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Toxic characteristics of Clarias gariepinus juveniles (Tuegels 1982) exposed to aqueous and ethanol pod extract of locust bean (Parkia biglobosa)

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Abstract: *Juveniles of Clarias gariepinus mean weight (10.69± 0.2g) and mean total length (9.64± 0.10cm) were exposed to aqueous and ethanol extracts of Parkia biglobosa pods (60, 70, 80, 90, and 100mg/l) over a period of 96hrs under the standard laboratory conditions. Exposure to the pod extract resulted in some physiological dysfunction of the test fish, such as signs of agitated behaviours, respiratory distress and abnormal nervous behaviours including eventual deaths more observed. Control fish neither died nor exhibited any unusual clinical signs. Percentage mortality of the fish was increasing with the increase in the concentration, however, mortality was higher on the fish exposed to aqueous extract of the P. biglobosa and it was concluding that both aqueous and ethanol pod extract of P. biglobosa are toxic to C. gariepinus juveniles with aqueous extract being more toxic.*

Keywords: *Clarias gariepinus, clinical signs, Parkia biglobosa, pod, toxicity.*

I. Introduction

Medicinal plants are the most ancient source of drugs for caring human and animal diseases, their use in the crude or refined form is of utmost interest in the efforts aimed at integrating herbal with orthodox medicine .Ichthyotoxic plants or poisonous plants are those plants that when touched or ingested in sufficient quantity can be harmful or fatal to fishes. The use of plant poisons to catch fish is still used in many places in the world today. A multitude of plant species are known to possess chemicals that are toxic to fish and evidence suggests that such plant species have different efforts depending on which variety of fish are targeted [1].

Fisher folks in Africa extensively use plants and plant products for capturing fish[2]; Fafioye et al.,[3]. Fish farmers and fisher folks haphazardly use various kinds and parts of these plants due to their narcotic, pesticidal and molluscidal properties in other to stupefy fish for easy catch also to clean up the aquatic ecosystems of some pests [4]. Parkia biglobosa is a perennial leguminous crop that is grown in the tropics. It belongs to the family of Parkia and has species that are grown in the sub-Saharan Africa [5]. The mature fruit of the locust bean occur in large bunches, of which the pod varies between 12 - 30cm by length and 12-25mm by breath [6]. P. biglobosa is tough and fibrous, enclosing a soft powdery yellowish pulp in which small seeds are embedded. The toxic parts of the plant known to certain piscicidal properties include the bark, pods, fresh seeds and the pulp[7]; [8].[9] gave the average length, width and thickness of P. biglobosa seed 10.80, 8.42 and 4.64 mm.

These plants that are poisonous to fish have been recorganized as effective alternative to harmful synthetic compounds [10] such as glyphosate[11]. This is because they have low toxicity against non target animals[12] while being more environmental friendly[13].

Various parts of the P. biglobosa tree are used to make tonics and ointments to treat many aliments[14];[15];[16]. The most significant products from P. biglobosa probably is food, but the husk is also sold in the market for various uses[17], especially in the dry season[18];[19]. The seed is also fermented into a spice called “dawa-dawa”, used for seasoning traditional soups[8];[20];[17].

Fish is very important to humans because it contains protein of very high quality and also has sufficient of all the essential amino acids required by the body for growth and maintenance. *Clarias gariepinus* is the most

cultured fish in Nigeria and indeed Africa and the third in the world[21]. Catfish comprises of the major family of food fish of economic value, not only in Nigeria but in the tropical and sub-tropical regions. Some of their outstanding characteristics include fast growth rate, high fecundity rate, ability to survive a wild range of water conditions and the ability to convert natural feedstuff into use.

Clarias gariepinus is hardy; due to the presence of arborescent air breathing organ, Omnivorous feeding habit, better feed conversion, and ability to withstand adverse environmental condition, high fecundity and ease of culture[22].

The objective of this study is to determine the toxic effect of aqueous and ethanolic pod extract of *P. biglobosa* and its median lethal concentration on *Clarias gariepinus* juveniles during a 96hrs exposure.

II. Materials and Methods

Study area: The research was conducted at the Fisheries resource laboratory of Department of Animal and Environmental Biology of Imo State University, Owerri Imo State Nigeria and Biology laboratory of Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.

2.1. Collection, Preparation and Extraction of plant (*P.biglobosa*)

950g of *Parkia biglobosa* pods were collected from Benue state and placed under the sun for five days for proper drying before grinding / blending into fine powder prior to extraction. Three litres of distilled water was used to soak 400.00 g of the fine powder of *P.biglobosa* pods over night, prior to filtration based on maceration method[23];[24] which was subsequently sun dried, yielding 200g of the sun dried aqueous extract. Four litres of ethanol absolute was used to soak 500.00 g of the fine powder of *P.biglobosa* pods over 48 hours period prior to filtration based on the same maceration method. This was subsequently concentrated to dryness over a 72 hours period, yielding 270.00 g of dry ethanol extract.

2.2. Collection and Preparation of experimental fish (*C. gariepinus*)

120 juveniles of *C.gariepinus* (10.69 ± 0.2g mean weight; mean ± SEM and 9.64± 0.10 cm mean total length; mean ± SEM) were purchased from commercial farm (Nina farms) at Aba, Abia State, Nigeria and they were acclimatized for five days under laboratory conditions. They were fed at 5% body weight daily with 2mm commercial catfish feed (Multi feed, Zelmach feed mill, Isreal), and the experimental water changed every other day. Feeding was stopped 48 hours before and throughout the 96 hours experimental period[25] in order to minimize interference by the stomach contents and wastes from exposed fish[26];[27].

2.3. Toxicity testing

A static bioassay was done[28] after performing a range finding test to obtain five graded concentrations [29];[27] of 60,70,80,90 and 100mg/l for both extracts of *P.biglobosa* pods on *C.gariepinus* juveniles with controls containing no extracts. 10 fishes each were randomly introduced into each of the reconstituted extracts which had been allowed to stand for 30 minutes[29], to ensure their proper mixing.

Clinical signs and deaths were monitored and recorded at the onset 24h, 48h, 72h and 96h respectively.The level of toxicity of the extracts was determined based on hazard rating for synthetic agrochemicals[30]. The maximum admissible toxicant concentrations for the extract were established for *C.gariepinus* juveniles by multiplying the estimated 96h LC_{50} by a constant 0.01-0.1[31]. Fish were considered dead when the opercula and tail movements stopped and there was no response to a gentle prodding. Dead fish were removed immediately from test solutions to avoid fouling the test media. Water quality parameters like dissolved oxygen, pH, hardness and temperature were monitored as contained in standard methods in [28]. Data was subjected to analysis of variance (ANOVA).

III. Results

The observation from the test carried out on the fishes showed that the toxic effects of aqueous and ethanol extracts of *P. biglobosa* pods on *C. gariepinus* juveniles were represented in the tables 1a,1b and 1c. Agitated behaviour and respiratory distress increased with increase in extract but decreased with exposure period for both extracts except for stunned posture, vertical positioning with exposed snout (VPES), air gulping and excessive mucus secretion that continued and increased with exposure period, while the observed abnormal

behaviour increased with increase in the quantity of both extracts on exposed fish except for sudden darts which decreased with exposure period.

The juveniles exposed to ethanol extract of *P. biglobosa* pods showed a higher mortality rate than those exposed aqueous extract of p. biglobosa. Fishes in the control group exhibited no unusual signs nor died throughout the 96hrs exposure period. The fishes that died are confirmed dead when it showed no response on pushing the experimental container gently, floating with side and change in colour to white and skin looks like its peeling. The number of mortality were recorded and the dead fish removed immediately from the bowl to avoid contamination of the water.

Table 1a: Agitated behavioural pattern of *C. gariepinus* juveniles exposed to varying concentrations of aqueous and ethanoic extracts of *P. biglobosa* pod.

Behavioural activity Pattern	Extracts	concentrations (Mg/l)					
		0	60	70	80	90	100
Aggression	A	-	-	-	-	+	++
	E	-	-	-	+	++	+++
Jumping	A	-	-	-	+	++	+++
	E	-	-	+	+	++	+++
Raised Dorsal Fin	A	-	-	-	+	++	+++
	E	-	-	+	++	+++	+++
Erratic Swimming	A	-	-	-	+	++	+++
	E	-	-	+	++	+++	+++
Frequent surface to Bottom movement	A	-	-	-	+	++	++
	E	-	-	-	+	++	+++
Stunned posture	A	-	+	++	++	+++	+++
	E	-	+	++	++	+++	+++

Table 1b: Respiratory distress rate of *C.gariepinus* juveniles exposed to varying concentrations of aqueous and ethanolic extracts of *P.biglobosa* pod

Behavioural activity Pattern	Extracts	concentrations (Mg/l)					
		0	60	70	80	90	100
Rapid Opercula Movement	A	-	-	-	-	+	++
	E	-	-	-	+	++	+++
Excessive Mucus Secretion	A	-	+	++	++	+++	+++
	E	-	+	++	++	+++	+++
Air Gulping	A	-	-	-	-	+	++
	E	-	-	-	+	++	++
Vertical Position with Exposed Snout	A	-	-	+	+	+	+
	E	-	-	+	++	++	+++

Table 1c: Abnormal nervous behavioural pattern of *C. gariepinus* juveniles on exposed to varying concentrations of aqueous and ethanoic extracts of *P. biglobosa* pod.

Behaviour activity Pattern	Extracts	concentrations (Mg/l) 0	60	70	80	90	100
Slothful and	**A**	-	-	-	+	+	++
swirling movement	**E**	-	-	-	+	+	++
Still state	**A**	-	+	++	++	+++	+++
	E	-	+	++	++	+++	+++
Change in Pigmentation	**A**	-	-	-	+	++	++
	E	-	-	+	++	+++	+++
Sudden dart	**A**	-	-	-	+	+	++
	E	-	-	-	+	+	++
Vertical, Angular or	**A**	-	+	++	+++	+++	+++
flat postures	**E**	-	+	++	+++	+++	+++
Death	**A**	-	+	+	++	++	+++
	E	-	+	++	+++	+++	+++

Keys:

A	=	Aqueous extract
E	=	Ethanol extract
-	=	None
+	=	Weak
++	=	Moderate
+++	=	Strong

IV. Discussion

From the result of this study, it was shown that both the aqueous and ethanol extracts of *P. biglobosa* pod are toxic to *C. gariepinus* juveniles and their toxicity increases along side with exposure period and increase in concentration. The level of toxicity also differs and this could be as a result of the method of preparation and extraction of the plant pods.

This study revealed that *C.gariepinus* juvenile exposed to aqueous and ethanol extract of *P.biglobosa* pod powder exhibited behavioural changes like, raised dorsal fin, erratic swimming, frequent surface to bottom movement, rapid opercula movement, vertical, angular or flat postures which are indications of physiological stress in fish. The dried Euphorbia heterophylla stem water extract was found to induce varying behavioural response in the fish. Also in an akin research by[32] reported agitated behaviours, respiratory distress and abnormal nervous behaviours when Oreochromis niloticus was exposed to aqueous and ethanol extracts of Ipomoea aquatica leaf at varying concentrations.

The exhibited clinical signs and eventual death of exposed fish may be due to direct poisoning leading to pathological alterations in their tissues and organs[33] and[34] or may indirectly be due to changes in the physiochemical conditions of their immediate external environment[35];[27]. The agitated behaviours correspond to both the contact and exertion phases of fish's response to toxicants exposures [36].

C.gariepinus juveniles exposed to various concentrations of aqueous and ethanol extracts of *P.biglobosa* were stressed progressively before death. This stressful behaviour had been reported in *C. gariepinus* juveniles exposed to aqueous extracts of *Blighia sapida* and *Kigelia africana*[37] and aqueous and ethanol extract of *Raphina vinifera*[3]. Increased physical activity, gulping of air, excessive secretion of mucus, erratic swimming, respiratory distress, sudden quick movement, change in colouration of fish, were associated

with P. biglobosa toxicity pod extract in this study. This is in line with the findings of[38] on *Oreochromis niloticus* exposed to trichloroform.

The abnormal nervous behaviours could be due to disruption of nervous system activity depending upon the part and the extent of such nervous involvement. This finding is in agreement with the reports of[39] and[40].

V. Conclusion

In conclusion, this work reviewed that the aqueous and ethanol extracts of *P.biglobosa* pods causes clinical behavioural changes and also threaten the life of the exposed *C.gariepinus* juveniles. Therefore P.biglobosa can be used to eradicate unwanted fishes and predators in the pond by farmers instead of using agrochemicals. Also the use of the plant (*P.biglobosa*) as fish bait by artisanal fishermen should be prohibited.

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