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Effect of Aqueous Leaf Extracts of *Lepidagathis alopecuroides* on the Behaviours and Mortality of Hybrid Catfish (*Heterobranchus bidorsalis* ♂ X *Clarias gariepinus* ♀) Fingerlings

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Abstract: Lethal renewable bioassay was conducted to evaluate the effect of aqueous extracts of leaves of *L. alopecuroides* (0.00, 0.5, 1.0, 1.5, 2.0, 2.25 mg/l) on the behaviours and mortality of African catfish hybrid (*H. bidorsalis* ♂ x *C. gariepinus* ♀) fingerlings (total length, 7.34±1.78cmSD; mean weight, 2.33±1.96 gSD). Exposed fish were hyperactive, swimming erratically, showed hyperventilation and rapid tail beat with increased mucus secretion on the skin and gills, gulped for air and became listless at he bottom of the aquaria before death occurred. Opercular beat frequency (OBF) and Tail beat frequency (TBF) increased with increase in the concentration of the chemical but decreased with exposure duration. The 96hrsLC_{50} and safe concentration values were 0.77 and 0.08 mg/l, respectively. The median lethal time (MLT_{50}) for the 0.50 and 2.25 mg/l concentration were 87.72 hrs and 29.72hrs, respectively. The positive linear correlation between mortality, concentration and exposure duration show that mortality was influence by exposure duration and concentration. The low lethal concentration values recorded in this study suggest that *L. alopecuroides* is highly toxic to catfish hybrid fingerlings.

Key words: *Lepidagathis alopecuroides*, African catfish hybrid, behaviour and mortality

INTRODUCTION

Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties (Cagauan *et al*., 1992). Plants from different families have been applied for catching fish all over the world. The toxic parts of plants employed as fish poisons include the roots, seeds, fruits, barks or leaves. Plant extracts used as piscicides in fisheries are considered advantageous when viewed against the backdrop of using persistent chemicals. Piscicidal plant are frequently used by fishfolks to catch fishes because they are readily available and highly toxic to fish. Frequent applications of high concentrations of these ichthyotoxins in water may have adverse effects not only on fish species but also on other aquatic fauna. Ichthyotoxic plants used for baiting or stupefying fish are often crushed and cast into stagnant, slow moving water or spread on mud flats to poison fish . Several studies have shown that these plant toxins at low concentrations are very toxic to all groups of aquatic fauna (Tiwari and Singh, 2003; Goktepe *et al.,* 2004*;* Kreutzweiser, *et al.,* 2004*);*

L. alopecuroides is a tropical shrub belonging to the family Acanthaceae and commonly found in the coastal countries of West Africa. The leaves are used to immobilized or kill fish in many communities of Cross River and Rivers States of Nigeria. In Rivers State it is used for quick kill of hardy fishes like mudskippers (Obomanu *et al*., 2007) and the clariids and in Cross River it is normally applied in pools to kill mostly tilapias and catfishes constituting the major source of dietary protein (Ekanem *et al*., 2003). The phytochemistry of the plant revealed it contains flavinoids, saponins tannins, glycosides and alkaloids (Obomanu *et al*., 2005). which may have accounted for the reported toxicity to mosquito larvae (Obomanu *et al*., 2006)

The leaves of the plant are indiscrimately used for catching fish in many water bodies in several parts of the country, yet there are no documented effects of the plant materials on important fish species such the clariids. Since the extracts demonstrate high levels of toxicity to fish, it becomes necessary to study the effects of aqueous extracts of the leaves of *L. alopecuriodes* on the behaviours (opercular beat frequency/min., OBF and tail beat frequency/min., TBF) and mortality of the hybrid fingerlings exposed to acute concentrations of the extracts.

MATERIALS AND METHODS

Fingerlings of hybrid (mean weight, 2.33±1.96 gSD; total length, 7.34±1.78 cmSD) fingerlings were obtained from Idomor Farms Ltd. Olomoro, Delta State, Nigeria. They were transported in plastic jerry cans to Fisheries

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Wet Laboratory, Rivers State University of Science and Technology, Port Harcourt, Nigera. Fish (50) was acclimated in 40*l* borehole water (characteristics: temperature, 26.71±1.92ºC; dissolved oxygen, 4.7±1.37 mg/l; pH, 7.12±1.21; total alkalinity, 18.3±2.32 mg/l as $CaCO_{3;}$ total hardness, 17.77±1.44 mg/l) per rectangular aquarium (60x 30 x30cm^3) for one week. The water in the aquaria was renewed daily and the fish were fed twice a day on a 45% crude protein at 1% body weight. Fishes were not fed 24hrs before the commencement of and during experiment to minimize contamination of the test media. The plant material was obtained from Ogbakiri in Emohua, Rivers State. They were sun dried for five days. The dried leaves were then pulverized with a sterile grinding machine and sieved with 100-micron sieve to obtain a fine powder. This was stored in dry airtight container.

A range finding test was conducted to determine the concentrations to be used in the actual experiment using standard procedure following the methods of APHA (1998). Based on the results of the range finding test five concentrations (0.50, 1.00, 1.50, 2.00 and 2.25 mg/l) of the extracts and a control (0.00 mg/l) were prepared in glass aquara in triplicates. Ten fish was randomly introduced into each of the aquaria. Test solutions and water in the control were renewed daily.

OBF and TBF and mortality were recorded at the 12th, 24th, 48th, 72nd and 96th hr. Other abnormal behaviours were also recorded. Dead fish was promptly removed from the aquaria to avoid contamination of the test media. Presence of mucus on the skin and gills of experiment was also checked by feeling with the fingers. Data obtained from the experiments (OBF min^{-1}, TBF min^{-1} and cumulative mortality) were subjected to ANOVA and differences among means were separated by Duncan multiple range test (Zar, 1984) at $p<0.05$. OBF min^{-1}, TBF min^{-1} and cumulative mortality were regressed on concentrations of the extracts and exposure duration and coefficient of determination and correlation coefficient recorded (Zar, 1984). An analysis of the lethal concentration (LCs) and median lethal time (MLTs) with associated confidence interval were done with Probit Analysis (Finney, 1971) using probit model. Safe concentrations at the various time intervals were obtained by multiplying the lethal concentration (LC_{50}) by a factor of 0.1 (EIFAC, 1983).

RESULTS

Hybrid fingerlings were stress progressively with time before death. The stress signs included erratic swimming, increased opercular ventilation, and increased mucus secretion on the skin and gills. The OBF and TBF of experimental fish at the various concentrations of the extracts declined with exposure duration (Fig. 1 and 2). The OBF became more variable from the 24thhr; whereas TBF was more variable from the 12th up to the 72nd h. Cumulative mortality was very variable at the duration of exposure (Fig. 3). At the exposure concentrations of the extracts OBF and TBF did not differ ($p>0.05$) but cumulative mortality did, $p<0.01$ (Table 1).

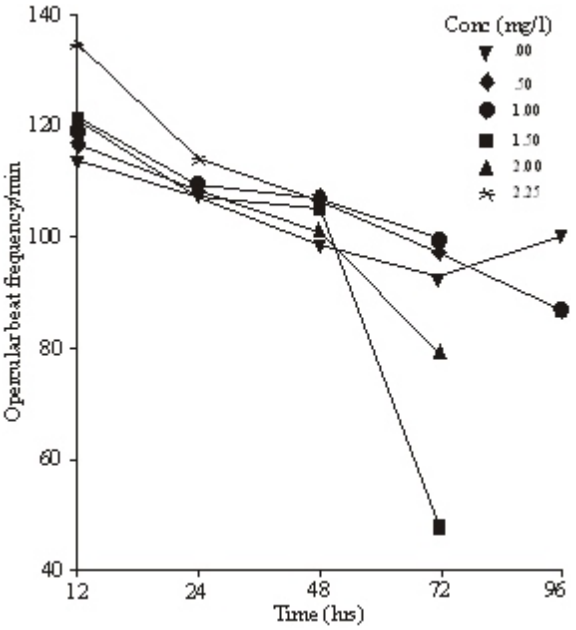

Fig. 1: Changes in the tail beat frequency of *H. bidorsalis* fingerlings under acute concentrations of aqueous extracts of *L. alopecuriodes* leaves.

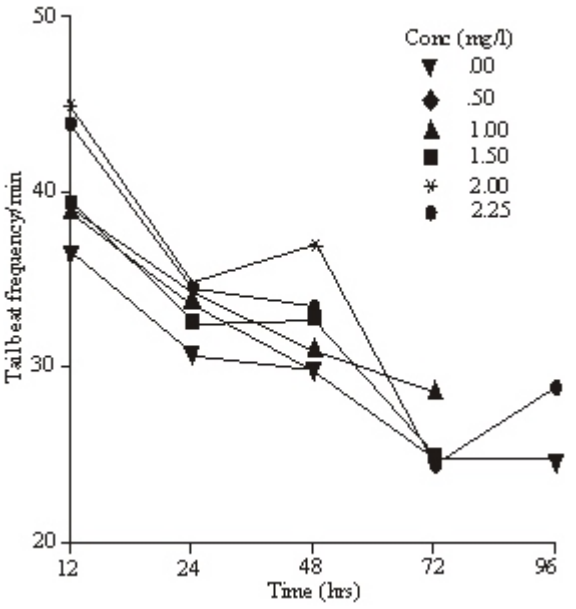

Fig. 2: Changes in the opercular beat frequency of *H. bidorsalis* under acute concentrations of aqueous extracts of *L. alopecuriodes* leaves

No mortality was recorded in the control, however, in treated fish mortality increased as concentration and exposure duration increased. One hundred (100%) of the fish was killed in 0.5, 2.0 and 2.25mg/l at the 96th, 48th,

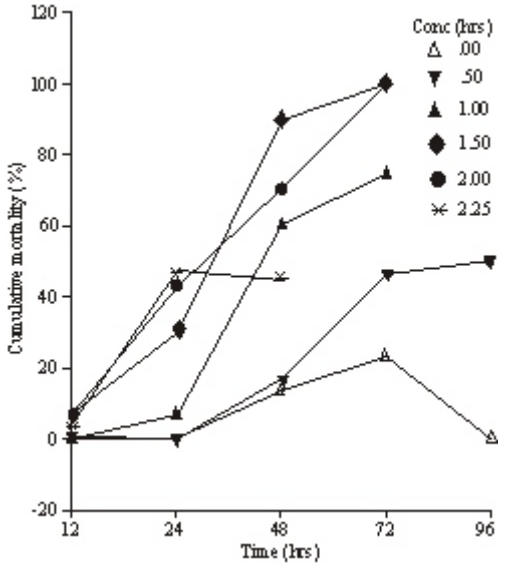

Fig. 3: Changes in cumulative mortality of *H. bidorsalis* under acute concentrations of aqueous extracts of *L. alopecuriodes* leaves

and 24th hours respectively (Fig. 3). Wide variations were recorded in the OBF, TBF and cumulative mortality at the various time intervals (Table 2). Regression analysis showed that the relationship between OBF, TBF and exposure duration was negative but positive with mortality (Table 2). That between OBF, TBF, mortality and exposure was positive (Table 2). The 24 and 96hr LC_{50} were 1.30 and 0.77mg/l and their 96hrs safe concentrations, 0.01 and 0.08 mg/l, respectively. The 24hrs LC_{50} for *L. alopecuriodes* was 1.95 times greater than the 96hrs LC_{50} value (Table 3). The mean lethal time (MLT_{50}) for 0.5, 1.5 and 2.25 mg/l were 83.57, 26.02 and 19.49hrs respectively (Table 4). The MLT_{50} decreased with increase in thconcentration of *L. alopecuriodes*.

DISCUSSION

Studies have revealed that fish exposed to toxicants usually exhibits some behavioral changes such as increased opercular rate, erratic swimming, mucus secretion and gulping for air before death (Davis, 1973; Nwanna *et al*., 2000). The pattern of behavioural changes observed in this study compared favorably with the records of Fafioye *et al.* (2004) when African catfish (*C. gariepinus*) was exposed to *Parkia biglobossa* and *Raphia vinefera* extracts and catfish hybrid fingerlings treated with cassava mill effluents (Oti, (2002). Increased concentrations of *L. alopecuriodes* led to increased OBF, TBF and mortality as was also similarly observed in *C. gariepinus* exposed to aqueous extracts of *Blighia sapida* and *Kigelia africana* (Onusiriuka and Ufodike, 1994), catfish hybrid exposed to *Thevetia peruviana* (Oti and Ukpabi, 2000), and 2,4ichlorophenyloxyacetic acid (Koffi, 2005) and quick killed of mudskippers (*Periophthalmus papillio*) exposed to *L. alopecuriodes* (Obomunu *et al*., 2007). The marked deviation in the rate of OBF and TBF from reference (control) suggests an adjustment in physical fitness as a result of the stress condition (Edwards and Fushur, 1991; Leight and Van Dolah, 1999).

The value of 96hrsLC_{50} of 0.59mg/l reported in this study is much lower than those earlier reported by Oti and Ukpabi (2000) and Fafioye *et al.* (2004) for some clariid species exposed to some other plant extracts. This observation implies that *L. alopecuriodes* is more toxic to African catfish hybrid than *T. peruviana*, *P. biglobossa* and *R. vinifera*. Mucus production and accumulation on the gills may have contributed immensely to the increase in the opercular ventilation and mortalities recorded in this study. Konar (1970) reported that accumulation of mucus on the gills reduces respiratory activity in fishes. This might be due to inability of the gills surface to actively carry out gaseous

Table 1: Opercular and tail beat frequency and cumulative mortality (%) of fingerlings of hybrid exposed to various concentrations of *L. alopecuroides* for 96hrs

Variable	Concentration of *L. alopecuroides* (mg/l)					
	0.00	0.50	1.0	1.50	2.00	2.25
OBF/min.	102.72	103.32	109.47	104.73	109.08	120.05
	±10.05^{b}	±13.41^{b}	±8.02^{b}	±25.82^{b}	±16.42^{b}	±20.70^{a}
TBF/min.	29.30	31.00	33.53	34.28	37.51	37.73
	±7.42^{b}	±6.09^{b}	±5.56^{ab}	±8.00^{ab}	±8.07^{a}	±9.90^{a}
Cum.mortality	0.00	22.67	31.82	45.00	40.00	30.00
	±0.00^{C}	±27.38^{ab}	±37.64^{a}	±45.04^{a}	±39.28^{a}	±32.95^{a}
	Duration of Exposure (hrs)					
	12	24	48	72	96	
OBF/min.	121.22	109.52	104.54	93.63	89.67	
	±10.05^{a}	±10.79^{b}	±10.51^{b}	±17.36^{c}	±7.99^{c}	
TBF/min.	40.42	33.43	31.53	25.30	26.75	
	±8.08^{a}	±3.80^{b}	±4.92^{bc}	±2.36^{d}	±7.25^{cd}	
Cum.mortality	2.78	20.59	43.57±	56.00	25.00b	
	±5.75^{d}	±27.04^{cd}	37.95^{ab}	±40.06^{a}	±29.50^{c}	

Means with the same superscript in the row are not significantly different (p>0.05)

Table 2: Regression lines for the prediction of the values of OBF/min., TBF/min. and cumulative mortality of Hybrid exposed to *L. alopecuriodes* for 96 hrs

Independent Variable	Dependent variable	Regression Equation	Coefficient of determination (r^2)	Correlation coeffeicient (r)	Significance level
Time	OBF	y=120.92-0.34x	0.84	0.91	***
Time.	TBF	y=39.45-0.16x	0.81	0.90	***
Time	Mort.	y=13.02+0.34x	0.30	0.55	ns
Conc.	OBF	y=101.28+5.75x	0.60	0.78	****
Conc.	TBF.	y=29.28+3.83x	0.98	0.99	***
Conc.	Mort.	y=11.29+14.03x	0.59	0.77	ns

Where y= independent variable (concentration, Time), x= dependent variable ($OBFmin^{-1}$, $TBFmin^{-1}$ and mortality).
F- test significance level: * 0.05, ** 0.01, *** 0.001,****0.0001,
ns= not significant r^2 = coefficient of determination, r= coefficient of correlation

Table 3: Lethal concentration (LCs) and safe conc. of *L. alopecuroides* on hybrid fingerlings for 96 hours

Exposure duration (hrs)	Lethal Conc. and associated 95% C.L			Safe Conc.	D/f	Probit equation	T.F	Sign. Level
	LC_5	LC_{50}	LC_{95}					
24	0.14 -(4.29-0.76)	1.15 (0.095-1.67)	2.17 (1.94-7.31)	0.12	3	Y= -1.87+1 .62x	1	**
48	-0.41 (0.00-0.00)	0.88 (0.00-0.00)	2.16 (0.00-0.00)	0.09	3	Y= -1.12 +1.28x	1.32	***
72	-0.34 (0.00-0.00)	0.66 (0.00-0.00)	1.64 (0.00-0.00)	0.07	3	Y= -1.09+ 1.66x	1.76	***
96	0.149 (0.26-0.33)	0.59 (0.46-0.70)	1.04 0.89-1.36)	0.06	3	Y= -2.20 + 3.71x	1.95	ns

C.L= Confidence Limit, D/F = Degree of freedom, LC= Lethal Concentration,
T.F= Toxicity factor =LC_{50} value at 24hrs/ LC_{50} value of any other periods

Table 4: Mean Lethal Times (MLT_{50} and MLT_{95}) and associated 95% confidence limit of hybrid exposed to *L. alopecuroides* for 96hrs

Conc. of extracts (mg/l)	MLT and associated 95% C.L		D/f	Probit equation	Sign.	R.T
	MLT_{50}	MLT_{95}				
0.5	87.719 (78.145-102.791)	144.13 (122.87-187.21)	3	Y=-2.558 +0.029x	ns	1
1.0	53.978 (48.336-59.872)	85.40 (76.90-98.74)	3	Y=-2.826 +0.052x	ns	1.63
1.5	53.98 (48.34-59.87)	85.40 (76.90-98.74)	3	Y=-2.826 +0.052x	ns	1.63
2.0	32.55 (15.75-47.89)	66.10 (49.96-126.18)	2	Y=-1.596 +0.049x	ns	2.69
2.25	29.72 (25.23-34.54)	52.68 (45.66-65.03)	3	Y=-2.129 +0.075x	ns	2.95

C.L= Confidence Limit, D/F = Degree of freedom, S.L =Significance Level;
MLT=Mean Lethal Time, R.T=Relative Time = MLT_{50} values at 0.5mg/l/; MLT_{50} value at any other concentration.

exchange. The observed restlessness and mortalities of the test fish might be due to the effect of flavinoids, alkaloids and saponins present in the extracts (Obomanu *et al*., 2005). Saponins are ichthyotoxins which destroys the erythrocytes and is assimilated directly through the gills (Elpel, 2000). Alkaloids on the other hand inhibit oxidative phosphorylation, blocks the mitochondrial enzymes, NADH ubiquinone reductase, hence impairing their oxygen consumption (Bocek, 1984; Tiwari and Singh 2003). Olaifa *et al.* (2004) and Omitoyin *et al*., (2006) reported a 96hrLC_{50} of copper as 0.67mg/l and lindane 0.38mg/l for *C. gariepinus* respectively stating that they are highly toxic. Hence *L. alopecuriodes* is highly toxic to African catfish hybrid. The mean lethal time (MLT_{50}) for 0.5, 1.5 and 2.25mg/l to fingerling to the fish were 83.57, 26.02 and 19.49hrs respectively, declined with toxicant concentrations indicating that the survival time for the fish declined with increase in the concentration of the toxicant.

This study revealed that *L. alopecuriodes* exerts piscicidal property and are highly toxic to the hybrid. It acts as respiratory poison possibly affecting the gills, impairing respiration and the various abnormal behaviours and eventually death. These effects on are directly proportional to the toxicant concentrations. Hence, the use of this toxicant in aquatic environment needs proper control to avoid reduction in fish production and non-target aquatic fauna. More study be carried out on the mode of action of the biocide in effecting the fast mortality recorded in the exposed fish.

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