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Full Length Research Paper

Toxicity of leaf powder of *Lepidagathis alopecuroides* to Nile tilapia *Oreochromis niloticus* Juveniles

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Oreochromis niloticus **juveniles (mean body weight, 13.99±0.38 g and length, 8.32±0.93 cm) were exposed in a daily renewal bioassay to different concentrations of *Lepidagathis alopecuroides* (0.00, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l), for 96 h to assess its toxicity. The opercular (obf), tail beat (obf) frequencies and mortalities were monitored during the exposure period. The pattern of response of OBF/min and TBF/min decrease with time of exposure, but increased with increase in concentration of the toxicant. Opecurlar beat frequency (OBF) per minute was more variable from the 24th h, whereas TBF were less variable throughout the duration of the exposure. The cumulative mortality of the fish increased with time and concentrations of the toxicants. Less than 50% mortality was recorded for 1.0 and 1.50 mg/l by the 96th h, while the threshold concentrations that killed 100% of exposed fish were 3.0, 2.5 and 2.0 mg/l at the 48th and 72nd h, respectively. Wide variations were recorded in OBF and mortality at the various times of exposure and concentrations. The relationship between OBF, TBF and time of exposure was negative and significant at ($p< 0.01$) but positive with mortality and insignificant. The 24 and 96 hrLC_{50} were 2.27 and 0.88 mg/l and their safe concentration were 0.23 and 0.09 mg/l, respectively. The mean lethal time decreased with increase in the concentration of *L. alopecuroides*. The results suggest that aqueous extracts of the leaves of *L.alopecuroides* has piscicidal property and are highly toxic to *O. niloticus.* Hence, care should be exercised in the use of the toxicant under culture conditions except for pond cleansing of unwanted fauna before stocking.**

Key words: Opercular beat frequency, tail beat frequency, mortality, *Lepidagathis alopecuroides* and *Oreochromis niloticus*.

INTRODUCTION

Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties (Cagauan and Arce, 1992). Plants from different families have been applied for catching fish, control of predators and reduction of overpopulation in aquaculture ponds all over the world and are considered advantageous when viewed against the backdrop of using persistent chemical (Van Andel, 2000; Tiwari and Singh, 2003). Frequent application of high concentrations these ichthyotoxins in water may have adverse effects not only on fish species but also on other aquatic fauna. Several studies have shown that plant toxins at very low concentrations are very toxic to all groups of aquatic fauna (Goktepe et al., 2004; Gabriel and Okey, 2009).

L.alopecuroides (Vahl) is a tropical shrub belonging to the family Acanthaceae commonly found in the coastal countries of West Africa. The leaves are used to immobilize fish in many communities of Rivers and Cross River

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States of Nigeria. In Rivers State it is used for quick kill of hardy fish like mudskippers (Obomanu et al., 2007) and in Cross River State it is normally applied in pools to kill mostly catfishes and tilapias constituting the major source of dietary protein (Ekanem et al., 2003). Phytochemical screening of the plant revealed the presence of alkaloids, tannins, saponins, glycosides and flavonoid (Obomanu et al., 2005) all of which have been proven to posses some form of toxic effect on organisms. The aqueous extract of the leaves of *L. alopecuroides* on the haematological, biochemical, behavioral (Opercular and tail beat frequencies) and mortality of African catfishes of various sizes have been reported (Gabriel and Okey, 2009: Keremah, et al., 2010).

Nile tilapia (*Oreochromis niloticus*) is important and commercially valued fish for the Nigerian fishery and aquaculture industry. They are widely cultured in ponds and occur naturally in freshwater and swamps where they are fished in large numbers at the onset of the dry season using plant poisons. Although, the toxicity level and lethal concentration of *L.alopecuroides* have been determine for African clarrids, no report on the toxicity of the plant to Nile tilapia. This study was there to determine the toxicity of leaf powder of *L. alopecuroides* to *Oreochromis niloticus* juveniles.

MATERIALS AND METHODS

Bioassay was conducted in the Fisheries Wet Laboratory, Cross Rivers University of Technology (CRUTECH). Two hundred and fifty *O. niloticus* juveniles (mean body weight, 13.99±0.38g and length, 8.32±0.93 cm) were obtained from CRUTECH fish Farm Obubra campus Cross River State, Nigeria and acclimated to Laboratory conditions using glass tanks (45 x 40 x 40cm) of 40 liters capacity, filled with 30 liters of tap water (characteristics: Temperature 26.52±1.21°C, DO_2 5.47± 0.56 mg/l, total alkalinity 16.19 ±0.97 mg/l as $CaCO_3$ and pH, 7.63± 2.11. The water in the containers was renewed daily and the fish were fed with commercial fish feed (Pellets) containing 35% crude protein at 1% of their body weight (FAO, 1986). Unconsumed feed and faecal wastes were removed and water replenished regularly as recommended by Oyelese and Faturoti (1995). Feeding was discontinued 24 h before the commencement of and during experiment, to minimize the contamination of the test aquaria. *L. alopecuroides* were procured from Ofumbongha, Obubra Cross Rivers State, where they are mostly use to kill fish in pools and streams. The leaves were sun dried, then pulverized with a sterile grinding machine and were then sieved with 100-micron sieve to obtain a fine powder. A range finding test was conducted according to OECD (2001) and Omitoyin, et al (2006), respectively to determine the concentrations of *L. alopecuroides* used in the definite test. The following concentrations 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l were prepared in triplicates from a stock solution and introduced into the glass aquaria. Ten fish each were randomly placed in each of the aquaria holding 30l of the test solution. Test media was renewed daily for through out the duration of the experiment. The behaviour Obf and Tbf, mortality and other external changes in the body of the test fish were observed and recorded at 12, 24, 48, 72 and 96 h of exposure time as described by Odiete (1995), and Omitoyin et al. (2006). Dead fish were promptly removed to avoid contamination of the test media.

Data obtained from the experiments were subjected to ANOVA using statistical package for the social sciences, (SPSS) version 13. Where differences exist, Duncan multiple range test separated them and F- test used for significant differences ($p < 0.05$) between the various treatments (Wahua, 1999). Correlation and regression between OBF min^{-1}, TBF min^{-1} and cumulative mortality against concentrations and time for the different species was done according to Zar (1996). An analysis of the lethal concentration (LCs) and median lethal time (MLTs) with associated confidence interval were done with Probit analysis. Safe concentrations at the various time intervals were obtained by multiplying the lethal concentration (LC_{50}) by a factor of 0.1 (EIFAC, 1983; Koesoemadinate, 1980).

RESULTS

O. niloticus juveniles exposed to *L. alopecuroides* at different concentrations shows initial distress swimming movements, rapid opercular movements, loss of balance, incessant gulping of air, excessive mucus secretion, unusual lethargy and fish settling at the bottom motionless with slow opercular movement and erratic swimming before death.

The abnormal behaviour displayed by the fish increased with increasing concentration of *L. alopecuroides* in water but decreased with increase in time of exposure.

The pattern of response of OBF/min and TBF/min decrease with time of exposure, but increased with increase in concentration of the toxicant (Figures 1 and 2). Opecurlar beat frequency (OBF) per minute was more variable from the 24^{th} h, whereas TBF were less variable throughout the duration of the exposure. The cumulative mortality of the fish increased with time and concentrations of the toxicants (Figure 3). Less than 50% mortality was recorded for 1.0 and 1.50 mg/l by the 96^{th} h, while the threshold concentrations that killed 100% of exposed fish were 3.0, 2.5 and 2.0 mg/l at the 48^{th} and 72^{nd} h, respectively. No mortality was recorded in the control (0.00 mg/l) group throughout the exposure period. Wide variations were recorded in OBF and mortality at the various times of exposure and concentrations (Tables 1 and 2). Regression analysis showed that the relationship between OBF, TBF and time of exposure was negative and significant at ($p< 0.01$) but positive with mortality and insignificant (Table 3). The 24 and 96 hrLC_{50} were 2.27 and 0.88 mg/l and their safe concentration were 0.23 and 0.09 mg/l, respectively (Table 4). The mean lethal time decreased with increase in the concentration of *L. alopecuroides*.

DISCUSSIONS

The water quality parameters recorded were within acceptable ranges for toxicity test (APHA, 1998). They may not have acted synergistically with the toxicant to affect the behavior as well as mortalities recorded in this study. Similar observations were reported by Onusiriuka and Ofodike (1994) in acute test solutions of Akee apple, *Blighia sapida* and *Kigelia Africana* extracts and Gabriel and Okey (2009) to acute concentrations of *L. alopecuroides* to which *C. gariepinus* were exposed.

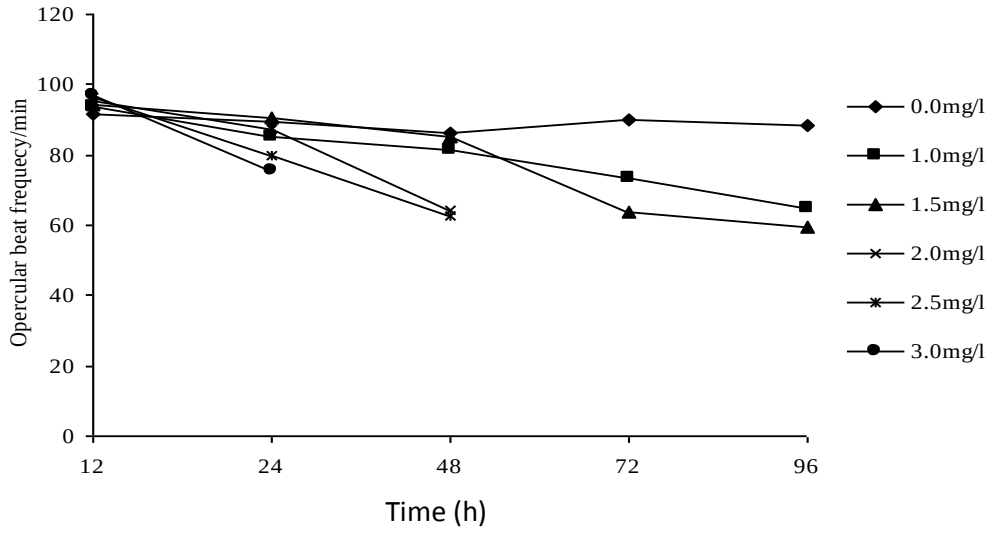

Figure 1. Changes in opercular beat frequency/min of *O. niloticus* juveniles exposed to aqueous extract of *L. alopecuroides*

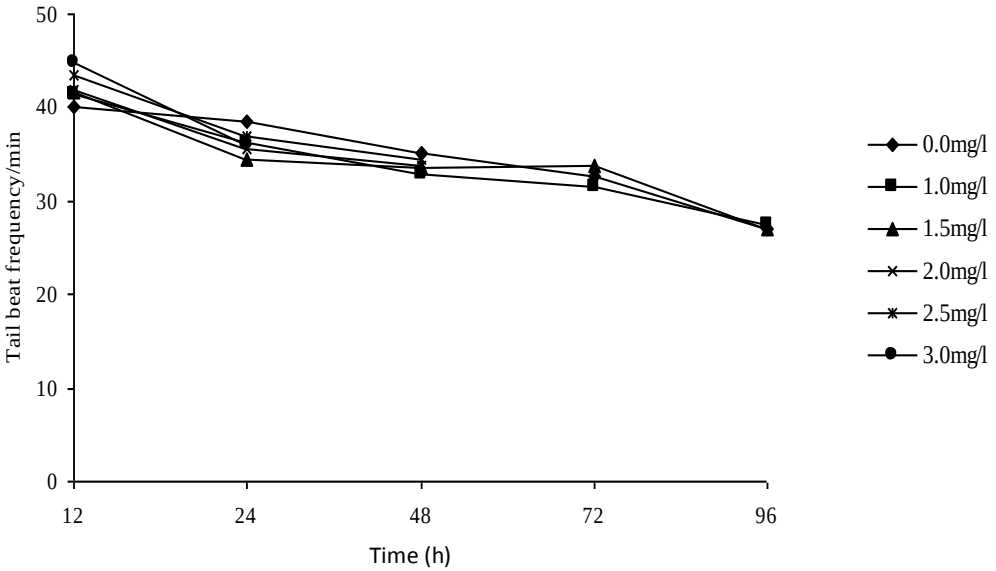

Figure 2. Changes in tail beat frequency/min of *O. niloticus* juveniles exposed to aqueous extract of *L.alopecuroides*.

Heath (1991) and Adeyemo et al., (2004) have reported changes in haemotological parameters and swimming activity of fish due to changes in the water quality parameters.

The behavioural pattern in this study revealed that fish exposed to toxicants usually exhibits some behavioral changes such as increased opercular and tail beat frequencies, mucus secretion and gulping for air (Nwanna et al., 2000). The behavioral changes observed in this study were similar to those reported for *O. mossambicus*, *T. niloticus* and *O. niloticus* exposed to leaf extracts of *Apodytes dimidiate* and *Thevitia nerifolia* (Sambasivam et al., 2003). This also compared favorably with those reported by Ayotunde and Offem (2005) and Gabriel and Okey (2009). Signs of agitated behaviour, respiratory distress and abnormal nervous behavours including eventual deaths were observed in *O. niloticus* exposed with ethanolic and aqueous extracts of *Ipomoea aquatica* leaves (Ayoola et al., 2011). The 96 hrLC_{50} (0.88 mg/l) recorded in this study was smaller than the 4.2 mg/l reported for aqueous extract of pawpaw seed powder (Ayotunde and Offem 2005) and 7.13 g/l reported for aqueous extract of *Euphorbia poisonii* leaves to tilapia *Oreochromis niloticus* fingerlings (Ayoola et al., 2011).

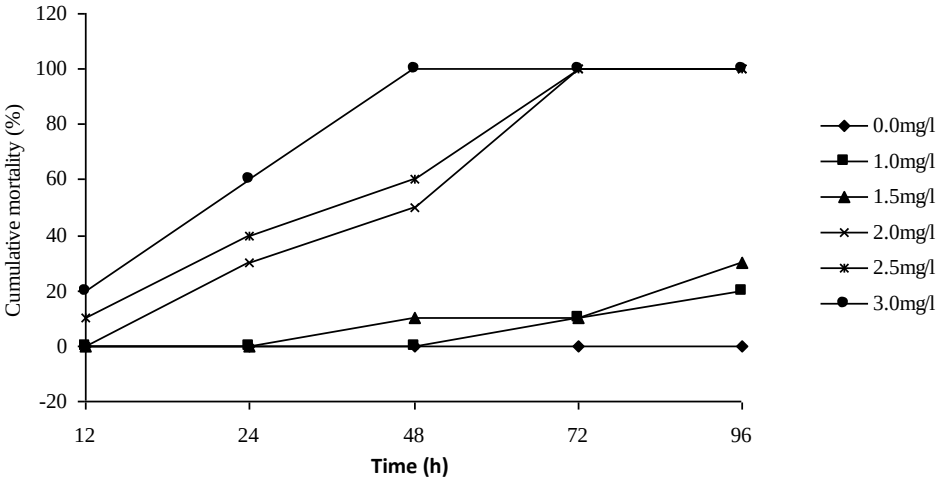

Figure 3. Changes in the cumulative mortality of *O. niloticus* juveniles exposed to aqueous extract of *L.alopecuroides.*

Table 1. Opercular and tail beat frequency and cumulative mortality (%) of *O. niloticus* juveniles exposed to aqueous extract of *L.alopecuroides.*

Variable	Concentration of *L. alopecuroides* (mg/l)					
	0.00	1.00	1.50	2.00	2.50	3.00
OBF/min	91.07±6.76^{a}	79.73±9.62b^{c}	78.71±9.62^{c}	81.84±5.57b^{c}	79.58±7.88b^{c}	86.41± 10.10^{b}
TBF/min	34.65±7.88^{b}	33.88± 7.25^{b}	34.13± 9.65^{b}	37.18± 4.77^{ab}	38.23± 6.39^{a}	40.35± 9.90^{a}
Mortality	0.00±0.00^{d}	6.33± 22.13^{d}	24.25 ±17.26^{c}	55.71± 27.13^{b}	84.29±24.50^{a}	98.35± 38.52^{a}

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

Table 2. Opercular and tail beat frequency and cumulative mortality (%) of *O. niloticus* juveniles at various durations of exposure to aqueous extract of *L.alopecuroides.*

Variable	Duration of exposure (h)				
	12	24	48	72	96
OBF/min	94.73± 8.15^{a}	84.68± 6.09^{b}	76.05± 8.00^{c}	75.79± 10.13^{c}	70.82± 11.66^{c}
TBF/min	42.19± 6.42^{d}	36.28± 5.56^{b}	34.02±8.44^{b}	32.65± 6.33^{b}	27.19± 9.90^{c}
Mortality	2.56± 6.33^{d}	22.39± 14.57^{c}	41.73± 27.14^{b}	56.25± 32.44^{a}	48.99± 26.13^{b}

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

Table 3. Regression lines f or the prediction of the values o f OBF/min., TBF/min. an d cumulative mortality of *O. niloticus* juveniles exposed to aqueous extract of *L.alopecuroides.*

Independent variable	Dependent variable	Regression equation	(r^2)	(r)	Significant level
Time	OBF	y=93.10-0.25x	0.92	0.82	0.05
Time.	TBF	y=42.09-0.15x	0.95	0.90	0.05
Time	Mort.	y=7.53+ 0.33x	0.55	0.30	ns
Conc.	OBF	y=85.53+1.50x	0.82	0.91	0.0001
Conc.	TBF.	y=32.97+5.00x	0.81	0.90	0.001
Conc.	Mort.	y=10.38+26.30x	0.90	0.81	0.05

Where y = independent variable (concentration, Time), x = dependent variable (OBFmin^{-1} , TBFmin^{-1} and mortality. r^2 = coefficient of determination, r = coefficient of correlation.

Table 4. Lethal (LCs) and safe concentrations and Mean Lethal Times (MLTs) and associated 95% confidence limit of *L. alopecuroides* on *O. niloticus* juveniles.

Exposure time (h)	Lethal Conc. and associated 95% C.L			Safe Conc.	T.F	Conc. (mg/l)	MLT and associated 95% C.L		R.T
	LC_5	LC_{50}	LC_{95}				MLT_{50}	MLT_{95}	
24	1.04 (0.15-0.28)	2.27 (40.95-70.06)	3.94 (1.94-7.31)	0.02	1	1.0	102.72 (87.15-96.47)	164.40 (46.72-65.03)	1
48	0.88 (12.45-43.70)	1.63 (0.10-1.67)	2.31 (0.00-0.00)	0.02	1.39	1.5	83.98 (66.70-99.05)	98.57 (43.59-56.08)	1.22
72	0.64 (65.08-78.45)	1.02 (0.00-0.00)	2.04 (23.34-56.89)	0.01	2.23	2.0	51.67 (11.95-13.13)	71.11 (79.60-98.74)	1.99
96	0.34 (23.90-78.65)	0.88 (0.46-0.70)	1.64 (1.44-6.30)	0.01	2.58	2.5	34.77 (36.08-79.09)	48.88 (41.58-69.81)	2.95
						3.0	19.94 (97.65-103.7)	35.93 (48.74-86.83)	5.15

LC= Lethal Concentration, T.F= Toxicity factor =LC50 value at 24hrs ÷ LC50 value of any other periods, C.L= Confidence MLT=Mean Lethal Time, R.T=Relative Time=MLT50value at 1mg/l ÷ MLT50 value at any other concentrations.

This indicates that *L. alopecuroides* is more toxic than pawpaw seed and *Euphorbia poisonii* to *O. niloticus*. The toxicity of *L. alopecuroides* to *O. niloticus* is higher than the result of Wade et al. (2002) who reported that the 96 hLc50 of 0.19 mg/l^{-1}, for the Nile tilapia *Oreochromis niloticus*, fingerlings exposed to effluent of cassava (*Manihot esculenta*) which may be attributed to the age of fish and the strength of the active ingredient in the toxicant.

Positive correlation between concentration (mg/l) and mortality agrees with observations of Tiwari and Singh (2003) in *C. carpio* exposed to ethanol extracts of *Nerium indicum.* Results of the regression of OBF on the concentration of the toxicant and time lend credence to the observation and claim by several authors that OBF is better tool for assessing acute toxicity of the plant extract than TBF based on the unit changed in each of these variables with that of the toxicant (Ekweozor et al., 2001;; Bobmanuel et al., 2006). This may explain why several authors prefer the use of obf to tbf in the study of stress responses of fish to the effects of toxicants, (Onusiriuka and Ufodike, 1994; Babatunde et al., 2000; Oti, 2002).

The lethality of the leave extract of *L. alopecuroides* to fish species had already been confirmed by previous studies (Obomanu et al., 2005, 2007; Gabriel and Okey, 2009). Since the toxicant was highly toxic to the exposed fishes, under field applications to catch fish by artisanal fishermen or clear ponds of unwanted tadpoles and amphibians which are major competitors and predators in fishponds (Nguenga et al., 2000), priced culture fishes like the test species may be adversely affected.

Conclusion

The results suggest that aqueous extracts of the leaves of *L. alopecuroides* has piscicidal property and are highly toxic to *O. niloticus.* From the toxicity tests *L. alopecuroides* powder concentration as low as 0.88 mgl^{-1} in the medium can be potentially hazardous to some fish species in freshwater. 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 cleansing of unwanted fauna before stocking. Therefore, acute toxicity data of the present study provide baseline information needed to develop models of *L. alopecuroides* powder effects on ecological systems.

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