ACUTE TOXICITY OF ETHANOLIC EXTRACT OF *Derris elliptica* ROOTS TO O*reochromis niloticus* FINGERLINGS

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Abstract

Replicate static 24-hour bioassay was conducted to determine the median lethal concentration (LC_{50}) and median lethal time (LT_{50}) for Nile Tilapia *Oreochromis niloticus* fingerlings to ethanolic extracts of dried roots of *Derris elliptica.* Six graded concentrations of 0 (Control), 93, 139.5, 186, 232.5 & 279 mg/litre of *Derris elliptica were used as treatments.* These were applied in the form of root powder solution to *O. niloticus* fingerlings in glass tanks A, B, C, D, E and F (where "A" served as the control). Ethanolic extracts of dried roots of *D. elliptica* were tested for toxicity under laboratory conditions with a 24h LC_{10} of 139.5mg/10L (weight/volume). Toxic reaction exhibited by the fish includes discouration, gulping for air, erratic swimming, loss of reflex, slow opercular movement and settling at the bottom motionless. Histological examination of *O. niloticus* fingerlings showed some pathological changes. Damage became severe with increasing concentration of the plant extracts.

Introduction

Derris elliptica (Family Papilionaceae) are widely available in the tropics and their twigs and roots have been used as natural piscicides in artisanal fisheries and aquaculture ponds in Nigeria. The use of plant piscicides such as *Tephrosia candida*, *Tephrosia purpurea, Mundulea sericea, Acacia pennata* (Weiss 1973), *Adenia cissampeliodes* (Morah 1985), *Tetrapleura tetraptera, Parkia filicoides, Tephrosia vogelii* is common among fish farmers in controlling pests and predators. Many plants contain chemicals, which have traditionally been used in fish culture in almost all parts of the world (Jenness 1967). The best-known plant species is *Derris*, which produces rotenone and species of *Tephrosia*, which contain tephrosin, a substance similar to rotenone. The active substance of the majority of plant poisons are resin, tannin, rotenone, saponin, etc. (Morah 1985). Indiscriminate use of piscicides poses a great risk to aquatic organisms, especially food fishes and consequently to humans. Therefore, a good control measure that will be effective in killing the target organism at high doses which is not hazardous to people and animals and the environment but easily available and economical should be sought. *Derris* plant contains rotenone and are used as piscicide and insecticide in Malaysia. This study was conducted to determine the 24-hour median lethal concentration (LC_{50}) for *Oreochromis niloticus* fingerlings exposed to *Derris* root powder extracts.

Materials and methods

Oreochromis niloticus fingerlings (mean weight, 2.7g) were obtained from a government freshwater pond in Ondo State, Nigeria and acclimated to laboratory conditions using glass tanks (45cm x 40cm x 40cm) of 30 litres capacity, filled with 10 litres of water, well aerated. The fingerlings were unfed for 24 hours prior to and during the experiment. Three separate static bioassay tests with aeration were conducted for 24 hours each as described by APHA (1975) in the range finding test. A completely randomized design was used in the experiment with 10 fish/10-litre freshwater. The *Derris* powder roots were sun-dried for 5 days and later milled and sieved with 100-micron sieve to obtain a fine root powder. A known weight of the powdered roots (180g) was packed into the soxhlet extractor using ethanol as solvent for the extraction, after which distillation of the solvent (ethanol) took place. About 20g of the ethanolic extract of *Derris elliptica* was obtained and dissolved in distilled water to form a stock solution of 250mg/L of the material, the following concentrations were prepared and introduced into each of the experimental glass tanks (A, B, C, D, E & F): 0.00 (control), 93, 139.5, 186, 232.5, 279 mg/L. Ten (10) fingelings of *O. niloticus* were introduced into each glass tank, replicated three times.

The experimental duration was 24 hours. The median lethal concentration (LC_{50}) values and its corresponding 95% confidence limits were conducted by probit analysis (Finney 19982). Fish mortality in each aquarium was monitored at 3-hour interval while percentage mortality and the time for 50% mortality were determined using standard methods. The behavior of the fish was observed after the introduction of the root powder extracts. Fish were sampled after mortality for histopathological analyses of the selected tissues (i.e. the gill, liver). Fish were randomly selected and dissected to extract the tissues. Gills were preserved in 10% formalin and liver in Bouin's fixative. Tissues were processed, sectioned and stained with hematoxylin and eosin, using standard histological techniques. Water quality was analyzed at the start and termination of the experiment. Dissolved oxygen was determined using a digital DO_2 meter. pH was determined using a digital pH meter (mettler Teledo 320), temperature was determined using mercury in glass thermometer. Hardness and conductivity were likewise measured. Results obtained were subjected to probit and logit analyses (Finney 1971) using SPSS 11.0 for windows XP on PC.

Results

Behavioural changes

O. *niloticus* exposed to *Derris* powder extracts displayed different behavior. Fish showed initial disturbed swimming movements, rapid opercular movements, loss of balance incessant gulping of air, blackening of the whole body, unusual lethargy and fish settling at the bottom motionless with slow opercular movements. There were no obvious changes in fish behavior in the lower concentrations for the first 3 hours of the experiment. The abnormal behaviors displayed by the fish increased with increasing concentrations of *Derris* powder in water; it decreased with time of exposure, then gradually reduced at higher concentrations.

Toxicity

Table 1 summarizes the range of the physicochemical parameters of control and treated fish observed during the experimental period. There was no significant difference among the different treatments in terms of physicochemical parameters measured. Alkalinity significantly increased throughout the experiment.

Table 1. Ranges of water quality parameters during experiments on the toxicity of Derris solution to *Oreochromis niloticus*

Parameter	Control	Derris solution
Temperature (^{o}C)	28.00-28.00	28.00-28.50
pH	7.64-7.64	7.64-7.68
Alkalinity (mg/L)	79.00-79.00	79.00-82.00
DO_2 (mg/L)	4.70-4.70	4.70-4.70
Conductivity (X104)	4.40-4.40	4.40-4.46

The LC_{50} values of *Derris* root powder extracts of different time intervals and the 95% confident intervals are presented in Table 2.

Table 2. Derris root powder median lethal concentrations (LC_{50}) and 95% confidence intervals.

Time (hour)	LC_{50}	95% Confidence Interval	
		lower	upper
.5	0.244	0.231	0.257
3	0.328	0.315	0.341
6	0.482	0.469	0.495
9	0.509	0.496	0.522
12	0.573	0.560	0.586
15	0.618	0.605	0.631
18	0.630	0.617	0.644
21	0.650	0.637	0.663
24	0.698	0.684	0.711

Histopathological changes

Gills of *O. niloticus* exposed to *Derris* root powder extracts exhibited varying degrees of epithelial hyperplasia among filaments of treated and untreated fish. Normal gill filaments (controls) are shown in Table 3. Slight congestion and gill alteration with slight separation of the epithelial layer from the supportive tissue were generally observed in fish exposed to 93mg/L *Derris* root extracts for 24hours. After 24 hours of exposure in 139.5mg/L *Derris* powder extract, vacuole formation and hyperplasia resulted in the lamellae while gills of *O. niloticus* exposed to high concentration of *Derris* root powder extract showed varying degrees of damage (186, 232.5 and 279mg/L *Derris*) such as high level of degeneration in the lamallae, anoxic injury and epithelial layer from capillaries were observed respectively, Table 3. The histostructure of liver from treatment A was normal

without necrosis, and without congestion. Livers of fish exposed to 93mg/L derris for 24 hours had no appreciable cellular changes except for some space formations whereas fish exposed to 139.5mg/L derris showed space formation in the tissue parenchyma and necrosis of liver parenchyma. Fatty necrosis, thickening of nuclear cell (pyknosis), vacuolar degeneration, struken and dense nucleus were observed (Table 3).

Table 3. Histopathological changes observed in the gill and liver of *O. niloticus* exposed to different concentrations of *Derris elliptica* ethanolic root extract for 24 hours.

Treatment Concentration (mg/l)	Organs	Epithelial hyperplasia	Congestion	Gill alteration	Cellular infiltration	Necrosis	Degeneration
A (O)	G	-	-	-	-	-	-
	L	-	-	-	-	-	-
B (93)	G	½	½	½	½	-	-
	L	-	½	-	-	½	-
C (139.5)	G	½	+	+	+	-	½
	L	-	+			+	
D (186)	G	+	+	++	+		+
	L		+			+	
E (232.5)	G	++	++	++			+
	L		++			++	
F (279)	G	++	++	++	++		++
	L		++		++	++	++

Legend
G = Gill
L = Liver
- = Completely absent
+ = Present
½ = Mild
+ + = Severe
= Treatments with no signs indicated no histopathological changes were observed

Discussion

Studies revealed that organisms exposed to toxicants usually exhibit changes in opercular rate, erratic, sudden jerky swimming movements and different behavioral activities as shown in this experiments which demonstrated to be a sensitive indicator of physiological stress in fish subjected to sub-lethal concentration of pollutants (Derris 1973). The behavioral responses obtained from the study compared favorably with the observation of Pascual et al (1994) when formalin at different concentrations were used on sea bass (*Lates calcarifer*) fry.

The observations in this study agreed with Lin and Liu (1990) who reported that clinical signs such as abnormal movement and high respiration rate in hybrid tilapia (*O. mossambicus*) induced by ammonia suggested neurological dysfunction and gill damage. The LC_{50} value of *Derris elliptica* observed in this study was found to be higher than those reported in the literature, this may be as a result of fish species, environmental factors, food or water parameters. WHO (1992) reported 96h – LC_{50} of 0.02 – 0.2mg/L for different fish species and for daphnids (Water Fleas) exposed to rotenone. Guerrero et al (1986) reported 96h LC_{50} of 10 – 20ppm for *O.niloticus* fingerlings exposed to *Derris* root powder.

The results showed that the 24-hour LC_{50} values are within the levels used in fishponds. Twenty four-hour (24h) LC_{50} are useful measures of relative acute lethal toxicity to organism under certain experimental conditions, but, these values do not represent safe concentration in natural habitats. High mortality occurred in fish showing severe gill epithelial hyperplasia, separation of the gill epithetial layers from supportive tissues, necrosis of liver hepatocytes. Gill alterations such as epithelial hyperplasia and separation of the epithelial layer from supportive tissues are usually directly related to gill function disorders, which may affect the physiology or cause the death of fish (Smart 1976).

Liver parenchymal necrosis, fatty degeneration, blood cell congestion and fibroses are non-specific liver lesions associated with pesticides toxicity (Cahn 1975). The histopathological alterations found in the gills and liver of *O. niloticus* seem to be all caused by the piscicide *(Derris* root powder extracts). Based on these results, *Orechromis niloticus* can tolerate the levels of *Derris* powder being used in fishponds. However, histological analyses of gills and liver showed pathological changes even at sub- lethal levels. Thus, an application factor of 0.1 is recommended to be multiplied with the 24-hour LC_{50} value to estimate the safe concentration of *Derris* roots powder extract for *O. niloticus* at 186mg/L.

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