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STUDIES ON ACUTE TOXICITY OF PISCICIDAL PLANT EXTRACTS (*TETRAPLEURA TETRAPTERA*) ON TILAPIA (*SAROTHERODON GALILAEUS*) FINGERLINGS

B.O. OMITOYIN, A.O OGUNSANMI AND B.T ADESINA
Department of Wildlife and Fisheries Management,
University of Ibadan, Ibadan, Oyo State, Nigeria.

Target Audience: Fish farmers, fishery scientists and toxicologist.

ABSTRACT

Ethanolic extracts of dried, ripe fruits of *Tetrapleura tetraptera* was used to test the toxicity tolerance in Tilapia *Sarotherodon galilaeus* fingerlings. Five graded concentration of 4.5, 10.0, 22.0, 45.0, 100mg /litre of water (weight/Volume) and a control 0mg/15l of water (represented by Aquaria A,B,C,D,E and F (where " A" served as a control). Ethanolic extracts of dried, ripe fruits of *T . tetraptera* were tested for toxicity under laboratory conditions with a 96h Lc 50 of 675mg/15l (weight/ Volume). The mean mortality percentages were 13.13, 26.66, 30.00, 50.00, and 100.00 % in the order of concentration of 4.5, 10.0, 22.0, 45.0, 100mg/litre (weight/Volume), respectively, while there was no mortality in the control treatment. There were significant differences (P<0.05) on the effect of concentration of *T. tetraptera* on total mortality percentage. The higher the concentration, the higher the mortality of the *S. galilaeus* fingerlings while toxicity of *T. tetraptera* reduced with time.

Histopathological examination of fish without ethanolic fruits extract showed that there was no lesion, but histopathological changes were observed in other fish subjected to different concentrations of ethanolic fruits extract of *T. tetraptera*.

***S. galilaeus* fingerlings succumbed only to higher concentrations of *T. teraptera* ethnolic fruits extract within 9-54 hours. Thus, it is possible to use the extract in selective eradication of aquatic organisms in nursery and rearing ponds before stocking.**

Key words : *Tetrapleura tetraptera,* toxicity, *Sarotherodon galilaeus.*

DESCRIPTION OF PROBLEM

In semi-intensive and extensive aquaculture systems, a common problem is the presence of wild organisms such as insect larvae, amphibian and snails. A conventional means of eradication is the use of biocides. Typical biocides are synthetic toxins like chlorinated hydrocarbon particularly (Eldrin, Dieldrin and Aldrin) and organophosphate thiometron(1). Similarly, DDT, 2, 4-D and sodium cyanide have been reported to be use in the eradication of predators and pests (2). But these are not generally recommended because of their toxicity to fish, other aquatic species and possible health hazard to the consumers (1,3).

Derris rootpowder, the most popular piscicide used in aquaculture to eradicate unwanted fish is expensive; whereas synthetic compounds such as Adrin and Eldrin that are sometime used, contaminate the environment (3). These biocides are expensive, stable in water and are threats to the integrity of the environment. Therefore, a good control measure that will be effective in killing the target organism at fairly high doses which is not injurious to people and animals, but easily available and economical, and quickly dissappear in aquatic environment without leaving any cumulative adverse or hazardous effect on water and its ecosystem, should be sought.

These qualities are possessed by some local plants in Nigeria. Thus they have a role in to play in the eradication of wild, resident species of fish and insect which may prey on or compete with fry and fingerlings of desired fish species prior to stocking. The dry fruits of *T. tetraptera* traditionally used as a spice in flavouring pepper soup in Sothern Nigeria contain toxic constituents such as hydrogen cyanide, phytates, oxalates, tannins and saponins in the fruit shell, pulp and seed (4, 5).

The physical and emulsifying properties of the powdered, aqueous, methanolic and ethanolic extracts of the ripe fruits of *T. tetraptera* (which have medicinal, molluscoidal and piscicidal properties have been investigated (4, 6, 7).

Based on the piscicidal property of *T. tetraptera* fruits, there is the need to evaluate the ethanolic extract of the fruits for its potential as fish poison. The aim of the present study was therefore to determine the acute toxicity (Lc50) of *T. tetraptera* ethanolic fruits extract and its effect on the gill, liver, intestine and brain of Tilapia (*Sarotherodon galilaeus*) fingerlings.

MATERIALS AND METHODS

The Nigerian variety of dried, ripe fruits of *T. tetraptera* was used for this study. The spices were sun-dried for 5 days and later milled with a grinding machine. The ground fruits were passed through 100 micron sieve to obtain the fine powder from the fruits. A known weight of the powdered fruits (995g) was packed into the soxhlet extractor, using ethanol as solvent for the exraction, after which the distillation of the solvent (ethanol) took place. About 189g of the ethanolic extract of *T. tetraptera* was obtained, this was used for the toxicity test following the standard recommended method for freshwater fish toxicity studies (8) . There were six treatment viz, A,B,C,D,E, and F with 3 replicates each. Five graded concentrations of *T. tetraptera* ethanolic fruits extract were prepared . The following graded concentration were prepared per/litre of water: 0.mg.4.5mg, 10mg, 22mg,45mg, and 100mg/*l* so that the grade concentration in weight volume were prepared as 0, 67.5, 150, 130, 675, and 1,500mg/15L of water respectively into the aquaria A,B,C,D,E and F treatments. Ten (10) fingerlings of *S. galilaeus* were introduced into 34*l* aquaria glass tank which were covered with netting material to prevent the fish from jumping out. The experimental duration was 96 hours.

Fish mortality in each tank was monitored at 3 hours intervals, while percentage mortality and the time for 50% mortality were determined, using standard methods. The behavioural pattern of the fish after the introduction of the fruits extract was also observed critically. Fish were sampled after mortality for histopathological analyses of the selected tissues (i.e. the gill, liver, intestine and brain). Fish were randomly selected and dissected to extract the tissues after which they were preserved in Bouin's fluid before being transferred into formalin.

Data obtained were to subjected to statistical analysis using one way analysis of variance and comparison of means was by Duncan Multiple Range(9).

RESULTS AND DISCUSSION

Results obtained show that there were significant differences ($P< 0.05$) in the mean mortality percentage of *S. galilaeus* fingerlings subjected to different concentrations of ethanolic fruit extracts of *T. tetraptera*. Table 1 shows the mean percentage mortality. Fish in treatment A had no mortality while those in treatment B-F reacted to varying concentrations of ethanolic fruit extract.

Treatment F had 100 % mortality which was significantly higher ($P < 0.05$) than fish exposed to treatments B, C, D and E . This was followed by treatment E with 50% mortality which was also significantly higher ($P<0.05$) than those in treatments B,C, and D. Although treatments B and C had 26.66 and 30.0 % mortality which were significantly higher than fish exposed to treatment B with 13.33% mortality, both treatments however showed no significant differences ($P <0.05$) from each other. The behaviour of fish in control treatment was normal, but fish in other treatments showed slow movements with fish settling at the bottom of the aquaria motionless for some minutes. Erratic swimming, decrease in operculum beat, loss of balance incessant gulping of air were also observed.

The stressful and erratic behaviour of *S. galilaeus* fingerlings tended to show feelings of respiratory impairment due to the toxic effect of *T.tetraptera* on the gills. Similar observation was reported for fish subjected to varying toxicants (10, 11, 12). Fishes became inactive at higher concentration in acute and chronic toxicity tests (13). '

Results of the histopathological changes observed in the brain, gill, liver and intestine of experimental fish are presented on Table 2.

There was discoloration of the brain, gill, liver and intestine of fish subjected to varying concentrations of the toxicant, while there was none in the organs of fish in the control treatment. Fish exposed to treatment B showed mild congestion, mononuclear cellular infiltration of the meninges, neuronal degeneration and demylinization of the white matter. Whereas fish exposed to treatments C, D and F showed severe brain damage.

The liver of fish in treatment A was normal without necrosis, haemosiderosis and without congestion. There was mild centrilobular and sinusoidal

Table 1: Mean mortality percentage of *Sarotheodon galilaeus* fingerlings subjected to different concentrations of *Tetrepleura tetraptera* ethanolic fruit extract for 96 hours

Treatment [(Conc In mg/(W/V)]	TIME OF EXPOSURE (HOURS)																																		Mean Mortality (%)
	0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96		
A(0)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		0
B(4.5)	-	-	-	-	-	-	2	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		13.33[a]
C(10.0)	-	-	-	-	3	-	-	2	1	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		26.66[b]
D(22.0)	-	-	-	-	3	2	1	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		30.00[b]
E(45.0)	-	-	-	2	1	-	3	-	-	2	1	-	-	2	1	-	-	-	3**	-	-	-	-	-	-	-	-	-	-	-	-	-	-		50.00[c]
F(100.0)	-	-	-	3	4	4	3	3	3	3	4	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		100.00

* Time for 100% mortality = 33hrs

** Time for 50% mortality = 54 hrs

Means with the same superscript on the vertical row are not significantly different (P>0.05)

congestion of the liver cells of fish in treatment B, however fish in treatments C, D and E showed congestion, but severe portal, centrilobular and sinusoidal congestions were more apparent in fish in treatment F.

Table 2: Histopathological changes observed in the brain, gill, liver and intestine of *Sarothroden galilaeus* Fingerlings subjected to different concentrations of *Tetrapleura tetraptera* ethanolic fruit extract for 96 hrs.

Treatment Concen– mg/l	Hours of Mortality	Organs	Congestion	Necrosis	Haemossi -derosis	Cellular Infiltration	Lesions	Neuronal Degeneration
A(10)	-	B	-	-	-	-	-	-
		G	-	-	-	-	-	-
		L	-	-	-	-	-	-
		I	-	-	-	-	-	-
B(4.5)	18	B	1/2			1/2	1/2	1/2
		G	1/2			1/2	1/2	
		L	1/2	1/2	1/2		1/2	
		I	-			1/2	1/2	
C(10.0)	36	B	+			+	+	+
		G	+			+	+	
		L	+	+	+		+	
		I				+	+	
D(22.0)	39	B	+			+	+	+
		G	+	-		+	+	
		L	+	+	+		+	
		I				+	+	
E(45.0)	54	B	++			+	+	++
		G	++			+	+	
		L	+	+	+		+	
		I				+	+	
F(10.0)	30	B	++			++	++	++
		G	++			++	++	
		L	++	++	++		++	
		I				++	++	

Legend

B	=	Brain
G	=	Gill
L	=	Liver
I	=	Intestine
-	=	completely absent
+	=	present
1/2	=	mild
++	=	severe

= Treatments with no signs indicated no histopathological changes were observed.

The gill of fish in treatment A was normal slender, with branching projections lined by highly vascularised simple cuboid epithelium, whereas fish in treatment C-F showed that there were destruction of cillous and branching epithelia cells and squamous metaplasia with fish in treatment F showing severe damage.

The intestine of fish in treatment A had villi wall, no dimidiation into intestinal lumen. While intestine of fish in treatment B had mild lesion in the wall of villi with moderate mononuclear cellular infiltration of the submucosa of the villi and intestinal wall. Fish in other treatments showed mononuclear cellular infiltration of the intestinal wall except those in treatment F with mononuclear cellular infiltration, villous collapsed and submucosa monuclear congestion.

The observed histological changes were as a result of various clinical factors. The higher the concentration of *Ttetraptera* the more severe the degree of damage to fish tissue. In the liver, necrosis and vacuolation of hepatocyte and blood cell congestion of liver parenchyma are said to be non specific liver lesions which are usually observed in toxic situation (14). Degeneration of neuron and consequent increase in interstitial tissue in fish exposed to pesticides, chemotherapeutants and other toxicants has been reported (14). Similar exposure of fish to toxicant affects the various organs directly by promoting metabolic abnormalities(15). Furthermore, histopathological damages observed in the tissues of fish subjected to various level of ethanolic fruit extract of *T.tetraptera* showed that it contains some toxic substances such as oxalates, tannins, hydrogen cyanide, ascorbic acid, alkaloid and saponins which have been reported to be poisonous to fish (4, 5).

CONCLUSION AND APPLICATIONS

1 The present study shows that *T. tetraptera* had a 96 hour Lc 50 of 45mg/l (weight/Volume).

2 At higher concentration i.e 100mg/l (weight/Volume),100% mortality was recorded at 33 hours. This indicates that the higher the concentration the higher the mortality rate at a given time.

3 Since *S. galilaeus* fingerlings only succumb to higher concentration of extract in a short period of time, it is possible to use the extract of *T. tetraptera* in selective eradication of unwanted fish both from nursery and rearing ponds before stocking.

4 *T. tetraptera* can also be used as fish toxicant in stock assessment studies without any dangerous environmental consequences.

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