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BEHAVIOURAL RESPONSES AND MORTALITY OF *Clarias gariepinus* EXPOSED TO ACUTE LEVELS OF *Lepidagathis alopecuroides* (Vahl)

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ABSTRACT

The toxicity of L. alopecuriodes dried leaves (0.50, 1.00, 1.50, 2.00 and 2.25mg/l) on African catfish, Clarias gariepinus (mean body weight, 2.33±1.98gSD); total length, 7.34±1.78cmSD) were assessed in a renewable bioassay after 96hrs of exposure. Toxic reactions exhibited by the fish included hyperventilation, erratic swimming, gasping for air, loss of reflex and death. Opercular beat frequency (OBF) and Tail beat frequency (TBF) decreased with increase in time of exposure but increased with concentration. Cumulative mortality increased with increase in both concentration and time of exposure. There were significant interactions between concentration and time on OBF. Concentration of the extract shows a positive linear correlation with OBF, TBF and mortality. The 96hrLC$_{50}$ and safe concentration were 0.653mg/l and 0.07mg/l, respectively, whereas the MLT$_{50}$ for the highest concentration (2.25mg/l) was 17.25(8.47-23.16 C.L.). The low 69hrLC$_{50}$ recorded for L. alopecuriodes on C. gariepinus indicates that it is highly toxic and the behavioural changes in the exposed fish suggest that these variables can be used to assess stress caused by the plant material even before dead occurs.

Key words: *Lepidagathis alopecuroides, Opercular beat frequency, Tail beat frequency, mortality*

INTRODUCTION

Many toxicants can disrupt the internal physiology of fish leading to complex behavioural changes which may be reflected in predator avoidance, reproduction, and social behaviours (Scott and Sloman, 2004). Since behaviour links physiological functions with ecological processes, behavioural indicators of toxicity appear ideal for assessing the effects of aquatic pollutants on fish populations. The main sources of freshwater pollution are from discharge of untreated wastes and industrial effluents, run-offs and drifts of herbicides/pesticides from agricultural fields and direct application to aquatic weeds, and the use of ichthyotoxins by fishery managers (Davies and Shelton, 1992; Duman, *et al.*; 2001).

Ichthyotoxic plants play significant roles in fisheries managements and aquaculture. For example they are used for assessment of fish stock (Brandt, 1984; Davies and Shelton 1992), stun and catch fish (Obomanu, *et al*., 2007), and pond cleansing of unwanted fish species and competitors (Nguenga, *et al.* 2000) and molluscs (Singh, *et al*., 2005). Assessment of the effects of these biocides on fish indicate that exposed fish demonstrated altered behaviours, disruptions of internal biochemical and physiological processes and in severe cases may lead to death (Omoniyi *et al.,* 2002*;* Fafioye *et al*., 2004*;* Singh and Singh, 2003; Tiwari and Singh, 2003). *Lepidagathis alopecuriodes* (Family Acanthaceae) is a shrub whose piscicidal potentials have been established in various parts of Nigeria (Reed *et al.,* 1967; Obomanu, *et al.,* 2005; 2007). It was reported to cause altered fish behaviours (Okey, 2007) and internal biochemistry (Emetonjor, 2006). Photochemical screening of the plant shows the presence of alkaloids, saponins, tannins, glycosides and flavonoids (Obomanu *et al*., 2005) which may have been responsible for the activity of the plant in fish.

Clarias gariepinus is one of the commercially important species next to tilapia in Nigeria and elsewhere in the developing world. The fish has fast growth rate and are desirable as food in tropical and sub-tropical countries, where it is extensively fished and cultured (Ugwumba and Ugwumba, 2003). The effects of pollutants on fish are assessed by acute and chronic toxicity tests (Heath, 1991). This is a quick and effective means of assessing the toxicity of commonly used piscicidal plants like *L. alopecueroides*. However, the toxicity and effects of the leaves of *L. alopecuroides* on the behaviours of *C*. *gariepinus* have been not been reported. This study was conducted to determine the behavioural responses such as opercular beat freguency (Obf), tail beat freguency, (Tbf) and lethal concentrations (LCs), median lethal times (MLTs) for freshwater catfish, *C. gariepinus* exposed to acute concentrations of aqueous extracts of leaves of *L. alopecuroides.*

MATERIALS AND METHODS

The African catfish, *C. gariepinus* fingerlings (mean body weight (2.33±1.98gSD) and length, (7.34±1.78cmSD) were obtained from Idomor Farm Ltd., Olomoro in Delta State, Nigeria and acclimated to laboratory conditions in rectangular glass aquaria for seven days. Bioassay was conducted in the Fisheries Wet Laboratory, Rivers State University of Science and Technology following the protocols in ASTM (1994) with few modifications. The water in the aquaria was renewed daily and the fish were fed with commercial fish feed (pellets) containing 40% crude protein at 1% of their body weight. Fish were exposed to 0.00 (control), 0.50, 1.00, 1.50, 2.00 and 2.25mg/l crude extracts of *L. alopecuroides* in triplicates in 10 fish/30l test solution per aquarium. The experiment lasted for 96 hours and test solutions and water in the control were renewed daily.

The behaviours (obf and tbf per min.), mortality and other external changes in the body of the test fish were observed and recorded at 12, 24, 48, 72 and 96hr of exposure duration. Dead fish were promptly removed to avoid contamination of the test media. The bodies of dead fish were also felt with the finger to assess the amount of mucus produced. Data obtained from the experiment were subjected to ANOVA and where differences existed, Duncan Multiple Range Test was used to separate them at 95% probability (Wahua, 1999). Obf, tbf and cumulative mortality were correlated and regressed on exposure concentrations and duration of exposure.

An analysis of the lethal concentrations (LCs) and median lethal times (MLTs) with associated confidence interval were done with probit analysis (Finney, 1971). Safe concentrations at the various time intervals were obtained by multiplying the lethal concentration (LC_{50}) by a factor of 0.1 and relative time (MLT_{50} at the lowest concentration/ MLT_{50} at any other concentration) were obtained according to Chukwu (2005).

RESULTS

Fish in the toxicant showed a variety of abnormal behaviours which included dash movements, rapid opercular beat rate, loss of balance, excessive mucus production before death The obf and tbf of exposed fish at the various concentrations of the toxicant declined with exposure duration (Figs. 1 and 2), however, the reverse was the case with mortality (Fig. 3). Less than 40% mortality was recorded for 0.50mg/*l* by the 96th hour, while the threshold concentrations that killed 100% of exposed fish were 2.0mg/l and 2.25mg/l both at the 48th hour. No mortality was recorded in the control during the exposure period. ANOVA (Table 2) showed that the obf ($p<0.05$) at the various concentrations of *L. alopecuroides* did not differ, but tbf ($p<0.01$) and cumulative mortality ($p< 0.001$) did. Obf ($p<0.001$), tbf ($p<0.001$) and cumulative mortality ($p< 0.001$) were impacted variously at the exposure durations. Interactions between the exposure time and concentrations of the toxicant affected mortality ($p< 0.001$), but not the obf and tbf, $p>0.05$ (Table 1). The association between obf ($r= 0.92$), tbf ($r= 0.97$) and concentration was positive, whereas it was negative with exposure duration (obf, $r= -0.99$; tbf, $r=-0.87$, Table 2). Mortality was positively correlated with concentration and exposure duration (Table 2).

The lethal effects of *L. alopecuroides* on *C. gariepinus* fingerlings expressed as LC_5, LC_{50} and LC_{95}, respectively for 24, 48, 72 and 96hr LC_{50} and associated 95% (EIFAC, 1983). Toxicity factor (LC_{50} value at 24hr/ LC_{50} value of any other period) confidence limits, C.L of the toxicant concentrations (Table 3) indicated that the range (0.08mg/l) of the values

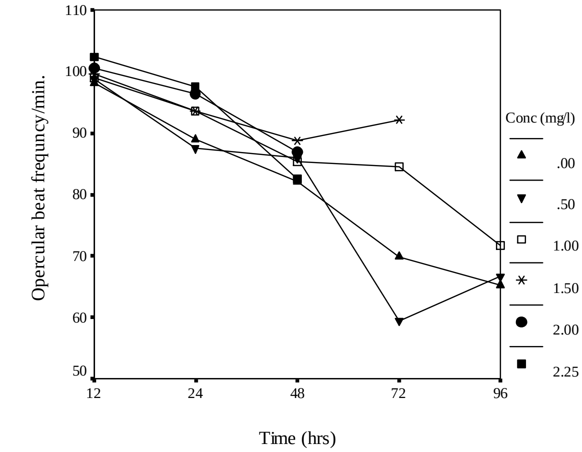

Fig.1. Effect of interactions between concentration of *L. alopecuriodes* on the opercular beat frequency of *C. gariepinus*

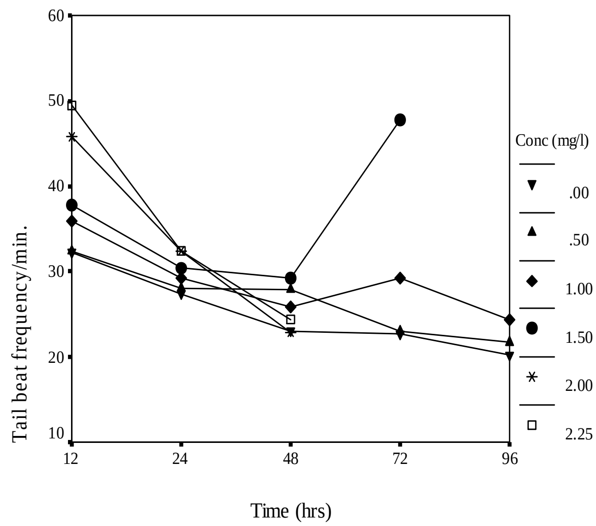

Fig.2. Effect of interactions between concentrations of *L. alopecuriodes* and time of exposure on the tail beat frequency of *C. gariepinus.*

the variation (0.08mg/l) between the 24hr (1.45mg/l) and 96hr LC_{50} (0.65mg/l) was very narrow. The safe concentrations at 96hrLC50 of *L. alopecuroides* (0.15mg/l for 24 hr; 0.07mg/l for 96 hour) on *C. gariepinus* fingerlings were very low. The regression equation indicated for any increase in the values of concentrations at the 24 and 96hr of exposure time, percentage cumulative mortality was expected to increase by amount of 1.15 and 4.22% respectively. The toxicity factor (T.F.) of the various concentrations increased with duration of exposure from 24 to 96hr. LC_{50} value at 24 hours was 2.2 times greater than that at 96hr.

The time it took for half of the exposed fish to die (MLT_{50}) at the various exposure concentrations decreased with duration, with the least concentration (0.5mg/l) killing half of the exposed fish at about 3.4x less than the time it took for 2.25mg/l of the toxicant (Table 4). The lethal concentration and MLT values appeared to have an inverse relationship with exposure concentrations and duration. Both LC_{50} and MLT_{50} values declined with increase in concentrations toxicant and exposure duration, respectively (Table 4).

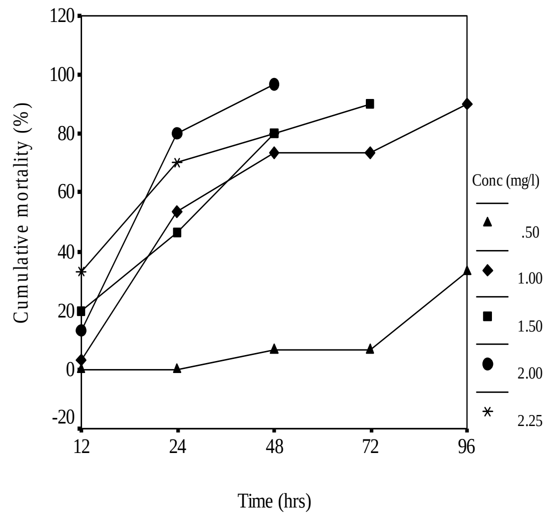

Fig.2. Effect of interactions between concentrations of *L. alopecuriodes* and time of exposure on cumulative mortality of *C. gariepinus*

Table 1.Obf and tbf and cumulative mortality (%) of *C. gariepinus* fingerlings exposed to various concentrations of *L. alopecuroides* for 96hrs

Variable	Concentrations of *L. alopecuroides* (mg/l)					
	0.00	0.50	1.0	1.50	2.00	2.25
Obf/min. ^{ns}.	80.90 ±16.76^{bc}	79.67 ±16.76^{c}	89.10 ±10.35^{ab}	93.73 ±9.63^{a}	94.62 ±8.76^{a}	97.46 ±7.88^{a}
Tbf/min.**	25.10 ±5.62^{c}	26.62 ±4.42^{c}	29.64 ±6.85b^{c}	34.03 ±7.70b^{c}	33.71 ±10.10^{ab}	38.56 ±8.96^{a}
Cum. mortality***	0.00 ±0.00^{b}	9.33 ±15.34^{b}	53.85 ±32.54^{a}	53.00 ±30.20^{a}	63.33 ±40.31^{a}	55.71 ±25.73^{a}
	Duration of exposure (hrs)					
	12	24	48	72	96	
Obf/min. ***	99.70 ±6.12^{a}	92.93 ±8.56^{ab}	85.57 ±9.32^{b}	73.56 ±13.16^{bc}	66.82 ±13.60^{c}	
Tbf/min. ***	38.93 ±10.26^{a}	30.01 ±4.73^{b}	25.71 ±4.05b^{c}	27.30 ±8.79^{b}	21.41 ±2.25^{c}	
Cum. mortality***	11.67 ±15.81^{d}	41.67 ±34.85^{b}	53.12 ±41.27^{a}	33.00 ±39.46^{bc}	27.14 ±33.52^{c}	

Obf-opercular beat frequency, Tbf-tail beat frequency; Cum. mort.-cumulative mortality (%). F-test significant level: ns- not significant, **- 0.01,***- 0.001. Means with the same superscript in the same row are not significantly different (p<0.05)

DISCUSSION

The study revealed that exposed fish showed stress responses such as increase opercula rate, erratic swimming, gasping for air and general hypersensitivity, shown to be sensitive indicators of physiological stress in fish subjected to pollutants (Davis, 1973). Plants biocides and other toxicants even at low concentrations are known to impair

Table 2. Regression lines for the prediction of the values of obf, tbf and cumulative mortality of *C. gariepinus* exposed to *L. alopecuroides* for 96 hrs

Independent Variable	Dependent variable	Regression equation	(r^2)	(r)	Sign.ificant level
Time	TBF	y=37.02-0.17x	0.76	0.87	**
Conc.	TBF	y=24.84 +5.63x	0.93	0.97	***
Time	OBF	y=103.48- 0.39x	0.99	0.99	***
Conc.	OBF	y=78.21 +8.72x	0.84	092	***
Time	Mort.	y=30.39 +0.06x	0.02	0.13	ns
Conc.	Mort.	y=5.58 +27.82x	0.80	0.89	ns

Where y= independent variable (concentration, Time), x= dependent variable ($OBFmin^{-1}$, $TBFmin^{-1}$ and mortality. F- test significance level: ns-non-significant, **- 0.01, ***- 0.001; r^2 = coefficient of determination, r= coefficient of correlation

Table 3. Relative toxicity of *L. alopecuroides* on *C. gariepinus* fingerlings for 96 hours.

Exposure Time(hrs)	Lethal Conc. and associated 95% C.L			Safe Conc.	Probit equation	T.F	S.L.
	LC_5	LC_{50}	LC_{95}				
24	0.023 (0.00-0.00)	1.454 (0.00-0.00)	2.884 (0.00-0.00)	0.15 (0.00-0.00)	Y=-1.67+1.15x	1	**
48	0.070 (0.00-0.00)	0.943 (0.00-0.00)	1.961 (0.00-0.00)	0.09 (0.00-0.00)	Y=-1.52+1.61x	1.54	***
72	0.213 (0.00-0.00)	0.879 (0.00)	1.545 (0.00)	0.09 (0.00-0.00)	Y=-2.17+2.45x	1.65	***
96	0.263 (0.00-0.00)	0.653 (0.00-0.00)	1.043 (0.00-0.00)	0.07 (0.00-0.00)	Y=-2.75+4.22x	2.23	ns

C.L= Confidence Limit, D/F = Degree of freedom, LC= Lethal Concentration, S.L = Significant level, T.F= Toxicity factor =LC_{50} value at 24hrs ÷ LC_{50} value of any other periods; F- test significance level: ns-non-significant, **- 0.01, ***- 0.001;

respiration (oxygen transfer) across the gill epithelia leading to death, depending on the exposure duration (Rach and Gingerich, 1986; Singer and Ramsay, 1994; Tiwari and Singh, 2003) The increased mucus secretion in exposed fish may be a defense response, an attempt to reduce entrance of the toxicant through the skin and gills into the body (Agbebe, *et al.,* 2000). However, copious mucus secretion which forms thin film on delicate and sensitive gill tissue may minimize exchange of gases, excretion of nitrogenous wastes. The periodic loss of motion by the exposed fish may be due to loss of muscular contraction as a result of the interference of the poison with the normal functioning of the nervous system and consequently the coordination of muscular activities (Girah, 1997; Gbem *et al*., 1990). The trend in obf in this study and several others is that it is usually raised, peaked and then fell with exposure concentration of the toxicants

Table 4. Mean Lethal Time and associated 95% confidence limit of *C. gariepinus* exposed to *L. alopecuroides* for 96hrs.

Conc. (mg/l)	MLT and associated 95% C.L		Probit equation	S.L.	R.T.
	MLT_{50}	MLT_{95}			
0.5	113.27 97.65-156.28	170.76 (137.28-275.78)	Y=-3.240 +0.03x	ns	1.00
1.0	38.562 (0.00-0.00)	98.18 (0.00-0.00)	Y=-1.06 +0.03x	**	2.94
1.5	27.77 (20.25-34.19)	68.09 (57.97-85.82)	Y=-1.13+ 0.04x	ns	4.08
2.0	20.49 167.89-13.78	38.70 (15.36-60.74)	Y=0.273+ 0.03x	ns	5.53
2.25	17.25 (8.47-23.16)	50.61 (41.578-69.81)	Y=-0.85 + 0.05x	ns	6.57

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 concentrations; F- test significance level: ns-non-significant, **- 0.01.

tested (Onusiriuka and Ufodike, 1994; Aguigwo, 1998; Oti, 2002).Increased opercular rate might be an attempt by the exposed fish to increase gaseous exchange across the gills to enhance internal metabolic rate. The pattern of obf in this study are similar to those reported by other authors for clariids under various stress conditions (Onusiriuka and Ufodike, 2000; Nwanna *et al.,* 2000; Omitoyin *et al*., 2006). The response pattern of obf and tbf of exposed fish with respect to duration and concentration of The toxicant in this study agrees with the findings of of Koffi (2005) who studied the acute effects of 2, 4-D an organophosphate, on *C. gariepinus* and its hybrid But it differed from the findings of Ekweozor *et al*. (2001), Gabriel and Kparobo (2002)and Onusiriuka and Ufodike (1994) in *C. gariepinus, O. niloticus*, *H. bidorsalis* and catfish hybrid exposed to fertilizer effluents, gramoxone, bark and the floral part of Akee apple, *Bligha sapida* and sausage plant, *Kigellia africana,* respectively. Behavioural responses of fish to most toxicants and differences in reaction times have been observed to be due to the effect of chemicals, their concentrations, species, size and specific environmental conditions (FAO, 1986). These stressful behaviours exhibited by the fish may be as a result of respiratory impairment due to the effect of rotenone and alkaloid on the gills and general metabolism of the exposed fish.

The LCs and MLTs values showed that aqueous leave extracts of *L. alopecuroides* was more toxic to *C. gariepinus* than some other plant extracts tested on a number of fish species (Alard, *et al.,* 1991; Chadhaury, *et al.,* 2001; Singh and Singh, 2000). The mortalities recorded for the species appeared to be influenced by the type of extract, exposure conditions, fish species and size of fish. Obomanu *et al.* (2006) reported that 0.22mg/l of *L. alopecuroides* produce 100% mortality for mosquito larvae and Suleiman (2006) reported same percentage for tadpoles within 24 hours of exposure. Hence, the aqueous leave extract of *L. alopecuroides* can be

effectively used to control tadpoles, adult amphibians, predators and mollucs that are known nuisances in culture ponds.
The inverse relationship between MLT and lethal concentrations and the toxicant concentrations and exposure duration for *C. gariepinus,* respectively, indicate that the survival time for the fish declined with increase in the concentration of the toxicant and exposure duration.

The results suggest that aqueous extracts of the leaves of L*. alopecuriodes* has piscicidal property and are highly toxic to *C. gariepinus.* The high potency of the plant extract may possibly be due to the ability of the components to impair oxygen transfer through the gills leading to death which rate was proportional to the toxicant concentration. Hence, care should be exercised in the use of the toxicant under culture conditions except for pond clean sing of unwanted fauna before stocking.

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