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Full Length Research Paper

Toxicity of *Parkia biglobosa* pod extract on *Clarias gariepinus* juveniles

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The toxicity of aqueous and ethanolic extracts of *Parkia biglobosa* pods (95, 85, 75, 65 and 55 mg/l) on *Clarias gariepinus* was investigated under laboratory condition over a 96 h exposure period. Fish exposed to both extracts exhibited clinical signs including eventual death but was not observed in the control fish. Weight change in fish was observed as well as the effect of the plant on the water parameters. However, clinical signs and death were more in fish exposed to the ethanolic extract. It was concluded that aqueous and ethanol extracts of *P. biglobosa* pods are toxic to *C. geriepinus* juveniles with the ethanol extract being more toxic, which shows that apart from the bark of *P. biglobosa*, the pods has piscicidal property and can be put into use in the control and management of fish ponds to eradicate predators by farmers.

Key words: *Parkia biglobosa,* pod, *Clarias gariepinus*, toxicity.

INTRODUCTION

Agrochemicals, such as pesticides, especially chlorinated hydrocarbons, are routinely employed as part of the integrated farming practice to protect crops and animals from insects, weeds and diseases. Widespread use of pesticide on farm is now a worldwide phenomenon (Omitoyin et al., 2006).

There are plants which contain chemicals which have traditionally been used to harvest fish in almost all parts of the world. Farmers in Nigeria have persistently and indiscriminately abused these natural plant piscicides by using much higher concentrations than necessary. Notable example of such plant piscicide is *Parkia biglobosa* (Fafioye et al., 2005) which is commonly found within the savannah belts of West Africa including Nigeria. The toxic parts of the plant known to contain piscicidial properties include the bark, pods, fresh seeds and the pulp (Bonkoungou, 1986; Campbell-Platt, 1980). This plant has been found to contain alkaloids, tannins and flavonoids (Omitoyin et al., 2006). Agricultural practices along river banks in Nigeria includes washing of pods of *P. biglobosa* off the seeds for dawa-dawa processing which could lead to a build up of toxic chemical concentrations in natural water, causing mass mortality of fish, contaminating the fresh water bodies and affecting non target organisms (Ayoola, 2008). The physical and chemical changes of aquatic environment such as reduction in water quality often cause some physiological changes in fish, thus, the water quality of an aquatic body is very crucial because, it determines the productivity and fish survival.

These plants that are poisonous to fish have been recognised as effective alternatives to harmful synthetic compounds (Fafioye et al., 2005) such as glyphosate (Ayoola, 2008). This is because they have low toxicity against non target animals (Chiayvareesajja et al., 1997) while being more environmental friendly (Marston and Hostettmann, 1985).

Widespread use of pesticide on farm is now a worldwide phenomenon (Omitoyin et al., 2006). The use of chlorinated hydrocarbon such as DDT, dieldrin and

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Table 1. Agitated behaviours of the test concentration (*Parkia biglobosa)* on *Clarias gariepinus* juveniles.

Clinical signs	Experimental group	0	55	65	75	85	95
Aggression	A	–	–	–	–	++	+++
	B	–	–	–	++	+++	+++
Jumping	A	–	–	–	++	+++	+++
	B	–	–	–	++	+++	+++
Stunned posture	A	–	–	++	++	+++	+++
	B	–	–	++	++	+++	+++
FSBM	A	–	–	–	+	++	+++
	B	–	–	+	+	++	+++

Frequent surface to bottom movements (FSBM), aqueous extract (A), ethanol extract (B), none (–), weak (+), moderate (++) and strong (+++).

lindane as pesticides has been documented. Pesticides currently in use are biocides that have high mammalian toxicity and necessitate considerable precautions in their application.

The use of piscicides as a tool in pond management during pond preparation to get rid of predators before fish stocking is an important tool (Harwood and Sytsma, 2003).

However, these chemicals may have negative effects on the environment, farmers and health (Ayoola and Ajani, 2007). Hence, there is a need to explore other environment and health-friendly fish toxicants such as botanical plants with piscicidal activity.

Clarias gariepinus which is hardy (Hogendoom, 1979; Olaifa et al., 2003) and indigenous to Africa (Rahaman et al., 1992), is now recognised as an important and commercial tropical catfish for aquaculture within the West African sub region (Clay, 1979; Anthony, 1982) including Nigeria where it is highly valued (Olaifa et al., 2003). This study is to investigate the effect of aqueous and ethanol extracts of *P. biglobosa* pod on *C. gariepinus* juveniles as well as on their immediate environment.

MATERIALS AND METHODS

P. biglobosa pods were collected and sun dried before blending them into fine powder prior to extraction. Six litres of distilled water was used to soak 1000 g of the fine powder of *P. biglobosa* pods over night, prior to filtration based on maceration method (Bentley, 1977; Ghani, 1990) which was subsequently oven dried, yielding 500 g (50.00% w/w) of the oven dried aqueous extract. Five litres of ethanol absolute was also used to soak 1300.00 g of the fine powder of *P. biglobosa* pods over a 48 h period prior to filtration based on same maceration method. This was subsequently concentrated to dryness over a 72 h period, yielding 750 g (57.69% w/w) of dry ethanol extract.

360 healthy juveniles of *C. gariepinus* was used for the toxicity test. The healthy juveniles of *C. gariepinus* were sourced from within Niger State and they were acclimatised for 5 days during which they were fed. Feeding was stopped 48 h before, and throughout the 96 h experimental period (Adeyemo, 2005) in order to minimize interference by the stomach contents of exposed fish and their waste in reconstituted extracts (Olufayo, 2009).

A static bioassay (APHA, 1995) was done after performing a range finding test to obtain five graded concentrations (Ayotunde and Ofem, 2008) of 55, 65, 75, 85, and 95 mg/l for aqueous and ethanol extract of *P. biglobosa* pods on *C. gariepinus* with control containing no extracts. Ten fishes each were randomly introduced into each of the reconstituted extracts which had been allowed to stand for 30 min (Usman et al., 2005). This is to allow for proper mixing of the reconstituted extracts. Clinical signs and deaths were promptly monitored and recorded over the 96 h exposure period.

Water quality parameter like dissolved oxygen, pH, hardness and temperature were monitored as contained in standard methods in APHA (1995). Data was subjected to analysis of variance (ANOVA)

RESULTS

C. gariepinus juveniles exposed to aqueous and ethanolic extract of *P. biglobosa* pod exhibited similar clinical sign such as agitated behaviour, respiratory distress and abnormal nervous behaviour (Tables 1 to 3). Agitated behaviour and respiratory distress increased with increasing extracts but decreased with exposure period except for stunned posture, vertical positioning with exposed snouts and excessive mucus secretion that persist and increased with exposure period, while abnormal nervous behaviours increased with both increasing extracts and exposure period except for sudden darts which decreased with exposure period.

Clinical signs and death were more in juveniles exposed to the ethanol extract of *P. biglobosa* pod than in fish exposed to aqueous extract of the same plant; there were no deaths or clinical signs in control fish. The death of the fish is confirmed by its floating on its side or failure to respond to stimulus even when touch with a forcep. The number of such fishes were recorded and removed from the test media to prevent contamination of the whole

Table 2. Respiratory distress of the test concentration (*Parkia biglobosa)* on *Clarias gariepinus* juveniles.

Clinical signs	Experimental group	0	55	65	75	85	95
Opercula movement	A	–	–	–	–	++	+++
	B	–	–	–	+	++	+++
Air gulping	A	–	–	–	–	+	++
	B	–	–	–	+	+	++
VPES	A	–	–	–	–	+	++
	B	–	–	–	+	+	++
EMS	A	–	+	++	++	+++	+++
	B	–	+	++	++	+++	+++

Vertical posture with exposed snouts (VPES), excessive mucus secretion (EMS), aqueous extract (A), ethanol extract (B), none (–), weak (+), moderate (++) and strong (+++).

Table 3. Abnormal nervous behaviour of the test concentration (*Parkia biglobosa)* on *Clarias gariepinus* juveniles.

Clinical signs	Experimental group	0	55	65	75	85	95
SSM	A	–	–	–	+	+	++
	B	–	–	–	+	+	++
Motionless state	A	–	+	++	++	+++	+++
	B	–	+	++	++	+++	+++
Sudden darts	A	–	–	–	–	+	++
	B	–	–	–	+	+	++
VAFP/DP	A	–	+	++	++	+++	+++
	B	–	+	++	++	+++	+++
Death	A	–	+	+	++	++	+++
	B	–	+	++	++	+++	+++

Sluggish and swirling movement (SSM), different postures (DP), vertical, angular or flat positions (VAFP), aqueous extract (A), ethanol extract (B), none (–), weak (+), moderate (++) and strong (+++).

media. The numbers of dead fishes were computed as percentage (%) mortality per period of exposure. This process was replicated to eliminate bias that may result due to handling, differences in size and weight, and other intrinsic physiological imbalance in the test organisms.

DISCUSSION

The result obtained from this study showed that both the aqueous and ethanolic extract of *P. biglobosa* had toxic effect on the juveniles of *C. gariepinus* and the effect of their toxicity increases with time of exposure.

The difference in the level of toxicity of the extracts could be as a result of the method of extraction used. In the case of aqueous extract, the alkaloids was extracted but the presence of water dilute it, hence reducing its potency, while the alkaloids of ethanolic extract obtained using ethanol as extracting medium remains almost undiluted.

Studies have shown that when the water quality is affected by toxicants, physiological changes will reflect in the values of one or more of the haematological parameter and swimming activity of the fish (Heath, 1991; Adeyemo, 2005).

Comparing both aqueous and ethanol extracts on the water quality, significant difference (P<.0001) was observed as shown in Table 5, which means *P. biglobosa* pods with different extracts has different effect on the physiochemical parameters of the water. Also the

Table 4. Weight change of *Clarias gariepinus* juveniles on exposure to *Parkia biglobosa* pod extract.

	Variable	Mean ± SD	t – value	Prob.	Remark
Weight	A	13.808 ± 4.02	- 2.55	0.012	S
	B	11.74 ± 4.18			

A = Initial weight, B = final weight. Significant difference (p < 0.0001) was observed on *Clarias gariepinus* juveniles before and after exposure to both extracts of *Parkia biglobosa* pod.

Table 5. T–test for comparing aqueous and ethanol extracts of *Parkia biglobosa* pod extract on the water quality on exposure to *Clarias gariepinus* juveniles.

Parameter	Extract	Mean ± SD	t – value	Prob.	Remark
pH	A	7.16 ± 0.13	20.32**	<0.0001	S
	B	6.59 ± 0.20			
DO_2	A	10.32 ± 0.62	26.22**	<0.0001	S
	B	6.72 ± 0.98			
Temperature	A	26.46 ± 0.10	– 81.58**	<0.0001	S
	B	28.37 ± 0.17			
Hardness	A	0.97 ± 0.09	2.49*	0.0140	S
	B	0.71 ± 0.87			
BOD	A	4.75 ± 1.85	6.46**	<0.0001	S
	B	2.52 ± 2.27			

A = Aqueous extract, B = ethanol extract. **Significant at 1%, * significant at 5%.

interaction between time and concentration (Tables 6 and 7) of the aqueous and ethanol extract of *P. biglobosa* pod was significantly difference (P<.0001) because there was continuous decrease in the water parameters when compared to the control in pH, DO2, hardness and B.O.D but for temperature which was significant at 5% and hardness for ethanol extract which was also significant at 5% which means there was no much difference in interaction between time and concentration. *C. gariepinus* juveniles exposed to various concen-trations of aqueous and ethanolic extract were stressed progressively with time before death. This stressful behaviour had been reported in *C. gariepinus* juveniles exposed to aqueous extracts of *Blighia sapida* and *Kigelia Africana* (Onusiriuka and Ufodike, 1994), and aqueous and ethanol extract of *Raphia vinifera* (Fafioye et al., 2004). The stressful behaviour exhibited by the fish may be as a result of respiratory impairment due to the effect of *P. biglobosa* pod extract as it affects the physiochemical parameter of the water since it is known to contain alkaloids (Omitoyin et. al., 2006). *C. gariepinus* became inactive at higher concentrations (55 to 95 mg/l) of the extract. This is similar to the report of *C. gariepinus* exposed to *R .vinifera* (Fafioye et al., 2004).

There was significant difference in weight of the fish before and after the exposure period as shown in table 4 and this may be due to the fact that feeding is stopped 48 hours before and during exposure period (Adeyemo, 2005) and this is done, so as to minimize interference by stomach content and their waste in reconstituted extract (Olufayo, 2009).Increased physical activity, convulsion, excess secretions of mucus, incessant gulping of air, erratic swimming, respiratory distress, paralysis, sudden quick movement, increase in opercula ventilation, and prior to death, darkening of fish, were associated with *P. biglobosa* toxicity in this study. This agreed with the findings of Abalaka and Auta (2010) on *Oreochromis niloticus* exposed to trichloroform. Omitoyin et al. (1999) reported similar observation in *Sarotherodon galilaeus Tetrapleura tetraptera*. The observed respiratory distress may have been due to both decreasing dissolved oxygen contents of reconstituted extract with decreasing ability of the exposed fish to respiration. Decreasing dissolved oxygen content of reconstituted extracts may be due to the continuous oxidative bio degradation of the constituents oxygen depletion is directly dependent upon the pollutants concentrations (Horsefall and Spiff, 1998). Excessive mucus secretions are natural defence mechanisms to coat their body surfaces to prevent or reduce absorption of offending toxicants

Table 6. Comparing interaction between time and concentration of aqueous extract of *Parkia biglobosa* pod.

Treatment	Parameter				
	pH	**DO_2**	**Temperature**	**Hardness**	**BOD**
Time (h)					
24	7.14^{b}	10.13^{c}	26.52^{a}	1.00^{a}	3.90^{c}
48	7.14^{b}	10.72^{a}	26.48^{ab}	0.95^{bc}	5.39^{b}
72	7.14^{b}	10.53^{b}	26.43^{b}	0.94^{c}	5.95^{a}
96	7.22^{a}	9.88^{d}	26.38^{c}	0.96^{b}	3.73^{d}
Concentration					
0	7.31^{a}	10.11^{d}	26.42^{c}	0.93^{cd}	4.94^{c}
55	7.21^{b}	10.79^{a}	26.45^{abc}	1.02^{a}	6.00^{a}
65	7.08^{e}	10.35b	26.48^{ab}	0.97^{b}	5.37^{b}
75	7.15^{c}	10.23^{c}	26.43^{bc}	0.96^{cb}	4.79^{d}
85	7.12^{d}	10.23^{c}	26.46^{abc}	0.97^{b}	4.08^{e}
95	7.09^{e}	10.23^{c}	26.50^{a}	0.91^{d}	3.32^{f}
Interaction T × C	<0.0001**	<0.0001**	0.0007*	<0.0001*	<0.0001*

**significant at 1%, *sign at 5%.

Table 7. Comparing interaction between time and concentration of ethanol extract of *Parkia biglobosa* pod.

Treatment	Parameter				
	pH	**DO_2**	**Temperature**	**Hardness**	**BOD**
Time (h)					
24	6.38^{d}	6.74^{b}	28.19^{d}	0.69^{ab}	3.05^{b}
48	6.47^{c}	6.67^{c}	28.29^{c}	1.08^{a}	1.29^{d}
72	6.65^{b}	7.03^{a}	28.45^{b}	0.40^{b}	3.37^{a}
96	6.83^{a}	6.43^{d}	28.53^{a}	0.66^{ab}	2.37^{c}
Concentration					
0	6.76^{a}	7.74^{a}	28.34^{a}	0.55^{a}	5.27^{a}
55	6.59^{c}	6.80^{c}	28.37^{a}	0.52^{a}	1.81^{d}
65	6.55^{d}	5.90^{f}	28.36^{a}	0.65^{a}	1.69^{e}
75	6.62^{b}	6.62^{d}	28.37^{a}	1.10^{a}	1.84^{d}
85	6.48^{f}	6.24^{e}	28.35^{a}	0.70^{a}	2.36^{b}
95	6.53^{e}	7.02^{b}	28.39^{a}	0.72^{a}	2.14^{c}
T × concentration	<0.0001**	<0.0001**	0.0008*	0.2212*	<0.0001**

**significant at 1%, *significant at 5%.

(Cagauan et al., 2004). The presence of excessive mucus secretions (Jothivel and Paul, 2008) could have hindered gaseous of both extracts. However, the extent of such dissolved activities (Sambasivarao, 1999) and may be responsible for the respiratory distress and death (Omitoyin et al., 2002). These findings of respiratory distress agree with the report of Ayoola (2008); also, the findings of excessive mucus secretions in exposed fish agree with the report of Jothivel and Paul (2008). Warren (1997) had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration, which will impair respiration, leading to asphyxiation.

Abnormal nervous behaviour could be due to the disruption of nervous system activity depending on the part and impact of the toxicants on fish (Fafioye et al., 2005) or may be due to biochemical body derangement including hepatic compromise (Fadina et al., 1991).

Conclusion

Generally, one could deduce from this research work that the introduction of *P. biglobosa* into water bodies would threaten the life and existence of fish. Therefore, this plant can actually be used as a biological control in eradicating predators and unwanted organisms in the ponds by farmers instead of using agrochemicals, but its use should be discouraged from being used to harvest fish from the wild and caution should be observed when it is being used as insecticides.

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