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Short Communication

Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles

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Acute toxicity tests were carried out with aqueous and ethanolic extracts of two botanicals on *Clarias gariepinus* juveniles. 96-h LC50 values for *Parkia biglobosa* aqueous (AEPB) and ethanolic extracts (EEPB) were 2.8 and 2.4 ppm, respectively. While for *Raphia vinifera* aqueous (AERV) and ethanolic extracts (EERV), the values were 3.4 and 3.2 ppm, respectively. The resulting 96-h LC50 values showed that extracts of *P. biglobosa* were more potent than the extracts of *R. vinifera* and that EEPB was the most toxic. Histopathological changes in liver and gill of exposed fish showed subtle cellular damages like necrosis, lesions, oedema and hepatocytes.

Key words: Botanicals, toxicity, juveniles, *Clarias gariepinus*.

INTRODUCTION

Botanicals are natural biocides (Burkill, 1985) and their contamination of natural waters has become inevitable in Nigeria because of recent wide use. Piscicidal plants like *Blighia sapida*, *Kigelia africana, Tetrapleura tetraptera, Raphia vinifera, Parkia biglobosa* and *Tephrosia vogelii* are frequently in use by the fisherfolks because they are highly potent (Fafioye, 2001). However, the presence of these botanicals in high concentrations may have adverse effects on aquatic organisms.

The African catfish, *Clarias gariepinus* is an ecologically important and commercially valued fish for the Nigerian fishing industry (Ita, 1980). These mudfish are frequently and widely cultured in ponds and they also occur freely in Nigerian natural fresh waters. The demand for this fish species by almost 75% of Nigerian population has necessisated the cropping of it in large number using poisons.

Based on the piscicidal properties of *P. biglobosa* barks and *R. vinifera* fruits, there is the need to evaluate the aqueous and ethanolic extracts of these plants for their potentials as fish poisons. The objective of this study was therefore to determine the acute toxicity (LC50) of the aqueous and ethanolic extracts of the two botanicals and their effects on the histopathology of the liver and gill of *C. gariepinus* juveniles.

MATERIAL AND METHODS

Fish

Clarias gariepinus juveniles (total length: between 14.5 and 17.1 cm) were purchased from Tumifeb Fish Farms, Ibadan, Nigeria and transported in oxygenated polythene bags to the Research Laboratory.

Four hundred fish juveniles were held in four rectangular concrete tanks (105 L) for 14 days acclimation. The tanks were filled to half their capacities with tap water, which had been allowed to stand for 24 h to allow for dechlorination. Feeding was administered at 2% body weight using Olaogun Fish Feed, Nigeria Limited (40% crude protein) twice (morning and evening) daily. Change of used water was done every other day to avoid pollution by fish exudes and food reminants.

Ethanolic extracts preparation

The Nigerian variety of dried, ripe fruits of *R. vinifera* and barks of *P. biglobosa* were used for this study. The fruits and barks were

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Table 1. Mortality of C. gariepinus juveniles exposed to aqueous and ethanolic extracts of R. vinifera with the LC50 values.

Concentration (ppm)	No of Fish/tank	Mortality/time (h)							
		AERV				EERV			
		24	48	72	96	24	48	72	96
0.0	10	0	0	0^{c}	0^{e}	0	0	0^{d}	0^{e}
0.8	10	0	0	0^{c}	0^{d}	0	0	0^{d}	0^{d}
2.4	10	10	10	20^{b}	20^{c}	10	10	30^{c}	40^{c}
3.2	10	20	20	30^{b}	40^{b}	20	30	40^{bc}	50^{b}
4.5	10	30	40	60^{a}	60^{b}	30	50	60^{ab}	70^{ab}
6.0	10	50	60	80^{a}	100^{a}	60	80	90^{a}	100^{a}
LC50		6.0	5.0	3.8	3.4	5.6	4.5	3.5	3.2

Values with the same superscripts vertically in a column are not significantly (P>0.05) different.

Table 2. Mortality of C. gariepinus juveniles exposed to aqueous and ethanolic extracts of P. biglobosa (AEPB and EEPB) with the LC50 values.

Concentration (ppm)	No of Fish/tank	Mortality/time (h)							
		AEPB				EEPB			
		24	48	72	96	24	48	72	96
0.0	10	0	0	0c	0^{e}	0	0	0^{d}	0^{e}
0.8	10	0	0	10^{c}	10^{d}	0	0	0^{d}	10^{d}
2.4	10	0	10	20^{c}	40^{c}	10	10	30^{c}	40^{c}
3.2	10	20	30	50^{b}	60^{c}	20	30	40^{bc}	50^{b}
4.5	10	40	40	60^{b}	80^{b}	50	60	80^{b}	90^{a}
6.0	10	50	70	90^{a}	100^{a}	60	80	100^{a}	100^{a}
LC 50		6.0	4.8	3.2	2.8	4.5	3.2	2.8	2.4

Values with the same superscripts vertically in a column are not significantly (P>0.05) different.

sun dried for 6 days and later milled separately with Phillips grinding machine. The ground botanicals were passed through 100-micron sieve to obtain fine powder. A known weight of each powdered botanical (880 g of *P. biglobosa*; 1000 g of *R. vinifera*) was packed into the soxhlet extractor, using ethanol (2 - 4 L) as solvent for the extraction, after which the distillation of the solvent took place. About 178 g of *P. biglobosa* and 196 g of *R. vinifera* ethanolic extracts (EEPB and EERV) were obtained and used for the toxicity testings.

Aqueous extracts preparation

Fresh fruits of *R. vinifera* and barks of *P. biglobosa* were collected and pounded using wooden mortal and pestle. 100 g of each pounded botanical were separately soaked in 10 L of dechlorinated water for 5 days to ferment. The prepared aqueous and ethanolic of both botanicals were used for the static toxicity tests following standard procedures (FAO, 1986). Five graded concentrations (6.0, 4.5, 3.2, 2.4 and 0.8 ppm) of the extracts were prepared in triplicates after range finding tests had been conducted.

Ten juveniles *C. gariepinus* were exposed in 36 glass aquaria (18-L capacity) and covered with netting material to prevent the fish from jumping out. The whole experimental set-up was aerated continuously using four electric aerators. Renewal of the test media was made at every 24 h for 4 days. The dechlorinated tap water used during the experiment had a temperature of 28.1±1.0^{o}C, pH 7.1±0.5, dissolved oxygen 5.4 ±1.3 ppm and total dissolved solutes 26.0±5.0 ppm (APHA, 1980).

Observations for mortality were made daily and death was assumed when fish failed to respond to mechanical stimulation. Dead fish were removed at each observation and were sampled for histopathological analyses of the gill and liver using the methods of Luna (1986). The 96–h LC50 values were calculated statistically (Steel et al., 1997).

RESULTS AND DISCUSSION

Clarias gariepinus juveniles were stressed progressively with time before death. The pattern of mortality was similar for various concentrations of the two botanicals. At higher concentration (3.2, 4.5 and 6.0 ppm), rate of mortality significantly (P< 0.05) increased from 20% at 24-h to 100% at 96-h (Tables 1 and 2). There was no mortality in the control (0.0 ppm) experiment.

The stressful behaviour of *C. gariepinus* juveniles tended t o show feelings of respiratory impairment due to the toxic effect of *P. biglobosa* and *R. vinifera* on the gills. Similar observation was reported for *C. gariepinus* exposed to water extracts of *Blighia sapida* and *Kigelia*

Tables 3. Histopathological changes observed in the gills and liver of *C. gariepinus* juveniles exposed to different concentrations of aqueous and ethanolic extracts of *P. biglobosa* and *R. vinifera* after 96 h exposure.

Conc. (ppm)	Organs	Nerc-rosis	Vancuo-lation	Lesion	Melano	Cong-estion	Oedema	Hepatocytes	Heamo-siderosis
0.0	Gill	-	-	-	-	-	-	-	-
	Liver	-	-	-	-	-	-	-	-
0.8	Gill			+		+	+	-	-
	Liver	+	+	+	+	+	+	+	-
2.4	Gill			++		++	+		
	Liver	+	++	++	+	++	++	+	+
3.2	Gill			++		++	++	-	-
	Liver	++	++	++	++	++	++	++	+
4.5	Gill			+++		++	++	++	-
	Liver	+++	+++	+++	++	+	++	++	+
6.0	Gill			+++	-	+++	++	-	-
	Liver	+++	+++	+++	+++	+++	+++	+++	++

- = Completely absent
+ = Present
++ = Mild
+++ = Severe
Conc. = Concentration
Melano = Melanomacrophages

NOTE: Treatments with no signs indicated no histopathological changes were observed.

africana (Onusiriuka and Ufodika, 1994). *C. gariepinus* became inactive at higher concentrations of the toxicants and this was similar to the reports of Omitoyin et al. (1999) on Nile tilapia and Fafioye and Adebisi (2000) on African catfish.

Histopathological changes observed in the gill and liver of experimental fish showed different lesions, which ranged from necrosis and vacuolation to diffused congestion of cells and melanomacrophages (Table 3). The observed histological changes were as a result of various clinical factors. The higher the concentrations of *P. biglobosa* and *R. vinifera*, the more severe the degree of damages to fish gill and liver.

The control gill had normal morphology of the hayaline cartilaginous rods in each filament, while the treated gill showed histological changes which included microthrombi in capillaries, oedema of the submucosa, degeneration of the lamella cartilage and focal lamellae epithelial hyperlasia. These histopathological changes in the gills are similar to epithelial damages caused by cadmium (Oronsaye, 1997) and endrin (Eller, 1971). The alterations produced may probably lead to several physiological stresses in the fish.

The control liver had normal internal arrangement components with presence of brownish granular pigment within the parenchyma. The treated liver showed disorganized hepatic cords with diffuse vacuolation of hepatocytes at the lower concentrations (0.8 and 2.4 ppm) of AERV, EERV, AEPB, and EEPB. At the higher concentrations (3.2, 4.5 and 6.0 ppm) more damages like fatty degeneration of the hypatocytes, haemosiderosis, coagulative necrosis and severe oedema occurred. These effects were also reported by Chinabut et al. (1978) using Dipterex on freshwater fisheries. Heath (1984) also confirmed the same effect in bluegill (*Lepomis macrochirus*) exposed to copper.

From the present studies, the results indicate that extracts of both plants are toxic to Clarias. *P. biglobosa* are more acute than extracts of *R. vinifera* to *C. gariepinus,* while the ethanolic extracts are more potent than the aqueous extracts.

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