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Acute Toxicity of Ethanol Extracts of Cocoa Bean Shell on *Sarotherodon galilaeus* Juveniles

[1]F.E. Olaifa, [2]R.A. Hamzat and [1]O.O. Oyetoyan
[1]Department of Wildlife and Fisheries Management,
University of Ibadan, Ibadan, Nigeria
[2]Crop Utilization Research Group, Cocoa Research Institute of Nigeria (CRIN),
P.M.B. 5244, Idi-ayunre, Ibadan, Nigeria

Abstract: An acute toxicity test of ethanol extract of cocoa bean shell (CBS), a by-product of cocoa processing was conducted on 250 *Sarotherodon gallilaeus* juveniles for 96 h. There were 5 treatments and one control with each having 3 replicates. The treatments were prepared as 10,000, 7500, 4167, 2315, 1286 and 0 mg L^{-1} (control). The aim of the experiment was to study the toxic effects of Ethanol extract of CBS. While, the control experiment produced no mortalities and adverse histopathological responses, there were reactions to the presence of CBS extract in all the treatments which included restlessness and mortality. The lethal concentrations at which fifty percent of the test population died (LC 50) obtained using the logarithm method were 7943, 5012 and 6310 mg L^{-1} for replicates 1, 2 and 3, respectively while the probit method gave 5878, 4865, 6103 mg L^{-1} for replicates 1, 2 and 3, respectively. Histopathological examination of control fish showed no lesions on all tissues examined. However, marked changes were observed in the liver in form of degenerative hepatocytes, multifocal aggregation of haemosiderin laden macrophages; in the gills as shortening of gill filaments and in the brain as spongiosis of white cerebral matter of fish subjected to various concentrations of CBS extract. It was concluded that ethanol CBS extract possessed piscicidal properties on *S. gallilaeus* which could be useful in culling stunted and unwanted fish populations from ponds before stocking.

Key words: Toxicity, cocoa bean shell, extract, fish

INTRODUCTION

Fish is a good source of animal protein in Nigeria complementing other animal products and has high protein quality (Omoniyi, 1995). The average annual fish supply (both domestic and importedddded) of about 750 tons in 1997 fell short of the demand estimated at 1.86 million tons annually (Otubusin, 1999).

Fish production through aquaculture has been identified as a practical and promising approach to meeting fish demand. Predatory animals like *Chaoborus* sp. larvae (Diptera: *Chaoboridae*), tadpoles, frogs, leeches and unwanted fish species compete with and feed on fry and fingerlings of stocked fish. This problem needs to be addressed in order to meet fish requirements through production (Omoniyi *et al*., 2002). It is a common practice in fish culture to use synthetic toxins including chlorinated hydrocarbons and organophosphates to eradicate predatory and competing species from the nursery, rearing and production ponds prior to stocking of preferred species. This application of synthetic toxins is not advisable due to pollution and their persistence in the environment (Mason, 1998). An alternative to synthetic toxins is the use of plant piscicides, which are less expensive, biodegradable and environmentally safer (Sudhanshu and Singh, 2004).

Cocoa is the leading foreign exchange earner in the non-oil export sector in Nigeria (Baba Ahmed, 1999). A major way the International Cocoa Organization is working towards the sustainability of the cocoa industry is through economic waste or by-product management (Central Bank of Nigeria Annual Peport, 1998). The cocoa bean is the only commercialized portion of the cocoa fruit (Lopez *et al*., 1985) and Cocoa Bean Shell (CBS) is a by product of the cocoa processing industry. Nigeria has 6 functioning cocoa processing factories (Ojo, 1999) and all have problems disposing of CBS, the thin husk immediately surrounding the cocoa bean makes up about 10 percent of the cocoa bean (Menon, 1982) and has an anti nutritional factor, theobromine which is about 1.3-2% (Abiola and Tewe, 1991). Theobromine belongs to the same naturally-occurring methylated xanthine group as

Corresponding Author: F.E. Olaifa, Department of Wildlife and Fisheries Management, University of Ibadan, Ibadan, Nigeria

caffeine found in coffee (Ching and Wong, 1986). In moderate quantities, theobromine acts as a stimulant like caffeine but intake greater than 0.0279/kg body weight is injurious to animals (Menon, 1982).

The objective of this study was to determine the acute toxicity of ethanol extract of CBS and histopathological effects on organs of *Sarotherodon galilaeus* juveniles.

MATERIALS AND METHODS

Fish: Two hundred and fifty *S. galilaeus* juveniles (mean weight 8±0.5 g and mean length 8.5±0.4 cm; top loading mettler balance, Ohaust-TM160, accuracy 99±0.1%) were purchased from a fish farm in Ibadan and kept in 20 L capacity plastic aquaria containing de-chlorinated water for 21 days to acclimatize, fed at 2% of their body weight twice daily and electrical aerators used to maintain dissolved oxygen levels. The experiment was conducted in the Laboratory of the Department of wildlife and Fisheries Management, university of Ibadan, Ibadan, Nigeria.

Cocoa bean shell extraction: Cocoa bean shell obtained from Cocoa Industries, Ikeja in Lagos State was sun dried for a week and milled. A 2 kg of the powdered CBS was packed into the soxhlet extractor, one kg at a time using 2-4 L of ethanol as solvent and distilled. 389 g of the CBS extract was obtained. A range finding test was conducted as described by Odiete (1999) using 54 juveniles and the percentage mortality recorded 3 hourly for 24 h.

Preparation of the stock solution: A stock solution of the cocoa bean shell extract with a concentration of 10 g L^{-1} was prepared by diluting 10 g of the extract in one litre of distilled water and test solutions were obtained by serial dilution.

Introduction of the toxicant and experimental design: Ten litre of water were measured into each plastic aquarium. A factor of 1.8 was used to obtain the final concentrations used for the definitive test (Reish and Oshida, 1987). The concentrations of CBS extract in each treatment were 10,000, 7500, 4167, 2315, 1286 and 0 mg L^{-1} representing treatments A-F, respectively. Each treatment had three replicates and the experiment lasted 96 h with no feeding or aeration. Renewal of the test medium was done every 24 h and mortality recorded 3 hourly. Dead fish were identified by lack of opercular, body movement or response to any stimulus and removed.

Water quality parameters: The physicho-chemical parameters of the water: Dissolved oxygen, temperature and pH were measured before introducing the CBS extract, immediately after introduction and at the end of the experiment as described by Boyd (1979).

Collection of samples for histopathological examination: At the end of the 96 h period, fish samples were collected from each treatment and dissected. The gills, livers, intestines, muscles and brains were removed, preserved in Bouin's solution and later examined for histopathological changes.

The data obtained were subjected to a one way analysis of variance at 5% level of probability. The Lethal concentration at which 50% of the test organisms died (LC 50) was determined using logarithm (Litchfield and Wilcoxon, 1949) and probit methods (Sprague, 1973). The Fisher's Least Significant Difference (LSD) was used as a follow-up test.

RESULTS AND DISCUSSION

The physicho-chemical parameters of the water before introducing the CBS extract were temperature 26.3°C, pH 7.05 and D.O. 6.24 mg L^{-1} and Table 1 shows the Physico-chemical characteristics of water during the experiment.

Fish behaviour: The results obtained showed that all the test organisms in the control experiment survived throughout the 96 h period. However, in the other treatments, there were reactions such as slow movement or death before which the fish became distressed and restless. The dead fish were covered with mucus and foam was observed on the surface of the water in the first 24 h of exposure to the CBS extract. The fish died with their mouths opened. However, 100% mortality was not recorded for any treatment. At low concentrations; no

Table 1: Physicho-chemical characteristics of the water immediately after Introducing CBS extract and after 96 h

Treatment	Temperature°C	Parameters pH	Dissolved oxygen(mg L^{-1})
A (10000 mg L^{-1})	27.1	7.28	4.70
B (7500 mg L^{-1})	27.0	7.20	4.88
C (4167 mg L^{-1})	26.8	7.15	5.62
D (2315 mg L^{-1})	26.7	7.13	5.87
E (1268 mg L^{-1})	26.6	7.03	6.28
F (0 mg L^{-1})	26.4	7.05	6.40
At 96 h			
A (10,000 mg L^{-1})	27.4	7.28	3.90
B (7500 mg L^{-1})	27.3	7.18	4.30
C (4167 mg L^{-1})	26.9	7.14	4.40
D (2315 mg L^{-1})	26.9	7.12	5.20
E (1268 mg L^{-1})	26.7	7.06	6.15
F (0 mg L^{-1})	26.5	7.05	6.20

Table 2: Number and percentage mortality of fish in 96 h

Concentration mg L^{-1}	Log concentration	No. of test fish/tank	Number of dead fish percent mortality					
			Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep3
A 10000	4.000	10	7	8	7	70	80	70
B 7500	3.875	10	5	6	5	50	60	50
C 4167	3.620	10	4	5	4	40	50	40
D 2315	3.365	10	3	2	2	30	20	2
E 1286	3.109	10	0	0	0	0	0	0
F 0	0	10	0	0	0	0	0	0

mortality was recorded indicating that high concentrations were needed to cause mortality (Table 2).

For the logarithm method, the LC50s were 7943, 5012 and 6310 mg L^{-1} for replicates 1, 2 and 3, respectively. The mean mortality in each treatment was converted to percentage mortality that was transformed into percent probit using a probit Table. The probit mortality curves (Fig. 1-3) were subjected to regression analysis and from the trend line, the 96-h LC 50 were estimated as 5878, 4865 and 6130 mg L^{-1} with a mean of 5526 mg L^{-1}.

The results were subjected to ANOVA and significant differences ($p<0.05$) were observed among the treatments. The fisher's least significant difference was 0.86 and compared to the treatment means, all the treatments were significantly different from one another except treatments E and F.

Histopathology: The gills, brain, muscle, intestine and liver of the control fish had normal morphological structure. Gills of fish exposed to 4167 mg L^{-1} CBS extract showed matting and shortening of filaments. Changes in liver structure appeared in all treatments containing CBS extract where multi focal aggregations of haemosiderin laden macrophages with diffuse fatty degeneration of hepatocytes were observed. This indicated that the CBS extract produced changes in the liver, the major organ of detoxification. Spongiosis of the brain was also observed in fish exposed to 1286 mg L^{-1} of the CBS extract.

The observed restlessness of the fish in the bioassay media could be due to the effect of theobromine which has stimulant properties similar to caffeine in coffee (Ching and Wong, 1986; Kabeer *et al*., 1980) reported that caffeine could bind to acetylcholine receptors in the nervous system leading to excitation and restlessness .Similar responses were observed in tobacco leaf dust extract exposed fishes (Omoniyi *et al*., 2002; Omitoyin *et al*., 1999) reported an LC50 of 45 mg L^{-1} for *Tetrapleura tetraptera* plant extracts in *S. gallilaeus* indicating that CBS extract is less toxic. The lesser toxicity of CBS extract could be advantageous because more CBS would be required to eradicate the same number of organisms leading to more utilization of the CBS which currently is

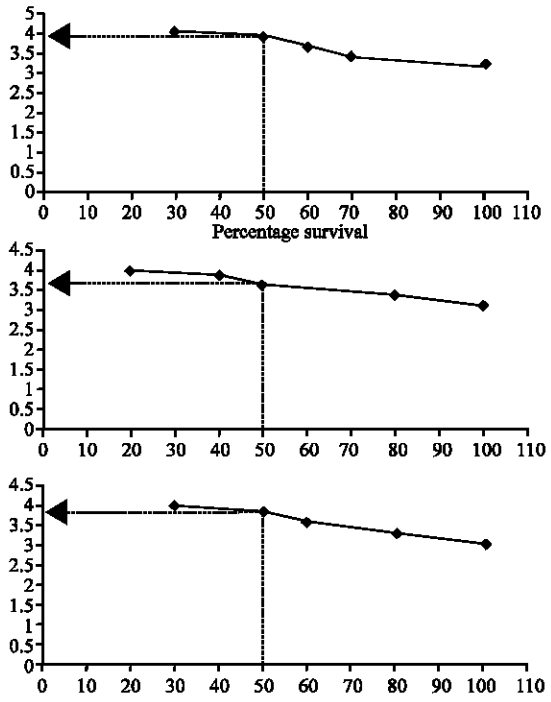

Fig. 1: Calculation of the 96 h LC 50 by the logarithmic method for the 3 replicates

little utilized. The histopathological changes in gills such as matting and shortening of gill filaments observed due to exposure could impair respiratory function by reducing the surface area for gaseous exchange. Increase in mucus secretion may reduce the oxygen uptake and cause suffocation.

The liver of the fish in the control had normal arrangement internally with the presence of brownish pigment within the parenchyma but the liver of the fish exposed to 10,000 mg L^{-1} CBS extract showed disorganized cords with fatty degeneration of hepatocytes. Other showed similar effects though to lesser extents. These effects were similar to those observed by Chinabut *et al.* (1978) using Dipterex on freshwater fisheries. Fafioye *et al.* (2004) observed similar effects on *Clarias gariepinus* exposed to *Parkia biglobosa* and *Raphia vinifera* extracts. The presence of

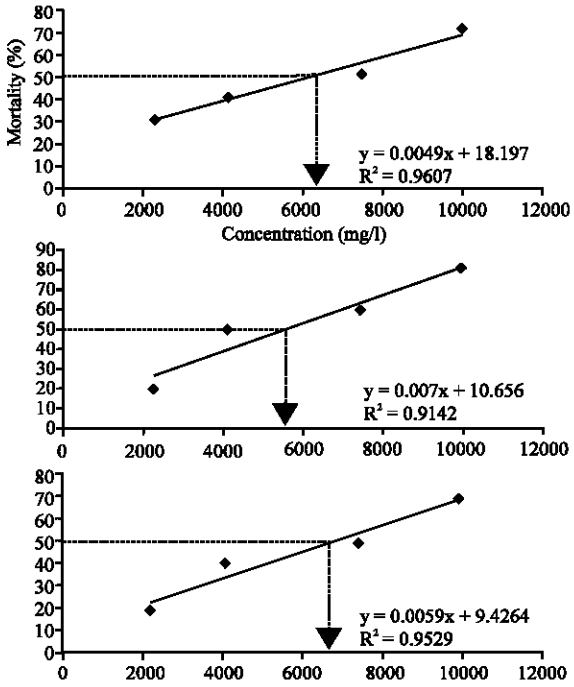

Fig. 2: Calculation of the 96 h LC 50 by the lprobit method for the 3 replicates

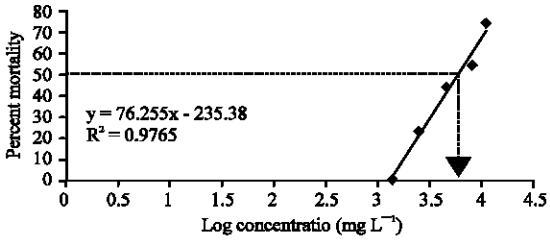

Fig. 3: Mean percent mortality versus log concentration

diffuse spongiosis of the cerebral white matter of the brain observed in the fish on 1286 mg L^{-1} CBS extract implies nervous disruption of the fish.

CONCLUSION AND RECOMMENDATION

All the anomalies observed in exposed fish showed that ethanol extract of CBS is toxic to *S. gallilaeus* and this property could be used to eradicate stunted and unwanted fish populations from the pond. The use of CBS extract would reduce the cost and effort involved in harvesting fish to be culled. It would also reduce the use of chlorinated hydrocarbon and organophosphates which pollute and may be persistent in the environment. Further studies using chronic and acute toxicity tests on *S. gallilaeus* and other fish species are recommended to establish and document the toxicity of ethanol and aqueous extracts of CBS on fish.

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