

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, 2.97, 4.45 and 6.67mg/1 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/1 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. cordifolia* in 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*, *Tephrosiavogelii*, *Acaciapennata*, *Tetrapleratetraptera*, *Munduleasericea*, *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, toxicology is concerned with the chemical nature, interaction with biological systems and safety evaluation of potentially poisonous materials (*Ojutiku et al.*, 2013). Toxicity could be acute or chronic, depending on the dosage nature and duration of effects of the toxin (*Fafioye et 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 molluscicidal 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 pesticidal 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

plant (Drought, heat/cold, mineral deficiencies, etc) and disease. Different varieties of the same plant species can also have different levels of toxins and nutritional value [8]. Some indigenous plant species in Nigeria with piscicidal potentials have been reported by Obot [21]. These include *Cassia alata*, *Erythrophloeovirens*, *Omphalocarpumelatum* and *Piptademastrumafricanum*. Others are *Albiziaferruginea*, *Albiziaadanthifolia*, *Strychnosaculeata* and *Tetrapleuratetraptera* (commonly called Aidon). These plants are all used as local fish poisons in different parts of Nigeria. The use of pesticides such as tea seed cake and derris root, and their toxic effects have been well documented [2]. However, these conventional pesticides are either not within the reach of the fish farmers, or their use may not be cost effective, especially in developing countries such as Nigeria. This study aims to determine the toxic effects of ethanolic extract of *Alchorneacordifolia* leaf on *Clariasgariepinus* post fingerlings with particular reference to determination of the LC50 and physico-chemical parameters.

Materials and Method

Study Area

The study was conducted at the Fisheries complex of the Department of Fisheries and Aquatic Environmental Management, University of Uyo, Nigeria. This area lies between Latitude 2°02'6"N and Longitude 7°55'19"E.

Collection and Acclimatization of *C. gariepinus* post-fingerlings

Five hundred (500) post-fingerlings (average weight 9.32 g) of *C. gariepinus* were procured from Safe Foods farm in Uyo, Akwalbom State and transported in five oxygenated polythene bags to the hatchery unit of the Department of Fisheries and Aquatic Environment Management, University of Uyo. The weight ranged from 6.6 to 12.8g, total length from 10.5 to 12.5cm. They were kept in the laboratory for acclimatization in transparent plastic tanks of length 22.6cm and width of 18.5cm. The experimental fish were adapted to laboratory condition for fourteen (14) days before being used in the experiment, as recommended by APHA [3]. The fishes were fed twice a day during the period of acclimatization according to APHA (2005) at 5% body weight with commercial feeds containing 45% crude protein. Water was changed twice daily to remove faecal materials and unconsumed feeds. The tanks were covered with netting material to prevent the fish from jumping out of the plastic container and to protect them from predators. Feeding was discontinued during the test period.

Collection and Identification of *A. cordifolia*

Fresh leaves of *A. cordifolia* were gathered from Vika Farms, Oron Road, Uyo, Akwalbom State, southern Nigeria. The plant was identified in the Department of Ecological Studies laboratory, Faculty of Science, University of Uyo, Akwalbom State, Nigeria. The leaves were oven-dried for 72 hours (3 days) at a constant temperature of 40 °C in the University of Uyo Fisheries Research Laboratory. The dried leaves were collected after 72 hours and pulverized separately using conventional mortar and pestle to powdery form.

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, 100mg/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. *Clariasgariepinus* 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, 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).

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 1: Physio-chemical parameters during 96hrs exposure of Aqueous extract of *Alchorneacordifolia* leaf to *Clariasgariepinus*post-fingerlings

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

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 *Clariasgariepinus* 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 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 LC_{50} 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 LC_{50} (96 hrs) of ethanolic extract of *A. cordifolia* is represented in Figure 1

Table 2: Mortality of *Clariasgariepinus*post-fingerlings exposed to aqueous extract of *Alchorneacordifolia* leaf

Concentration (mg/L)	Number of fish	Total mortality (96hrs)	% mortality rate
1.31	10	2	20
1.96	10	5	50
2.97	10	7	70
4.45	10	8	80
6.67	10	10	100

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 Fafioyee et al., (2004) when African catfish (*Clarias gariepinus*) was exposed to *Parkia biglobosa* and *Raphiavinefera* extracts. Increased concentrations of *Alchornea cordifolia* leaf to erratic swimming, air gulping, discoloration, loss of body equilibrium and mortality as was also similarly observed in *Clarias gariepinus* exposed to aqueous extracts of *Blighiasapida* and *Kigelia africana* [24]. The marked deviation in the rate of swimming, discoloration and air gulping suggests an adjustment in physical fitness as a result of

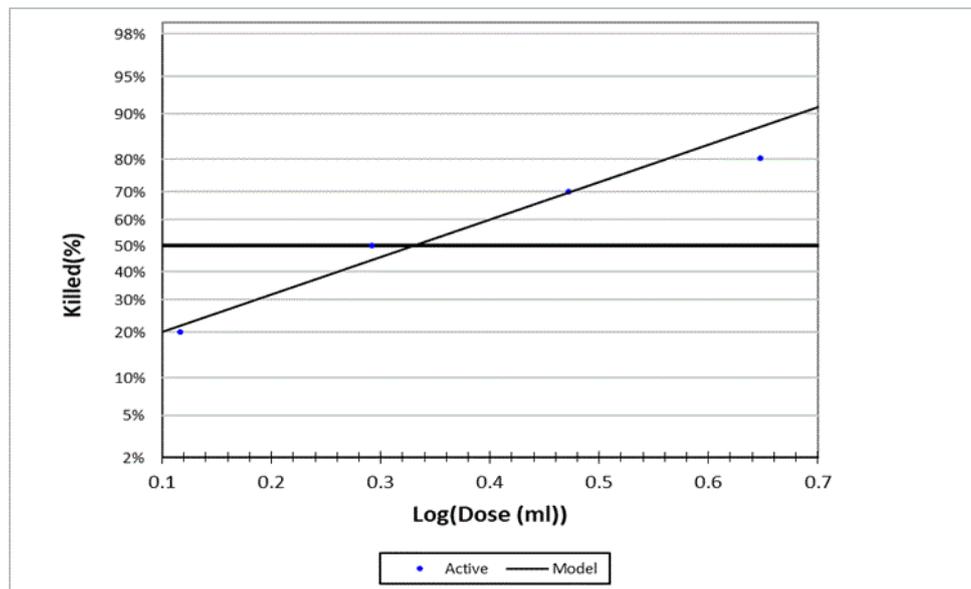


Figure 1: Graphical illustrating of 96hoursLC₅₀ of *Alchornea cordifolia* by probit method for *Clarias gariepinus* post-fingerlings

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; $\text{Log}^{-1}[0.33] = 2.138 \text{ mg/L}$.

Safe concentration is given by $\text{LC}_{50} \times \text{safe factor}$ giving $2.138 \times 0.1 = 0.2138 \text{ mg/}$

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/l 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 *Thevetiaperuviana*, *Parkiabiglobassa* and *Raphiaviniferaplant* extracts and a bit higher than that reported by Gabriel and Okey, on hybrid catfish exposed to *Lepidagatalopecuriodes* [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. alopecuriodes*. 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, cardiac glycosides, terpenoids/steroids, phloba and de-oxy 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 (Svobodova 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.

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.

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