Journal of Animal and Veterinary Advances 9 (6): 1068-1072, 2010
ISSN: 1680-5593

Toxicity of Aqueous and Ethanol Extracts of *Parkia biglobosa* Pods on *Clarias gariepinus* Juveniles

[1]S.E. Abalaka and [2]J. Auta
[1]Department of Veterinary Pathology and Microbiology,
[2]Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria

Abstract: The toxicity of aqueous and ethanol extracts of *Parkia biglobosa* pods (55, 65, 75, 85 and 95 mgL^{-1}) on *Clarias gariepinus* juveniles was investigated over a 96 h exposure period. Fish exposed to both extracts exhibited clinical signs of agitated behaviours, respiratory distress and abnormal nervous behaviours and deaths were recorded in some fish exposed to both extracts. No unusual clinical manifestations or deaths were recorded in control fish. However, signs and deaths were more in fish exposed to the ethanol extracts. The 96 h LC_{50} values for aqueous and ethanol extracts were 296.14 and 225.48 mgL^{-1}, respectively. Both extracts are of minimal toxicity and the maximum toxicant admissible concentrations for aqueous and ethanol extracts ranged between 2.96-29.61 mgL^{-1} and between 2.26-22.55 mgL^{-1}, respectively. It was concluded that aqueous and ethanol extracts of *P. biglobosa* pods are toxic to *C. gariepinus* juveniles with the ethanol extract being more toxic.

Key words: *Parkia biglobosa* pods, *Clarias gariepinus*, toxicity, juveniles, clinical manifestations, lethal concentrations, maximum admissible toxicant concentrations

INTRODUCTION

The demand for fish in Nigeria is increasing by the day because of short supply of other animal protein. This is due to the myriads of problems facing the Nigerian livestock and poultry industry (Adekunmisi *et al*., 2004). Fish farming or aquaculture has been described as the world's fastest growing food production system (Kureshy *et al*., 2000) and has a lso been identified as a practical and promising approach to meeting the fish demand (Olaifa *et al*., 2008) outside actual fishing. However, this sector is facing some challenges (Spadling *et al*., 1997; Adekunmisi *et al*., 2004; Assiah *et al*., 1997; Taiwo and Odunaiya, 2004) thereby making fishing the only viable alternative where both conventional and unconventional methods are employed for the sole aim of obtaining fishes from the vast water bodies for human consumption. This includes the exploitation of synthetic compounds which bio-accumulates and persist in both target and non-target animals (Arasta *et al*., 1996) with untold consequences for man and aquatic environment (Fafioye and Jeje, 2000).

Plant piscicides or plants that are poisonous to fishes have been recognized as effective alternatives to these harmful synthetic compounds (Dahiya *et al*., 2000; Fafioye, 2005). This is because they have lower toxicity against non-target animals (Chiayvareesajja *et al*., 1997), while being more human and environmental friendly (Marston and Hostettmann, 1985). Notable example of such plant piscicide is *Parkia biglobosa* (Fafioye, 2005), which is commonly found within the savannah belts of West Africa including Nigeria. *Clarias gariepinus* which is hardy (Hogendoorn, 1979; Olaifa *et al*., 2003) and indigenous to Africa (Rahman *et al*., 1992) is now recognized as important 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 therefore, aimed at determining the clinical manifestations and lethal concentrations of the toxicities of aqueous and ethanol extracts of *P. biglobosa* pods to *C. gariepinus* juveniles in view of the fact that there has not been any such rersearch before now.

MATERIALS AND METHODS

The dried pods of *P. biglobosa* were collected and later blended into fine powder. Maceration method (Bentley, 1977) was used to extract about 429 g (45.90% w/w) of the freeze-dried aqueous extract after soaking 1000 g of this fine powder with 6 L of distilled water over night prior to filtration and freeze drying. Same

Corresponding Author: S.E. Abalaka, Department of Veterinary Pathology and Microbiology, Ahmadu Bello University, Zaria, Nigeria

method was used to obtain 725 g (57.56% w/w) of the ethanol extract after soaking 1260 g of this fine powder in a separation funnel over a 48 h (h) period with 5 L of absolute ethanol prior to filtration and concentration to dryness in an evaporation dish over a 72 h period.

Juvenile *C. gariepinus* (25.09±0.52 g mean weight; mean±SEM and 15.38±0.10 cm mean total lengths; mean±SEM) were purchased and acclimatized for two weeks under laboratory conditions while being fed ad libitum daily with commercial catfish feed (Multi feed, Zelmach feed mill, Israel). Pond water was changed every other day, while acclimatization mortality was <5%. Feeding was stopped 48 h before and through out the 96 h experimental period (Adeyemo, 2005). This was to minimize interference by both the stomach contents of exposed fish and their wastes in reconstituted extracts (Olufayo, 2009).

A static bioassay (APHA, 1985) was performed for the toxicity testing after conducting a pilot study to obtain five graded concentrations (Ayotunde and Ofem, 2008) of 55, 65, 75, 85 and 95 mgL^{-1} for aqueous and ethanol extracts of *P. biglobosa* pods on *C. gariepinus* juveniles, respectively. Ten fish 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. Control aquaria contained no extracts. Clinical signs and deaths were promptly monitored and recorded over the 96 h exposure period.

The Lethal Concentration (LC50) for each extract over the entire 96 h exposure period was determined by subjecting results to Probit and logit analyses (Finney, 1971). The level of toxicity of both extracts was determined based on hazard rating for synthetic agrochemicals (Louis *et al*., 1996). The maximum admissible toxicant concentrations for both extracts were established for *C. gariepinus* juveniles by multiplying the estimated 96 h LC_{50} by a constant 0.01-0.1 (Koesoemadinata, 2000).

RESULTS

Juvenile *C. gariepinus* exposed to both aqueous and ethanol extracts of *P. biglobosa* pods exhibited similar signs of agitated behaviours, respiratory distress and abnormal nervous behaviours respectively (Table 1-3). Agitated behavious increased with increasing extracts concentrations but decreased with exposure period except for the stunned posture that persisted through out the exposure period. The exhibited signs of respiratory distress were directly proportional to increasing extracts concentrations but indirectly proportional to exposure period except for vertical positioning with exposed snouts and excessive mucus secretion whose intensity increased with exposure period. The exhibited abnormal nervous behaviours increased with both increasing extracts concentrations and exposure period except for sudden darts whose intensity decreased with exposure period. Even though clinical manifestations were similar in fish exposed to both extracts, signs and death were more in *C. gariepinus* juveniles exposed to the ethanol extracts of *P. bigloboss* pods than in fish exposed to their aqueous counterparts while there were no deaths or any form of unusual clinical manifestations in control fish. The probit and logit analyses for determining the 96 h LC_{50} for both extracts were as presented in Fig. 1 and 2

Table 1: Agitated behaviours

		Extracts concentrations ($mg\ L^{-1}$)					
Clinical signs	Exp. group	0	55	65	75	85	95
Aggression	A	-	-	-	-	++	+++
	B	-	-	-	+	++	+++
Jumping	A	-	-	+	++	+++	+++
	B	-	-	+	++	+++	+++
Stunned posture	A	-	-	++	++	+++	+++
	B	-	-	++	++	+++	+++
FSBM	A	-	-	-	+	++	+++
	B	-	-	+	+	++	+++

Experimental group (Exp. group), Frequent Surface to Bottom Movements (FSBM), Aqueous extract (A), Ethanol extract (B), None (-), Weak (+), Moderate (++) and Strong (+++)

Table 2: Respiratory distress

		Extracts concentrations ($mg\ L^{-1}$)					
Clinical signs	Exp. group	0	55	65	75	85	95
Opercula movement	A	-	-	-	+	++	+++
	B	-	-	-	+	++	+++
Air gulping	A	-	-	-	-	+	++
	B	-	-	-	+	+	++
VPES	A	-	-	-	-	+	++
	B	-	-	-	+	+	++
EMS	A	-	+	++	++	+++	+++
	B	-	+	++	++	+++	+++

Experimental group (Exp. group), 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

		Extracts concentrations ($mg\ L^{-1}$)					
Clinical signs	Exp. group	0	55	65	75	85	95
SSM	A	-	-	-	+	+	++
	B	-	-	-	+	+	++
State of motionless	A	-	+	++	++	+++	+++
	B	-	+	++	++	+++	+++
Sudden darts	A	-	-	-	-	+	++
	B	-	-	-	+	+	++
DP	A	-	+	++	++	+++	+++
	B	-	+	++	++	+++	+++
Death	A	-	+	++	++	+++	+++
	B	-	+	++	++	+++	+++

Experimental group (Exp. group), Sluggish and Swirling Movements (SSM), Different Postures (DP), Aqueous extract (A), ethanol extract (B), None (-), Weak (+), Moderate (++) and Strong (+++)

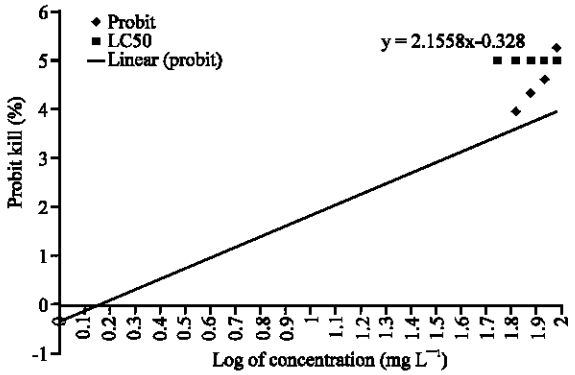

Fig. 1: Log of concentrations of aqueous extracts of *P. biglobosa* pods and its probit value in exposed *C. gariepinus* juvenile (96 h)

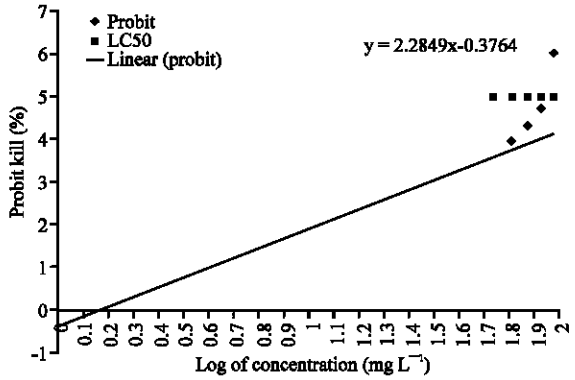

Fig. 2: Log of concentrations of ethanol extracts of *P. biglobosa* pods and its probit value in exposed *C. gariepinus* juvenile (96 h)

and the 96 h LC_{50} values for aqueous and ethanol extracts of *P. biglobosa* pods were 296.14 and 225.48 mgL^{-1}, respectively. The maximum toxicant admissible concentrations ranged between 2.96-29.61 mgL^{-1} for the aqueous extract and between 2.26-22.55 mgL^{-1} for the ethanol extract, respectively.

DISCUSSION

The exhibited agitated behaviours were fish flight response from making contact with reconstituted extracts so as to prevent the absorption of offending extracts. The findings agree with reports of Omoniyi *et al.* (2002) and Usman *et al.* (2005). The observed respiratory distress may have been due to both decreasing dissolved oxygen content of reconstituted extracts with decreasing capacity and/or ability of exposed fish to oxygenate their blood via respiration. The decreasing dissolved oxygen content of reconstituted extracts with exposure period may be due to the nature of phytochemical constituents of aqueous and ethanol extracts of *P. biglobosa* pods coupled with their subsequent continuous oxidative bio-degradation over time. However, the extent of such oxygen depletion due to the continuous oxidative bio-degradation of both extracts may depend on such extract concentrations. The decreasing capacity and/or ability to respire may be due to pathological alterations in the gills and skin (the primary and secondary respiratory structures) of exposed fish where the extent of such decreasing incapacitation could be directly proportional to the level of pathological compromise of these vital respiratory structures. Excessive mucus secretions are natural defense mechanisms by exposed fish to coat their body surfaces in order to prevent and/or reduce the absorption of offending toxicants (Cagauan *et al.*, 2004). However, such excessive mucus secretions are reported to reduce respiratory activity in fishes (Konar, 1975) which together with decreasing oxygen content of reconstituted extracts results in the creation of hypoxic states in exposed fishes (Usman *et al.*, 2005) leading to subsequent respiratory distress and deaths in exposed fishes (Omitoyin *et al.*, 1999). The findings of exhibited excessive mucus secretions in exposed fish agree with the report of Jothivel and Paul (2008). Abnormal nervous behaviours are associated with the impacts of the toxicants on fishes (Fafioye, 2005). This may be due to nervous system involvement or failure (Ufodike and Omoregie, 1994; Oti and Ukpabi, 2000) or may be due to biochemical body derangement including hepatic compromise (Fadina *et al.*, 1991).

The findings agree with the reports of Omitoyin *et al.* (1999), Omoniyi *et al.* (2002) and Ayotunde and Ofem (2008). The observed increasing state of inactivity with increasing extracts concentrations and exposure period are considered normal in both acute and chronic toxicity testing (Kulakkattolickal and Kramer, 1997).

The estimated 96 h LC_{50} values were due to the toxicities of both extracts on exposed fish. These values were much higher than 96 h LC_{50} values of 0.36 and 13.18 mg L^{-1} reported by Omitoyin *et al.* (2006) and Usman *et al.* (2005), respectively meaning that the pollutants used by these researchers were more toxic to *C. gariepinus* juveniles than aqueous and ethanol extracts of *P. biglobosa* pods. Even though, the ethanol extract of *P. biglobosa* pods was more toxic compared to their aqueous counterparts from their estimated 96 h LC_{50} values and exhibited clinical signs, both extracts of *P. bigloobsa* pods are of minimal toxicity to exposed *C. gariepinus* juveniles based on the hazard ratings for synthetic agrochemicals (Louis *et al.*, 1996). The findings of ethanol extract being more toxic to xposed *C. gariepinus* juveniles agree with the report of

Fafioye *et al.* (2004). The estimated maximum toxicant admissible concentrations for aqueous and ethanol extracts of *P. biglobosa* pods were concentration ranges which will not have any adverse effect on exposed fishes or the physicochemical constituents of their immediate external environment (Lesnikov, 1979).

CONCLUSION

In this study, we observed clinical signs and deaths of exposed *C. gariepinus* juveniles including the established 96 h LC_{50} values for both extracts implies that the aqueous and ethanol extracts of *P. biglobosa* pods are toxic and therefore, can be used as effective piscicide in place of synthetic compounds to harvest fishes from our abundant water bodies.

REFERENCES

APHA, 1985. Standard Methods for Examination of Water and Waste Water. 16th Edn., American Public Health Association, New York, pp: 1193.

Adekunmisi, A.A., H.A. Awojobi, M.A.S. Mulero and M.A. Onigemo, 2004. Performance of *Clarias gariepinus* fed poultry offal meal as a replacement for fishmeal. Nig. J. Anim. Prod., 31: 286-290.

Adeyemo, O.K., 2005. Haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus*. Afr. J. Biomed. Res., 8: 179-183.

Anthony, A.D., 1982. Identification of Nigerian Freshwater Fishes. University of Jos Press, Jos, Nigria, pp: 21.

Arasta, T., V.S. Bais and P. Thakur, 1996. Effects of Nuvan on some biological parameters of Indian catfish, Mystus vittatus. J. Environ. Biol., 17: 167-169.

Assiah, V.E., V.S. Ton and H. Aldin, 1997. Small Scale Fresh Water Fish Farming. 2nd Edn., Agromisa Press, Netherlands, ISBN: 90-77073-83-3, pp: 8.

Ayotunde, E.O. and B.O. Ofem, 2008. Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to adult Nile tilapia (*Oreochromis niloticus* Linna 1757). Afr. J. Biotechnol., 7: 2267-2274.

Bentley, A.O., 1977. Bentleys Textbook of Pharmaceutics. 8th End., Bailliére Tindall, London, ISBN: 10: 0702003913, pp: 177-180.

Cagauan, A.G., M.C. Galaites and L.J. Fajardo, 2004. Evaluation of botanical piscicides on Nile Tilapia (*Oreochromis niloticus* L.) and mosquito fish (*Gambusia affinis* Baird and Girard). Proceedings of the 6th International Symposium on Tilapia in Aquaculture, Sept. 12-16, Manila, Philippines, American Tilapia Association and Philippine Bureau of Fisheries and Aquatic Resources, pp: 179-187.

Chiayvareesajja, S., J. Chiayvareesajja, N. Rittibhonbhun and P. Wiriyachttra, 1997. The toxicity of five Thai plants to aquatic organisms. Asian Fish. Sci., 9: 261-267.

Clay, D., 1979. Population biology, growth and feeding of the African catfish (*Clarias gariepinus*) with special reference to juveniles and their importance in fish culture. Arch. Hydrobiol., 87: 453-482.

Dahiya, S., A. Kuar and N. Jain, 2000. Studies on diflouridation of water with Eichornia plant. J. Environ. Biol., 21: 337-340.

Fadina, O.O., V.O. Taiwo and A.O. Ogunsanmi, 1991. The effects of single and repetitive oral administration of common pesticides and alcohol on rabbits. Trop. Vet., 17: 97-106.

Fafioye, O.O. and C.Y. Jeje, 2000. Toxicity of the herbicides primextra and gramoxone on two tilipine fish. Biosci. Res. Comm., 12: 407-413.

Fafioye, O.O., 2005. Plants with piscicidal activities in South Western Nigeria. Turk. J. Fish. Aqua. Sci., 5: 91-97.

Fafioye, O.O., A.A. Adebisi and S.O. Fagade, 2004. Toxicity of *Parkia biglobosa* and Raphia vinifera extracts on *Clarias gariepinus* juveniles. Afr. J. Biotechnol., 3: 627-630.

Finney, D.J., 1971. Probit Analysis. 3rd Edn., Cambridge University Press, Cambridge, UK.

Hogendoorn, H., 1979. Controlled propagation of Africa catfish, Clarias lazera (C&V).1. Reproductive biology and field experiment. Aquaculture, 17: 323-333.

Jothivel, N. and V.I. Paul, 2008. Evaluation of the acute toxicity of the seeds of *Anamirta cocculus* (Linn.) and its piscicidal effect on three species of freshwater fish. The Internet J. Toxicol. 5 (1). http://www.ispub.com/ostia/index.php?xmlPrinter=true&xmlFilePath=journals/ijto/vol5n1/fish.xml.

Koesoemadinata, S., 2000. Acute toxicity of the insecticide formulation of endosulphan, chlorpyrifos and chlorofluazuron to three freshwater fish species and freshwater giant prawn. J. Penelitian Perlikan Indonesia, 4: 36-43.

Konar, S.K., 1975. Pesticides and aquatic ecosystems. Indian J. Fish, 22: 80-85.

Kulakkattolickal, A.T. and D.I. Kramer, 1997. The role of air breathing in the resistance of bimodally respiring fish to water. J. Fish Biol., 32: 119-127.

Kureshy, N., D.A. Davis and C.R. Arnold, 2000. Partial replacement of fish and bone meal, flash-dried poultry by-product meal and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. North Am. J. Aquac., 62: 266-272.

Lesnikov, L.A., 1979. Elaboration of Standards of Acceptable Concentration of Pollutant in the Water of Fish Culture Units. Sbornik Nauènych Trudov, GosNIORCH, Leningrad, pp: 3-41.

Louis, A.H., L.W. Diana, H. Patricia and R.S. Elizabeth, 1996. Pesticides and Aquatic Animals: A Guide to Reducing Impacts on Aquatic Systems, Virginia Cooperative Extension. Virginia State University, Virginia, pp: 24.

Marston, A. and K. Hostettmann, 1985. Plant molluscicides. Phytochemistry, 24: 639-652.

Olaifa, F.E., A.K. Olaifa and O.O. lewis, 2003. Toxic stress of lead on *Clarias gariepinus* (African catfish) fingerlings. Afr. J. Biomed. Res., 6: 101-104.

Olaifa, F.E., O.O. Oyetoyan and R.A. Hamzat, 2008. Acute toxicity of ethanol extracts of cocoa bean shell on *Sarotherodon galilaeus* juveniles. J. Fisheries Int., 3: 56-60.

Olufayo, M.O., 2009. Haematological characteristics of *Clarias gariepinus* (Burchell, 1822) juveniles exposed to Derris elliptica root powder. Afr. J. Food Agric. Nutr. Dev., 9: 920-933.

Omitoyin, B.O., A.O. Ogunsami and B.T. Adesina, 1999. Studies on acute toxicity of piscicidal plant extracts (*Tetrapleura tetraptera*) on Tilapia (*Sarotherodon gailaeus*) fingerlings. Trop. J. Anim. Sci. 2: 191-197.

Omitoyin, B.O., E.K. Ajani, B.T. Adesina and C.N.F. Okuagu, 2006. Toxicity of lindane (Gamma Hexachloro-Cyclottexane) to *Clarias gariepinus* (Burchell, 1822). World J. Zool., 1: 57-63.

Omoniyi, I.A., O. Agbon and S.A. Sodunke, 2002. Effects of lethal and sub-lethal concentrations of Tobacco (*Nicotiana tobacum*) leaf dust extraction on weight and haematological changes in *Clarias gariepinus* (Burchell). J. Applied Sci. Environ. Manage., 6: 37-41.

Oti, E.E. and U.H. Ukpabi, 2000. Acute toxicity of milk extracts *Thevetia peruviana* (Persoon) to African freshwater catfish, *Clarias gariepinus* (Tengels). Afr. J. Sci. Technol., 1: 132-138.

Rahman, M.M., I. Varga and S.N. Choudhury, 1992. Manual on African Magur (*Clarias gariepinus*) Culture in Bangladesh. FAO-UNDP, Dhaka, Bangladesh, pp: 1-45.

Spadling, M., F. Blasco and C. Field, 1997. World Mangrove Atlas. International Society for Mangrove Ecosystem, Okinawa, Japan.

Taiwo, I.O. and O. Odunaiya, 2004. Sex ratio and fecundity of *Chrysichthys nigrodigitatus* (Family-Claroteidae) from Asejire Lake, Nigeria. Nig. J. Anim. Prod. 31: 135-143.

Ufodike, E.B.C. and E. Omoregie, 1994. Acute toxicity of water extracts of barks of *Balanites aegyptica* and *Kigelia africana* to *Oreochromis nitoticus* (L.). Aquac. Fish. Manage., 25: 873-879.

Usman, J.I., J. Auta, A.K. Adamu and M.S. Abubakar, 2005. Toxicity of methanol extract of *Euphorbia laterifolia* (Schum and Thann) to the juveniles of the African catfish (*Clarias gariepinus*) (Teugels). Chem. Class J., 2: 59-61.