TOXICITY OF AFRICAN LOCUST BEAN EFFLUENT ON *Oreochromis niloticus*

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ABSTRACT

The effects of 96 h sub-lethal concentrations of African locust bean effluent on *Oreochromis niloticus* were studied using plastic buckets. Packed cell volume (PCV) and histological study of gills and liver were the tissue chemistry parameters investigated. The control buckets contained no effluent. Results revealed increase in ESR (Erythrocyte Sedimentation Rate) decrease in heamoglobin, red blood cell and PCV Value of the fish exposed to all sub-lethal concentrations. Damage to liver and gills were noticed in some of the sub-lethal concentrations of all the effluent. Normal situation was observed in all the examined tissues of fish from the control buckets. The African locust bean effluent is toxic on *O. niloticus* and the lethal concentrations of African locust bean effluent deducted from the 96 hours acute toxicity effect ranged from 3ml.

INTRODUCTION

Effluent discharges constitute the greatest pollution problem that Nigeria has ever experienced (Sikoki & Kolo, 1993). The effluent from these agriculture and industrial products has continued to affect both man and aquatic ecosystems directly or indirectly. Industrial effluent discharges exert great impact on the aquatic system even at low concentrations. Krous *et al.*(1982) and Ofojekwu *et al.*(1990) used different test organisms, different periods of exposure to pollutants, waters of different quality and different ways of reporting the results. The acute toxicity of Rogor® was determined for the juveniles of the freshwater fish, Nile tilapia (*Oreochromis niloticus*) by Annune & Ajike (1999) and it was suggested that osmoregulatory and respiratory incapacitation through mucus accumulation and epithelial detachment were the major causes of fish mortality following exposure to Rogor®. Exposure of the *O. niloticus* to sub-lethal concentrations of malachite green led to severe physiological impairment of fish after a 10 week exposure period (Omoregie *et al.*, 1992).

The African locust bean (*Parkia biglobosa*) is a source of protein in diets of Nigerians, and in the fermented form, it is one of the most important food condiments in the entire savanna region of West and Central Africa. Locust beans are boiled for about an hour to soften the seed coat for removal; the hulls are removed followed by washing besides rivers, and boiling for additional 10-12 hours to soften the cotyledons. The cotyledons are allowed to undergo wild fermentation for about 2 to 3 days during which the characteristic colour (brown) and odour (ammeruated) are developed. The effluents generated during African locust bean production may have harmful effect on water bodies as well as organisms that inhabit such water bodies. The effluent may be introduced deliberately or accidentally into the aquatic ecosystem during washing besides rivers impairing the quality of the water and making it unfavourable for aquatic life. The environment of the fish is often altered by toxic substances and this results in fish kills) especially when the concentration is higher than what the homeostasis of the fish can control. Information on the effects of sub-lethal and lethal exposure of freshwater species to African locust bean and its effluent is limited (Ahmed *et al.*, 2005), hence this study is to evaluate the effects of sub-lethal concentrations of *P. biglobosa* effluent on the behaviour, survival, some haematological parameters, and histology of the gills and liver of *O. niloticus* fingerlings.

MATERIALS AND METHODS

Altogether, 240 live *O. niloticus* fingerlings were purchased from Agricultural Development Project (ADP) fish farm at Alagbaka, Akure. African locust bean effluent was obtained from a local producer at Oke-Aro Titun in Akure. The fingerlings were transported to Fisheries laboratory of FUTA and placed in 100ml of water inside conico-cylindrical plastic containers for 48 hours to acclimate to laboratory conditions. Feeding was discontinued during the acclimation period in order to avoid pollution of the water with feed wastes. The fingerlings were weighed individually using a top-loading Mettler balance and randomly distributed into each container containing 100ml of water at ten fingerlings per container. A range-finding test was conducted using ten containers, each filled with 100ml of water prior to the introduction of the effluent. Each of the five varying concentrations (5ml, 10ml, 15ml, 20ml and 25ml) of African locust bean effluent was introduced with a pipette into the containers at 09.00 hr and the mixture was stirred. Each stock solution treatment was replicated twice.

Two replicates of the control treatment were also prepared by placing ten fingerlings in each of the two containers filled with 100ml of water without the effluent.

This was followed by a 96-hour definitive test conducted using ten conico-cylindrical plastic containers each filled with 100ml of water. Each of the five stock solution varying concentrations (1ml, 2ml, 3ml, 4ml and 5ml) of African locust bean effluent was introduced with a pipette into the containers at 09.00 hours and the mixture was stirred. Stock solution and control treatments were replicated twice. A total of 120 *O. niloticus* fingerlings (mean wt. 2.15g) were used for this test. The number of dead and living fingerlings in each plastic container was recoded every 24 hours starting at 08.00 h, during which the behaviour of the fingerlings was monitored. Observations made include mortality rate, lack of movement and lack of reaction to gentle prodding as described by Ward & Parrish (1982). The percentage mortality in each concentration was determined and the LC_{50} value was determined by the probit-logit transformation method. Water quality parameters such as temperature, P^H and dissolved oxygen (DO_2) concentration were determined at 24 hour intervals using standard methods described by APHA (1989) during 96-hour test. Immediately after death, gills and liver of the fingerlings were excised upon dissecting the fish for histological examination according to standard procedures. Blood samples were collected from two fingerlings from each of the treatment media immediately after death by caudal puncture into 2.5ml syringe already treated with ethylene amino acetic acid (EDTA) to prevent coagulation. Packed cell volume (PCV), haemoglobin concentration, leucocytes count and the erythrocyte count were estimated using various appropriate laboratory methods as described by Blaxhall & Daisley (1973). Data collected were subjected to the two-way analysis of variance (ANOVA) test (P = 0.05). Differences among the means were separated by Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Temperature and dissolved oxygen changed with the time while pH increased steadily. Dissolved oxygen concentration declined continuously, leading to stressful conditions on the fishes (Table 1). Eileen *et al.*(1991) also suggested that fish mortality tests are affected by temperature, dissolved oxygen concentration, pH and the duration of exposure. Fish mortality occurred in all concentrations (1, 2, 3, 4 or 5 ml) of the test toxicant as shown in Table 2. No fish died after 24 hours of exposure. No mortality was observed on exposure of tilapia fingerlings to 1 ml or 2 ml of effluent throughout the experiment however, mortality was found between 24 and 96 hours of exposure to 3 – 5 ml. Generally, after the application of the effluent, most of the fishes gradually became imbalanced and sank to the bottom within two hours of exposure. They later came to the surface and engulfed air by projecting their snout above the water level. At this time they lost their swimming ability. Opercular movements slowed down after one hour exposure.

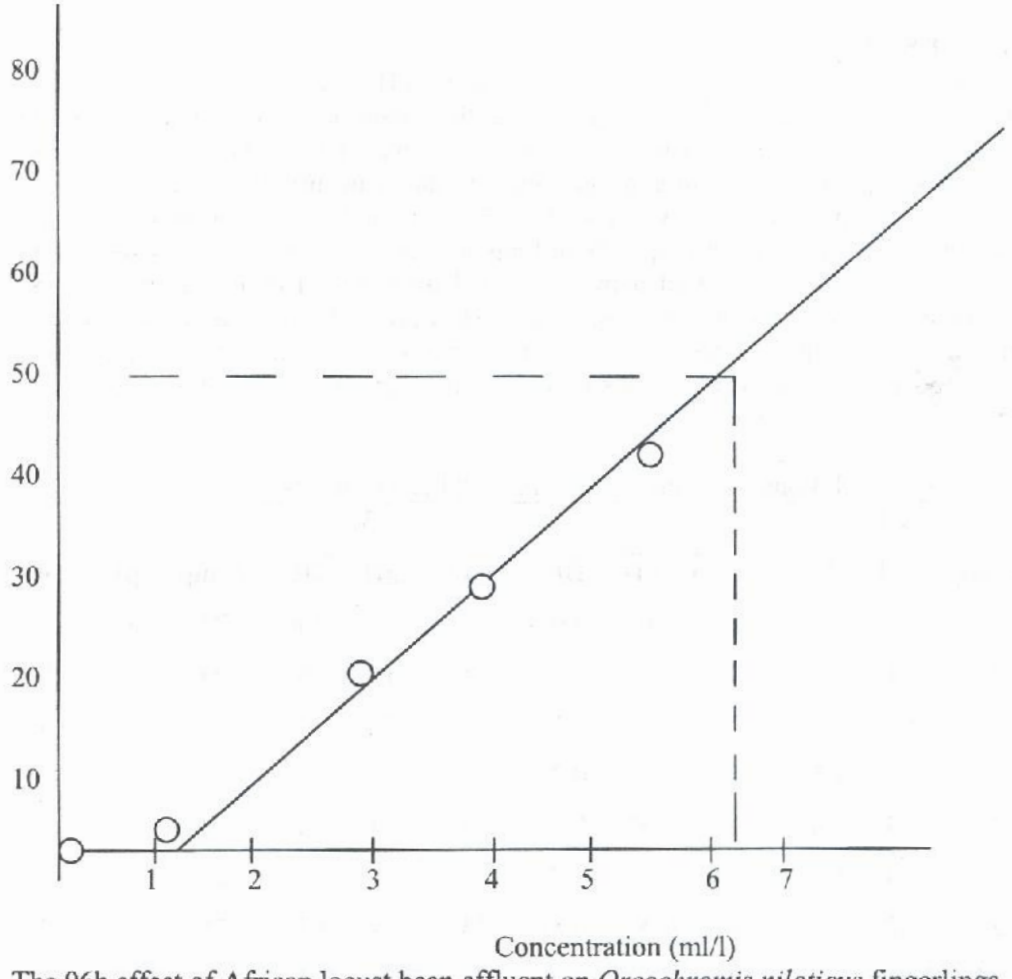
Table 1. Water quality of test solutions containing African locust bean effluents.

Concentration	Day 1			Day 2			Day 3			Day 4		
ml/l	**Temp**	**pH**	**DO**	**Temp**	**pH**	**DO**	**Temp**	**pH**	**DO**	**Temp**	**pH**	**DO**
0	25	7.65	4.8	25	7.68	4.0	25	7.70	3.8	24	7.80	3.1
0	25	7.64	4.4	23	7.62	3.9	25	7.65	3.6	24	7.78	2.9
1	25	8.01	2.6	25	7.85	0.6	26	7.75	1.0	24	7.69	0.9
1	25	7.52	1.7	25	7.55	0.7	25	7.74	1.1	25	7.62	0.9
2	25	7.65	1.4	24	7.63	0.9	25	7.70	0.6	24	7.70	0.6
2	25	7.70	1.2	25	7.65	0.7	24	7.72	0.5	24	7.68	0.6
3	25	7.52	1.0	25	7.53	0.5	23	7.43	0.5	25	7.80	0.3
3	24	7.65	0.8	24	7.65	0.6	23	7.66	0.4	25	7.77	0.1
4	25	7.60	0.9	24	7.73	0.4	25	7.75	0.5	25	7.74	0.2
4	25	8.02	0.7	25	7.75	0.3	25	7.73	0.4	25	7.72	0.1
5	25	7.66	0.3	24	8.01	0.1	24	7.78	0.1	25	8.01	0.1
5	25	7.82	0.2	24	7.98	0.1	25	7.90	0.1	25	8.01	0.1

Table 2. Mortality of *Oreochromis niloticus* exposed to African locust bean effluent for 96 hours.

		6 hours		24 hours		48 hours		72 hours		96 hours	
Concentration	% mortality	Live	Death	Live	Death	Live	Death	Live	Death	Live	Death
5ml	20%	10	-	9	1	9	-	8	1	8	-
10ml	70%	8	2	7	1	7	-	6	1	3	3
15ml	70%	10	-	7	3	6	1	6	-	3	3
20ml	90%	8	2	3		3	-	2	1	1	1
25ml	100%	8	2	3	5	1	2	0	1	-	-

The exposure of tilapia to 5 and 10ml of African locust bean effluent resulted in 20% and 70% mortality, respectively, within 96 hours while exposure to 15, 20 and 25 ml resulted in 50%, 60%, 80% and 100% mortality, respectively, within the exposure period. The 96 h LC_{50} obtained graphically (Fig 1) was 8ml. The 96 hr LC_{50} values are shown in Figure 1. No mortality was observed in tilapia exposed to 1 or 2 ml; on exposure to 3 - 5 ml, no fish died between 6 and 24 hours, however mortality occurred between 48 and 96 hours of exposure. At 5ml concentration, the highest mortality (30%) occurred between 48 and 96 hours of exposure. There was no mortality in the control treatments and fish in this group did not show any abnormal behaviour.



The 96h effect of African locust bean effluent on *Oreochromis niloticus* fingerlings.

The concentration of the African locust bean effluent used for the haematological test were 1ml, 2ml, 3ml, 4ml and 5ml for 96 hours, and theresults are presented in Table 3.

Table 3. Haematological value of *Oreochromis niloticus* fingerlings

Concentration of test media	ESR (mm)	PCV (%)	RBC (mm^3)	Hb (g/mm^3)
Control	4.0	30	15300000	26
1 ml	5.0	28	1470000	24
2 ml	5.5	28	1500000	26
3 ml	6.0	25	13500000	23
4 ml	6.5	24	1348000	23
5 ml	7.5	21	1160000	21

Hb, RBC and PCV values of tilapia fingerlings exposed to 1, 2, 3, 4 or 5 ml of the effluent decreased significantly compared to those of the control fish while the RBC counts increased at the end of the exposure period compared to those recorded in the control. Exposure of *O. niloticus* to sub-lethal concentrations of African locust bean caused a significant decrease in PCV, RBC and Hb of the fish. Similar reduction in blood parameters was reported by Sampath *et al.*(1993) and Omoregie *et al.*(1994) when *O. niloticus* was exposed to polluted environment under laboratory conditions. The significant reduction in these parameters is an indication of severe anaemia caused by the effluent on the exposed fish. Anaemic response could be due to the destruction of erythrocyte production (Wintrobe,1978; Omoregie,1995), haemodilution (Sampath *et al.*,1993), as well as the destruction of intestinal cells (Gardner & Yerich,1970). Similarly, Rambhaskar & Rao (1990) noted that changes in haematological parameters due to unfavourable exogenous factors such as adverse water quality, overstocking, and starvation are indices of ill health in cultivated fish. Ayotunde (1997) also reported that stress factors result in changes in haematological characteristics in fish. It is concluded that some parameters, and organs are affected negatively by increasing concentrations of the effluent and this will guide in policy formulations against water pollution.

The gills and liver were examined to assess the toxicological effect of the African locust bean effluent on them. The various organs were exposed to different level of concentrations of the toxicants (1, 2, 3, 4 or 5 ml). Mucus accumulation was observed on body surfaces and gill filaments of dead fish. Examination of the gills of untreated (control) *O. niloticus* fingerlings revealed a normal gill filament consisting of primary lamellae with its arrays of delicate secondary lamellae, primary epithelium and secondary epithelium covering the primary and secondary lamellae respectively and no vacuolation. There was little or no discernible change in gill structure of *O. niloticus* fingerlings exposed to low concentrations (1 and 2 ml) of the effluent. At higher concentrations however (3 and 5 ml) plates 10 and 12, the gill structure showed detachment of the epithelial cells in both the primary and secondary lamellae, there was also vacuolation of the filament and influx of cells into the tissue which resulted into degeneration of the tissue. Histological studies revealed that low concentrations (1-2 ml) of the locust bean effluent did not cause any histological damage to *O. niloticus* liver. In higher concentration (3-5 ml), the liver exhibited histological changes as indicated by loss of nuclei in some hepatocytes, which also did not show distinct cytoplasmic boundaries as seen in the control fish liver. There was space formation in the parenchyma tissue and the nuclear cells had thick dark look (pykuosis) as seen in plate 3-5. The cellular arrangement of the liver cells was also distorted. At some places, necrotic zones were observed where complete cell death was evident. Infiltration was also clearly evident. Lesions were more prominent in the central zone of the liver than in the periphery. Histological examination revealed that from the treated fish species, the gills were swollen and the lamellae were extensively fused and congested with blood. Similar observations were made by Onwumere (1986), who expressed that the histology of liver and gills of *O. niloticus* fingerlings exposed to 30, 40 and 50% effluent from the NNPC Refinery at Kaduna showed that the gills were swollen and bulged the opercula. Aderiye (1998) also stated that the gill structure of *O. niloticus* treated with petrol and engine oil mixture was fused together and that there was extensive hyperplasia and separation of the epithelial layer from the supportive tissues. In the liver, degeneration vacuole formation and irregular nuclear was noted and these was observed in newborn guppies, *Lebistes reticulates* (Crandall & Goodnight,1962), disintegration and necrosis in common carp, *Cyprinus carpio* (Wong *et al.*,1977) and vacuolation, necrosis and appearance of some globular bodies in *Puntius eonchonius* (Kumar & Pant,1981) due to zinc toxicity were reported.

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