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Evaluation of the Ichthyotoxicity of Dioscorea Dumetorum on the African Catfish, Clarias Gariepinus Fingerlings and Anuran Tadpoles

Nsikak NseAkanse[1], Blessing Julius Oribhabor [2]
[1,2] *Department of Fisheries and Aquatic Environmental Management, University of Uyo, PMB 1017, Uyo, AkwaIbom State, Nigeria*

ABSTRACT: *Dioscorea Dumetorumtubers are used in stupefying fish for easy harvesting in Akwa Ibom State, Nigeria specifically in Annang part of the state. D. dumetorum belongs to the family of Dioscoreaceae and is commonly known as bitter yam. This study was conducted to determine the bioactive components and evaluate the acutetoxicity of the aqueous extract of the D. dumetorum on C. aariepinus fingerlings and anuran tadpoles. The 96hLC50 values of D. dumetorum tuber aqueous extracts were determined in the laboratory under static bioassay conditions against C. aariepinus fingerlings and anuran tadpoles. Range finding bioassays were conducted to get the range of concentrations for the definitive bioassays. The range of concentrations of test media for C. aariepinus fingerlngs was 0.6-5mgl^{-1} while that of anuran tadpoles was 1.6-3.8mgl^{-1}. The median lethal concentrations (LC50) were determined using probit analysis. The 96hLC50 value of the test plant against exposed fingerlings was 2.153mgl^{-1} with lower and upper confidence limits of 1.869 and 2.485mgl^{-1} respectively while that of tadpoles was 2.538mgl^{-1} with lower and upper confidence limits of 2.295 and 2.771 mgl-^{1} respectively. Unpaired t-test showed that there was no significant difference (p>0.05) between the toxicity of the test plant to C. aariepinus fingerlings and that of anuran tadpoles. The mean water quality parameters were within the optimal range requirement for the test species. The study indicated that D. dumetorumexerted piscicidal property on C. aariepinus fingerlings and anuran tadpoles. The indiscriminate use of this plant to catch fish by local fishermen should be discouraged.*

Keywords: *Dioscorea dumetorum, Clarias aariepinus, anuran tadpoles, ichthyotoxicity, static bioassay.*

I. INTRODUCTION

The use of plant materials in many part of the world is a traditional practice. Plants are used for different purposes because some plants contain compound of various classes that have insecticidal, piscicidal and molluscicidal properties (Cagauan *et al.*, 1992). Plants from different families have been applied for catching fish, control of predatorsand reduction of overpopulation in aquaculture ponds all over the world and are considered advantageous when viewed against the backdrop of using persistent chemicals (Van Andel, 2000; Tiwari and Singh, 2003). Ichthyotoxicplants containsuch toxic constituents as saponins, coumarins, cyanogens, glycosides, alkaloids and 2-cyclopentenones etc (Morah, 1986). Excessive application of high concentrations of these plant extracts in water may have adverse effects, not only on fish species but also on non-target aquatic fauna. Plant extracts are called botanicals and when toxic to fish are called piscicides (Fafioye, 2005). Piscicidal plants are frequently used by fisher folks to catch fishes because they are readily available, biodegradable and leave no residues in the environment and are easily reversed in fish subjected to chronic concentration (Onusiriuka and Ufodike,1994) and highly toxic to fish. Fish poison plants are known to cause decrease in dissolved oxygen and physiological changes in the fish which eventually lead to the death of aquatic life (Morah *et al.,* 2015; 2016). Several studies have shown that plant toxins at very low concentrations are very toxic to groups of aquatic fauna (Goktepe *et al*., 2004; Gabriel and Okey, 2009).

D. dumetorum is among the first four varieties of yam that are indigenous to Africa and probably Nigeria (Coursey, 1967; Martins and Ruberte, 1975; Onwueme, 1978; Okonkwo, 1985; FAO/WHO/UNU, 1985). It occurs widely throughout Africa, predominantly in the tropics (Luka *et al.,* 2012). *D. dumetorum*belongs to the family of Dioscoreaceae and commonly known as bitter yam. The plant could be eaten only in times of food scarcity. It is prepared by slicing and so king in water for at least three days before cooking which makes it part of the traditional food system. The tubers are used in stupefying fish for easy harvesting in Akwa Ibom State, Nigeria specifically in Annang part of the state. Dioscoretine, an alkaloid present in the yam extract has been reported to possess hypoglycemic effect (Iwu *et al*., 1990). In "folk medicine," extracts of *D. dumetorum* tuber have been used in the treatment of diabetes mellitus because of its hypoglycaemic effect (Luka *et*

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al., 2012). *D. dumetorum*tuber also contains diosgenin which is used as a precursor in the commercial synthesis of sex hormones, birth control pills (Crabbe, 1979).

C. aariepinus is widely and naturally distributed in all parts of Africa. Ecologically, it requires calm waters like lakes, ponds, and pools but may occur in fast flowing streams and rivers. It is an air-breather capable of tolerating a wide fluctuations of dissolved oxygen and other extreme environmental conditions because of possession of accessory breathing organs which enables it to breathe in air when exposed to adverse environmental condition like lack of dissolved oxygen. It is one of the most important aquaculture candidates because of its ability to tolerate a wide range of environmental conditions, high stocking densities under culture conditions, fast growth rate, disease resistance, acceptability of artificial feed, high fecundity, good taste and meat quality, ease of artificial breeding and high market value (Morah,2016).Tadpoles are young frogs or toads found in tropical countries like Nigeria. They normally breed in shallow, still, fresh water such as ponds with breeding commencing in March (Udofia *et al.,* 2013). The adults congregate in the ponds while the males compete for females and their courtship ritual involves croaking and a successful male grasps the female under the forelegs (Udofia *et al.,* 2013). The females, which are generally larger than the males, lay between 1,000 and 2,000 eggs (Eccleston, 2008).Adults feed on invertebrate of a suitable size, they do not feed during the breeding season (Kuzmin, 2008).

Knowledge of the piscicidal potentials of *D. dumetorum* has been known among fisherfolks, but there is no documented information on its toxicity against *C. gariepinus* and anuran tadpoles. The present work was therefore conducted to determine the bioactive constituents and evaluate the acute toxicity of the aqueous extract of *D. dumetorum*on *C .aariepinus* and anuran tadpoles under static bioassay conditions.

II. MATERIALS AND METHODS

The 96h LC_{50} values of *D. dumetorum* tuber aqueous extracts were determined in static bioassays against *C. aariepinus* fingerlings and anuran tadpoles between August and November 2015 at the Department of Fisheries and Aquatic Environmental Management laboratory, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The study area is geographically located at latitude 5°2′26″N and longitude 7°55'19″ E. *C. aariepinus* fingerlings reared under controlled condition free of pollutants were obtained from Akanse's Fish Farm,Edem Idim Ibesit, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria and transported to the laboratory in a plastic container (30l volume, 52cm diameter 50cm depth) with water from the site of collection. The experimental tadpoles were obtained with a long handled scoop net from a pond in the same environment and transported to the laboratory under same condition. In the laboratory, the fish fingerlings and tadpoles were kept in holding plastic containers (30l volume, 52cm surface diameter, 34cm width and 20cm depth) half filled with dechlorinated borehole water. The fingerlings and tadpoles were kept in the containers for at least one week, to allow them acclimate to laboratory conditions (29°C ± 1°C) before using them in bioassays. About 100 individuals were kept in each container. During this period of acclimation, the fishes were fed twice daily (mornings and evenings) with coppens feed at 5% of their body weight while the tadpoles were fed with algae throughout the period. During this period of acclimation, the water in the aquaria was changed every 48hours to ensure good health of the experimental organisms while uneaten feed were removed by siphoning to avoid fouling. Also, dead and weak individuals were immediately removed and the total mortality recorded during the acclimation period was less than 5% (Adeyemo, 2005). Acclimation of test organisms to laboratory conditions and experimental procedures were in accordance with guidelines for bioassay techniques (APHA, 1998).

The tubers of *D. dumetorum* were procured from Edem Idim Ibesit, Oruk Anam Local Government Area, Akwa Ibom State, Nigeria. The samples were washed with clean water to free them from sand and debris. They were cut and sundried to constant weight. The dried cubes were pulverized with a sterile manual grinding machine and then sieved with 100 micron sieve to obtain the fine powder (Fafioye *et al*., 2004). The samples produced were stored in air tight containers. Subsequently, fifty grams (50g) of the test plant was dissolved in 500ml of dechlorinated water for 24 hours. Thereafter, the mixture was filtered through Whatman's filter paper (No.1) and that served as the stock solution for the experiment. The prepared aqueous extract of the test plant was refrigerated and used for the static bioassay tests following standard procedures (FAO, 1986).

Clean plastic containers (20l volume, 31cm surface diameter, 31cm width and 19cm depth) were employed in all

bioassays. A predetermined volume of the test compound was pipetted into a measuring cylinder and made up to 1l by adding appropriate units of dechlorinated borehole water as diluents, to achieve the desired concentration of the test compound. Active specimens of about the same size (mean weight 3.04 ± 2.05g; mean length 2.92 ± 1.97cm) for *C. aariepinus* fingerlings and (mean weight 0.01 ± 0.008g; mean length 0.38 ± 0.041cm) for anuran tadpoles were randomly assigned to bioassay containers, already containing the test media prepared. In all bioassays, a total of 10 active animals were placed in each container. Tests were run at several concentrations and untreated controls. In each treatment, there were two replicates. Test animals were exposed to several concentrations of each test compound after range-finding bioassays were conducted (Table 1).

Table 1: Toxicant concentrations to which C. gariepinus fingerlings and anuran tadpoles were exposed

Test Animal	Concentrations ($mg\ l^{-1}$)
C. gariepinus	0.6, 1, 1.4, 1.8, 2, 2.6, 3,4 and 5
Anuran Tadpoles	1.6, 2, 2.5, 2.6, 2.8, 3.2 and 3.8

Mortality assessments were made by examining each animal separately every 24 hours over a 96 hour experimental period. ***C. aariepinus*** fingerling was considered dead when respiratory and tail movements stopped, and no response to gentle prodding with a rod. Anuran tadpole was considered dead if it sunk to the bottom of the test medium and no response to gentle prodding with a rod.

Water temperature, conductivity, dissolved oxygen, pH, ammonia, alkalinity and hardness were determined in the acclimation media, untreated control and each test-compound-treated medium at the beginning (0 hr) and end (96 hr) of each bioassay. Temperature was determined by using mercury in-glass thermometer, conductivity by conductivity meter (Hannah product Model H19812 – 5), dissolved oxygen by using HANNA dissolved oxygen meter model H19146, pH by pH meter (HANNA product model HA989), hardness by the EDTA titrimetric method, alkalinity by titrimetric method, and ammonia colorimetrically using ammonia test kits. The physical and chemical parameters of acclimation media were maintained optimally and are summarized in Table 2.

Table 2: Summary of the physical and chemical parameters of the acclimation media

Physical and chemical parameters	Mean ± S. E. (*C. gariepinus*)	Mean ± S. E. (Tadpoles)
pH	6.8±0.09	6.49±0.112
Dissolved oxygen ($mg\ l^{-1}$)	5.02±0.25	8.37±0.70
Temperature (°C)	25.37±0.2	26.01±0.34
Conductivity (µg/cm)	101.57±2.03	147.29±9.16
Ammonia ($mg\ l^{-1}$)	0.63±0.08	3.49±0.05
Alkalinity ($mg\ l^{-1} CaCO_3$)	65.43±1.94	22.86±1.01
Hardness ($mg\ l^{-1} CaCO_3$)	4.14±0.21	9.4±0.15

SE = Standard error

The toxicity data based on quantal response (mortality) was analysed by probit analysis (Finney, 1971). The analysis, including the equation for probit line, and unpaired t-test used to test for significance on toxicity of the test plant between ***C. aariepinus*** and tadpoles was achieved via computer programme using IBM SPSS Statistics 20. Indices of toxicity/susceptibility level were based on the 96h LC50 values.

The extract of ***D. dumetorum*** tuber was screened to identify its constituents of bioactive compounds (Alkaloids, flavonoids, saponins, tannins, phytate, glycosides and oxalate) through preliminary phytochemical screening as described in literature (McCance and Widdowson, 1935; Cuilei, 1982; Sofowore, 1984 and Ejikeme et al., 2014).

III. RESULT

Physical and chemical parameters of the test media

The physical and chemical parameters of the test media are summarized in Table 3 below. When ***D. dumetorum*** was tested against ***C. aariepinus***, the physical and chemical parameter data over 96h period, showed that D. dumetorum caused increase in pH, conductivity, ammonia and alkalinity, while temperature, dissolved oxygen and hardness decreased (Table 3). A test of ***D. dumetorum*** against tadpoles indicated decrease in temperature, dissolved oxygen, alkalinity and hardness. But there was an increase in pH, conductivity and ammonia.

Table 3: The physical and chemical parameters of test media for ***C. aariepinus*** fingerlings and anuran tadpoles

	Mean ± S.E			
Parameters	*C. gariepinus* fingerlings		Anuran tadpoles	
	0Hr	96Hr	0Hr	96Hr
Temperature (0C)	28.41 ± 0.11 (28.0 – 28.9)	27.6 ± 0.31 (26.1 – 28.7)	27.6 ± 0.29 (6.20 – 28.6)	27.1± 0.31 (26.0 – 28.3)
pH	8.44 ± 0.25 (7.5 – 9.1)	9.26 ± 0.20 (8.31 – 9.10)	8.10 ± 0.40 (6.6 – 10.0)	9.84 ± 0.29 (8.0 – 10.4)
Dissolved Oxygen (mg l^{-1})	8.58 ± 0.33 (7.5 – 9.9)	7.56 ± 0.53 (5.0 – 9.9)	7.97 ± 0.41 (6.24 – 9.89)	6.87 ± 0.83 (4.43 – 11.9)
Conductivity (µS/cm)	111.3 ± 2.88 (100.0 -130.0)	135.2 ± 8.11 (111.0 – 187.0)	115.8 ± 9.92 (106.0 – 203.0)	173.6 ± 8.94 (132.0 – 209.0)
Ammonia (mg l^{-1})	0.55 ± 0.04 (0.3 – 0.72)	0.66 ± 0.05 (0.41 – 0.91)	0.55 ± 0.04 (0.34 – 0.66)	1.39 ± 0.76 (0.44 – 0.68)
Alkalinity(mg l^{-1} CaCO3)	39.9 ± 2.29 (32.3 -53.3)	66.6 ± 10.3 (18.0 – 120.0)	64.1 ± 5.96 (45.3 – 100.0)	57.8 ± 5.51 (33.3 – 80.9)
Hardness (mg l^{-1} CaCO3)	5.96 ± 0.43 (4.3 – 8.2)	5.65 ± 0.36 (4.21 – 7.8)	6.86 ± 0.80 (2.0 – 9.0)	5.82 ± 0.71 (3.02 – 8.0)

The minimum and maximum values are represented in parentheses

Acute toxicity of *D. dumetorum* tuber against *C. gariepinus* fingerlings and anuran tadpoles

Based on 96h LC50, ***D. dumetorum*** tuber was slightly more toxic against C. aariepinus fingerlings than the anuran tadpoles. The computed 96h LC50 values for fingerlings and tadpoles being 2.153 mgl^{-1} and 2.538 mgl^{-1} respectively. Computed toxicity factor based on 96h LC50 values showed that D. dumetorum was 0.85 times more toxic against ***C. aariepinus*** fingerlings than anuran tadpoles (Table 4). However, unpaired t-test showed that there was no significant difference ($p>0.05$) between the toxicity of the test plant to ***C. aariepinus*** fingerlings and that of anuran tadpoles (Table 5).The log-dose probit graph depicting the relative toxicity of ***D. dumetorum*** against ***C. aariepinus*** and tadpoles based on the 96hr values were non-parallel (Fig. 1).

Table 4: Comparative toxicities of the D. dumetorum tuber against C. aariepinus fingerlings and anurantadpoles

D. dumetorum plant tuber	96hLC_{50} (95% CL) mg l^{-1}	Slope ± S.E	D.F	Regression Equation (Probit|Response)	T.F
C. gariepinus fingerlings	2.153 (1.869 – 2.485)	3.563 ± 0.497	7	Y= -1.186 + 3.563X	1
Anuran tadpoles	2.538 (2.295 - 2.771)	5.561 ± 0.973	5	Y= -2.250 + 5.561X	0.85

L.C = lethal concentration, C.L = 95% confident limit, T.F = toxicity factor, S.E = standard error, D.F = degree of freedom

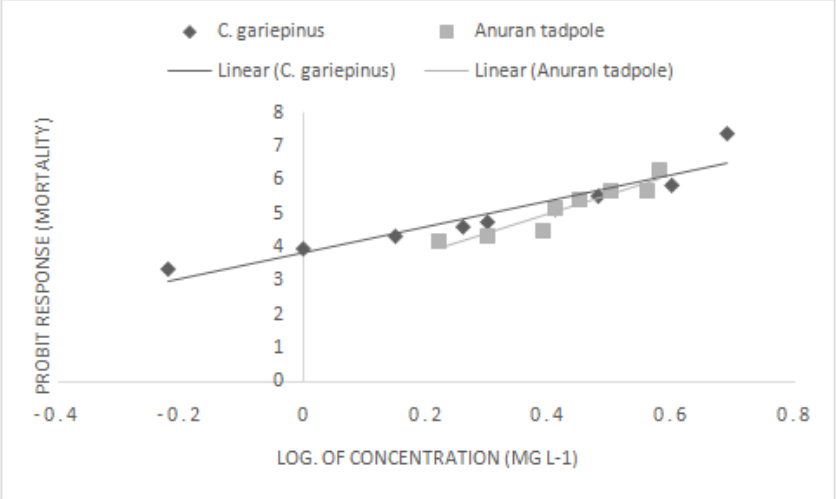

Fig. 1: Log-dose probit graph depicting the relative toxicity of D. dumetorum against C. aariepinus and anuran tadpoles based on 96hr values under static bioassay

Table 5: Test of significance for the 96h LC50values of D. dumetorum between C. aariepinus fingerlings and anuran tadpoles

Unpaired t-test				
Variables	Standard Error Mean	t	Sig. (2-tailed)	t-test probability
C. gariepinus-Tadpoles	0.477	-0.678	0.508	p>0.05

α = 0.05

Some phytochemical constituents of the test *D. dumetorum* plant tuber

The results of phytoconstituents analysis conducted on ***D. dumetorum*** tuber aqueous extracts revealed the presence of some bioactive components such as flavonoids, saponins, phythates, glycosides and oxalates (Table 6). Flavonoids was slightly present, while saponins, phythates, glycoside and oxalates were moderately present.

Table 6: Phytochemical components of D. dumetorum tuber aqueous extract

Bioactive Constituents	*D. dumetorum*tuber aqueous extract	
Flavonoids	+	
Saponins	++	
Phythates	++	
Glycosides	++	
Oxalates	+++	

++ = Moderately Present; + =Slightly Present

IV. DISCUSSION

Hazardous organic chemicals tend to have adverse effects on the aquatic environment and the life they support. Results from this study showed that *C. aariepinus fingerlings and anuran tadpoles* exposed to toxicants usually exhibit some behavioral changes such as increase in opercular rate, erratic swimming, mucus secretion and gulping for air before death (Davis, 1973; Nwanna *et al*., 2000). The pattern of behavioural changes observed in this study compared favorably with the records of Fafioye *et al*. (2004) when African catfish (*C. aariepinus*) was exposed to *Parkia biglobossa* and *Raphia vinefera* extracts and catfish hybrid fingerlings treated with cassava mill effluents (Oti, 2002). This study revealed that *D. dumetorum* tuber subjected to phytochemical screening contain some bioactive components such as flavaniods, saponins, phythates, glycosides and oxylates. These active ingredients are known to

be toxic to fish and other aquatic organisms even at low concentrations (Tiwari and Singh, 2003; Goktepe *et al.,* 2004; Kreutzweiser *et al.,* 2004).

The physical and chemical parameters investigated were within acceptable ranges for toxicity test (APHA, 1998). The mean parameters were within the range earlier reported as optimal requirement for African catfishes(Boyd, 1979; Viveen *et al*., 1985; Adeniji and Ovie, 1989). This indicates that the parameters did not influence the toxicity of *D.dumetorum,* hence, it might not have contributed to the mortality of the test species. This is in agreement with earlier work which exposed *C. aariepinus*to to Akee apple and sausage plant extracts and indicated no significant difference ($P<0.05$) in the water quality parameters(Onusiruika and Ufodike, 1994).. The test species, ***C. aariepinus*** fingerlings and anuran tadpoles at various concentrations of the extracts were stressed progressively within the 96 hour period before death. Plant toxins even at low concentrations are known to elicit mucus production on the body and gills of the fish (Annune *et al.,* 1994; Ayuba and Ofojekwu, 2002). Increased mucus secretion in fish exposed to toxicants is a defense response by which fish attempts to reduce entrance of the toxicant through the skin and gill surfaces. The mucus forms a thin film on the delicate and sensitive gill tissue thus minimizing exchange of gases, particularly intake of oxygen (Agbede *et al*., 2012). Before death, the exposed test species were motionless possibly due to the 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 (Gbem *et al.,* 1990; Keremah *et al*., 2010).

The 96hLC_{50} values of 2.153mg $1^{-1}$and 2.538mg $1^{-1}$for *C. aariepinus* fingerlings and anuran tadpoles respectively are greatly highly toxic conditions. *D. dumetorum* toxicity in the present study is less than extremely toxic range earlier reported for acute toxicity of lyophilized aqueous extract of *Psychotriamicrophylla*on *C. aariepinus* (Oti *et al*., 2004)and another study that reported 0.36mg $1^{-1}$for acute toxicity of *Tetrapleuratetraptera* on the tilapia, *Sarotherodian galilaeus*(Omitoyin *et al*., 1999).It is however significantly and extremely more toxic than those earlier reported for other highly toxic ichthyotoxic plants tested on clariid species (Onusiriuka and Ufodike, 2000; Oti and Ukpabi, 2000; Ayuba and Ofojeku, 2002; Abalaka and Auta, 2010). The observed differences in the present study and those of earlier workers could be attributed to the type and part of the plants used, size and type of fish, environmental factors, water parameters and selective action of the plant toxicants.Although it has been reported that ichthyotoxic plants besides fish have effects on other living things like water snakes and frogs in water(Seyaniand and Chiotha, 1990; Dartay *et al*., 2007),no literature exists on the toxicity of ichthyotoxic plants on tadpoles. However, little literature existing on the toxicity of pesticides on tadpoles reported LC_{50}values as follows: 7.5mg 1^{-1} for malathion acute toxicity on tadpoles of *Duttaphrynus melanostictus*(David and Kartheek, 2015), range of 1.25 and 5.9mg 1^{-1} for tadpoles of *Rana, Bufo* and *Hyla speires* Relyea, 2004) and 2.137 mg 1^{-1} for tadpoles of *Rana boylii*(Sparling and Fellers, 2007).

C. gariepinus fingerlings were more susceptible to the test plant than anuran tadpoles. Generally, the differential susceptibility of the organisms to chemicals has been widely investigated by earlier workers and shown to depend on factors like cuticuler disposition to penetration by the toxicant in question, the rate of enzymatic break down, excretion of the compound and availability of physiological storage mechanism in the organism (Rand 1995).

Tadpoles and adult amphibians are major competitors and predators in fish ponds (Nguenga *et al.,* 2000). This study showed that *D. dumetorum* exerted piscicidal property and can be applied in ponds to eradicate predators, competitors and unwanted fish populations. The use of the extracts to clear ponds may be more preferable to other chemicals since they are biodegradable in nature with the tendency of degrading faster without bioaccumulation in the organism. However, indiscriminate use of the toxicant to catch fish should be discouraged.

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