Effects of Locust Bean (Parkia biglobosa) Effluent on the Histology of Clarias gariepinus Juveniles (Burchell, 1822)

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ABSTRACT

The poor nature of most agricultural processing practices in Nigeria have had adverse effect on our environment and the aquatic habitat are of no exception. This study was conducted to ascertain the median lethal concentration of the effluent within the 96 hours period and the histological damage on the liver and gills of Clarias gariepinus juveniles. Juveniles of the C. gariepinus (mean weight 30±2.5 g) were exposed to 87.5, 88.0, 88.5, 89.0, 89.5 and 90.0 mL of the locust bean (Parkia biglobosa) mill effluent under the standard laboratory conditions. Data on mortality corresponding to the various concentrations were subjected to the probit and logit transformations and used to calculate the LC50 graphically. The result shows the toxicity of the locust bean effluent to C. gariepinus juveniles as 0.089 mg L−1 (treatment 4) during the period of study. The results obtained on water physical chemistry shows that there were no significant difference between the controlled and the treated media nor between the treatments because values obtained fall within the accepted water quality standard for fish culture. Exposure to the effluent resulted in a number of physiological dysfunction of the test fish, such as rupture and erosion of the gills, leading to reduction in the respiratory surface area of the filament, that can cause the death of the fish as well as impairment of oxygen-carbon dioxide exchange. Observation of the fish under the tests revealed unbalanced swimming patterns, loss of reflex, rapid opercular movements and gasping for air. Histopathological examination of the gill and liver of the treated fish showed signs of damage, ranging from hypoxia, lesions, necrosis, fibrosis, cell stasis and hyperplasia.

Key words: Locust bean, effluent, Clarias gariepinus, histology, concentration

INTRODUCTION

There has been a growing concern on the method of processing most agricultural products, due to their adverse impact on the environment, especially the aquatic habitat. The African locust bean (Parkia biglobosa) is a perennial leguminous crop that is grown in the tropics. It belongs to the family of Parkia and has species that are grown in the sub-Saharan Africa (NAS, 1979). The mature fruit of the locust bean occur in large bunches, of which the pod varies between 12 and 30 cm by length and 12-25 mm by breadth (Yadkin, 1985). It is tough and fibrous, enclosing a soft powdery yellowish pulp in which small seeds are embedded. Olajide and Ade-Omowaye (1999) gave the average length, width and thickness of locust bean seed as 10.80, 8.42 and 4.64 mm.
Locust bean effluent, a waste product from the fermentation process of African locust bean may be harmful to aquatic organisms. Regardless of its taste it is also a major source of protein, oil, carbohydrates, vitamins and minerals. It is known to contain 30-40% protein, 31-40% oil, 11.7-15.4% carbohydrate, 8.82-94% crude fibre, 4.40-5.38% ash, Nitrogen Free Extracts (NFE) of 37 and 93.7% content of dry matter (Campbell-Platt, 1980).

It is also reported that the locust bean husk combined with cassava peelings can be used to control root rot nematode of sugar cane (Salawu, 1988). The powder of locust bean oil can also serve as a good pesticide for the control of dried fish beetles (Odeyemi et al., 2000).

The processing involves the shelling of matured pods, steaming, dehulling and subsequent fermentation into various forms of condiments (Adewumi, 1997).

Clark (1992) defined toxicity as a measure of how poisonous a substance is or how large a dose is required to kill or change an organism, hence toxicity test is used to determine and establish potential toxicological effects of certain chemical substances and other harmful materials on organisms. This may be short-term or long-term depending on the problem to be solved and the objectives for carrying out the study.

A wide variety of plants are known to be potentially toxic because they contain allelochemicals like glycosides, tannins, cyanides, alkaloids, phytic acid, hemagglutinins and saponins upon hydrolysis (Aletor, 1993).

Odeyemi et al. (2000) gave the list of toxicants contained in locust bean (Parkia biglobosa) pods as saponin, tannins, alkaloids and cardiac glycosides.

Catfish comprises of the major family of food fish of economic value, not only in Nigeria but in the tropics and the sub-tropical region. Some of their outstanding characteristics include fast growth rate, high fecundity rate, ability to survive a wide range of water conditions and the ability to convert natural feedstuffs into use.

Oti (2002) gave the median lethal concentration (LC50) of cassava mill effluent to Clarias gariepinus fingerlings over 96 h duration at 50.12 mg L⁻¹ while Ayuba and Ofoekwu (2002) gave LC50 of Datura inoxia (Jimson’s weed) over 96 h as 125.89-384.59 mg L⁻¹ for the Clarias gariepinus fingerings.

The objective of this study is to conduct and determine the effects and toxicity of locust bean effluent to the Clarias gariepinus juveniles, especially on the histology of the gills and the liver.

MATERIALS AND METHODS

Study area: The research was conducted at the fisheries Laboratory Federal University of Technology, Akure, South-West of Nigeria.

Sources of collection and preparation: The 16.08 kg locust bean seeds (Parkia biglobosa) were purchased from the Oba market, Akure, Ondo State, Nigeria. The seed were introduced into the 135 L water and boiled overnight. The seeds after cooling were collected, dehulled and allowed to ferment after 48 h. the waste water of fermentation was then collected prior to the start of the experiment.

Data collection procedure: A total of 250 live Clarias gariepinus juveniles of mean weight 30 g±0.2 were purchased from the Ondo State Agricultural Development Programme (ADP) hatchery and subsequently transported in white plastic containers to the fisheries laboratory for the experiment. The fishes were acclimatized to laboratory conditions for 7 days prior to the toxicity
test. Feeding was stopped 48 h prior to the commencement of the experiment to make the test media free of debris and dissolved solids data on fish mortality and behavioural patterns were recorded in each of the treatments. The LC50 of the locust bean effluent which kills 50% of the test organisms within 96 h was determined using the probit-logit transformation method (Finey, 1982).

**Water quality parameters**: Temperature, pH and Dissolved Oxygen (DO) were conducted using the standard methods as described by APHA (1987). Temperature was determined by using the mercury in glass thermometer calibrated in degree centigrade (°C).

pH was determined by the use of pH meter (Model No Mettler Toledo, 320).

The Dissolved Oxygen (DO) was determined by using the digital oxygen meter (Model Jenway, 9071).

**Experimental design and layout**: The layout of the design is:

\[ Y_{ij} = \mu + T_j + E_{ij} \]

Where:
- \( Y_{ij} \) = Individual observations
- \( \mu \) = General mean
- \( T_j \) = Effect of the jth treatment
- \( E_{ij} \) = Experimental error containing all the uncontrollable sources of variation

A total of one hundred and forty (140) *Clarias gariepinus* juveniles were selected by size and uses for the range finding test. Treatment consists of 50, 60, 70, 80, 90 and 100 mg L\(^{-1}\) of the effluent into each of the 14 zplastic containers containing 10 L aerated water and the *Clarias gariepinus* juveniles, placed under laboratory conduction.

Subsequently, another set of six treatments and the control consisting of 87.5, 88.5, 89.0, 89.5, 90.0 and 0.00 mg L\(^{-1}\) of the locust bean effluent were duplicated to each of the 15 L plastic containers for the definitive test. Each of the ten *Clarias gariepinus* juveniles were introduced into each of the 14 plastic containers (Two of which are the control) and continuously aerated for the entire 96 h.

**Statistical analysis**: Data collected were subjected to one-way Analysis of Variance (ANOVA) at p<0.05. Differences among means were separated using the Duncan (1955) test. Data also on LC50 96 h were determined using the probit-logit transformation method (Finey, 1982).

**Explanation of probit-logit transformation method**: This method involves the use of two variables to determined the most suitable range for LC50 within a specified period of 96 h, through certain established values in the probability table and the corresponding Figures in the logarithm. This time the concentration values were converted to logarithm numbers while the specific mortality rates were derived from the already corresponding probability table.

Histology examination of the liver and gills starts with excising both organs by dissection. Dissected tissues of the liver and gills were fixed in 10% formalin and preserved for 3 days. The fixed tissues were dehydrated in graded levels of alcohol and cleared in 50/50 mixture of alcohol
and xylene for consecutive 6 h. This was followed with the impregnating of the specimens in molten wax placed in an oven for 6 h and embedded in petri dishes covered with paraffin wax. The specimens were later mounted in wooden blocks and sectioned properly into thin sizes with microtome and then stained later. The sectioned organs were mounted on a glass slide and observed under the light microscope (model Olympus 399817, Japan). Photographs of stained section were taken and interpreted accordingly.

RESULTS AND DISCUSSION

The levels of the physicochemical parameters of the experimental water are shown in Table 1. Most of the water quality parameters in the containers were relatively constant during the duration of the experiment. The water temperature varied from 26-27°C, dissolved oxygen varied from 6.4-8.8 mg L⁻¹ while the water pH was between 6.82-6.70.

Observation from the tests carried on the fishes showed them exhibiting lots of erratic swimming movement, serious opercula movements, air surfacing and loss of reflex, especially at the higher concentrations (Table 2). At stage mortality were noticed in some of the concentrations which is in proportion to the level of the effluents.

The LC₅₀ values obtained from the fish exposed to locust bean effluent within the 96 h is shown as 0.089 mg L⁻¹ (Fig. 1). The impact of the locust bean effluent on the liver and gills of the fish showed pathological changes except the controls. The result showed varying degrees of epithelial hyperplasia, fusion of lamellae and separation of the respiratory epithelium.

The liver had fibrosis, congestion of the sinusoids and necrotic pancreatic acinar tissue.

Generally the sampling of the gills and liver was done because of their physiological importance to the absorption and metabolism of chemicals (Roberts, 1989). Furthermore, gills perform numerous functions which include respiration, osmoregulation, excretion of nitrogenous waste products and acid-base balance. Therefore, functional impairment of gills caused by pollutants can significantly damage the health of fish, as fish gills are considered appropriate indicators of water pollution levels (Alazemi et al., 1996), as well as target organs for detoxification process (Cardoso et al., 1996). The liver on its own is the primary organ for detoxification of organic xenobiotics, with wide varieties of pollutants and other toxic by-products tending to accumulate in high concentrations within it and thereby suffering from harmful effects (Metelev et al., 1971). Alterations in the liver serves as useful markers of exposure to environmental stress.

The LC₅₀ of locust bean effluent to *Clarias gariepinus* juveniles (0.089 mg L⁻¹) obtained from this study is different from the result of other workers for some species of fish using different toxicants. This could be due to the nature of each toxicant, age and size of fish and the genetic composition (Nwanna et al., 2003; Cruz et al., 1988).

<table>
<thead>
<tr>
<th>Water parameter</th>
<th>Control</th>
<th>8.75</th>
<th>8.80</th>
<th>8.85</th>
<th>8.90</th>
<th>8.95</th>
<th>9.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean temperature (°C)</td>
<td>26.00°</td>
<td>26.00°</td>
<td>26.00°</td>
<td>26.50°</td>
<td>27.00°</td>
<td>27.00°</td>
<td>27.00°</td>
</tr>
<tr>
<td>Mean pH</td>
<td>6.70°</td>
<td>6.70°</td>
<td>6.60°</td>
<td>6.60°</td>
<td>6.60°</td>
<td>6.60°</td>
<td>6.50°</td>
</tr>
<tr>
<td>Mean dissolved oxygen concentration</td>
<td>6.80°</td>
<td>6.80°</td>
<td>6.70°</td>
<td>6.68°</td>
<td>6.60°</td>
<td>6.50°</td>
<td>6.40°</td>
</tr>
</tbody>
</table>

Values in the same row followed by the same superscript are not significantly different at p<0.05
Table 2: Behaviour of *C. gariepinus* exposed to the varying concentrations of the *Parkia biglobosa* effluent.

<table>
<thead>
<tr>
<th>Behaviour activity</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. biglobosa (ml L(^{-1}))</td>
<td>P. biglobosa (ml L(^{-1}))</td>
<td>P. biglobosa (ml L(^{-1}))</td>
<td>P. biglobosa (ml L(^{-1}))</td>
</tr>
<tr>
<td>Pattern</td>
<td>8.75</td>
<td>8.80</td>
<td>8.85</td>
<td>8.90</td>
</tr>
<tr>
<td>Erratic swimming</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Rapid opercula</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Surfacing movements</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Loss of reflex</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Change in pigmentation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: Positive response, -: Negative response
The results obtained on water physical chemistry shows that there was no much difference between the controlled and the treated media nor between the treatments because the values obtained all fall within the accepted water quality standard for fish culture (Boyd, 1981). The water temperature recorded during the experimental period agrees with those of Lloyd (1992), who found optimum temperature level of 20-30°C optimal for the rearing of catfish. The DO concentration recorded during the experiment was within the range 5-8 mg L⁻¹ optimal for fish growth (Boyd, 1981). The pH range of the water recorded in this study (6.62-6.70) fail short of those recommended by Boyd and Lichtkoppler (1985) that water pH values of about 6.5-9.0 at day break are considered best for fish production. The progressive decrease of both pH and DO with increase in temperature could be attributable to the fact that the effluent contains carbon dioxide CO₂ which invariably reduced the water pH and dissolved oxygen, hence the increase in water temperature.

Organisms exposed to the toxicants usually exhibit changes in opercular movements, surfacing behaviour and disturbed swimming movements stressing a sensitive indicator of physiological stress and avoidance response to the effluent. The direct proportionality increase in stress and hyperactivities with increase in the concentrations of the toxicant is the same (Drummond et al., 1973). Aguiwgo (1998) reported that the toxicity of cassava leaf extracts (Manihot sp.) became lethal to Clarias anguillaris as the concentration of the toxicant increases. This affects the gill lamellae, leading to a number of mortalities as a result of rupturer and erosion of the gills.

The gills of the Clarias gariepinus exposed to locust bean effluent showed varying degrees of epithelial hyperplasia among normal gill filaments (control). Generally, hyperplasia in fish was more pronounced towards the tip of the filament exposed to the higher concentration towards the end of the 96 h. Hyperplasia resulted in the fusion of the lamella leading to the reduction in the respiratory surface area of the filaments. Gills of Clarias gariepinus exposed to the different levels of the effluent showed varying degrees of damage. All fishes exposed to the different levels of the effluent showed separation of the respiratory epithelium from underlying supportive tissue. This phenomenon is related to the gill function disorders that affect the physiology or cause the death of fish (Smart, 1976). These are associated with asphyxiation and impairment of oxygen-carbon dioxide exchange (Mitchell et al., 1978). Livers of fish being exposed showed fibrosis, congestion of sinuoid with necrotic pancreatic acinar tissue together with congestion with red blood corpuscles which demonstrates stasis effect. Liver parenchymal necrosis, blood cell congestion and fibrosis are signs of lesions common with toxic substances (Solangi and Overstreet, 1982). Rana and Raizada (1989) while carrying out acute short term
bioassay on *Labeo rohita* with tannery and textile dye industry effluents showed severe histological alteration in the liver tissue of fish exposed to the effluents.

**CONCLUSION AND RECOMMENDATION**

Fish farming is a major contributor of animal protein requirements in Nigeria and in the diaspora, with catfish (*Clarias gariepinus*) and the tilapia (*Oreochromis niloticus*) as the most culturable species. But the greatest limitation is the indiscriminate and accidental discharge of some of the locally processed products, like the locust bean effluent.

Based on the results of the research and the discussions that were highlighted, it could be concluded that the locust bean (*Parkia biglobosa*) effluent poses a serious threat to our aquatic life if not properly addressed. It is very clear that the investigation carried out on the gills and liver showed signs of damage because of the high toxic nature of the effluent (0.089 mg L⁻¹). This therefore might be helpful in the future in assessing the toxic composition of the locust bean, especially the wastewater.

As a short-term measure, efforts should be geared towards reducing the impact of effluent to our aquatic habitat. These include educating our farmers and cautioning them against indiscriminate and accidental discharge of some locally processed products, as well as enacting stricter policies and laws as a means of curtailing the source of obnoxious discharge to our environment.

**REFERENCES**


