Environtropica, March 2016 Vols. 12 & 13, 28-34 ISSN 1597-815X

Piscicidal Effects of Aqueous Leaf Extract of *Calotropis procera* on *Clarias gariepinus* Fingerlings

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Accepted on January 11, 2016

Abstract

The presence of unwanted predatory fish in newly stocked fish ponds constitute a nuisance and also compete with fingerlings for food in earthen ponds. An ichthyotoxin of plant origin, aqueous extract of *Calotropis procera* (Common name: Apple of Sodom) was tested on *Clarias gariepinus* fingerlings to evaluate its potential use in the eradication of unwanted fish from culture systems. The experiment with concentrations of 0 (control), 10, 20, 40 and 80 mg/L was carried out using static renewal bioassay techniques in transparent glass tanks in triplicates. Data on temperature, pH, dissolved oxygen (DO), conductivity and total dissolved solids (TDS) were collected and analyzed. Probit analysis was used to determine median lethal concentration (LC_{50}). The 48-hr LC_{50} obtained was 35.76 ml/L. The 95% confidence limit of 48-hr LC_{50} was found to be 25.708 ml/L for lower limit and 51.140 ml/L for upper limit. The study showed that leaf extract of *C. procera* can be used to clear fish ponds before stocking. It is recommended for use because it is natural, biodegradable and does not persist in the environment.

Keywords: *Calotropis procera,* Toxicity, Stocking, Predatory, Fish farm

Introduction

One of the problems confronting aquaculture today is the high incidence of unwanted fish in culture ponds. This is more pronounced in earthen ponds. Sometimes, unwanted fish may enter fish ponds through water supply inlets, predatory birds or along with fish seed brought into fish farm during stocking. Mortality from unwanted fish can account for up to 40% losses in the commercial fish and shrimps harvest (Pillay and Kutty, 2001). These unwanted fish may be predatory and feed on newly stocked fingerlings, or may compete with fingerlings for food which invariably create problems for the stocked fish in the pond (Omoniyi *et al*., 2002).

Unwanted fish can be eliminated at harvest when a pond is completely drained and dried. However, Chakroff (1976) reported the use of piscicides as effective in ponds which cannot be completely drained and dried ensuring total eradication of unwanted fishes in the pond. The use of piscicides in pond management during pond preparation to rid off predators before fish stocking is a recommended practice to obtain good yield (Agbon *et al*., 2004).

Piscicides used in aquaculture could be natural (plant source) or synthetic (chemical source). Fafioye *et al.* (2005) reported that synthetic piscicides are laden with problems such as non-degradability, environmental persistence, pest resurgence and detrimental effects on non-target organisms. Plants are an inexhaustible source of structurally diverse and biologically active substances (Batabyal *et al.,* 2007). Natural plant piscicides, on the other hand, do not create problems like those associated with synthetic ones. Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties and the active ingredients are extracted from flowers, bark, pulp, seeds, roots, leaves and even the entire plant (Wang and Huffman, 1991; Agbon *et al.*, 2004; Sirivam *et al.,* 2004; Fafioye *et al.*, 2005; Sarder *et al.*, 2008; Karunamoorthi *et al.*, 2009; Agbebi *et al.*, 2012).

Phytochemical analyses of piscicidal plants showed that they contain diverse toxic substances such as rotenones, saponins, flavonoids, alkaloids, glycosides, tannins, oxalic acids, solanine, selenium, nicotine, pyrethrum, ryania, coumerin, and resin (Wang and Huffaman, 1991; Al Ashaal *et al.*, 2010). These substances are toxic to fish and other aquatic organisms at high concentrations and fade out within a short time (Adewunmi, 1990).

Pisicicidal plants like *Blighia sapida, Kigelia africana, Tetrapleura tetraptera, Raphia vinifera, Parkia biglobosa and Tephtosia vogelli* are commonly used by fisherfolks to catch fishes because they are highly potent against fishes (Fafioye, *et al.,* 2004). Agbon, *et al.* (2002) and Omoniyi *et al.* (2002) reported the toxicity of *Nicotiana tobaccum* to *Oreochromis niloticus* and *Clarias gariepinus* fingerlings respectively while Agbebi *et al.* (2012) reported the use of *Euphobia kamerunica* in the control of saprolegnia in fish hatchery. Omoniyi *et al.* (2013) documented that a number of factors influence the response of organisms to toxicity tests which include age, disease, water quality, stage in life history, pollutant interaction, nutrient status, reproductive stage and species interaction.

Calotropis procera belongs to the family Asclepiadaceae and it is a soft woody evergreen perennial shrub. This plant has few branches and relatively few leaves concentrated near the growing tips. A copious white sap referred to as the latex flows whenever the stems or leaves are cut. The plant is commonly found in Asian temperate region (Arabian Peninsula), Asia-tropical (Indian subcontinent and Indo-China) and Africa (North, Northeast, East tropical, West Central and West tropical), particularly the semi-arid regions of Bauchi, Borno, Kano, Kaduna and most parts of Northern Nigeria (Sharma *et al.,* 1997; Ahmed *et al.,* 2005). In view of the inherent advantage in the use of botanical piscicides, there is a dearth of information on *C. procera* as a potential candidate for use in aquaculture. This study was therefore carried out to determine the toxicity of aqueous extract of *C. procera* on *C. gariepinus* fingerlings.

Materials and methods

The test fish species, *Clarias gariepinus* (African mud catfish), of mean weight 3.2 ± 0.04 g and mean total length of 7.6 ± 0.3 cm, were obtained from the Fish Hatchery of Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta. Two hundred and sixty apparently healthy fingerlings of African mud catfish were collected and distributed randomly into batches of 10 each and placed in a 20-litre capacity transparent container according to Ward and Parrish (1982) and European Inland Fisheries Advisory

Commission (1983). The fingerlings were acclimated to laboratory conditions for a week (Ward and Parrish, 1982) and were fed with 30% crude protein pelleted feed. The test fish were not fed 24 hours before the commencement and throughout the period of the experiment (Ward and Parrish, 1982). Experimental fish were introduced into the text containers within 1 hour after the toxicant was added to the dilution water (Ward and Parrish, 1982).

The plant, *Calotropis procera* was obtained from the Botanical garden of the Department of Biological Sciences, Federal University of Agriculture, Abeokuta. The leaves of the plant were collected between 09.00 and 10.00 hours. The fresh leaves of *C. procera* were washed, weighed, blended in 200 ml of distilled water using Binatone® electric blender and allowed to soak for 24 hours.. The mixture was sieved through a No. 1 Whatman filter paper as described by Agbon *et al*. (2004). The filtrate (stock solution), was then diluted (v/v) serially to the required level of concentration.

Rectangular transparent glass tanks, each measuring 60 x 30 x 25 cm, were used. The tank was covered with 1mm mesh size net on a wooden frame. Experimental water was obtained from uncontaminated bore hole. Physico-chemical parameters of the experimental water were monitored. Water temperature (ºC), pH, conductivity (µS/cm) and total dissolved solids (TDS, mg/L) were monitored using Hannah multipurpose pocket instrument (Model H198129) and recorded. Dissolved oxygen (mg/L) was monitored by REX dissolved oxygen meter.

Acute toxicity tests were conducted to determine the lethal concentrations of *C. procera,* on *C. gariepinus,* after 48 hours exposure in the bioassay. Four different treatment concentrations were used with a control. The treatment concentrations in logarithmic intervals (EIFAC,1983) of 0 ml/L (control), 10 ml/L, 20 ml/L, 40 ml/L, 80 ml/L were used. All treatments were in triplicate (Ward and Parrish, 1982) and behavioural responses of the test fish to the piscicide at different concentrations were observed and recorded. The mean values of mortality figures were subjected to probit analysis using USEPA Ver 1.5 software.

Results and Discussion

The physico-chemical parameters of the bioassay media monitored during the study are presented in Table 1. The values obtained for water quality parameters observed were within the normal tolerance range of the test fish for optimal metabolic activities as reported by Boyd, (1990) and Agbon *et al*. (2013). No significant difference $P>0.05$ was observed between the treatments. The pH values observed increased with increasing levels of plant extract concentrations. This may be an indication that *C. procera* extract is not acidic but alkaline in nature. This finding agrees with that reported for *E. kamerunica* by Agbebi *et al.* (2012).

The dose response of the fish to the plant extract is presented in Table 2 while Figure 1 shows Lethal Concentrations (LC) values at different percentages of mortalities. The EPA Probit Analysis Program output is presented below:

Chi - Square for Heterogeneity (calculated) = 5.128
Chi - Square for Heterogeneity (tabular value at 0.05 level) = 5.991
Mu = 1.559939
Sigma = 0.284653

Parameter	Estimate	Std. Err.	95% Confidence Limits
Intercept	-0.480147	1.431547	(-3.285980, 2.325686)
Slope	3.513052	0.920459	(1.708952, 5.317152)

Theoretical Spontaneous Response Rate = 0.0000

Table 1: Mean water parameters observed during 48 hrs exposure of *C. gariepinus* to *C. procera* leaf extract

Concentration (ml/L)	Temperature (ºC)	pH	DO (mg/L)	Conductivity (µS/cm)	TDS (mg/L)
Control	29.0 ± 0.02	7.4 ± 0.53	5.80 ±0.12	33 ± 0.06	16 ± 0.03
10	29.0 ± 0.11	7.5 ± 0.07	5.90 ± 0.09	32 ± 0.03	17 ± 0.01
20	29.5 ± 0.01	7.6 ± 0.02	5.90 ± 0.05	32 ± 0.13	16 ±0.03
40	29.5 ± 0.02	8.0 ± 0.04	5.70 ± 0.14	32 ± 0.08	16 ± 0.05
80	29.0 ± 0.04	8.1 ± 0.03	5.70 ± 0.07	32 ± 0.06	16 ±0.01

NB: DO = Dissolved oxygen; TDS = Total dissolved solids

Table 2: Fish mortality during 48 hrs exposure to *C. procera* leaf extract

Conc. (ml/L)	Mean Number of fish per container	Mean Mortality (48hr)
10	10	1
20	10	1
40	10	4
80	10	10
Control (0 ml/L)	10	0

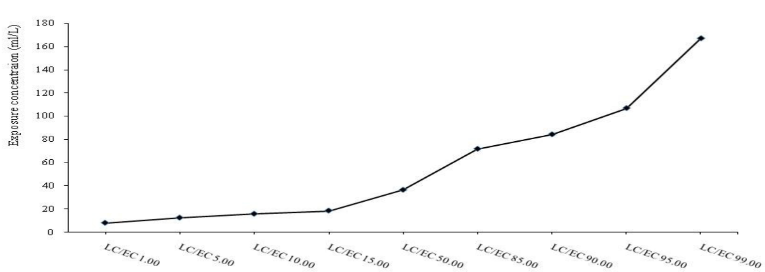

Figure 1: Estimated LC/EC values of *Calotropis procera* leaf extract on *C. gariepinus* in 48 hrs exposture.

The fish exhibited stressful behaviours which were higher as the concentration of toxicant increased. Gradual decrease in activity with time was observed until a state of calmness followed by subsequent death. Fafioye *et al.* (2001) and Rahman *et al.* (2002) reported similar behaviour on *C. gariepinus* and *C. punctatus* respectively. Mortalities were observed in all the treatments except in control medium. Increase in fish mortality with increasing concentration of the toxicant in the medium showed a dose-response relation which has been reported by Agbon *et al.* (2002) and Omoniyi *et al.* (2013). The 48-hr LC_{50} obtained was 36.303 ml/L of *C. procera* leaf extract as shown in Figure 1, with 95% confidence limit of 25.51 ml/L for lower limit and 54.14 ml/L for upper limit. At 48-hour LC_{50} experimental fish displayed erratic swimming and changes in behaviour was observed.

The LC_{50} value obtained in this study indicates that aqueous extract from *C. procera* was moderately toxic to fingerlings of *C. gariepinus* when compared to other ichthyotoxic plants used by farmers in the eradication of unwanted organisms in aquaculture ponds (Omoniyi *et al.*, 2002; Agbon *et al.*, 2004; Caguan, *et al.,* 2004; Ashraf *et al.,* 2010; Ayotunde *et al.,* 2010; Keremah *et al.,* 2010; Agbebi *et al.,* 2012). However, Ayuba *et al.* (2012) reported that *C. gariepinus* fingerlings exposed to *Datura innoxia* leaf extract had LC_{50} of 120.23 mg/L. This showed that *C. procera* was more toxic than *Datura innoxia* leaf extract.

Conclusion

In view of the results obtained in this study, aqueous leaf extract of *C. procera* can be used to control unwanted fish in aquaculture practices. The ready availability of *C. procera*, its low toxicity and biodegradability revealed the plant potentiality as a good candidate for natural piscicide and should be included in the inventory of plants with piscicidal properties. Further research needs to be carried out on this plant's use in aquaculture, especially a comparative study of this plant with other documented plants used as piscicides.

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