The Toxicity of Ethanolic Extract of Alchornea cordifolia Leaf on Clarias gariepinus Fingerlings

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Abstract

Acute toxicity effects of ethanol extract of Alchornea cordifolia leaf on Clarias gariepinus fingerlings was investigated over a 96hr exposure period as a potential organic pesticide. A static toxicity bioassay was performed after preliminary trail tests (range finding test) were conducted. Five hundred (500) post-fingerlings of Clarias gariepinus were distributed randomly in duplicate concentrations. The test fishes were treated with concentrations of 1.31, 1.96, 4.45 and 6.67mg/l of Alchorneacordifolia. Exposure to the plant toxicant caused visible behavioural changes which include erratic swimming, air gulping, discoloration, loss of body equilibrium, settlement at the bottom and death. Mortality was recorded in some of the exposed fish while the LC50 lethal concentration of 2.138mg/l was established and safe concentration was established as 0.2138mg/l. There were significant changes (p˃0.05) in the water quality parameters except electrical conductivity, the unstable behaviour of the fish must have been as a result of irritation from the toxicant. Therefore, the use of A. cordifoliain fish harvesting should be regulated and not allowed to gain access unnecessarily into the aquatic ecosystem and regulatory bodies should maintain the safe concentration of 0.2138mg/l.

Keywords: Toxicity; concentration; mortality; stress

Introduction

There are many indigenous sources of botanical fish toxicants in Nigeria that are extremely toxic to a wide range of animals including fish; some of these plants include: Derris elliptica, Tephrosiavogelli, Acaciapennata, Tetrapleratetraptera, Mund uleasericea,Boerhaviacoccinea[22]. The introduction of these plant extracts in the aquatic ecosystems could eventually lead to physiological stress in aquatic organisms and ultimately reduction in aquatic productivity [22]. In broader sense, toxicity is concerned with the chemical nature, interaction with biological systems and safety evaluation of potentially poisonous materials (Ojuikutuet al., 2013). Toxicity could be acute or chronic, depending on the dosage nature and duration of effects of the toxin (Fafioyeet al., 2004). Fish toxicants (pesticides) can be herbal or synthetic. Synthetic pesticides are not degradable, and hence pose the problem of environmental resistance, pest resurgence and could have detrimental effects on non-target organisms [10]. Plants pesticides on the other hand are easily biodegradable and leave no residues in the environment and are easily reversed in fish subjected to chronic concentrations [23,24]. Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscidal properties (Wang and Huffman, 1991). These can be extracted from flowers, baric, pulp, seeds, roots, leaves, and even the entire plants [26]. Several plants belonging to different families, which possess a number of compounds as saponins, tannins, alkaloids, di- and tri-terpenoids have high piscicidal activity and used in freshwater bodies to control harmful snails, disease causing insects, such as mosquito larvae and weed fishes [27,28].Botanicals can be natural biocides and their contamination of natural waters has become inevitable in Nigeria because of recent wide use[6]. Plants are chemical factories. Unlike animals having the luxury of teeth and claws and legs to help them get out of a tight spot, most plants spend their lives in one place and have evolved to rely upon elaborate chemical defenses to ward off unwanted predators. For this reason, plants have in their arsenal an amazing army of thousands of chemicals noxious or toxic to bacteria, fungi, insects, herbivores, and even humans. Fortunately, this chemical diversity also includes many compounds that are beneficial to humans: vitamins, nutrients, antioxidants, anticarcinogens, and many other compounds with medicinal value [17-19]. The knowledge and use of toxic plants is vital for the current technologically unsophisticated human populations. This applies especially to ichthyotoxic plants [15]. Piscicidal plants like Blighasapida, Kigeliaafricana, Tetrapleuratetraptera, Raphiavinifera, Parkiabiglobosa and Tephrosiavogelli are frequently in use by the fisher folks because they are highly potent [9].In many regions of the world, plant originated fish poisons (ichthyotoxic plants) are used to stun or kill fish [7]. The efficacy of plant extracts is due to the presence of one or more biologically active compound. Pharmacological assays have shown that the activity is not always due to the main components, but the minor ones, or even the synergism of all the active compound [14]. Toxic concentrations in a plant can be dramatically affected by environment’s stress on the
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Preparation of ethanolic leaf extract of Alchornea cordifolia

After pulverizing, the powdered form was sent to the Department of Pharmacy Laboratory for ethanolic extraction. The leaves of the assay plant were extracted in the Pharmacognosy and Natural Medicine Laboratory of the Department of Pharmacy, University of Uyo using conc. ethanol of 99.98% as a medium. The pulverized leaves were macerated in 3 litres of diluted ethanol for 72 hours, the ethanol was diluted using the formula C1V1 = C2V2 to 96% [1]. The ethanolic suspension was filtered using a filter net and filter paper and the extract was evaporated in a water bath at 40° Celsius for 48 hours and stored in a beaker covered with aluminum foil for bioassay immediately after the evaporation was complete.

Bioassay Procedure

After acclimatization, five (5) different concentrations (0.1mg/l, 1.0mg/l, 10.0mg/l, 100.0mg/l, and 1000mg/l of the extracts were prepared in duplicates and range finding test were conducted. The 0.0mg/l served as the control for the experiments. Clarias gariepinus post fingerlings were batch-weighed with a top-loading Mettler Balance (Mettler Toledo) and distributed randomly in duplicate per treatment. Each of the concentration was added to 20 litres of water in each of the rectangular plastic tanks containing ten (10) fishes each. The plastic tanks were covered with netting material to prevent fish from jumping out; there was no aeration, no water change nor feeding throughout the test period which lasted for 96 hours [1]. The behavioral responses (reaction) of the test fishes in each tank were monitored for 96 hours. Any fish found floating on the water surface without any movement was considered dead. Such fish is thus removed immediately from the affected tank and the time of mortality was recorded against the concentration of the extract introduced. After the range test, definitive test concentrations were derived using the highest observed effect concentration (HOEC) divided by a spacing factor of 1.5 [1]. The new concentrations of 1.31mg/l, 2.96mg/l, 2.97mg/l, 4.45mg/l, and 6.67mg/l were added to 20 litres of water in each of the rectangular plastic tanks containing ten (10) fishes each. The 96 hrs LC50 was calculated by logistic regression.

Water Quality Parameters

The water quality parameters such as dissolved oxygen, temperature and pH were observed before and after the experiment. Dissolved oxygen and temperature were measured using DO meter (H19461) in mg/l and thermometer in °C units respectively while pH was measured using a pen type pH meter (pH-009 111). Statistical Analysis

Water quality parameters were subjected to one-way analysis of variance (ANOVA) at 0.05 probability level to test for significant difference. Results with p<0.05 were considered significantly different [29]. The statistical analysis was done using IBM SPSS Inc. (Windows version 22.0).
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Results

Physico-Chemical Parameters

The result of the physico-chemical parameters of the experimental media is indicated in Table 1. There is significant difference (p<0.05) in the mean values obtained for temperature, dissolved oxygen, pH and total dissolved solute after the experiment while there is no significant difference in electrical conductivity. After the experiment water quality ranges were; temperature (27.83 – 29.00), DO (2.28 – 4.88), pH (2.91 – 5.08), TDS (42.05 – 52.43) and EC (3.69 – 4.00).

<table>
<thead>
<tr>
<th>Conc. (mg/l)</th>
<th>Temperature (°C)</th>
<th>DO (mg/l)</th>
<th>pH</th>
<th>TDS (mg/l)</th>
<th>EC (µS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.83 ± 0.05a</td>
<td>4.88 ± 0.11a</td>
<td>5.08 ± 0.03a</td>
<td>42.05 ± 0.05b</td>
<td>4.00 ± 0.00a</td>
</tr>
<tr>
<td>1.31</td>
<td>27.90 ± 0.04a</td>
<td>4.88 ± 0.05c</td>
<td>4.75 ± 0.06a</td>
<td>42.25 ± 0.06b</td>
<td>4.01 ± 0.01c</td>
</tr>
<tr>
<td>1.96</td>
<td>28.05 ± 0.03ab</td>
<td>4.23 ± 0.26bc</td>
<td>4.13 ± 0.23bc</td>
<td>42.73 ± 0.08b</td>
<td>3.99 ± 0.01bc</td>
</tr>
<tr>
<td>2.97</td>
<td>28.18 ± 0.03bc</td>
<td>2.83 ± 0.69bc</td>
<td>3.90 ± 0.23bc</td>
<td>48.60 ± 2.04a</td>
<td>4.00 ± 0.01bc</td>
</tr>
<tr>
<td>4.45</td>
<td>28.45 ± 0.05bc</td>
<td>2.70 ± 0.73bc</td>
<td>3.39 ± 0.24cd</td>
<td>49.83 ± 2.31a</td>
<td>3.99 ± 0.01bc</td>
</tr>
<tr>
<td>6.67</td>
<td>29.00 ± 0.19a</td>
<td>2.28 ± 0.84e</td>
<td>2.91 ± 0.24de</td>
<td>52.43 ± 2.81a</td>
<td>3.69 ± 0.23f</td>
</tr>
</tbody>
</table>

Means within same column with same superscript are not significantly different (p>0.05)

Legend: DO: Dissolved oxygen  TDS: Total dissolved solids  EC: Electrical conductivity

General behavioural changes of Clarias gariepinus post-fingerlings exposed to aqueous extract of Alchornea cordifolia leaf

Exposure to Alchornea cordifolia caused visible behavioural changes in Clarias gariepinus for both range finding and definitive tests. After 60 minutes, the changes in the behaviour of the fish order of occurrence include, settling at the bottom of the tank motionless, followed by erratic swimming, respiratory distress exhibited by coming towards the surface to gulp air before death. These behavioral changes displayed by the fish in response to the effects of the toxicants were more pronounced in tanks containing higher concentrations, but decreased with increase in time of exposure. However, fish in the control group did not exhibit any of these behavioral changes.

Mortality of Clarias gariepinus exposed to aqueous extract of Alchornea cordifolia leaf

The mortality of C. gariepinus post-fingerlings exposed to Alchornea cordifolia for 96 hours is shown in Table 2. No mortality was observed in the group of fish in the control experiment while mortality occurred in fish exposed to other varying concentrations of the toxicant. Mortality increased with increasing concentration of the extract showing a dose-dependent relationship. 100% mortality was observed in the group of fish exposed to 6.67ml/l while 20% mortality was recorded in the group of fish exposed to 1.31ml/l. The mean value of 96-hour LC50 of the leaf extract of Alchornea cordifolia to the test fish is shown in Table 2. The result of the mortality recorded for Clarias gariepinus post-fingerlings exposed to Alchornea cordifolia aqueous leaf extract for 96 hours at concentrations of 1.96mg/L, 2.97mg/L and 4.45mg/L were 50%, 70% and 80% respectively. The LC50 (96 hrs) of ethanolic extract of A. cordifolia is represented in Figure 1.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Number of fish</th>
<th>Total mortality (96hrs)</th>
<th>% mortality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.31</td>
<td>10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>1.96</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>2.97</td>
<td>10</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>4.45</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>6.67</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

Studies have shown that fish exposed to toxicants exhibited some behavioral changes such as increased opercula beat rate, erratic swimming, mucus secretion and air gulping before death [20]. The pattern of behavioral changes observed in this study compared favorably with the report of Fafioye et al., (2004) when African catfish (Clarias gariepinus) was exposed to Parkiabiglobosa and Raphiavinefera extracts. Increased concentrations of Alchornea cordifolia leaf to erratic swimming, air gulping, discoloration, loss of body equilibrium and mortality was also similarly observed in Clarias gariepinus exposed to aqueous extracts of Blighiasapida and Kigeliaafricana[24]. The marked deviation in the rate of swimming, discoloration and air gulping suggests an adjustment in physical fitness as a result of...
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Figure 1: Graphical illustrating of 96 hours LC50 of Alchornea cordifolia by probit method for Clarias gariepinus post-fingerlings

Legend: Graph of Logistic regression of Killed by Log (Dose (ml)) (Linear scale)
Using the value of intersection 0.33. Therefore, LC50 is given by: Log-1[0.33] = 2.138 mg/L.
Safe concentration is given by LC50 × safe factor giving 2.138 × 0.1=0.2138 mg/L.

the stress condition. It was also observed that on the day of the experiment, survived fishes were found swimming normally. This suggests that the effect of the toxicant may have subsided with time. The values of 96hrsLC50 of 2.138mg/1 of the leaf extract reported in this study is far lower than those earlier reported by Oti and Ukpabi and Fafioye et al., for some clarias species exposed to Thevetia peruviana, Parkia biglobosa and Raphia vinifera plant extracts and a bit higher than that reported by Gabriel and Okey, on hybrid catfish exposed to Lepidagatil salopereioides[11,13,25]. This observation implies that A. cordifolia is more toxic to African catfish Clarias gariepinus than T. peruviana, P. biglobosa and R. Vinifera. The lower value obtained by Gabriel and Okey, (2009) might be as a result of the genetically modified state of the hybrid catfish which of course might have made it a bit weaker than the hardy C. gariepinus rather than due to the higher toxicity of L. alopecurioides. The observed restlessness and mortalities of the test fish might be due to the effect of flavonoids, alkaloids and saponins present in the extracts or that of other phytochemical constituents of this toxicant which include tannin, calcium oxalates, terpenoids/steroids, phloba and de-xy sugars. Saponins (known for the formation of foams in aqueous solutions) are ichthyotoxins which destroys the erythrocytes and is assimilated directly through the gills [12]. Alkaloids on the other hand inhibit oxidative phosphorylation, blocks the mitochondrial enzymes, Nicotinamide Adenine Dinucleotide (NADH) ubiquinone reductase. Hence impairing their oxygen consumption [16,28].

Conclusion

Behavioural responses of fish to most toxicants and differences in reaction times have been observed to be due to the constituents of the toxin and their concentrations, species and size of fish and specific environmental conditions (FAO, 1981). The recorded responses for the test fishes in this study are in accordance with earlier reports of other authors for clariids under various stress conditions. Buckley, identified four main phases in the responses of fish to toxicants: the contact phase (brief period of excitability), exertion (visible avoidance characterized by fast swimming, leaping and attempts to jump out of the toxicant), loss of equilibrium and lethal (death) phase when opercula movement and response to tactile stimuli ceased completely[4,5]. Mortality of Clarias gariepinus post-fingerlings is due to the toxicity of the plant extract. The abnormal behaviours tend to suggest some nervous disorder and insufficient oxygen supply. The abnormal responses for example, increased ventilator rate and erratic swimming, and increased surfacing among others may increase the energy demand for metabolism beyond normal, leading to fatigue and stress (Svobodov et al., 1993). High mortality rate by C. gariepinus fingerlings showed typical reaction to toxicants hence the conclusion that A. cordifolia leaf extracts are highly toxic to fish but the long term effects of the plant extract is yet unknown. The LC50 from the present study was observed to be 2.138 mg/l of A. cordifolia while the safe limit was 0.2138 mg/l.
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Recommendation

From existing literatures, the piscicidal potential have been established, therefore it is necessary to know the long-term growth response of test organisms to this plant extract thus, long term growth response test should be conducted to know if the toxicant has any effect on the growth of fish. The constituents of the plant extract are biodegradable and thus diminish within a short period after exposure; hence can be used a biological control in eradicating predators and unwanted organisms in ponds by farmers instead of using agrochemicals. Finally, the use of plant extracts as fish harvesting method should be regulated and not allowed to gain access unnecessarily into the aquatic ecosystem because they have been found to alter the aquatic biota and one might not know their long term effect.

References