetrapleura tetraptera is a known piscicides (Fafioye 2005) and a molluscicides (Lekana-Douki et al., 2011; Aladesanmi 2007; Adekunle 2001). The leaf contains active ingredients like aridanin, tannins, flavonoids, umbelliferone and ferulic acid (Aladesanmi 2007).

Clariid catfish constitute the main food fish family of economic value in Africa (Adebayo and Fagbenro 2004). It’s one of the vital genera in the family Clariidae. Clarias gariepinus is the most cultured fish in Nigeria and indeed Africa and the third in the world (Haylor 1992). It is hardy: due to the presence of arborescent air breathing organ, omnivorius feeding habit, better growth rate, better feed conversion, ability to withstand adverse environmental
condition, high fecundity and ease of culture (Hecht et al., 1996). The fish is of a high demand because of its high quality and better taste of its flesh (Sogbesan and Ugwumba, 2008). Hence this study was undertaken to determine the median lethal concentration of Tetrapleura tetraptera leaf powder on Clarias gariepinus juveniles during the exposure period (96 hours) and to establish the influence of Tetrapleura tetraptera leaf powder on the blood parameters of C. gariepinus juveniles.

MATERIALS AND METHODS

Two hundred apparently healthy fingerlings of Clarias gariepinus juvenile mixed sex and the same brood stock, mean weight (46.68±0.62g) were procured from Ministry of Agriculture, Forestry and Fisheries Resources, Alagbaka, Akure, Ondo State.

They were transported live to Fisheries Management laboratory of Ekiti State University, Ado Ekiti, Ekiti State in a 50 litre capacity plastic container, half filled with pond water between 1700-1730h. They were later stocked in rectangular fibre tanks (75 x 40 x 40) cm, 60-litre capacity where they were allowed to acclimatize for 7 days. Ten C. gariepinus juveniles (46.68±0.62g) were stocked into each tank, with three replicates per treatment. Tetrapleura tetraptera leaves were collected along Ilokun village settlement, Ekiti State, Nigeria, it was shade-dried at ambient and milled into fine particle size (< 250 µm); and kept in a dry, clean, air-tight transparent plastic container.

The treatments are: Treatment 1, 1.0g T. tetraptera /L-1 of water; Treatment 2, 1.6g T. tetraptera /L-1 of water; Treatment 3, 2.2g T. tetraptera /L-1 of water; Treatment 4, 2.8g T. tetraptera /L-1 of water and Control, 0 g T. tetraptera /L-1 of water. Prior to the commencement of the experiment, the fish were starved for 2 days, to minimize waste in the test media and to prevent organic decomposition and oxygen depletion during blood collection. Temperature, pH, dissolved oxygen, and conductivity level were determined using standard methods and readings were taken at 24 h interval for 96 h.

Fish samples were gently removed (with care) per treatment using a hand net in order to avoid stress; they were anaesthetized and weighed using a Metler Top Loading balance (Model P13 8001); the fish showed no symptoms of stress or diseases. Blood samples were collected from each treatment group. About 5-10ml of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant; it was properly mixed and kept in the refrigerator for haematological analysis. Packed-Cell Volume (PCV) was determined thus: heparinized micro-haematocrit capillary tubes were filled with blood and centrifuged for 5 minutes at 15,000 rpm. PCV was calculated using a micro-haematocrit reader and it was expressed as a percentage (Svobodova et al., 1991). Haemoglobin concentration was determined using the cyanomethaemoglobin method. 2 ml of blood was pipetted and mixed with the diluent; the mixture was centrifuged to remove suspended cellular materials and the readings were made in a spectrophotometer. Erythrocyte count was made using the methods of (Svobodova et al., 1991); plasma obtained from the samples used in PCV determination, was put into Goldberg's Refractometer (Model 10400A) at 200C and the total plasma protein was determined by direct reading (gm/100 ml). Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing the haemoglobin content in g/100ml by the PCV/100ml of blood. MCH was determined from the haemoglobin value (Hb) and from the erythrocyte count (Bouck and Ball (1966).

RESULTS

The following behaviours were exhibited during the definitive test; loss of balance, respiratory distress (hyperventilation), erratic swimming (vertical/spiral uncoordinated swimming movement), irregular operculum and tail frequencies, loss of reflex and settling at the bottom which revealed sensitive indicators of physiological stress in fish.

Fish mortality at varying concentrations increased with increasing concentration of Tetraplura tetraptera leaf powder. All fish in the control tank survived the experimental duration of 96 hours.

There was significant loss of fish with increase in Tetraplura tetraptera leaf concentration (P < 0.05). The LC50 was determined graphically to be 1.60g Tetraplura tetraptera leaf powder / L-1 of water.

Water samples were collected weekly at a depth from each fibre tank. Dissolve oxygen (DO2) and temperature were measured using oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Spring, OH, USA) and mercury in glass thermometer, respectively. pH was measured with pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). In all treatments, DO2 concentrations decrease with the increase in the concentration of Tetraplura tetraptera leaf powder from 0.1-2.8 mg/L, water temperature average was 27.6 °C, pH value ranged from 6.3 - 8.6. All the water quality parameters
were within the acceptable range for fish growth (Environment Protection Authority, EPA (2003)). There was significant reduction \((p<0.05)\) in the values of the blood parameters of \(C.\) gariepinus juveniles after exposure to Tetraplura tetraptera leaf powder concentration for 96 h. Pack cell volume reduces from 26.50±1.50 in the control to 15.02±0.05 in Treatment 4(T4), white blood cell reduces from 25.45±3.80 g mm\(^{-1}\) in the control to 20.03±0.03 g mm\(^{-1}\) in Treatment 4(T4), which is the highest concentration,2.8 g L\(^{-1}\) Tetraplura tetraptera leaf powder concentration, the red blood cell reduces from 3.21±0.40 in the control to 1.55±0.35 in T4, platelet decreased from 91.00±5.00 in control to 76.02±0.03 in Treatment 4(T4).While, Neutrophil increase from 33.00±2.00% in the control to 64.50±0.05% in Treatment 4(T4), Lymphocyte decreases from 66.5±2.50 (%) in control to 34.00±0.02 (%) in Treatment 4 (T4) which is the highest concentration. Finally, monocyte increases from 1.50±0.50 (%) in the control to 2.02±0.03 (%) in Treatment 4(T4).

**Figure 1**
Effect of Tetraplura tetraptera leaf powder on mortality of Clarias gariepinus juveniles

<table>
<thead>
<tr>
<th>HAEMATOLOGICAL PARAMETERS</th>
<th>Control</th>
<th>T4 (2g/L)</th>
<th>T4 (2.8g/L)</th>
<th>T4 (3.6g/L)</th>
<th>T4 (4.4g/L)</th>
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</thead>
<tbody>
<tr>
<td>PCV (g%)</td>
<td>26.36±1.36</td>
<td>17.69±0.63</td>
<td>15.36±0.63</td>
<td>13.05±0.63</td>
<td>13.02±0.05</td>
</tr>
<tr>
<td>WBC (10^3/µL)</td>
<td>3.41±3.00</td>
<td>2.65±0.05</td>
<td>1.62±0.01</td>
<td>1.50±0.01</td>
<td>1.50±0.01</td>
</tr>
<tr>
<td>RBC (10^6/µL)</td>
<td>3.21±0.40</td>
<td>2.65±0.05</td>
<td>1.62±0.01</td>
<td>1.50±0.01</td>
<td>1.50±0.01</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>31.01±2.00</td>
<td>18.03±1.90</td>
<td>12.38±1.36</td>
<td>9.62±0.30</td>
<td>6.02±0.30</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>33.00±2.00</td>
<td>33.00±2.00</td>
<td>33.00±2.00</td>
<td>33.00±2.00</td>
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<tr>
<td>Lymphocytes (%)</td>
<td>66.5±2.50</td>
<td>66.5±2.50</td>
<td>66.5±2.50</td>
<td>66.5±2.50</td>
<td>66.5±2.50</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>1.50±0.50</td>
<td>1.50±0.50</td>
<td>1.50±0.50</td>
<td>1.50±0.50</td>
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</table>

*Mean ± Standard Error

**DISCUSSION**

This study revealed that Clarias gariepinus juveniles exposed to Tetraplura tetraptera leaf powder exhibits marked behavioural changes like hyperventilation, erratic swimming (vertical/spiral uncoordinated swimming movement), irregular operculum and tail frequencies, loss of reflex and settling at the bottom which are indicators of physiological stress in fish. In a similar study, Kori-Siakpere and Oviroh (2011) reported similar behavioural changes in Clarias gariepinus subjected to Nicotiana tobaccum leaf dust toxicity. Also in similar study by Dan Ologe and Sogbesan (2007) where the piscicidal potential of Euphorbia heterophylla was tested on Barbus Occidentalis fingerlings. The dried Euphorbia heterophylla stem water extract was found to induce varying behavioral response in the fish. An akin research by Ayoola et al., (2011) reported agitated behaviours, respiratory distress and abnormal nervous behaviors when Oreochromis niloticus juveniles was exposed to aqueous and ethanolic extracts of Ipomoea aquatica leaf at varying concentrations. Fish mortality increases with increase in concentration of Tetraplura tetraptera leaf powder. This study corroborates the study by Jegede and Olanrewaju (2012) where the piscicidal effect of Nicotiana tobaccum leaf dust on African giant catfish fingerling was investigated. The result revealed that the percentage mortality of the test organisms (African
giant catfish) fingerlings followed a regular pattern, as concentration of Nicotiana tobaccum increases, mortality also increase.

Exposure of C. gariepinus juveniles to Tetrapleura tetraptera leaf powder concentrations for 96 h clearly destroys the order in the blood parameters when compared with the control. Pack cell volume reduces from 26.50+ 1.50 in the control to 15.02+0.05 in Treatment 4(T4), white blood cell reduces from 25.45+ 3.80 g mm-1 in the control to 20.03+0.03 g mm-1 in Treatment 4(T4), which is the highest concentration, 2.8 g L-1 Tetrapleura tetraptera leaf powder concentration, the red blood cell reduces from 3.21+0.40 in the control to 1.55+0.35 in T4, platelet decreased from 91.00+5.00 in control to 76.02+0.03 in Treatment 4(T4),while lymphocyte also decreases with increase in Tetrapleura tetraptera leaf powder concentration. In a similar study by Olufayo and Jatto(2011) where the haematology response of Oreochromis niloticus juveniles were exposed to Nicotiana tobaccum leaf dust was investigated. It was reported that Erythrocyte, Haemoglobin values and Pack Cell Volume (PCV) values decreased with increasing concentrations of tobacco leaf dust. The reduction in some of the blood parameters is an indication of anaemia, which is a condition characterized by deficiency of haemoglobin, PCV and erythrocyte (Mason et al., 1994). Kecceci et al., (1998) also corroborated this by reporting that haematological values are indirect pointers to the health of live stocks (broiler chicken).

CONCLUSION

Finally, this study revealed the median lethal level (LC50) of Clarias gariepinus juveniles exposed to Tetrapleura tetraptera leaf dust toxicity to be 0.60g/L and has also shown the various alterations in haematological parameters, hence knowledge of this could help in fish health management and water quality management.

References

Bouck R. G and R. C (1966) Ball Influence of capture methods on blood characteristics and mortality in rainbow trout (Salmo garneri) Trans Am. Fish Soc. p 16


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