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Full Length Research Paper

Acute and sub-acute toxicities of five plant extracts on white tilapia, *Oreochromis niloticus* (Trewavas)

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Abstract

Acute and sub-acute toxicities of five plants'(Almond – *Terminalia catappa,* Pawpaw – *Carica papaya,* Neem – *Azadirachta indica,* Tobacco – *Nicotiana tobacum* and Cassava – *Manihot esculenta*) extracts on *O. niloticus* were conducted for 28 days to evaluate LC50s, fish behavior and heamatological indices of white tilapia in glass aquaria tanks. While the acute test lasted 48 hours, the sub-acute test lasted 28 days. The concentrations used for acute test were Almond = 15, 2.0, 2.5, 3.5 g/l, Cassava = 0.5, 1.0, 1.5, 2.5 g/l, Neem = 0.5, 1.5, 2.0, 2.5 g/l, Pawpaw = 1.0, 2.0, 3.0, 4.0 g/l, Tobacco = 0.25, 0.5, 1.5, 2.5 g/l and a control (0.0 g/l), while those used for sub-acute tests were Almond= 0.5, 0.7, 0.9, 1.0 g/l, Cassava = 0.15, 0.2, 0.35, 0.45 g/l, Neem = 0.10, 0.20, 0.35, 0.50 g/l, Pawpaw = 0.3, 0.5, 0.7, 1.0 g/l, Tobacco = 0.10, 0.15, 0.20, 0.25 g/l and a control (0.0 g/l). The mean values for the 48-h LC50s of the different plants' concentrations on *O. niloticus* were 0.83 g/l, *N. tobaccum*, 1.22 g/l *M. esculenta*, 1.64 g/l *A. indica,* 2.16 g/l *C. papaya* and 2.44 g/l *T. catappa* logarithmically. There were significant differences (P<0.05) in all the concentrations as mortality increased with time of exposure. The sub-acute test showed varied heamatological indices such as pcv, hb, tp, rbc, and glucose which were significantly different (P<0.05) and mcv, mch and mchc that were not significantly different (P>0.05) among the treatments. Also, fish became anaemic and stressful in the exposed plant botanicals and the severity was directly proportional to the active doses of each plant concentrations. The overall results showed the potency of the five botanicals in the order of tobacco>cassava>neem>pawpaw>almond.

Keywords: Acute, sub-acute, toxicity, plant botanicals, haematology.

INTRODUCTION

Fish has very low calorie, it is more easily digested than other animal protein source and it contains rich vitamins and minerals than meat (Egan *et al*., 1981). However fish in the wild are not enough for world population consumption hence the need for aquaculture to meet the demand. Problems associated with aquaculture include predatory animals like tadpoles, frogs, water snakes, leeches and weed fish species which predate on fry and fingerling of stocked fish. To overcome this predicament chemicals are used on nursery, rearing and production ponds to kill off the menace animals. But the outcome of this practice often results to long contamination of the ponds and non-target organisms including man who is the last consumer of fish (Mason 1993, Fafioye and Adeogun, 2009).

The use of specific natural biocides with pesticide properties derived from plants by artisanal fisher folks have been widely reported in Nigeria (Reed *et al*., 1967; Fafioye, 2005). Amongst the commonest plants used as piscicides in the south-western Nigeria are Almond – *Terminalia catappa,* Pawpaw – *Carica papaya,* Neem – *Azadirachta indica,* Tobacco – *Nicotiana tobacum* and Cassava – *Manihot esculenta.* The effects of these plants on fish safety have not been extensively studied, while the choice of the fish, *Oreochromis niloticus*, Trewavas is based on its being highly cultivable species and amenable to laboratory work. Also, since the effect of pollutants on fish can be assessed by heamatological indices for routine procedure in environmental monitoring toxicological research and fish health condition (Blaxhall, 1972; Sampath *et al*., 1993; Fafioye and Adebisi, 2000a; Fafioye, 2005). Therefore, this study was conducted to investigate:

a. impact of physio - chemical parameters of the used water on *Oreochromis niloticus* mortality,
b. comparative lethal concentrations at which 50%

(LC50) of *O. niloticus* exposed to the five different plant biocides die and

c. toxicity of the plants' extracts on the blood metabolite indices of *O. niloticus*

MATERIALS AND METHODS

Fish procurement-1000 fingerlings (mean body weight 7.11± 0.32 g) were procured from Oyo State Fish Production Farms, Agodi, and Ibadan, Nigeria and transported in five oxygenated polythene bags to the laboratory. They were held in ten large holding glass tanks (45.00L) half filled with dechlorinated well water and acclimatized for 7 days. They were fed twice daily with pelleted commercial feed (40% crude protein) at 3% body weight, while the used water was changed daily.

Plant procurement/ extract preparation- Five plants used are *Nicotiana tobacum, Terminalia catappa, Carica papaya, Azadirachta indica* and *Manihot esculenta*. The dried leaves of each plant except *Nicotiana tobacum* purchased from Oje Market, Ibadan, Oyo State, Nigeria were obtained from the Botanical Garden, University of Ibadan, Nigeria. The various leaves were oven-dried to a constant weight at 60 ^{o}C in a Gallenkamp oven. Each was separately powdered with Philips electric blender and stored in dry air-tight container. A stock solution of each powdered leaf extract with a concentration of 25 g/l was prepared and test solutions were obtained from it by serial dilutions. The volume of each test solution used in the toxicity per replicate in all treatments for each plant was 25 litres.

Test procedure- There were four concentrations and a control in two replicates with 20 fish per tank for each plant extract.

a. Range finding test: this is a pilot test to discover the different concentration of the test solutions. Test concentrations of 0.5, 2.0, 4.0 and 8.0 g/l of the various five plant species were used with each concentration having 5 fish and it lasted for 24 hours. Concentrations for the definitive tests were derived from those used for range finding tests.

b. The definitive test concentrations for acute test are: *T. catappa* = 15, 2.0, 2.5, 3.5 g/l, *M. esculenta* = 0.5, 1.0, 1.5, 2.5 g/l, *A. indica* = 0.5, 1.5, 2.0, 2.5 g/l, *C. papaya* = 1.0, 2.0, 3.0, 4.0 g/l, *N. tobacum* = 0.25, 0.5, 1.5, 2.5 g/l and control = 0.0 g/l.

48h LC_{50} Test: 20 fish per tank of the different concentrations of each botanical were used for the acute toxicity test. Fish behavior was monitored and recorded prior to death. Each solution was renewed daily using Buikema *et al* (1982) method, while LC_{50} was calculated logarithmically (Steel and Torrie, 1980).

c. Sub-acute test concentrations: Almond= 0.5, 0.7, 0.9, 1.0 g/l, Cassava = 0.15, 0.2, 0.35, 0.45 g/l, Neem = 0.10, 0.20, 0.35, 0.50 g/l, Pawpaw = 0.3, 0.5, 0.7, 1.0 g/l, Tobacco = 0.10, 0.15, 0.20, 0.25 g/l and control = 0.00 g/l

Sub-acute toxicity test: 10 fish per concentration were used. Feeding with Coppens feed was administered once at 3% body weight daily. The test solutions were changed every other day and the experiment lasted for 28 days.

Physio-chemical parameters: Dissolved oxygen (DO), temperature, pH, alkalinity, conductivity, etc. were taken weekly. Dissolved oxygen and pH were measured with Jenway digital probes, temperature with mercury in glass thermometer, conductivity with TOA model CM-205 conductivity meter and total ammonium with TOA model TM-5s ion meter.

Haematology: - Blood was collected from fish by cardiac puncture using hypodermic needle and syringe (2ml) into EDTA vials and the haematological parameters (heamoglobin (hb), packed cell volume (pcv), red blood cell (rbc), mean corpuscular volume (mcv), mean corpuscular heamoglobin (mch), mean corpuscular heamoglobin concentration (mchc), total protein and glucose) estimated as described by Blaxhall and Daisley (1973).

Statistical analysis: Each treatment values were analyzed using a two-way ANOVA at 5% probability.

RESULTS

Physio-chemical parameters: There were both slight and wide fluctuations in the physio-chemical parameters of the used water and control experiment (Table 1). While the slight fluctuations were not significantly different ($p<0.05$) and could not have produced serious effect on fish mortality, the wide fluctuations produced significant difference ($p>0.05$) since they might have been altered by the experiment.

The acute toxicity test showed fish mortalities in all the treatment tanks except the control. The percentage mean mortalities recorded for the five plant piscicides exposed to *O. niloticus* for 48 hours were shown on Tables 2a-e. However, fish showed stressful conditions of abnormal behaviours prior to death and mucus secretion on gills of moribund fish. These behavioural responses are dose dependent as the actions reduced with decreasing toxicant concentrations. By 48 hours, *Nicotiana tobaccum* and *Manihot esculenta* produced 100 % mortality *Azadiractha indica* and *Carica papaya* produced 90 % mortality, while *Terminalia catappa* recorded 80 % mortality. Mean values for the 48-hour median lethal concentrations (LC_{50}) were 0.83 g/l *N. tobaccum*, 1.22 g/l *M. esculenta*, 1.64 g/l *A. indica*, 2,16 g/l *C. papaya* and 2.44 g/l *T. catappa* logarithmically. There was significant difference ($p>0.05$) in all the concentrations as mortality increased with time of exposure. The results showed the potency of the five botanicals in order of *N. tobaccum* > *M. esculenta* > *A. indica* > *C. papaya* > *T. catappa*.

Sub-Acute Effect on Haematology: The effects of the five different botanicals on *O. niloticus* haematological parameters are shown in Tables 3a-e. There were

Table 1. Mean values of water quality parameters used for the experiment

Parameter	Used water		FEPA 2010 standard
	Control	After experiment	
Dissolved oxygen (mg/L)	6.80	3.50*	7.5
Temperature (^{o}C)	27.50	27.00	<40
Ammonium (mg/L)	3.80	1.60*	_
pH	6.40	6.25	6-9
Conductivity (us/cm)	138.50	160.70*	_
Total hardness (mg/L)	50	112*	5000
Total solid(mg/L)	262	285*	1000
Total suspended solids	100	121	_
Total dissolved solids	228	125*	1500
Alkalinity(mg/L)	102.5	164.5*	500
Chloride (mg/L)	48.33	54.65	600

Key *=significant P>0.05

Table 2a-e. Percentage mean mortality recorded for the five plant piscicides exposed to *Oreochromis niloticus* for 48 hours duration

Plant piscides	Conc (g/L)	LC50	%mortality/hour					
			0	1	12	24	36	48
Tobacco *Nicotiana tobaccum*	0.00	0.83	0	0	0	0	0	0
	0.25		0	0	5	10	15	25
	0.50		0	5	10	20	25	40
	1.50		0	10	25	40	65	80
	2.50		0	15	30	60	80	100
Neem *Azadirachta indica*	0.00	1.64	0	0	0	0	0	0
	0.50		0	0	5	5	10	20
	1.50		0	5	5	15	20	35
	2.00		0	10	15	30	40	65
	2.50		0	10	25	40	60	90
Pawpaw *Carica papaya*	0.00	2.16	0	0	0	0	0	0
	1.00		0	0	5	10	20	30
	2.00		0	5	10	15	30	40
	3.00		0	15	20	35	45	70
	4.00		0	15	25	40	65	90
Cassava *Manihot esculenta*	0.00	1.22	0	0	0	0	0	0
	0.50		0	0	5	5	10	20
	1.00		0	0	10	15	25	45
	1.50		0	10	20	35	45	60
	2.50		0	15	25	45	65	100
Almond *Terminalia catappa*	0.00	2.44	0	0	0	0	0	0
	1.50		0	0	5	5	15	25
	2.00		0	5	5	20	35	45
	2.50		0	10	15	30	45	65
	3.50		0	15	20	40	60	80S

significant differences ($p<0.05$) among the treatments except for the mchc in tobacco and cassava (Tables 3a and c), mcv, mch and mchc in almond and pawpaw (Tables 3b and d) and mcv and mchc in neem (Table 3e) which showed no differences. However, the values of the haematological parameters of the fish exposed to

Table 3a-e. Comparative toxicities of five plant biocides on white tilapia, *Oreochromis niloticus*

a tobacco

Heamatological parameters	Conc. control	Of 0.10	Tobacco 0.15	Leaves 0.20	Extracts (g/i) 0.25
PCV (%)	11.50	11.00	10.50	9.50	8.00
Hb (g/dl)	3.30	3.10	2.60	2.10	2.00
Rbc (mm^3*10^6)	1.25	1.20	1.15	1.00	0.90
MCV (μ/m^3)	78.50	78.50	78.00	76.50	73.50
MCh (µ/mg)	25.50	25.50	25.00	23.50	23.00
MChC (%)	30.50	30.50	30.00	29.50	29.50
TP (mg/dl)	15.00	14.50	14.00	12.50	10.00
Glucose (mg/dl)	13.00	11.00	10.00	8.50	7.00

b almond

Heamatological parameters	Conc. control	Of 0.50	Almond 0.70	Leaves 0.90	Extracts (g/i) 1.00
PCV (%)	11.50	11.40	11.30	11.00	10.40
Hb (g/dl)	3.30	3.30	3.10	3.00	2.60
Rbc (mm^3*10^6)	1.25	1.24	1.20	1.15	1.10
Mcv (μ/m^3)	78.50	78.50	78.30	78.00	77.60
Mch (µ/mg)	25.50	25.50	25.30	25.10	24.70
MChC (%)	30.50	30.50	30.40	30.30	30.00
Tp (mg/dl)	15.00	15.00	14.60	14.00	13.00
Glucose (mg/dl)	13.00	12.00	11.80	10.50	10.20

c cassava

Heamatological parameters	Conc. control	Of 0.15	Cassava 0.20	Leaves 0.35	Extracts (g/i) 0.45
PCV (%)	11.50	11.20	10.70	10.20	9.00
Hb (g/dl)	3.30	3.20	2.80	2.30	2.10
Rbc (mm^3*10^6)	1.25	1.22	1.18	1.13	0.95
Mcv (μ/m^3)	78.50	78.50	78.10	77.50	75.00
Mch (µ/mg)	25.50	25.50	25.20	24.00	23.50
Mchc (%)	30.50	30.50	30.20	29.80	29.60
Tp (mg/dl)	15.00	14.70	14.20	13.00	12.20
Glucose (mg/dl)	13.00	12.50	11.00	9.00	8.00

d pawpaw

Heamatological parameters	Conc. control	Of 0.30	Pawpaw 0.50	Leaves 0.70	Extracts (g/i) 1.00
PCV (%)	11.50	11.35	11.20	11.00	10.10
Hb (g/dl)	3.30	3.30	3.00	2.80	2.50
Rbc (mm^3*10^6)	1.25	1.24	1.20	1.14	1.00
Mcv (μ/m^3)	78.50	78.50	78.30	77.80	77.20
Mch (µ/mg)	25.50	25.50	25.30	25.00	24.50
Mchc (%)	30.50	30.50	30.35	30.10	29.80
Tp (mg/dl)	15.00	15.00	14.50	13.80	12.30
Glucose (mg/dl)	13.00	12.80	11.50	10.30	10.00

concentrations of tobacco were the least, while those of the almond were the largest. This showed that extract of tobacco was the most potent, while that of almond was the least.

DISCUSSION

The slight fluctuations of the physio-chemical parameters in the different treatments showed no significant

e neem

Heamatological parameters	Conc. control	Of 0.10	Neem 0.20	Leaves 0.35	Extracts (g/i) 0.50
PCV (%)	11.50	11.30	11.00	10.50	9.80
Hb (g/dl)	3.30	3.15	2.90	2.35	2.20
Rbc (mm^3*10^6)	1.25	1.23	1.18	1.10	0.98
Mcv (μ/m^3)	78.50	78.50	78.20	77.70	76.20
Mch (µ/mg)	25.50	25.50	25.25	24.60	24.00
Mchc (%)	30.50	30.50	30.30	30.00	29.65
Tp (mg/dl)	15.00	14.85	14.35	13.50	12.60
Glucose (mg/dl)	13.00	12.60	11.20	10.10	9.50

difference and the effects on this study could be negligible. However, the wide fluctuations with significant difference might have been altered by the experiment and hence produced deleterious effect such as stressful conditions of abnormal behaviours prior to death and mucus secretion on the gills of the moribund fish. Konar (1970), Steels (1983), Fafioye and Jeje (2000) and Fafioye (2002) reported that accumulation of mucus on fish gills reduces respiratory activity in fish and this might have accounted for mortality. Tobacco had been reported to have nicotine (Hassal, 1982) which binds to acetyl-choline receptors in the nervous system thus causing the excitation (Omoniyi *et al*., 2002).

The lethal toxicities of the various botanicals on *Oreochromis niloticus* produced contrasting LC_{50} results in line with the differences in the potenticity of the plants' extracts (Steel and Torrie, 1980). The 48-hour LC_{50} recorded for the five botanicals were either above or lower than the values of other workers. For instance, the 48-hour LC_{50} of tobacco on *O. niloticus* was 0.83 g/l as against 1.09 g/l recorded for the same fish by Agbon *et al* (2002) thus indicating that this result is more acute. The high lethality might be attributed to partly tolerance/vulnerability of the size and type of fish and the more potent ingredients of tobacco used. However, the toxicities of other plants' extracts on fish having similar results have been reported such as *Blighia sapida* and *Kigelia africana* on *C. gariepinus* (Onusiriuka and Ufodike, 1994 and 1998), *Parkia biglobosa* and *Raphia vinifera* on *C. gariepinus* and Tilapia (Fafioye *et al.,* 2004), Tobacco on *O. niloticus* and *C. gariepinus* (Agbon *et al*., 2002 and Omoniyi *et al*., 2002), *Raphia hookeri* on *C. gariepinus* (Adeogun *et al*., 2002).

The sub-lethal toxicity on the haematology of *O. niloticus* which revealed statistically significant ($p<0.05$) decreases in values was common in exposed fish to chronic concentrations of toxicants. Similar reduction of haematological indices had been reported by Omoregie *et al* (1994), Fafioye and Adebisi (2000), Fafioye and Jeje (2001), Adeogun *et al* (2002), Fafioye (2002, 2005 and 2007), in their respective experiments on fish. Moreover, the reduction in these blood metabolites is an indication of anaemia caused by exposure to the extracts of the used botanicals. This anaemic response of fish might be attributed to destruction of erythrocyte or inhibition of erythrocyte production (Wintrobe, 1978) or haemodilution (Sampath *et al*., 1993).

This study confirms that the extracts of the five botanicals exert piscicidal property, which means that they can be used on pond management to eradicate stunted and weed fish populations for healthy production of desirable fish species for the nation.

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