International Journal of Sciences

Research Article Volume 8 – May 2019 (05)

Histological Alterations in the Gills, Skin and Liver of Adult Nile Tilapia, *Oreochromis niloticus* (Linnaeus I779) Exposed to Jatropha Curcas Seed Powder

I. C. Adene[1]

[1]Department of Fisheries and aquaculture, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria

Abstract: Adult Nile Tiapia, *Oreochromis niloticus* was exposed to *Jatropha curcas* seed powder, a multipurpose plant used for demarcation, boundaries and the seeds for biodiesel and traditional medication. The histological alteration of the gill, skin and liver were studied at sub-lethal concentration of 133.3,200, 266.7, 333.3, 400 and 0 as control. The pathological lesions observed on the gill are Lamella degeneration, Hyperthrophy of the gill arc and severe necrosis of gill filament, Submucosal Congestion, Severe erosion of the secondary lamellae and cartilage at the base. The lesions on the skin are necrosis, dermal cell erosion and cellular infiltration by mononuclear cells. In the liver, moderate degeneration of periportal, diffuse vacuolation of hepatocytes and congested portal area were observed. The alteration on each of the organ became severe with increase in concentration of *Jatropha curcas* seed powder.

Keywords: Histology, Gill, Skin, Liver, Nile Tilapia, *Jatropha curcas*

Introduction:

Pollution is the major problem facing aquatic environment, the organisms in this environment are greatly affected. The immune system of aquatic organisms, such as fish, is continuously affected by periodic or unexpected changes of their environment. Adverse environmental situations may acutely or chronically stress the health of fish, altering some of their biochemical parameters and suppressing their innate and adaptive immune responses (Giron-peres *et. al*.,2007).

Histological changes are very sensitive thing that provide a better assessment of fish health, as well as the effects of pollution on each biochemical parameter. The histological changes that occur in the organs of fish have been integrated with the impact of various stressors (microbial pathogens, toxic compounds, nutritional and adverse environmental conditions). *Jatropha curcas* plant (Euphorbiaceae) contains a variety of biologically active phytochemicals such as proteins, peptides and diter-penes exhibiting a spectrum of biological activities (Devappa *et al*., 2010b, 2011). However, the seeds contain toxic phytochemicals called phorbol esters (PEs) (Haas *et al*., 2002; Rakshit *et al.*, 2011). *Jatropha curcas L*. is an oil-bearing shrub, widely distributed in Nigeria and many Latin American, Asian, and African countries (Gubitz *et al*., 1999). Majority of cultivation of J*atropha curcas* is being done in developing countries (GEXSI, 2008).

Recently, the non-edible *Jatropha curcas* has been hailed as one of the world's most sustainable biofuel crops. The *Jatropha* seed oil has gained tremendous interest as a feedstock for biodiesel production (Makkar and Becker, 2009; Devappa *et al*., 2010a).Among the different species of *Jatropha*, *Jatropha curcas* has a wide range of uses and promises various significant benefits to human and industry. Extracts from this species have been shown to have anti-tumor activity, the leaves can be used as a remedy for malaria and high fever (Gubitz *et al*., 1999; Henning, 1997) the seeds can be used in treatment of constipation and the sap was found effective in accelerating wound healing procedure (Gubitz *et al*., 1999). Species of the genes *Jatropha* are known to be very toxic. Several cases of *J. curcas* nut poisoning in humans after accidental consumption of seeds have been recorded. Symptoms such as giddiness, emesis and diarrhea have been reported (Makkar & Becker, 1998).

Nile tilapia is a tropical species that prefers to live in shallow water. The lower and upper lethal temperatures for Nile tilapia are 11-12 °C and 42 °C, respectively, while the preferred temperature ranges from 31 to 36 °C. *Oreochromis niloticus* is a relatively large cichlid fish, which is native to Africa

Published at: http://www.ijsciences.com/pub/issue/2019-05/
DOI: 10.18483/ijSci.1897; Online ISSN: 2305-3925; Print ISSN: 2410-4477

I. C. Adene (Correspondence)
+

from Egypt south to East and Central Africa and as far west as Gambia. It is also native to Israel, and numerous introduced populations exist outside its natural range (e.g. Brazil). According to Fagbenro (2002), it is presently farmed in the tropics, and sub-tropics of all continents, and occasionally where warm water is available, such as thermal effluent or geo-thermal spring.

Majority of the African freshwater aquaculture fishes that is of tropical origin breed naturally during the flood/ raining season which in most cases comes with increase in volume and turbidity of water. The seeds of *J. curcas* are normally washed by erosion from the gardens and farms into the rivers and ponds. In this case the seed can be swallowed by adult fish or crushed by some amphibians and crustaceans which assist in the release of the toxic substances such as the phorbol ester, the saponnis and the curcins that can be responsible for the toxification of the aquatic environment.

Materials and Methods

The experiment was conducted under standard static bioassay procedure (Reish and Oshida, 1987; American Public Health Association, 1987). Which involved carefully controlled environmental conditions as to define the response of the test fishes to *J. curcas* seed powder. The experiment was conducted at the Fish Farm (Obakekere) of the Federal University of Technology, Akure.

Collection of Experimental Fishes

Apparently healthy adult *O. niloticus* (total length 11.5cm-20cm, weighing 41.6g-105.5g) were collected from Ondo State Agricultural Development Project (ADP) farm, Akure Ondo State, Nigeria, and acclimated for one week in the farm inside rectangular concrete tanks, (75cm x 45cm x 45cm) container of 121.5 litres capacity, filled with 50litres unchlorinated well water. The fish were fed with pelleted fish diet containing 40% crude protein, during the acclimation period. Feeding was discontinued after 48 hours before the commencement of the experiment to minimize the production of waste in the test container.

Preparation of *Jatropha curcas* Seed Powder

Large quantities of freshly mature seeds of *J curcas* were collected from a private garden at No.12 Clerk Quarters, Owo, Nigeria. The seed powder was prepared according to the method described by Prince (2000). The seeds were sun-dried at ambient temperature (25-28^{oc} over three days), seed coats and wings were manually removed. The white kernel was ground to fine powder, using the coffle mill attachment of a Moulinex domestic blender. The powder was kept in the freezer in an air tight cellophane bag for later use. The behaviour pattern and mortality of the test fishes in each tank was monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours and every four hours, for the rest of the 24 hours, and once every 24 h until 96 h. Dead fish were removed immediately with a scoop net.

Toxicity Test

Range Finding Test: preliminary 24-hours range finding test was conducted for the experimental fishes following static bioassay procedures described by Parrish (1985) to determine the toxic range of *J. curcas* seeds to the experimental fishes. There were six treatments comprising three replicates, hence 36 tanks were used. A batch of ten of each of the experimental fishes were batch weighed and stocked into each tank (75cm x 45cm x 45cm) filled with 30L of unchlorinated well water. The seed powder was introduced into each tank at 400mg/l, 466.67mg/l, 533mg/l, 600mg/l and 666.67mg/l with a control of 0 mg/l. The behaviour and mortality of the test fish in each tank were monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours and every four hours for the rest of the 24hours period.

Definitive Test: Based on the results from the range finding (Lethal toxicity) test described above, 96 hours definitive (sub-lethal toxicity) tests following static bioassay procedures described by Parrish (1985) was carried out**.** There were six treatments comprising three replicates, hence 18 tanks were used. A batch of ten of each of the experimental fishes were batch weighed and stocked into each tank (75cm x 45cm x 45cm) each filled with 30L of unchlorinated well water. The seed powder was introduced into each tank at 133.3mg/l, 200mg/l, 266.7mg/l, 333.3mg/land 400mg/l. Test fish were not fed throughout the 96hours that the experiment lasted. Mortality of the test fish in each tank were monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours, every four hours for the next 24 hours and every 24hours for the remaining 96 hours. The inability of fish to respond to external stimuli was used as an index of death. Dead fish were removed immediately with a scoop net to avoid contamination due to rotting. Apart from monitoring and recording mortality the fish behaviour such as erratic swimming, air gulping and loss of reflex were also monitored.

Histological examination of test Organs

At the end of the experiment, one fish per treatment, that is, three fish per concentration were sampled after 96hours of exposure to *J. curcas* seed powder for histological analysis, the fish was anesthetized in aerated buffered tricaine methanesulfonate (MSS

222) to depress the CNS so as to reduce the impact of pain. The fish was sacrificed and dissected to remove the gill, liver and skin. The organs were fixed in 100% formalin for three days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry (1976). In graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding.

The organs were then embedded in molten wax. Tissue were sectioned into thin sections (5-7μm), by means of a rotatory microtome and were dehydrated and stained with harris haematoxylin-eosin (H&E) stain, Bancroft and Cook (1994), using a microtone and each section was cleared by placing in warm water (38ºC), where it was picked with clean slide and oven-dried at 58ºC for 30 minutes to melt the wax. Slides containing sectioned materials/tissue was cleared using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each.

The section was stained in haematoxyline eosin for ten minutes. The stained slide were observed under a light microscope at varying X100 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a digital camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P).

Result and Discussion

Table 1: Summary of Histological changes observed in adult *O. niloticus* exposed to *Jatropha curcas* seed powder for 96h.

CONCENTRATION(mg/l)	GILL (Plate 7)	SKIN (Plate 8)	LIVER (Plate 9)
CONTROL	Normal gill no lesion was observed	Skin is normal no lesion was observed.	No lesion observed.
133.3	Part of the gill arch filament and lamella degenerated	Normal skin. No visible lesion.	Periportal degenerate moderately.
200	Hyperthrophy of the gill arc and severe necrosis of gill filament.	Normal skin, No visible lesion.	Mild diffuse vacuolar degeneration.
266.7	Submucosal Congestion. Severe erosion of the secondary lamellae .	Normal skin No visible lesion.	Diffuse vacuolation of hepatocytes.
333.3	Severe submucosal congestion and absent of secondary lamellae.	Necrosis and dermal cell erosion	Diffuse vacuolation of hepatocytes
400	Observation of cartilage at the base and also it is devoid of lamellae.	Necrosis of the skin with cellular infiltration by mononuclear cells.	Severe diffuse hepatic vacuolar degeneration and congested portal area.

PLATES 1(A-F): Histological change observed in the gill of adult *O. niloticus* exposed to different concentration of *J. curcas*

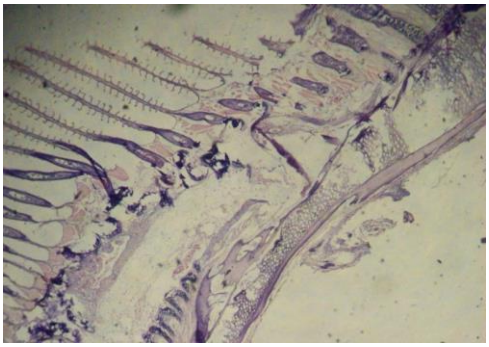

Plate 1a: Gill of adult *O. niloticus* in the control tank showing normal gill with no lesion.(X100)

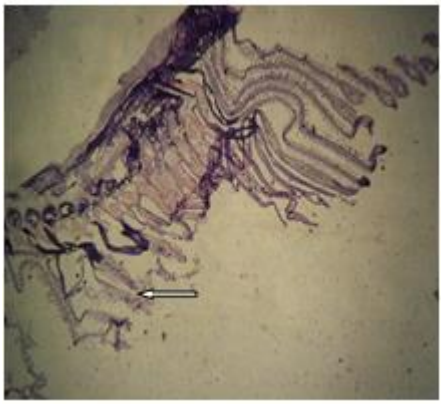

Plate 1b: Gill of adult *O. niloticus* exposed to 133.3mg/l of *J. curcas* showing part of the gill arch filament and lamella degenerated.(X100)

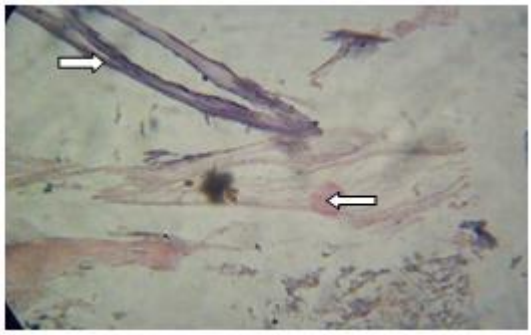

Plate 1c: Gill of adult *O. niloticus* exposed to 200mg/l of *J. curcas* showing hyperthrophy of the gill arc and severe necrosis of gill filament.(X100)

Plate1d: Gill of adult *O. niloticus* exposed to 266.7mg/l of *J. curcas* showing submucosal congestion and severe erosion of the secondary lamellae.(X100)

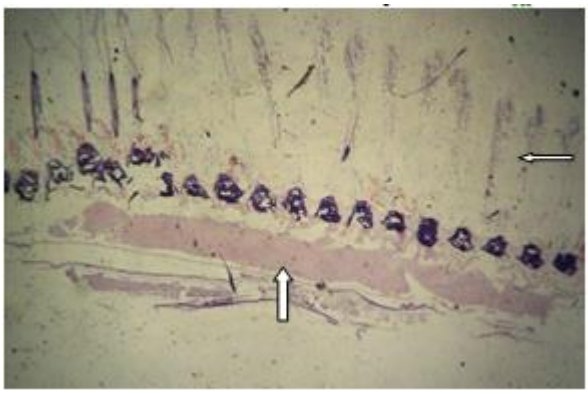

Plate 1e: Gill of adult *O. niloticus* exposed to 333.3mg/l of *J. curcas* showing severe submucosal congestion and absent of secondary lamellae.(X100)

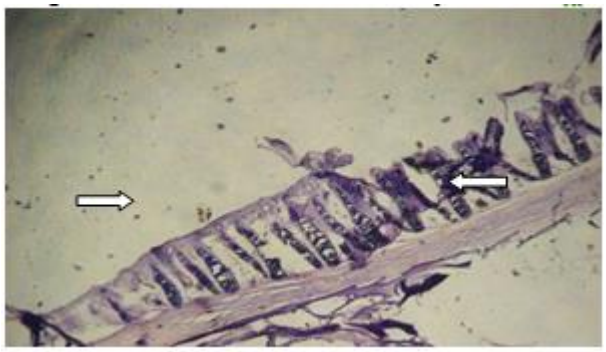

Plate 1f: Gill of adult *O. niloticus* exposed to 400mg/l of *J. curcas* showing cartilage at the base of the gill and devoid of lamellae.(X100)

PLATE 2(A-F): Histological change observed in the skin of adult *O. niloticus* exposed to different concentration of *J. curcas* seed powder

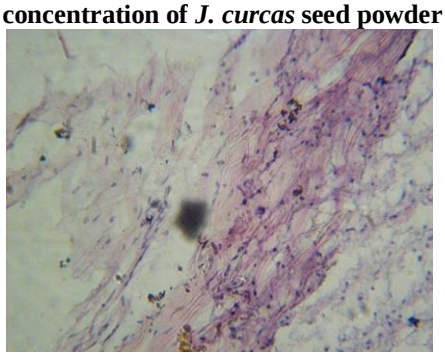

Plate 2a: Skin of *O. niloticus* in the control tank showing normal skin with no lesion. (X100)

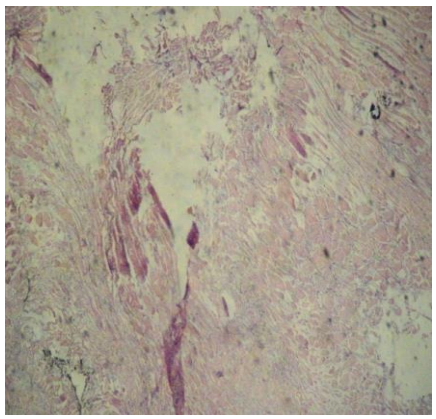

Plate 2b: Skin of adult *O. niloticus* exposed to 133.3mg/l of *J. curcas* showing no lesion.(X100)

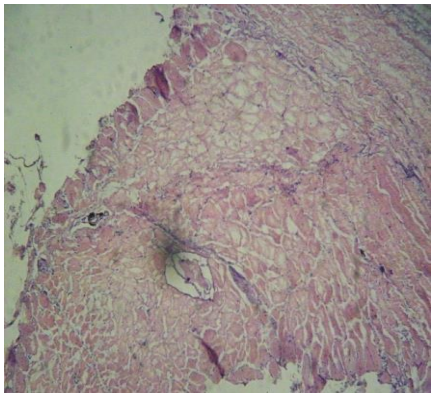

Plate 2c: Skin of adult *O. niloticus* exposed to 200mg/l of *J. curcas* showing no lesion.(X100)

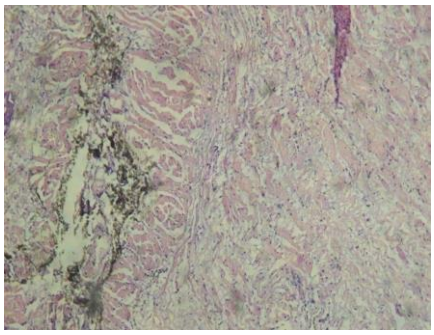

Plate 2d: Skin of adult *O. niloticus* exposed to 266.7mg/l of *J. curcas* showing no lesion.(X100)

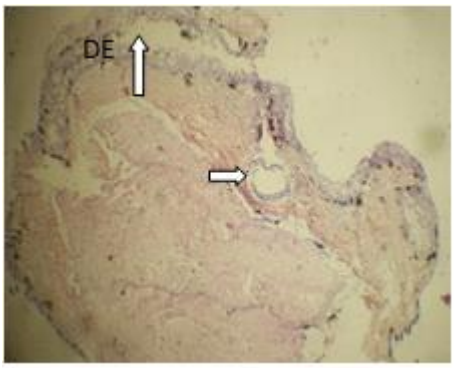

Plate 2e: Skin of adult *O. niloticus* exposed to 333.3mg/l of *J. curcas* showing necrosis and dermal cell erosion. (X100)

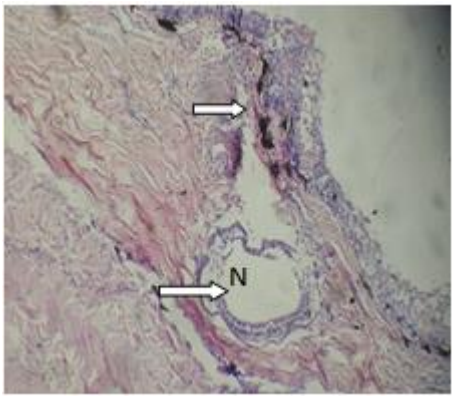

Plate 2f: Skin of adult *O. niloticus* exposed to 400mg/l of *J. curcas* showing necrosis and cellular infiltration by mononuclear cells. (X100)

PLATES 3(A-F): Histological change in the liver of adult *O. niloticus* exposed to different concentration of *J. curcas*

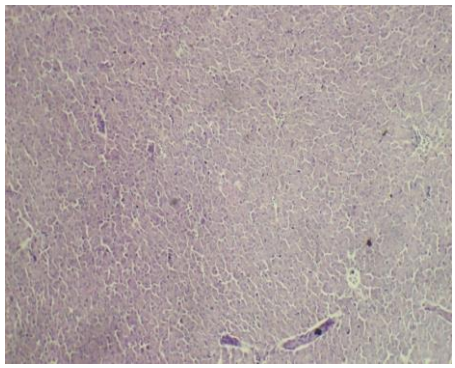

Plate 3a : Liver of adult *O. niloticus* in the control tank with no lesion. (X100)

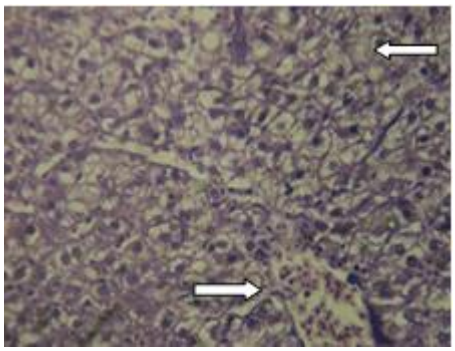

Plate 3b: Liver of adult *O. niloticus* exposed to 133.3mg/l of *J. curcas* showing moderately degenerated periportal. (X100)

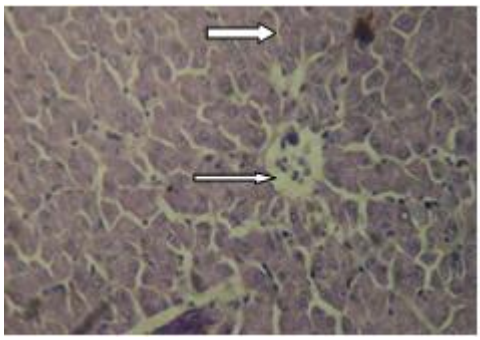

Plate 3c: Liver of adult *O. niloticus* exposed to 200mg/l of *J. curcas* showing mild diffuse vacuolar degeneration.(X100)

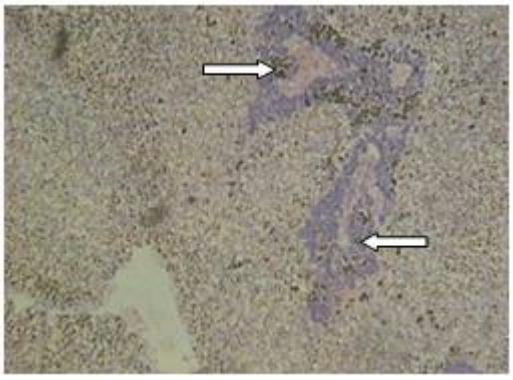

Plate 3d: Liver of adult *O. niloticus* exposed to 266.7mg/l of *J. curcas* showing diffuse vacuolation of hepatocytes . (X100)

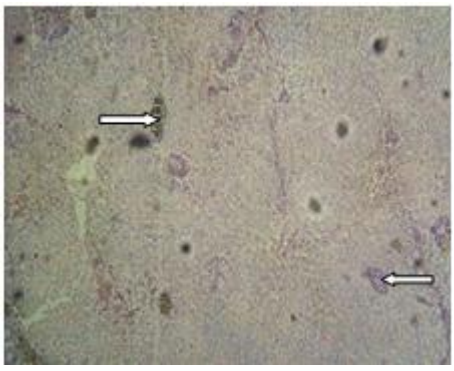

Plate 3e: Liver of adult *O. niloticus* exposed to 333.3mg/l of *J. curcas* showing diffuse vacuolation of hepatocytes . (X100)

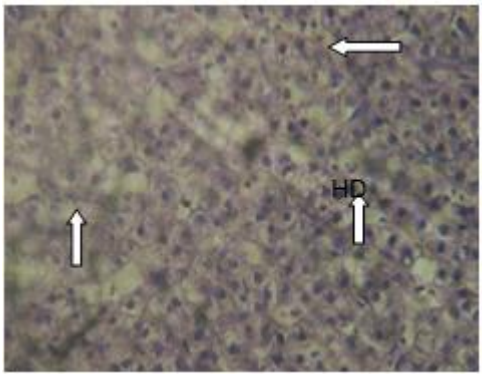

Plate 3f: Liver of adult *O. niloticus* exposed to 400mg/l of *J. curcas* showing Severe diffuse hepatic vacuolar degeneration and congestion of portal area.(X100)

Toxicity bioassays are mostly used to predict a toxicant influence and fate on the aquatic organisms. In this study the LC_{50} of *J.curcas* for adult catfish *C. gariepinus* at 96h is 314.43mg/l. The admissible toxicant concentration range for 96 h LC_{50} is between 3.1443mg/l - 31.443mg/l, these value was derived by multiplying a constant 0.01-0.1 by 96 h LC_{50} (Koesoemadinata, 2000).

Table1 shows the summary of histological changes in the adults Nile tilapia exposed to different concentration of *J. curcas* seed powder. The histological pictures on plate 1-3 present the changes that were observed in adult tilapia when treated with 133.3mg/l, 200mg/l, 266.7mg/l, 333.3mg/l, 400mg/l and 0mg/l as control. The result revealed changes in the gill, skin and Liver of the fish in the treatments with increase in concentration and time of exposure. Pathological alterations in gills and skin have been reported in fish exposed to toxicants: Lindesjöö and Thulin, 1994 (Histopathology of skin and gills of fish in pulp mill effluents.); Das and Mukherjee, 2000; Carp (Labeo rohita exposed to hexachlorocyclohexane); Soufy *et al.*, 2007(Chronic exposure of Tilapia to carbofuran pesticides.); Abalaka & Auta, 2010 (Exposure of *Clarias gariepinus* to aqueous extract of *Parkia biglobosa Pods*), Ayotunde *et al.*, 2011a,b (Adult *O. niloticus* exposed to aqueous extract of *Moringa oleifera* seed powder and Histology of adult *Clarias gariepinus* exposed to *Carica papaya* seed powder) including their devastating effects on exposed fish ability to respire (Obomanu, *et al.*, 2007; Abalaka & Auta, 2010).

Fish exposed to the highest concentration revealed severe alterations in organs examined. Alterations caused by *J. curcas* to the gills include: Degeneration of gill filaments and lamellae, hyperthrophy and necrosis of the arc, submocosal congestion and erosion of the secondary lamella which denotes gill functional disorders that may affect the fish physiology or cause death of the fish.

In the skin, necrosis, cell erosion and cellular infiltrations were observed. According to Silitonga *et al*. (2011) *Jatropha's* toxicity may present potential environmental and public health problems because of the curcanoleic acid contained in the seed oil which may promote skin cancer and that the oil can cause skin irritation. Therefore, the observations on the skin may be due to the curcanoleic acid in the seed oil as the skin is in direct contact with the environment.

The observation in the liver showed that the damage of the liver cells increased with increasing concentration and duration of exposure to the toxicant which include: Periportal degeneration, hepatic vacuolar degeneration, vacuolation of hepatocytes which are usually related to liver functional disorder, which may affect the physiology and caused death and spaces within the cell protoplasm filled with fluid.

The histological changes detected seem to have been caused by the toxicant *J. curcas*, while the mortality

recorded could be due to the malfunctioning of the gills and the disorder of the liver. The results showed that *J. curcas* is toxic to adult *O. niloticus* and that the survival of *O. niloticus* was directly related to the concentration of *J. curcas* in solution.

Conclusively, the toxicity effect of *J. curcas* seed powder on adult *O. niloticus* fish had a positive correlation with exposure. Although the toxicant can be less toxic when compare with other toxicant but the bioaccumulation effect in the body of any exposed fish can be potentially hazardous especially the freshwater fish. Therefore, acute toxicity data of the present study provide baseline information needed to develop models of *J. curcas* seed effects on ecological systems.

References

1. Abalaka, A. L and Auta J. (2010): Toxicity of Aqueous and Ethanol Extracts of Parkia biglobosa Pods on Clarias gariepinus Juveniles. Journal of Animal and Veterinary Advances,Vol. 9 Issue 6, 2010. Pg 1068-1072.
2. APHA, (1987) : Standard method for examination of wastewater and water (17th ed. Washington D. C) 8910 Pp.
3. Ayotunde, E.O, Fagbenro, O.A and Adebayo,O.T (2011a): Histological Changes in *Oreochromis niloticus* (Linnaeus I779) Exposed to Aqueous Extract of *Moringa oleifera* Seeds Powder. Turkish Journal of Fisheries and Aquatic Sciences 11: 37-43.
4. Ayotunde, E.O., Offem, B.O and Bekeh, A.F.(2011b): Toxicity of *Carica papaya* Linn: Haematological and Piscicidal Effect on adult Catfish (*Clarias gariepinus*) Journal of Fisheries and Aquatic Science, 6: 291-308.
5. Das, B.K. and Mukherjee, S. (2000): A histopathological study of carp (Labeo rohita) exposed to hexachlorocyclohexane. Veterinarski Arhiv. 70 (4):169-180.
6. Devappa, R.K.,Makkar,H.P.S and Becker,K.(2010a): Quality of biodiesel prepared from phorbol Ester extracted *Jatropha curcas* oil. J.Am.OilChem.Soc.87,697–704.
7. Devappa,R.K.,Makkar,H.P.S and Becker,K.,(2010b): Nutritional, biochemical, and pharmaceutical Potential of proteins and peptides from Jatropha: review. J. Agric. Food Chem. 58,6543–6555.
8. Devappa, R.K., Rajesh, S.K., Kumar V., Makkar, H. P.S. and Becker, K. (2011): Activities of *Jatropha curcas* phorbol esters in various bioassays. Ecotoxicol. Environ. Saf. doi:10.1016/j.ecoenv.2011.11.002.
9. Fagbenro, O.A (2002) : Tilapia: Fish for thought. Inaugural lecture series 32. Delivered at Federal University of Technology, Akure. Pp.77.
10. GEXSI, (2008): Availablefrom /http://www.Jatropha-platform.org/documents/GEXSI Global-Jatropha-Study_FULL-REPORT.pdfS.
11. Giro´n-Pe´rez M.I., Santene, A., Gonzalez-jaime, F., Casas-solis, J., Hernandez-coronado, M., Peregrina-sandoval, J., Takemura A. and Zaitseva, G (2007): Immunotoxicity and hepatic function evaluation in Nile tilapia cells (*Oreochromis niloticus*) exposed to diazinon. In fish and shellfish immunology 23 (2007) 760-769.
12. Gubitz, G.M., Mittelbach, M., Trabi, M., (1999): Exploitation of the tropical oil seed plant *Jatropha curcas L*. Bioresour. Technol. 67, 73–82.
13. Haas, W., Strerk, H and Mittelbach, M.(2002): Novel12-deoxy-16-hydroxyphorbol diesters isolates from the seed oil of Jatropha curcas. J.Nat.Prod.65, 1434–1440.
14. Henning, R.,(1997): Fuel Production Improves Food Production: The Jatropha Project in Mali. Proceedings from the symposium Jatropha 97, Managua, Nicaragua
15. Hughes, G. M., and Perry, S. F (1976): Morphometric study of trout gills: A light microscopic method for the evaluation of pollutant action. J. Exp. Bio., 63: 447-460
16. 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.
17. Lindesjöö, E. and Thulin, J (1994): Histopathology of skin and gills of fish in pulp mill effluents. Diseases of Aquatic Organisms.18: 81-93.
18. Makkar, H.P.S., Becker, K., Schmook, B., (1998): Edible provenances of *Jatropha curcas* from Quintana Roo state of Mexico and effect of roasting on antinutrient and toxic factors in seeds. Plant Foods Hum. Nutr. 52, 31–36.
19. Makkar, H.P.S., Becker, K.,(2009): *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. Eur. J. Lipid Sci. Technol. 111,773–787.
20. Obomanu, F.G., Ogbalu, O.K., Gabriel, U.U., Fekarurhobo, S.G.K. and Abadi, S.U (2007): Piscicidal effects of Lepidagathis alopecuroides on Mudskipper, *Periothalmus papillio* from the Niger Delta. Research Journal of Applied Sciences. 2 (4): 382-387.
21. Rakshit K.,Sanjay, K.R, Vikas Kumar,HarinderP.S.Makkarand KlausBecker (2011):Activities of *Jatropha curcas* phorbol esters in various bioassays. Ecotoxicology and Environmental Safety .
22. Reish, D. J. and Oshida, P. S. (1986): Manual of methods in aquaticenvironment research. Part 10. Short term static bioassays. FAO Fish Tech. Pap. (247), 62.
23. Silitonga, A. S., Atabani, A.E., Mahlia,T.M.I., Masjuki, H.H., Badruddin, I.A and Mekhilef, S. (2011): A review on prospect of *Jatropha curcas* for biodiesel in Indonesia. Renewable and Sustainable Energy Reviews 15 (2011) 3733–3756.