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Exploitation of Ethanol Extract of *Adenium obesum* Stem Bark as a Potent Organic Piscicide

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Abstract: The possible use of ethanol extract of *Adenium obesum* stem bark as a potent organic piscicide, against unwanted predatory animals and/or fish in fish ponds prior to the stocking of the desired fish was investigated via the exposure of the hardy air-breathing *Clarias gariepinus* to the extract over a 96 h exposure period. *Clarias gariepinus* of 265.50±4.03 g mean weight and 32.85±0.16 cm mean total length were exposed to 0.00, 6.25, 7.50, 8.20, 8.80 and 9.30 mg L^{-1} of the extract in a static acute toxicity bioassay after performing a range finding test. The extract contained some toxicologically bio-active substances. Changes in the fish culture water quality were within acceptable limits. The exposed fish showed signs of restlessness and hyperactivity along with signs of respiratory and nervous compromise, as well as the observed mortality in some of the exposed fish whose appearance and intensity were concentrations and exposure period-dependent. However, no signs of toxicity were observed in the unexposed control fish. The median lethal concentration of the extract in the exposed fish over the 96 h exposure period was 7.15 mg L^{-1}. Therefore, at this or much higher concentrations, the extract will be a potent organic piscicide that can be used to eradicate unwanted predatory animals, especially fish from ponds prior to the stocking of desired fish.

Key words: *Adenium obesum*, *Clarias gariepinus*, toxicity, median lethal concentration, pond management, piscicidal plant

INTRODUCTION

There has been a global demand for fish within the last 30 years from 45 million metric ton (mt) in 1973 to about 91 million mt in 1997 (Delgado *et al*., 2003) and this is further expected to rise to about 183 million mt by year 2030 (Ye, 1999). This is because fish has become one of the most important sources of available animal protein (Jan *et al*., 2012) that may be the sole accessible and/or affordable sources of animal protein for poor households in urban and semi-urban areas (Bene and Heck, 2005). This is most important in developing countries where fish is comparatively cheaper to either beef or chicken or mutton which is even usually in short supply (Omoniyi, 1995; Amisah *et al*., 2008). The increased global demand has led to increased fishing efforts in wild aquatic environments, especially in the developing countries. However, the global landings of wild-caught fish are thought to be nearing and/or exceeding their sustainable levels resulting in a worldwide resort to fish farming or aquaculture (Faturoti, 1999; Jourie, 2006; Omitoyin, 2007).

Aquaculture is the fastest growing food sector in the world, accounting for an estimated 43% of all fish consumed by humans globally (Allsopp *et al*., 2008). However, aquaculture growth is still very slow in Africa as it is currently faced with numerous challenges (FAO, 2011; Peteri *et al*., 1992; Masser *et al*., 1999; Omitoyin, 2005). Notable amongst these challenges is the issues of predatory animals like tadpoles, frogs, leeches and unwanted fish species that competes with and feed on the fry and fingerlings of stocked fish (Olaifa *et al*., 2008; Fafioye, 2012), including competition for available oxygen (Kumar, 1992). The use of synthetic compounds to clear out these predatory animals and/or fish has been a common practice amongst aquaculturists (Marking, 1992) but synthetic compounds cause heavy environmental pollution (Mondal *et al*., 2007) with harmful residues in aquatic environments (Koesomadinata, 1980; Cagauan and Arce, 1992). That is why attention is now focused on the use of piscicidal plants or plants that are poisonous to fish in place of these synthetic compounds (Marston and Hostettmann, 1985; Dahiya *et al*., 2000). This is because

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piscicidal plants are easily bio-degraded, less severe and are comparatively safer toward humans and the environment (Kela *et al*., 1989; Stalin *et al*., 2008).

Adenium obesum is a known piscicidal plant (Adamu *et al*., 2005; Oyen, 2008). The plant is distinct with swollen base, twisted branches and characteristic showy flowers which gives it the name Desert rose (McLaughlin and Garofalo, 2002; Bawden-Davies, 2010). The plant is slow growing but long lived (McLaughlin and Garofalo, 2002) and produces sticky clear or white latex (Rowley, 1983; Oyen, 2008). Although, the plant occurs throughout the Sahel region of Africa, spreading downward towards Central Africa and Northward into Arabia (Plaizier, 1980; Arbonnier, 2004), it is found worldwide where it is usually cultivated for ornamental purposes (Oyen, 2008; Hastuti *et al*., 2009).

Even though, *Clarias gariepinus* has Pan-African distribution (De Graaf and Janssen, 1996), it is absent from the Maghreb, Upper and Lower Guinea and Cape Provinces (FAO, 2011). The fish is hardy (Olaifa *et al*., 2003) and can tolerate harsh aquatic conditions (Hogendoorn, 1979; Eyo and Ezechie, 2004). Therefore, any piscicidal plant that kills this fish species might kill some of these unwanted predatory animals and/or fish before the stocking of the desired fish. This is in addition to the apparent dearth of information on the toxicity of *A. obesum* stem bark to exposed fish, especially the air-breathing *C. gariepinus* which has created the need for this study. The research aimed to ascertain the toxicity of the ethanol extract of *A. obesum* stem bark in the exposed adult *C. gariepinus*, as a possible tool for the eradication of unwanted animals and/or fish from ponds prior to the stocking of desired fish.

MATERIALS AND METHODS

Plant collection and preparation: *Adenium obesum* were collected from the open fields of Rurum town, Rano Local Government Area, Kano State, Nigeria within the months of January to April, 2011. These were authenticated at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Nigeria with Voucher No. 1386. The barks were removed, sun-dried and pounded into powder ready for the extraction process.

A total of 3.95 kg of the pulverized *A. obesum* stem bark was macerated in 21 L of ethanol (96.0% vol. Sigma-Aldrich® Inc., St. Louis, MO 63178, USA) based on the method of Bentley (1977) and Ghani (1990) over a 72 h period. The filtrates were then concentrated to dryness in evaporation dishes by allowing the ethanol to evaporate at room temperature until constant weights were obtained as described by Abu-Dahab and Afifi (2007).

Phytochemical screening: A preliminary phytochemical screening of the ethanol extract of *A. obesum* stem bark for tannins, steroids and triterpens, glycosides, saponins, flavonoids and anthraquinones (Trease and Evans, 1983) and alkaloids (Harbone, 1998) was carried out.

Physicochemical analysis: Fish culture water quality parameters was monitored and recorded prior to fish introduction and daily throughout the 96 h exposure period. The modified method of Winkler-Azide (Lind, 1979; APHA, 1985) was used to determine the dissolved oxygen content of the fish culture water. Similarly, the pH, temperature, electrical conductivity and the total dissolved solids were determined using the Hanna Combo portable hand instrument (Hi 98129, Hanna Instruments, Mauritius).

Fish toxicity bioassay: Adult *Clarias gariepinus* were purchased from a commercial catfish farm (Fannasson Investments Limited, Kano, Nigeria). The fish were authenticated at the Fishery Section, Department of Biological Sciences, ABU, Zaria, Nigeria. The fish were acclimatized for 21 days before the start of the experiment under natural day and night photo-periods. Pond water was changed completely once in every 3 days. The experimental fish were fed to satiation twice daily with 6 mm Coppens® fish feed for aquaculture (Coppens® International bv., 5700 AM Helmond, Holland). The fish were observed for disease conditions and mortality throughout the acclimatization period as recommended in the OECD (1992) guideline No. 203. The exposed fish were not fed 24 h prior to and during the 96 h exposure period in order to prevent interference from stomach contents and wastes in the fish culture water (Smith *et al*., 2007; Olufayo, 2009). The exposed and the unexposed (control) fish were monitored with prompt recordings of all observations at 24 (0, 3, 6 and 12 h), 48, 72 and 96 h during the exposure period.

Clarias gariepinus (265.50±4.03 g mean weight and 32.85±0.16 cm mean total length) were used for the static acute toxicity bioassay over the 96 h exposure period (OECD, 1992) in triplicates. This was after a preliminary concentration range finding test was performed (Fafioye, 2001) to determined 5 different ethanol extract concentrations of *A. obesum* stem bark that was used (0.0, 6.25, 7.50, 8.20, 8.80 and 9.30 mg L^{-1}). The median lethal concentration (LC_{50}) of the ethanol extract of *A. obesum* stem bark in the exposed *C. gariepinus* over the 96 h exposure period was established based on the Probit and logit analysis method (Finney, 1971). Fish mortality represented extract toxicity. The exposed fish were considered dead if there were no visible opercula movements or no visible reactions when touched (OECD, 1992).

Statistical analysis: Data were expressed as mean (±SEM) and subjected to ANOVA and Tukey's multiple comparison test for statistical significance at $p<0.05$ using GraphPad software programme (GraphPad Prism, version 4.0, San Diego, California, USA).

RESULTS AND DISCUSSION

The ethanol extract of *A. obesum* stem bark had no significant ($p>0.05$) effects on the physicochemical parameters of the fish culture water over the 96 h exposure period as shown in Table 1. Although, the observed changes were not concentration dependent, the extract caused decreases in the temperature, pH and the dissolved oxygen but increases in total dissolved solids and the electrical conductivity of the fish culture water with increasing extract concentrations compared to the respective controls. The preliminary phytochemical screening of the extract revealed the presence of tannins, alkaloids, saponins, steroids and triterpens, glycosides, flavonoids, cardiac glycosides but no anthraquinones as shown in Table 2.

The exposed fish showed signs of toxicity in the forms of restlessness, erratic movements and repeated attempts to jump out of the fish culture water in addition to excessive mucus secretion. Frequent opercula movements and air gulping with snouts exposures were also exhibited by the exposed fish. Other signs of toxicity were those of increasing states of motionlessness and the adoption of different postures with sudden darts, swirling movements and loss of balance, including the observed mortality in some of the exposed fish. These signs were directly related to the concentrations of the extract. None of these signs of toxicity were observed in the unexposed control fish. The LC_{50} of the ethanol extract of *A. obesum* stem bark in the exposed *C. gariepinus* over the 96 h exposure period was established to be 7.15 mgL^{-1} with a 96.95% degree of fits (R^2) as shown in Fig. 1. Mean mortality increased significantly ($p<0.05$) with increased extract concentrations.

The observed changes in the physicochemical parameters of the fish culture water were all within the acceptable limits for the growth and survival of *C. gariepinus* (Viveen *et al*., 1985; Peteri *et al*., 1992; Hogendoorn, 1979; Chapman, 2000). The preliminary phytochemical screening of the extract revealed the presence of some toxicologically active bio-active substances (Da Rocha *et al*., 2001; Bent and Ko, 2004). This is because saponins which are directly absorbed through the gills, haemolyses erythrocytes (Elpel, 2000), due to their ability to lower the surface tension between the aqueous and the lipid phases of the membranes of erythrocytes resulting in the emulsification of their lipid components with Na^+ and water influx and K^+ efflux until they ruptures to release their haemoglobin contents (Hostettmann and Marston, 1995). The toxicity might have also been through the impairment of oxygen consumption in the exposed fish as saponins are also reported to lower the surface tension of reconstituted extracts with the formation of colloidal substances within them (Geidam *et al*., 2007; Armstrong, 2008). This is in addition to the fact that saponins are also known to cause structural damages in the respiratory and intestinal epithelia of exposed fish (Roy *et al*., 1990).

The toxicity of tannins might be through their protein coagulative property (Davidson, 1979), especially gill epithelia, thereby causing respiratory failure or asphyxiation in the exposed fish (Idris, 2012). Tannins are also known to bind and inhibit endogenous proteins like the digestive enzymes (Kumar and Singh, 1984) in addition to causing intestinal damage and interference with iron absorption (Butler, 1989). Impaired oxygen consumption via the inhibition of oxidative phosphorylation involving the blockage of mitochondrial enzyme, NADH ubiquinone reductase has been ascribed to alkaloids in exposed fish (Bocek, 1984; Tiwari and Singh, 2003). This agreed with the reported inhibition of pyridine-linked substrates oxidation suggestive of interference with oxidative phosphorylation in ticks exposed to the aqueous extract of *A. obesum* stem bark (Mgbojikwe, 2000). The observed signs of toxicity might have also been due to excessive inhibition of the Sodium-Potassium pump (Na^+-ATPase pump) caused by the cardiac glycosides thereby causing a variety of severe arrhythmias resulting in blocked cardiac activity, decreased cardiac output and death in some of the exposed fish (Knight and Walter, 2001).

Table 1: The physicochemical parameters of the fish culture water for the 96 h exposure period

Extract conc. (mgL^{-1})	Physicochemical parameters				
	Temperature (°C)	pH	Dissolved oxygen ($mg\ L^{-1}$)	Total dissolved solids (ppm)	Electrical conductivity ($\mu\ Scm^{-1}$)
9.30	24.34±0.14	7.20±0.06	3.15±0.80	348.90±6.08	697.40±11.61
8.80	24.47±0.13	7.16±0.04	3.96±0.67	352.80±6.77	706.10±13.33
8.20	24.57±0.15	7.14±0.04	3.59±0.62	349.80±7.39	697.80±12.09
7.50	24.57±0.17	7.16±0.04	3.81±0.65	347.90±5.70	699.70±11.74
6.25	24.63±0.16	7.17±0.04	4.22±0.47	345.60±4.81	688.70±9.850
0.00 (Control)	24.65±0.18	7.22±0.04	4.56±0.63	341.80±7.61	680.30±14.70

Conc. = Concentration

Table 2: Preliminary phytochemical screening of the ethanol extract of *Adenium obesum* stem bark

		Extract solution	
Parameters	Tests	Observations	Indication
Saponins	Frothing	Honey comb forth	+
Tannins	Ferric chloride	Green precipitate	+
Alkaloids	Meyer	Creamy precipitate	+
Glycosides	Fehling's solution	Brick red precipitate	+
Cardiac glycoside	Kella-Killiant	Purple brown inter-phase	+
Flavonoids	Sodium hydroxide	Yellow colour	+
Carbohydrates	Molisch	Purple precipitate	+
Steroids and triterpenes	Lieberman-Buchard	Reddish brown color	+
Anthraquinones	Borntrager	Transparent	-

+ = Present; - = Absent

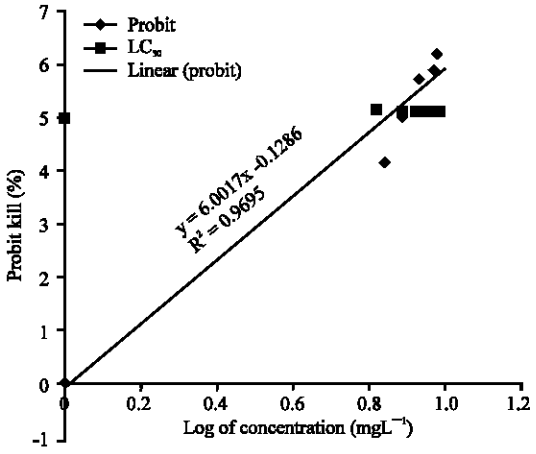

Fig. 1: Log of concentration of the ethanol extract of *Adenium obesum* stem bark and the probit values in *Clarias gariepinus* over the 96 h exposure period

The initial agitations and restlessness characterized by erratic movements and repeated attempts of the fish to jump out of the culture water were manoeuvres to escape from the toxic fish culture water. This is because fish responds to perceived noxious contamination by avoiding the area containing the chemical (Kane *et al*., 2005) in attempts to prevent the continuous absorption of the toxicant present within the fish culture water. Similar hyperactivity and uncoordinated movements were reported in ticks exposed to the aqueous extract of *A. obesum* stem bark (Mgbojikwe, 2000).

The increased opercula movements were due to decreased efficiency of oxygen uptake and transport and/or increased metabolic rate (Thomas and Rice, 1975). The observed frequent air gulping and snout exposures were attempts to free gill respiration for aerial respiration in order to limit continuous gill contact with the toxic aquatic environment. This is an aquatic surface respiration in response to low oxygen that predisposes affected to easy catch and/or predation (Reebs, 2009). The observed nervous disorders might be due to brain cytochrome c oxidase inhibition leading to cytotoxic hypoxia with resultant changes in the electrical activity of the brain associated with the maintenance of equilibrium (Rao and Rao, 1987). It might have also been due to acetylcholinesterase inhibition leading to acetylcholine accumulation at synaptic junctions with subsequent hyperstimulation (Halappa and David, 2009). This is because Mgbojikwe (2000), reported acetylcholinesterase activity inhibition in ticks exposed to the aqueous extract of *A. obesum* stem bark.

The presence of mortality in some of the exposed fish was indicative of the toxicity of the extract in the exposed fish as reinforced by the established LC_{50} value of 7.15 mgL^{-1}. The decrease in mean mortality with increase exposure period showed the acute nature of the toxicity. The subsequent decline might be due to the bio-transformation of the toxic bio-active constituents of the extract over time (Kela *et al*., 1989; Fafioye, 2005), especially as the extract concentrations were not maintained daily throughout the exposure period. The ethanol extract of *A. obesum* stem bark was highly toxic to the exposed *C. gariepinus* because the lower the LC_{50}, the higher the toxicity or sensitivity to test organisms and vice versa (Buikema *et al*., 1982) meaning that at higher LC_{50}, greater concentrations are required to the produce 50% mortality in test organisms and vice versa (Hilmy *et al*., 1985; Eisler and Gardner, 1973). The high toxicity might be the reason behind the global use of the powdered roots or a decoction of the bark and the leaves of the plant to poison fish (Arbonnier, 2004; Adamu *et al*., 2005; Oyen, 2008; Bawden-Davis, 2010).

CONCLUSION

The ethanol extract of *A. obesum* stem bark was highly toxic to the exposed *C. gariepinus*. The utilization of the LC_{50} or much higher concentrations of the extract will make it a potent organic piscicide that can be used to eradicate predatory animals, especially unwanted fish from ponds prior to the stocking of the desired fish.

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