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Piscicidal Effects of *Lepidagathis alopecuroides* on Mudskipper, *Periophthalmus papillio* from the Niger Delta

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Abstract: The piscicidal effects of dried leaves of *Lepidagathis alopecuroides* (1.0, 2.0, 4.0 and 6.0 g L^{-1}) on mudskipper, *Periophthalmus papillio* (mean total weight, 17.29±5.69 g, SD) were assessed in a non-renewable bioassay after 12 h exposure. Exposed fish showed dose-dependent hyperactivity with increased opercular and tail beat frequency with copious accumulation of mucus on the gills and skin. Time to death ranged from 18-37 min and the cidal action of the plant were influenced by the exposure concentration ($p<0.001$) and weight of the fish ($p<0.01$). Pathological changes were recorded only in the gill, stomach and brain of exposed fish and appeared to be dose-dependent. There was necrosis of epithelial cells of the mucosa lining, extensive loss of mucosa glands, oedema of submucosa and loss of fatty tissues in the intestine. The gill lamellae were clubbed and atrophied with extensive necrosis, hyperaemia and hyperplasia of mucus cells. The oedematous distensions in the lamellae were infiltrated by mono- and polymorpholonuclear leucocytes. Mild to severe oedamatous distensions were observed in the brain of exposed fish. However, the gill appeared to be the main site of action of the plant material. The high toxicity of the plant material to mudskipper seems to suggest that non-target species may not be spared during application in the environment.

Key words: *Lepidagathis alopecuroides*, mudskipper, *Periophthalmus papillio*, piscicidal effect, histopathological studies, Niger Delta

INTRODUCTION

Piscicides or ichthyotoxins are used in many parts of the world to stupefy or kill fish. Tropical America and Africa and Australasia regions have rich floral diversity and some of these have been widely used as molluscicide (Singh and Singh, 2005a), for pond cleansing-control of predatory fish (Tiwari and Singh, 2003) and baiting of fish (Ibrahim *et al*., 2000). For fish baiting or poisoning the plant materials are often crushed and thrown into rivers or pools with slow moving water or spread on mud flats at low tide. Several plant materials have been shown to be toxic to zooplanktons (Kreutzweiser *et al*., 2004), shrimps (Goktepe *et al*., 2004), commercial fish species both in the laboratory and field studies (Singh and Singh, 2002, Sambasivam *et al*. 2003; Tiwari and Singh, 2003), produced genotoxic effects in fish, for example *Anabas testiduneus* (Guha and Khuda-Bukhsh, 2003) changes in the enzyme profiles, for example, acetylcholinerase, acid and alkaline phosphatase in *Channa marulius* (Sigh and Singh, 2005b) and immuno-stimulation in exposed fish (Logambal and Michael, 2000).

The tropical shrub *L. alopecuroides* belonging to the family Acanthaceae can be found in the coastal countries of West Africa. Ground aerial parts of *L. alopecuroides* for example the leaves are used to immobilize and kill mudskippers, *Periophthalmus papillio* and other fish species in many communities in Rivers and Cross River States of Nigeria. In Rivers State, the practice is particularly common in communities where mudskippers constitute a major source of dietary protein. Sometimes when mudskippers are caught alive, to effect a quick-kill, the fish is usually mixed with ground leaves of *L. alopecuroides*. The plant is also used to treat abdominal pains and diarrhoea suggesting that it possesses antimicrobial activity (Obomanu *et al*., 2005). Besides, the aqueous extract of the plant demonstrated larvicidal action against *Anopheles gambiae* and *Culex quinquefasciatus* (Obomanu *et al*., 2006). The present study was undertaken to evaluate the piscicidal action of the plant on *Perophthalmus paillio,* including the histopathological changes in the gills, intestines and brain using a quick-kill method usually employed by the local fishermen.

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MATERIALS AND METHODS

Two hundred *P. papillio* were collected by trap nets along Elechi Creek, Port Harcourt, in the Niger Delta. They were transported in plastic aquaria to the laboratory, Department Chemistry, Rivers State University of Science and Technology, Port Harcourt. They were weighed and acclimated in 5% salinity brackish water drawn from source in aerated tanks (60×30×30 cm^3) which were tilted 30° to ensure that the fish were not submerged in water. Known weights of the dried leaves of *L. alopecuroides* in brackishwater from the source were made up to the required volume to give concentrations of 1.0, 2.0, 4.0 and 6.0 g L^{-1}. The range of concentrations were chosen to effect quick kill of the fish,

During exposure, the tanks were kept at an angle of about 30° so that fish were not submerged. Nine fish each were exposed in triplicate groups in 6 L of each toxicant concentration and the control (0.0 g L^{-1}) for 12 h. Abnormal behavioural responses, the time to death of each fish and mucus accumulation on the skin and gills of exposed fish after death were recorded. Dead fish were immediately removed from the toxicant and the weight of the fish in each of the exposure concentrations and the control were taken and grouped into four mean size groups viz: 10, 15, 20 and 25 g.

One dead fish from each of the exposure concentrations was dissected and the intestines, gills and brain excised and fixed in 10% formol saline. At the end of the 12 h, one live fish from the control was also dissected and the organs fixed as above. Thin (5 μ) cryostat sections were cut and processed using standard histological techniques. The sections were stained with haematoxylin and eosin, examined under light microscope and the histopathological features were recorded.

Time-to-death for all the size groups was plotted against the various concentrations of L. *alopecuroides*. Also time to death was plotted against the different size groups in each of the concentrations and a trend line fitted. Time-to-death of the various size groups at the various concentrations were subjected to Anova and differences among means were separated at 0.05% probability by Student Neuman Keuls method (Wahua, 1999).

RESULTS

On introduction into the toxicant, the fish showed hyperactivity with increased opercular and tail beat in a bid to escape from the toxicant. But there was a gradual decrease in the swimming activity with time until fish appeared calm and then died. These responses were concentration dependent. There was also a dose-dependent increase in mucus accumulation on the gills and skin of the dead fish, which was absent in the control.

Anova showed that the size of fish ($p<0.01$) and concentration of L. *alopecuroides* ($p<0.001$) influenced the time to death of *P. papillio* (Table 1). The mortality trend of the various sizes of exposed fish declined with concentration of the toxicant (Fig. 1). Time to death at the various concentrations of the plant material increased with increasing size of the fish, but declined with increase in the concentration of the toxicant (Table 1, Fig. 2a-e). The mortality trend graphs for the individual toxicant concentrations in Fig. 2 showed that only in 1.0 g L^{-1} (Fig. 1) that the line of best fit was polynomial. The rest showed direct linear relationship between weight of fish

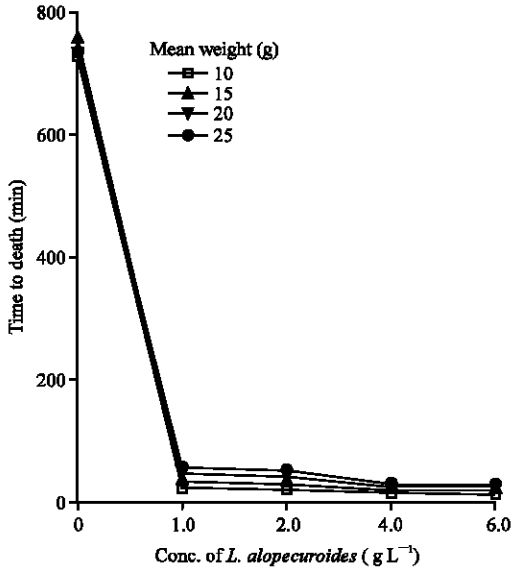

Fig. 1: Mortality trend of *P. papillio* exposed to various concentrations of *L. alopecuroides* (g L^{-1})

Table 1: Time to death (mins) of different sizes of *P. papillio* exposed to various concentrations of *L. alopecuroides* for 12 h

	Time to death (min)				
Weight (g)	10	15	20	25	
	121.44±255.95[d]	162.69±290.76[c]	147.78±262.96[b]	732.63±47.14[a]	
Conc.	0.0	1.0	2.0	4.0	6.0
	737.92±42.27[a]	37.12±12.60[b]	33.41±12.24[c]	22.94±6.93[d]	18.94±6.37[e]

Mean in the same row with similar superscipts are not significantly different ($p<0.05$)

Table 2: Pathological changes in the organs of *P. papillio* exposed to *L. alopecuroides* (g L^{-1}) for 12 h

	Conc. of *L. alopecuroides* (g L^{-1})				
Organ	0.0	1.0	2.0	4.0	6.0
Intestine	Normal	Mild necrosis of epithelial linings of the mucosa and goblet cells, loss of fatty tissues and extensive loss of intestinal glands	Mild necrosis of epithelial linings of the mucosa and goblet cells, hypoplasia of intestinal glands	Fatty tissues normal, hypoplasia of intestinal glands, moderate necrosis of epithelial cells lining the mucosa and oedema fluid in the submucosa infiltrated by mono- and polymorpho-nuclear leucocytes	Severe necrosis of the epithelial cells lining the lamina propria and goblet cells. The oedema was more severe compared with 4.0 g L^{-1}. Loss of fatty tissues and extensive loss of digestive glands.
Gill	Normal	Extensive necrosis of epithelial cells with oedema in the primary and secondary lamellae. The oedema was infiltrated by mono-and polymorphonuclear leucocytes. There was mild hyperplasia of mucus cells and clubbing of the secondary lamellae.	The changes were similar but were mild to moderate compared to that recorded in 1.0 g L^{-1}	There was severe hyperplasia of mucus cells and extensive lesions in the secondary and primary lamellae. The secondary lamellae were clubbed and atrophied. There were large scale oedematous distensions in the lamellae. The oedematous fluid was infiltrated by mono- and polymorphonuclear leucocytes.	Pathological changes in the gills were similar but severe than those recorded for 4.0 g L^{-1}. Besides, there was hyperaemia of the secondary lamellae
Brain	Normal	Slight oedema	Mild oedema	Moderate oedema	Severe oedema

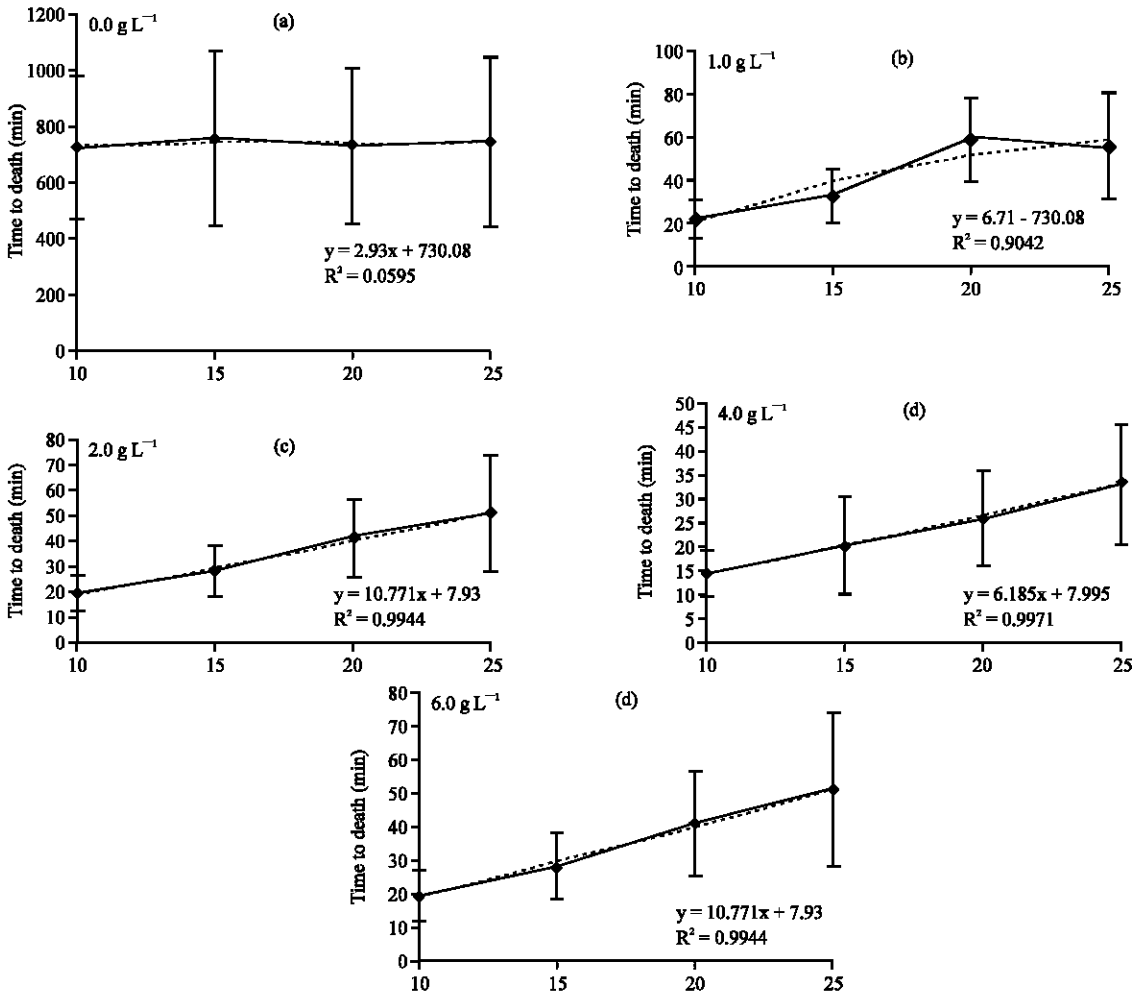

Fig. 2: Relationship between weight (g) and time to death (min) of *P. papillio* at different concentrations of dried plant extract of the leaves of *L. alocuperoides*

and time to death. The pathological changes in all the organs studied in the exposed fish appeared to be dose-dependent. However, fish in the control showed no pathological alterations in these organs (Table 2). There was necrosis of epithelial cells of the mucosa lining, extensive loss of mucosa glands, oedema of submucosa and loss of fatty tissues in the intestine. The gills suffered extensive necrosis of epithelial cells of the secondary lamellae, hyperplasia of mucus cells, oedematous distension in the lamellae that were infiltrated by mono- and polymorphonuclear leucocytes. There was clubbing and atrophy of the secondary lamellae and hyperaemia of the same. Mild to severe oedematous distensions were observed in the brain of exposed fish.

DISCUSSION

The behavioural responses of *P. papillio* in all the concentrations of the plant material before death were similar to those reported for *O. mossambicus, T. nilotica and O. nilotica* exposed to aqueous leaf extracts of *Apodytes dimidiata* and *Threvetia nerifolia* (Blakenbury and Appleton, 1997; Sambasivam *et al*., 2003), *Tephrosia vogelii* and *Justica extensa* (Ibrahim *et al*., 2000) and tobacco, *Nicotiana tobaccum* (Agbon *et al*., 2002), respectively. Altered behavioural changes under toxicant exposure may have negative impacts on the courtship behaviours, feeding and reproduction of the fish. The influence of size, dose and time on the mortality response recorded in *P. papillio* exposed to *L. alopecuroides* was similarly reported by Ibrahim *et al.* (2000) in fish poisoned by *Tephrosia vogelii* and *Justica extensa* and *Channa punctatus* treated with *Euphorbia royleana, Jatropha gossypifolia*, *Nerium indicum* and *Threvetia peruviana* (Singh and Singh, 2002) under laboratory and field conditions. The time to death for the different sizes of the fish (18.94-37.12 min) and the short time (18.94 min) required by the highest concentration to effect 100% mortality may suggest that the plant was highly toxic to *P. papillio*. More susceptible species in the environment such as the gobiids, *Aplochyleicthys* sp. shrimps and crabs may suffer more severely than the hardy mudskipper.

Different species of plants employed as piscicides have different effects, depending on the species of fish targeted (Van Andel, 2000). The active principles in the plant part used (leaves, seeds, kernel and bark) have varying potencies and modes of action depending on whether it is applied directly and the forms of extracts (aqueous or alcohol) used (Sambasivam *et al*., 2003). Phytochemical screening of *L. alopecuroides* showed the presence of alkaloids, saponins, tannins, cardiac glycosides and flavonoids (Obomanu *et al*., 2005). The two main groups of phytochemicals that occur in most plants used for stunning fish, the rotenones and saponins, as well as a third group of plants which liberate cyanide in the water, account for nearly all varieties of fish poisons, although plants with sufficient levels of ichthyoethereol, triterpene and other ichthyotoxins are also used (Béarez, 1998). Rotenone® is an alkaloid toxin and a flavonoid. Bocek (1984) observed that rotenone inhibits oxidative phosphorylation, the specific site of action being the electron transport system where it blocks the mitochondrial enzyme, NADH ubiquinone reductase. It stuns fish by impairing their oxygen consumption, thereby forcing the fish to the surface.

Accumulation of mucus on the gills and skin and extensive necrosis, clubbing and atrophy of the gill lamella of exposed *P. papillio* have grave implications for respiration of the fish. This is because the epidermis and epithelial cells of the gills of mudskippers are highly adapted for respiration. The mean diffusion distance between the capillary endothelial cells and surface of the epidermis range between 1.5-15.4 μm (Wilson, *et al*. 1997; Park, 2002; Park *et al*., 2000; 2006). Mudskippers like *Periophthalmodon schlosseri* and *Periophthalmus magnuspinnatus* can absorb gaseous oxygen through the blood rich membranes at the back of the mouth and the throat (buccopharygeal cavity) and through the skin that are richly supplied with blood rich capillaries as long as the skin is wet (Low *et al*., 1990; Park *et al*., 2006). *P. schlosseri* has unusual gill structure adapted for gaseous and ionic exchange. The inner opercular lining and part of the leading edge of the filaments have interperitoneal capillaries, which provide suitable gaseous exchange surface than the thickened lamellae with its restricted interlamellae spaces found in other fish species (Wilson *et al*., 1997). Besides, the arrangement of the respiratory and ionic exchange epithelia is opposite that found in all other fish in which the lamellae typically function in gaseous exchange and gill filament in ion regulation. Mudskippers may rely mainly on the gills or skin for gaseous exchange.

The structure of the gill and skin of *P. papillio* and their dependence on these for gaseous exchange and ion regulation may be similar to those of the above species. It is possible then that the quick action of the toxicant in effecting death in the fish may be due to interference with gaseous exchange in both the gills and skin by the accumulation of mucus and distortion of gill architecture. This may lead to an internal toxic environment resulting from accumulation of nitrogenous wastes in the body which in excess may result in death. But the effects, particularly on the gills may have been responsible for

the death of exposed fish. Studies have shown that under toxicant exposure, where several organs are studied, histopathological changes are first evident and most severe in the gills than in any other organs (Neskovic *e t al*., 1996; Poleksic and Karan, 1999). Heath observed that when death occurs in fish under toxicant action, it is usually due to the failure in the gill function.

In the process of attempting to escape from the toxicant, the fish may have swallowed the toxin leading to necrosis of the epithelial lining of the small intestine, extensive loss of goblet cells and intestinal glands and oedematous fluid in the submucosa of the intestinal wall which may have interfered with normal functions of the intestine-digestion and absorption. Production of digestive enzymes and function of the same, absorption and peristaltic movement of ingested food may be impaired with grave implications for the fish. Exposure of *Channa punctatus* to sublethal concentrations of endosulfan and quinalphos for one hour, 15 and 30 days, respectively, disrupted intestinal glucose transport (Sastry and Siddiqui, 1982). The decrease was concentration-dependent with the endosulfan being more effective in reducing the glucose transport rate. *P. papillio* may suffer similarly with the pathological changes recorded in the intestine, the main site of absorption.

Oedematous distension in the brain of exposed fish would have resulted from ischaemia and subsequent necrosis of brain cells. This will impair impulse transmission and distort the overall physiology of the fish. Anti-cholinesterase activities have been reported in nervous tissues of *C. marulius* exposed to latex of *E. rolyeana* and *J. gossypifolia*, *N. indicum* and *T. peruviana* (Singh and Singh, 2005b) and in *C. punctatus* treated with neem (Tiwari and Singh, 2003). Avoidance behaviours in mudskipper, *P. cantonensis* were shown to be mediated by c-fos protein expression in the brain. Substantial increase in the c-fos protein expression was recorded in the diencephalon and a more prominent expression in the pons and medulla under stress of agitation (Wai *et al*., 2006). Hence, avoidance behaviours and spatial movement which are mediated by the brain may be impaired in *P. papillio* by exposure to the toxicant.

This study revealed that leaves of *L. alopecuroides* are highly toxic to mudskipper, *P. papillio.* Quick kill can be achieved within 18-37 min. using 1.0-6.0 g L^{-1} dried leaves of the plant depending on the size of the fish which significantly affected the time to death. In the quick kill method, the gill appeared to be the main site of action of the plant material. However, the high toxicity of the plant material to mudskipper seems to suggest that non-target species may not be spared during application in the environment. Hence the use of the toxicant for fishing should be discouraged.

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