New Clues in Sciences 1 (2011) 55-68

Available at woaj

New Clues in Sciences

Journal homepage: www.woaj.org/ISE

# ACUTE TOXICITY AND HISTOPATHOLOGY OF NILE TILAPIA (*Oreochromis niloticus*) FINGERLINGS EXPOSED TO AQUEOUS AND ETHANOLIC EXTRACTS OF *Euphorbia poissonii* LEAVES

**Ayoola, Simeon Oluwatoyin**

Department of Marine Sciences, University of Lagos, Akoka, Yaba, Lagos State, Nigeria

A R T I C L E I N F O

*Article history:*
Received:
Revised:
Accepted:
Available online:

*Keywords:*
Acute Toxicity,
Histopathology,
*Oreochromis niloticus*,
*Euphorbia poissonii*

A B S T R A C T

The lethal effect of aqueous and ethanolic leaf extracts of *Euphorbia poissonii* on Nile Tilapia, *Oreochromis niloticus* were investigated. Five concentrations of each extract were tested. The concentrations for aqueous were; 20g/L, 40g/L, 80g/L, 160g/L and 320g/L. The concentration for the ethanolic were; 0.01g/L,0.1g/L,1.0g/L,10.0g/L and 100g/L. The randomized ANOVA for toxicity of the extracts against *Oreochromis niloticus* showed that there were significant differences between all treatments ($P< 0.05$). The 96hours $LC_{50}$ values of the aqueous and ethanolic leave extracts were 7.13g/L and 0.031g/L respectively. These results showed that the ethanolic extracts were more toxic than the aqueous extracts of *Euphorbia poissonii*. Histopathological effects were observed in the gill and muscle of *Oreochromis niloticus* in all concentrations of the aqueous and ethanolic extracts. Effects in the ethanolic extracts include generalized sub mucosal congestion and severe stunting of the secondary lamella in the gills and lesions on the muscles. The effect in the aqueous extracts includes degeneration of gills and necrosis on the muscle. It is also seen that aqueous and ethanolic extracts of *Euphorbia poissonii* have varying histopathological effects on *Oreochromis niloticus* fingerlings with increasing effects in higher concentration. The direct use of *Euphorbia poissonii* should be discouraged in a pond system except as a biological control to disinfect the pond from predators and it is to be used in low concentrations.



## 1. Introduction

Botanical fish toxicants are often used to control competing species in fish production especially in small water bodies enclosures, to eradicate fish, to control parasites and to conserve or restore native species but their uses are not encouraged because of their toxicity to aquatic organisms[1].Increase in the world's population has led to an increased application of technology to explore and exploit natural resources thereby increasing industrialization for the purpose of production of more food, goods and services[2]. Industrialization and modernization have been consciously used by government to create employment and make life longer and more comfortable. Environmental problem arose out of

* Corresponding author.
email address: sayoola@unilag.edu.ng.

man's quest to solve social problem, therefore industrialization and its consequent rapid population growth and concentration are the major contributions to environmental problems in both developed and developing countries[3]. The magnitude of the problem varies from country to country depending on various factors; including the stage of industrial development and the degree of enforcement of environmental regulation [3] .There are many sources of botanical fish toxicants in Nigeria that is extremely toxic to a wide range of animals including fish [1]. Plants are virtually inexhaustible source of structurally diverse biologically active substances [2].Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscidal properties unlike synthetic chemical pesticides which leave harmful residues in the aquatic environment [4]. Botanical insecticides are believed to be more environmental friendly compared to synthetic chemicals because they are easily biodegraded and leave no residues in the environment. Since, some of the pesticidal compounds present in plants are also toxic to fishes, botanical pesticides have potential to be used as piscicide to eradicate unwanted fish in the pond. Fish farmers in Nigeria have persistently and indiscrimately abused these natural plant piscicides by using much higher concentrations than necessary, causing mass mortality of fish in ponds, contaminating the water body and affecting non target organisms [5].

The physical and chemical changes in aquatic environment often cause some physiological changes in fish, thus, the water quality of an aquatic body is very crucial because it determines the productivity and other parameters necessary for the fish survival [5]. Many countries including Nigeria have legislated against the use of chemical poisons in aquatic systems and instead have policies favouring the use of natural biodegradable alternatives to remove unwanted fish species in aquatic systems.

Botanicals are natural biocides [6]and their contamination of natural water has become inevitable in Nigeria because of recent wide use. Piscicidal plants *Blighia sapida, Kigelia Africana, Tetrapleura tetraptera, Raphia vinifera, Parkia biglobosa and Tephrosia vogelii* are frequently used by fisher folks because they are highly potent [5].

According to [7], some substances when present in low concentrations, may not cause acute detectable effects in organisms, but may in the long run reduce their life span. In recent years, there has been an increasing awareness of genotoxic and cytotoxic potential of a wide variety of chemicals to which human population is exposed either environmentally or occupationally [8]. Toxicant stress also induces biochemical changes which may lead to disturbances in metabolism. The changes such as reduction in protein, glycogen contents of the tissue and inhibition of activity of some important enzymes at cellular level have led to retardation of growth, reduction in the fecundity and longevity of organisms [8]. Aquatic organisms in open aquatic systems such as lagoons are constantly exposed to pollutants that are present in the environment. [6] showed that for aquatic organisms to develop and survive well, the conditions of the environment in which they live is of fundamental importance. The introduction of any substance in the water produces changes in its quality, which are not always favourable to the development and survival of aquatic organisms.

Within the last decade, the use of fish as appropriate models for genetic monitoring of toxic chemicals such as botanicals in aquatic environment has become popular everywhere in the world. Standard toxicity tests performed are used extensively to predict the effects of chemicals in aquatic ecosystem [8].

In this study an attempt has been made to evaluate the toxicity of *Euphorbia poissonii* on *Oreochromis niloticus*. Since fish often respond to toxicants in a manner similar to higher invertebrates, they can be used to screen for chemicals that have potential to cause teratogenic and carcinogenic effects in humans [9]. Due to enormous role played by this organism in food chain and nutrient recirculation in the environment, it is therefore useful to evaluate the histopathological changes that occur as a result of piscicides pollution since the aquatic environment serves as filter bed for pollutants. Test organisms to be used for acute toxicity test must be ecologically important, occupy trophic position leading to humans or other important species, and have adequate background biology, be widely distributed, be genetically stable, have its early stages (larvae, fry, and juveniles) available throughout the year and be sensitive [6]. Tilapia (*Oreochromis niloticus*) is one of the most important freshwater finfish in aquaculture world. Among the numerous regions now inhabited by tilapia, many are under threat from various pollutants, especially botanical pollutant. This fish species is commonly used in experimental work for its rusticity and good adaptation to the captivity conditions [10]. As a result of their great adaptability, high fecundity and rapid growth they are used extensively for fish culture. This study intends to determine the toxic effects of extracts of *Euphorbia poissonii* on fingerlings of *Oreochromis niloticus*, using the acute toxicity test and to investigate the histopathological changes in the exposed fish.

## 2. Materials and Methods

A total of 500 healthy *Oreochromis niloticus* fingerlings were used for this study .The fingerlings were 2.5cm in size. The fish were brought from Ani-

mashaun farm in Badagry and was transported to the University of Lagos, Nigeria. In the laboratory, the fingerlings were transferred into stock tanks of 113cm length, breadth of 54cm and depth of 80cm.The stock tank was quarter filled with dechlorinated water.

*Oreochromis niloticus* fingerlings were allowed to acclimatize to laboratory condition for 24 hours in the stock tank. The water in the stock tank was changed every 3 days to remove debris and accumulated metabolic wastes.

Water in the stock tank was aerated by electric aerator model (AC9902) to prevent accumulation of toxic waste. The fingerlings were fed throughout the period of acclimatization.

For the maintenance of the stock and Serial dilution for bioassay test, dechlorinated tap water was used.

## PREPARATION OF TOXICANT SOLUTION

The leaves of *Euphorbia poissonii* was used for this study. The leaves were obtained from the botanical garden of the University of Lagos; they were collected and dried before blending them into powder prior to extraction. Eight liters of distilled water was used to soak 1500g of the leaves for 72 hrs prior to filtration based on using the maceration method which was subsequently concentrated to dryness yielding 620.0g of the dried aqueous extract was used for the bioassay test.

In ethanolic extract, six litres of ethanol was used to soak 1,650g of the powder of *Euphorbia poissonni* over a period of 72 hrs period prior to filtration based on the same maceration method which was subsequently concentrated to dryness yielding 750g of dry ethanol extract and was kept in the laboratory at room temperature.

*Oreochromis niloticus* fingerlings of approximately the same size were gently caught using a hand net in order to avoid stress, into bioassay tanks from an acclimatize batch

Test animals were taken as dead if failed to move their bodies. They float or sink into bottom when probed gently with a glass rod. During assessment for mortality each fish was removed from a test media with a pair of forceps, placed a clean empty petri dish and recorded.

## PRELIMINARY TEST

The concentration ranges chosen for the preliminary test of aqueous and ethanolic extract *of Euphorbia poissonii* on *Oreochromis niloticus* fingerlings were 1000mg/L, 500mg/L, 100mg/L, 10mg/L, 1.0mg/L. Ten fish were randomly introduced into each of the reconstituted extract and each concentration was set in triplicate with control containing 1000ml of dilution water to serve as control.

## DEFINITIVE TEST

The concentration ranges chosen for the aqueous extract for the toxicity test on *Oreochromis niloticus* after the preliminary test were 320mg/L, 160mg/L, 80mg/L, 40mg/L, 20mg/L and 0mg/L used as the control. The concentration used for the ethanolic extract on *Oreochromis niloticus* after the preliminary test were 100mg/L, 10mg/L, 0.1mg/L, 0.01mg/L, 0.001mg/L. The duration of the experiments was 96 hours. After 24hrs daily the $LC_{50}$ determination was conducted using a modified method [11]. The fish were starved in order to minimize waste production. The distress behaviour and the deaths were closely monitored and recorded from the onset of the experiment 3h,6h, 12h, 24h, 48h, 72h,and 96h respectively. During 96hours exposure, salinity was measured by using a hand refractometer. pH was measured by the use of Hanna instrument pH 211-micro processor pH meters. Dissolved oxygen (DO) was measured with DO meter (model EUTECH DO 600); water temperature was determined by simple mercury in glass thermometer, calibrated in centigrade (oC).

## HISTOPATHOLOGY

Fishes that died after 96 hours of the bioassay test were put aside for histopathological analysis. The gill, liver and muscle of the fish were removed from both control and experimental group. These organs were fixed in Bouin's fluid in other to avoid autolysis.

## STATISTICAL ANALYSIS

The quantal response (mortality) was analysed by probit analysis [12]. The log dose values for $LC_5$, $LC_{50}$ and $LC_{95}$ were obtained and tabulated. Graph of Probit values were plotted against logdose values using the line of best fit for a straight curve. The following indices of toxicity and their 95% confidence unit derived from a computer statistical programme SPSS 10.0 were:

$LC_{95}$ value (lethal concentration that causes the death/mortality of 95% of the exposed population).

$LC_{50}$ value (lethal concentration that causes the death/mortality of 50% of the exposed population).

$LC_5$ (lethal concentration that causes the death/ mortality of 5% of the exposed population).

One Way Analysis of Variance (ANOVA) and comparison of means by Student Newman Keul's (SNK) test were used to test for statistical differences.

## 3. RESULTS

**EFFECT OF AQUEOUS EXTRACT OF *Euphorbia poissonii ON Oreochromis niloticus***

The result of the acute toxicity test of aqueous extract of the leaf of *E.poissonii* against *Oreochromis niloticus* fingerlings at 24hrs, 48hrs, 72hrs and 96hrs of exposure period is shown in Table 1.

Figure 1 shows the graph of probit response and log – dose drawn from the probit line equation tables. The $LC_{50}$ values obtained at 24hrs, 48hrs, 72hrs and 96hrs for aqueous extract were 192.73mg/L, 93.82mg/L, 52.58mg/L and 7.13mg/L respectively.

The randomized analysis variance (ANOVA) showed there was significant difference ($P<0.05$) between all the concentrations at 24, 48, 72 and 96 hrs of exposure. Using the Student Newman – Keul's (SNK) test ($P<0.05$) shown in Table 2, the mean quantal response of 40mg/L was significantly different from the control at 24, 48, 72 and 96 hrs exposure. At 48 and 72hrs exposure period, 40mg/L and 80mg/L showed no significant difference so also is the case for 80mg/L and 160mg/L at 96 hrs exposure period. The physico-chemical parameters of aqueous extract of *Euphorbia poissoni* on *Oreochromis niloticus* are presented in table 3.

**TABLE 1. ACUTE TOXICITY EFFECT OF AQUEOUS EXTRACT OF THE LEAVES OF *Euphorbia poissonii* AGAINST *Oreochromis niloticus* fingerling AT 24, 48, 72 AND 96 HOURS EXPOSURE**

| Exposure time (hrs) | $LC_{50}$ (95% C.L mg/L) | $LC_{95}$ (95% C.L mg/L) | $LC_5$ (95% C.L mg/L) | Slope ± S.E | D.F | Probit Line Equation |
|---|---|---|---|---|---|---|
| 24 | 192.73 | 76.342 | 13.148 | 1.411±0.291 | 3 | Y=1.777 + 1.411X |
| 48 | 93.82 | 54.987 | 0.705 | 0.774±0.249 | 3 | Y=3.473 + 0.774X |
| 72 | 52.58 | 98.341 | 0.066 | 0.567±0.245 | 3 | Y=4.024 + 0.567X |
| 96 | 7.13 | 45.562 | 0.009 | 0.571±0.262 | 3 | Y=4.513 + 0.571X |

L.C = Lethal concentration
S.E = Standard Error
D.F = Degree of freedom
C.L = Confidential Limit

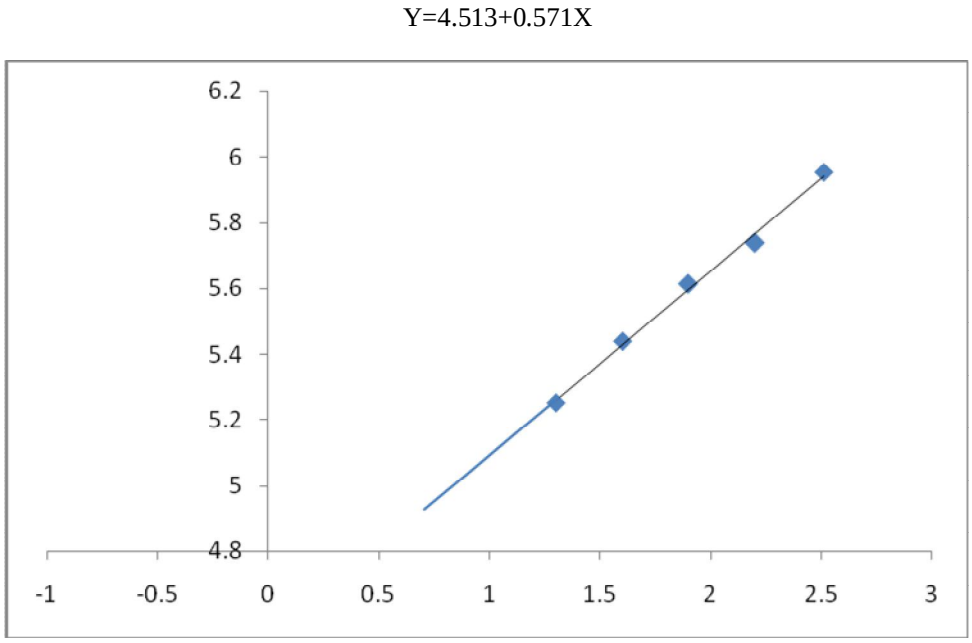


**Figure1.Linear relationship between probit response and log concentration of Aqueous Extract *of Euphorbia poissonii* on fingerlings of *O. niloticus***

**TABLE 2. PERCENTAGE MEAN RESPONSE OF *Oreochromis niloticus* EXPOSED TO DIFFERENT CONCENTRATION OF AQUEOUS EXTRACT *Euphorbia poissonii FOR* 96hrs**

| Concentration mg/L | Number of Organisms | Percentage Mortality (%) Time (hrs) | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| Control | 30 | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| 20.0 | 30 | $0^{a}$ | $27^{b}$ | $40^{b}$ | $60^{b}$ |
| 40.0 | 30 | $23^{b}$ | $43^{c}$ | $50^{b}$ | $67^{bc}$ |
| 80.0 | 30 | $37^{c}$ | $47^{c}$ | $50^{b}$ | $73^{cd}$ |
| 160.0 | 30 | $50^{d}$ | $60^{d}$ | $63^{c}$ | $77^{cd}$ |
| 320.0 | 30 | $53^{d}$ | $63^{d}$ | $67^{c}$ | $83^{d}$ |

Means followed by the same superscript letter in a column are not significantly different in the SNK test (P>0.05)

**TABLE 3. Mean physico-chemical parameters of the test concentrations (*Euphorbia poissonii*) on *Oreochromis niloticus* Aqueous extracts**

| Concentration mg/L | Physico chemical Parameters | | | |
|---|---|---|---|---|
| | Dissolved Oxygen (mg/L) | Salinity ($^{0}/_{00}$) | pH | Temp.$^{0}$C |
| 0 | 5.8±0.1 | 0 | 7.0 | 26.0±0.2 |
| 20.0 | 5.8±0.1 | 0 | 6.9±0.1 | 26±0.1 |
| 40.0 | 5.4±0.1 | 0 | 6.9±0.1 | 27±0.2 |
| 80.0 | 5.0±0.1 | 0 | 6.7±0.2 | 27±0.2 |
| 160.0 | 5.0±0.1 | 0 | 6.7±0.2 | 27±0.1 |
| 320.0 | 5.0±0.1 | 0 | 6.5±0.1 | 27±0.1 |

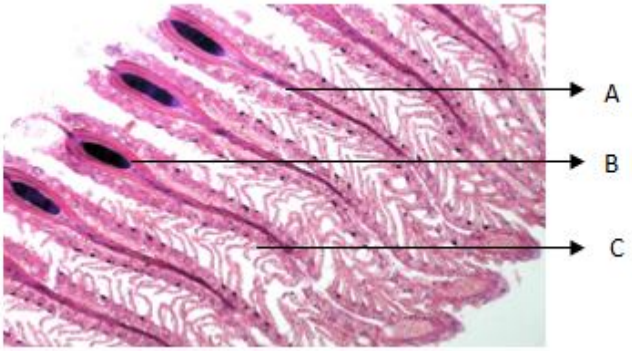


Plate 1a: Gills of *O. niloticus.* The 96-h exposed in the control group showing. No lesion, no necrosis.A, gill raker, B, nucleolus and C, filament

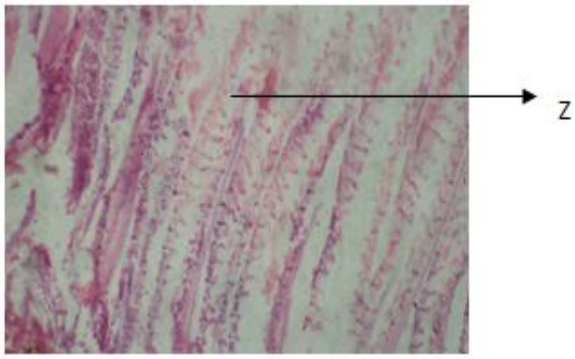


Plate 1b: Gills of *O. niloticus*. The 96-h exposed at 20g/L (aq.extract of *E.poissonii*) showing early stage of degeneration.

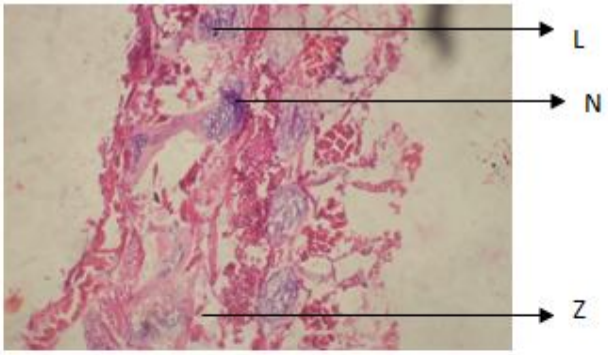


Plate 1c: Gills of *O. niloticus*. The 96-h exposed at 40g/L (aq.extract of *E.poissonii*) showing lesion, necrosis and lamellae degeneration

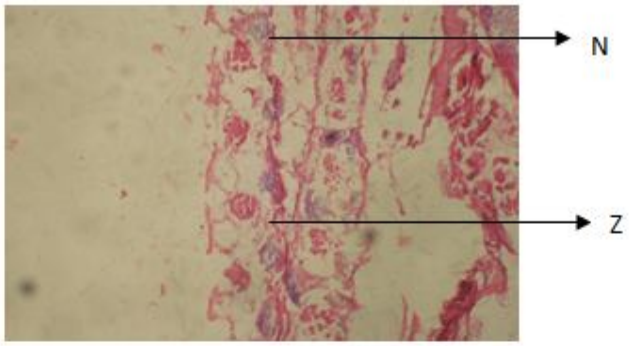


Plate 1d: Gills of *O. niloticus*. The 96-h exposed at 80g/L (aq.extract of *E.poissonii*) showing more lesion and lamellae degeneration

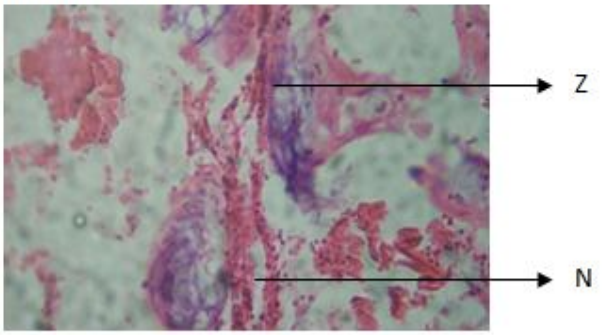


Plate 1e: Gills of *O. niloticus*. The 96-h exposed at 160g/L (aq.extract of *E.poissonii*) showing necrosis and gill degeneration.

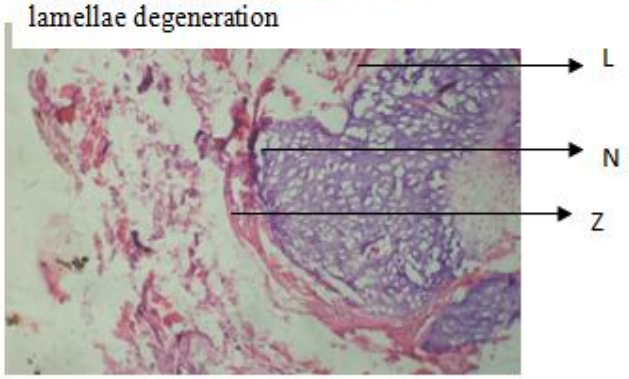


Plate 1f: Gills of *O. niloticus*. The 96-h exposed at 320g/L (aq.extract of *E.poissonii*) showing severe lesion, lamellae degeneration and necrosis.

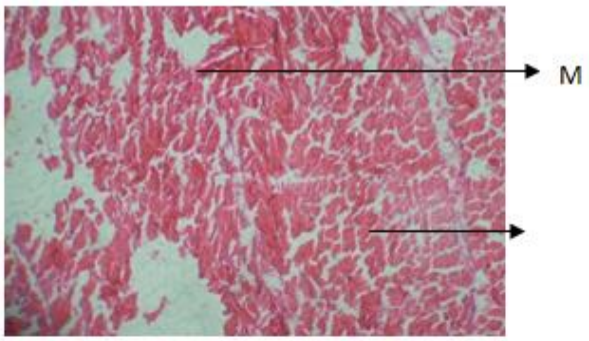


Plate 2a: Muscle of *O. niloticus*. The 96-h exposed in the control group showing. No lesion.

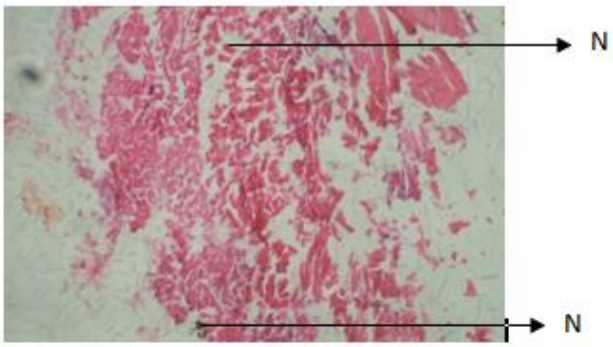


Plate 2b: Muscle of *O. niloticus*. The 96-h exposed at 20g/L (aq.extract of *E.poissonii*) showing mild cellular infiltration with some lesion

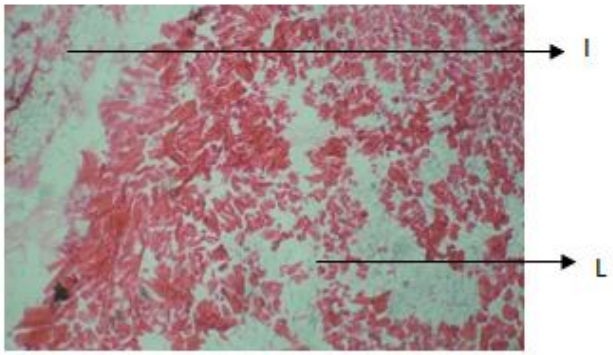


Plate 2c: Muscle of *O. niloticus*. The 96-h exposed to 40g/L (aq.extract of *E.poissonii*) showing mild cellular infiltration with some lesion

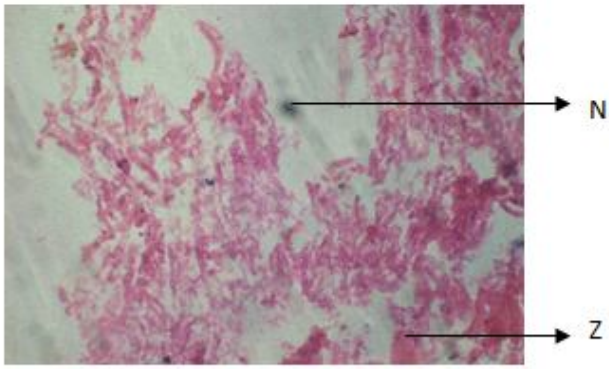


Plate 2d: Muscle of *O. niloticus*. The 96-h exposed to 80g/L (aq.extract of *E.poissonii*) showing cellular degeneration and necrosis

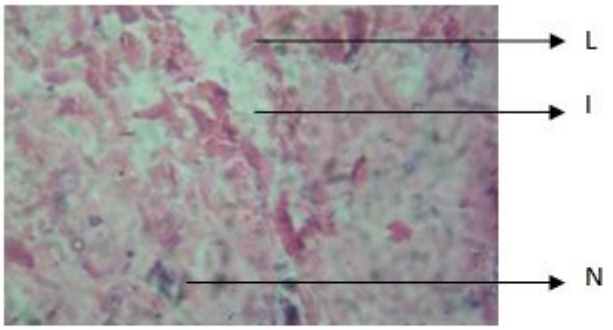


Plate 2e: Muscle of *O. niloticus*. The 96-h exposed to 160g/L (aq.extract of *E.poissonii*) showing cellular infiltration, necrosis and lesion.

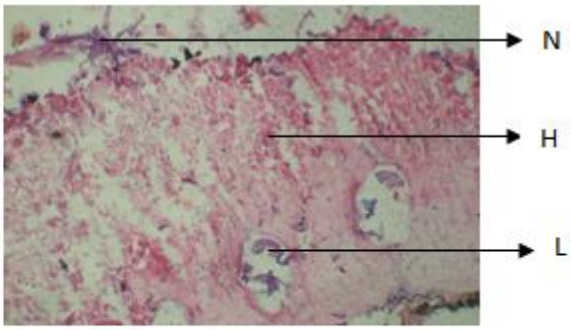


Plate 2f: Muscle of *O. niloticus*. The 96-h exposed to 320g/L (aq.extract of *E.poissonii*) showing cellular haemorrhage, necrosis and lesion

**EFFECT OF ETHANOLIC EXTRACT OF *Euphorbia poissonii on Oreochromis niloticus***

The result of the acute toxicity test of ethanolic extract of the leaf of *E. poissonii* against *Oreochromis niloticus* fingerlings at 24hrs, 48hrs, 72hrs and 96hrs of exposure period is shown in Table 4.

Figure 2 shows the graph of probit response and log – dose drawn from the probit line equation tables. The $LC_{50}$ values obtained at 24hrs, 48hrs, 72hrs and 96hrs for ethanolic aqueous extract were 95.470mg/L, 2.910mg/L, 4.341mg/L and 0.031mg/L respectively.

The randomized analysis variance (ANOVA) showed there was significant difference ($P<0.05$) between all the concentrations at 24, 48, 72 and 96 hrs of exposure. Using the Student Newman – Keul's (SNK) test ($P<0.05$) shown in Table 5, the mean quantal response of 0.01mg/L was significantly different from the control at 24, 48, 72 and 96 hrs exposure. At 48 and 72hrs exposure period, 1.0mg/L and 10mg/L showed no significant difference but showed a significant difference at 96 hrs exposure period. The physico-chemical parameters of ethanolic extract of *Euphorbia poissoni* on *Oreochromis niloticus* are presented in table 6.

**TABLE 4. ACUTE TOXICITY EFFECT OF ETHANOLIC EXTRACT OF THE LEAF OF *Euphorbia poissonii* AGAINST *Oreochromis niloticus* FINGERLINGS AT 24, 48, 72 AND 96 HOURS**

| Exposure time (hrs) | $LC_{50}$ (95% C.L mg/L) | $LC_{95}$ (95% C.L mg/L) | $LC_5$ (95% C.L mg/L) | Slope± S.E | D.F | Probit Line Equation |
|---|---|---|---|---|---|---|
| 24 | 95.47 | 65.234 | 0.00 | 0.24±0.078 | 3 | Y=4.525 +0.240X |
| 48 | 2.910 | 92.412 | 0.00 | 0.11±0.073 | 3 | Y=4.490+ 0.110X |
| 72 | 4.341 | 87.564 | 0.00 | 0.16±0.074 | 3 | Y=4.896+ 0.163X |
| 96 | 0.031 | 41.458 | 0.00 | 0.24±0.077 | 3 | Y=4.640+ 0.238X |

L.C = Lethal concentration
S.E = Standard Error
D.F = Degree of freedom
C.L = Confidential Limit

**TABLE 5. PERCENTAGE MEAN RESPONSE OF *Oreochromis niloticus* EXPOSED TO DIFFERENT CONCENTRATION OF ETHANOLIC EXTRACT *Euphorbia poissonii* FOR 96hrs**

| Concentration mg/L | Number of Organisms | Percentage Mortality (%) Time (hrs) | | | |
|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 |
| Control | 30 | $0^{a}$ | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| 0.01 | 30 | $17^{b}$ | $37^{b}$ | $27^{b}$ | $47^{b}$ |
| 0.10 | 30 | $23^{bc}$ | $47^{c}$ | $47^{c}$ | $53^{bc}$ |
| 1.00 | 30 | $33^{cd}$ | $50^{cd}$ | $50^{cd}$ | $63^{cd}$ |
| 10.0 | 30 | $40^{de}$ | $50^{cd}$ | $50^{cd}$ | $73^{de}$ |
| 100 | 30 | $50^{e}$ | $57^{d}$ | $57^{e}$ | $80^{e}$ |

Means followed by the same superscript letter in a column are not significantly different in the SNK test ($P>0.05$)

Y=4.64+0.24X

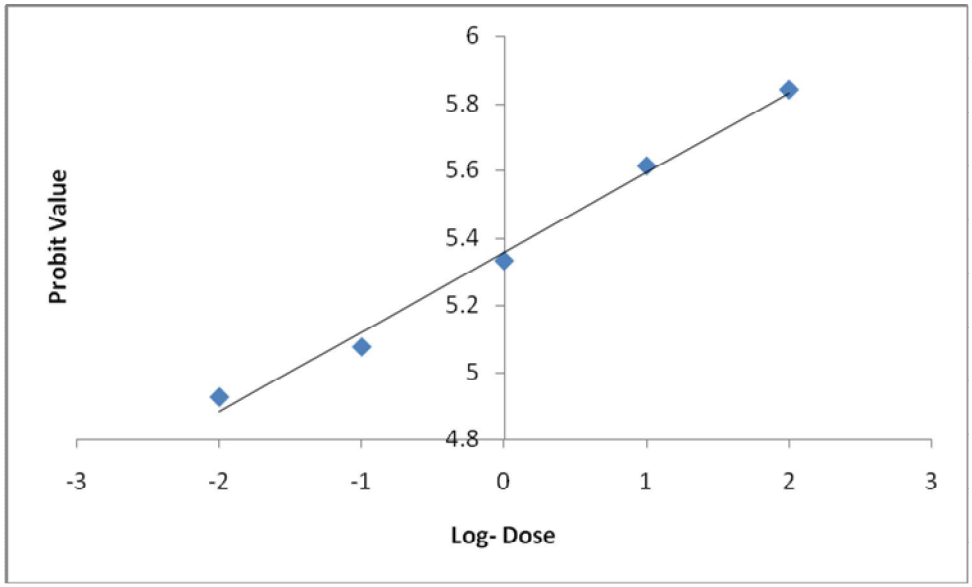


**Figure2. Linear relationship between probit response and log concentration of Ethanolic Extract of *Euphorbia poissonii* on fingerlings of *O. niloticus***

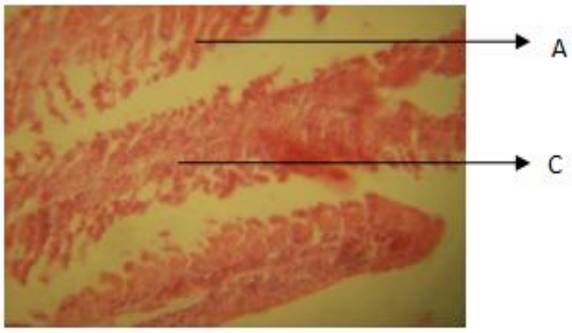


Plate 1a: Gill of *O. niloticus* the 96h exposed in the control group. No lesion, A, gill raker, C, gill filament

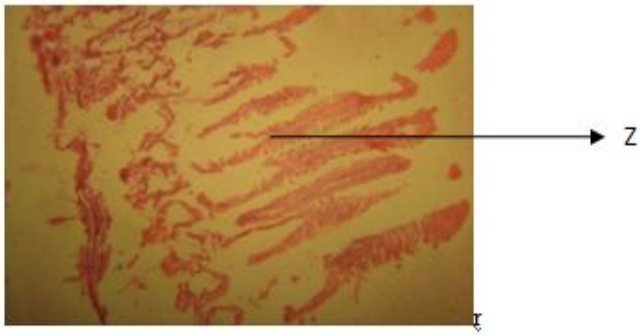


Plate 1b: Gill of *O.niloticus* the 96h exposed at 0.01mg/L (Ethanolic extract of *E.poissonii*) showing early stage of degeneration.

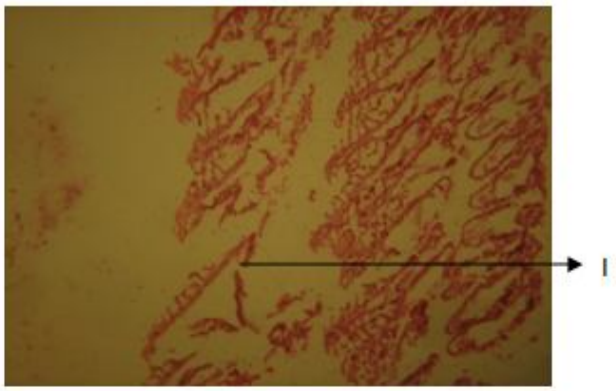


Plate1c: Gill of *O.niloticus* the 96h exposed at 0.10mg/L (ethanolic extract of *E.poissonii)* showing cellular infiltration

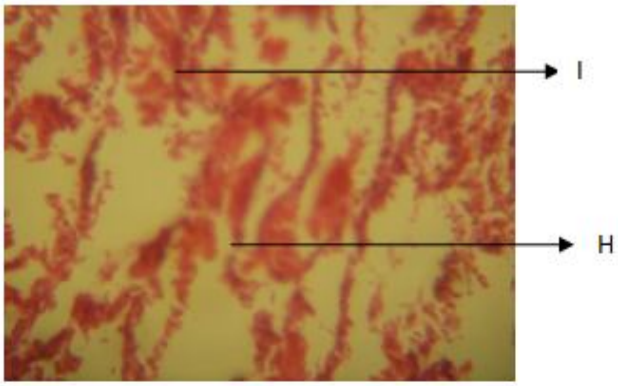


Plate1d: Gill of *O.niloticus the* 96h exposed at 1.0mg/L (ethanolic extract of *E.poissonii)* haemorrhage and infiltration.

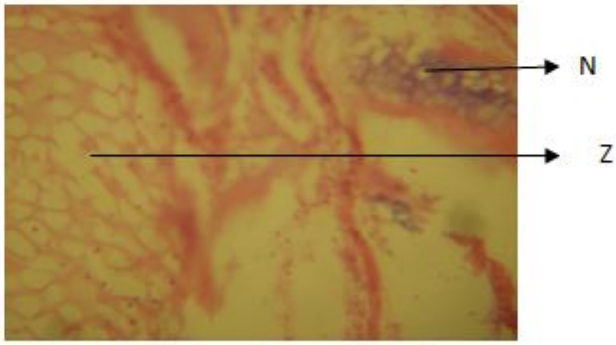


Plate 1e: Gill of *O.niloticus*.The 96h exposed at 10.0mg/L(ethanolic extract of *E.poissonii)*showing necrosis and degenerated Lamellae

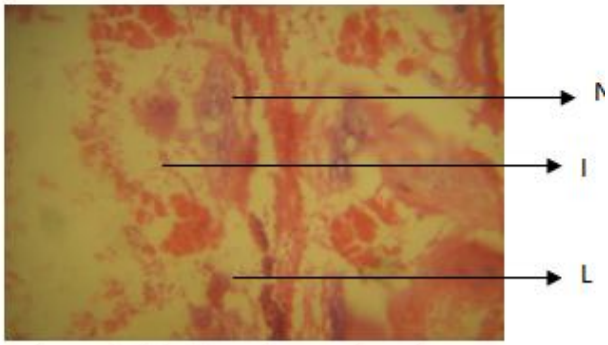


Plate 1f: Gill of *O.niloticus*.The 96h exposed at 100mg/L (ethanolic extract of *E.poissonii*) showing severe necrosis, infiltration and cellular degeneration.

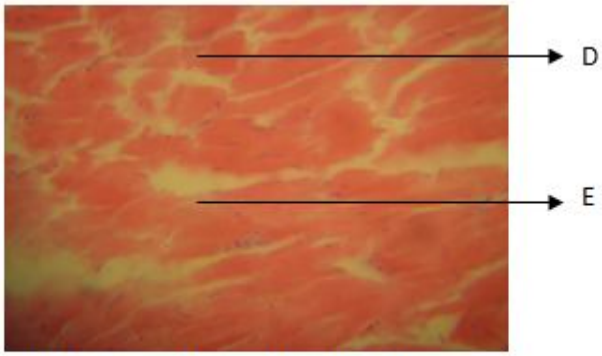


Plate2a: Muscle of *O. niloticus*.The 96h exposed in the control group showing no lesion, no necrosis and no degeneration.

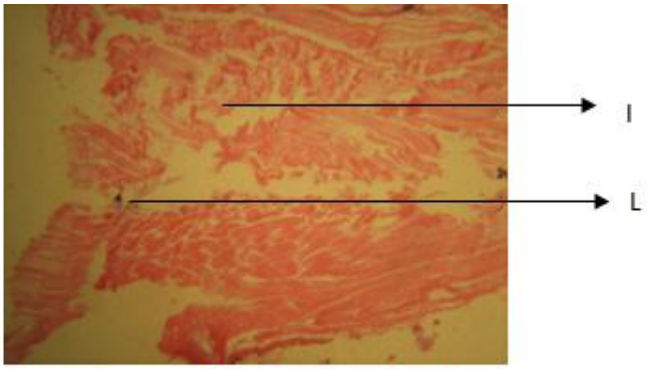


Plate2b: Muscle of *O.niloticus*.The 96h exposed at 0.01mg/L (ethanolic extract of *E.poissonii*)showing mild cellular infiltration and slight lesions.

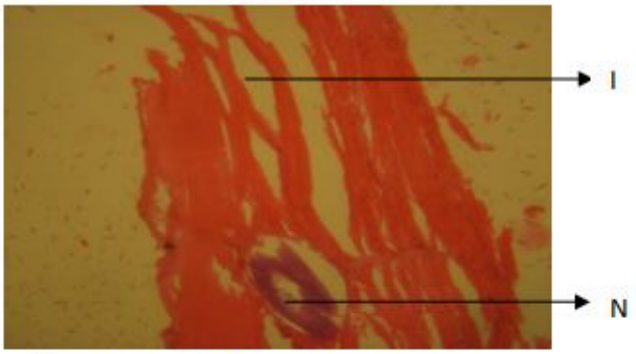


Plate2c: Muscle of *O.niloticus*.The 96h exposed to 0.10mg/L(ethanolic extract of *E.poissonii*)showing necrosis and infiltration

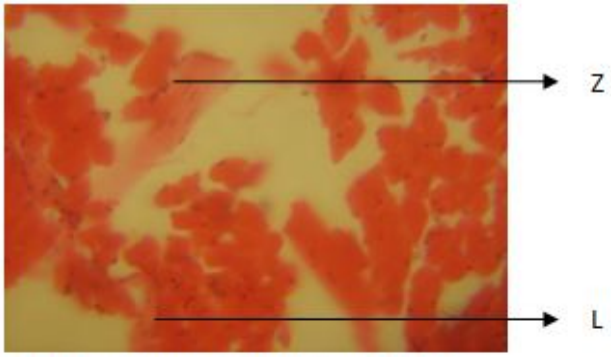


Plate2d: Muscle of *O.niloticus*.The 96h exposed to 1.0mg/L(ethanolic extract of *E.poissonii*) showing cellular degeneration and Lesion.

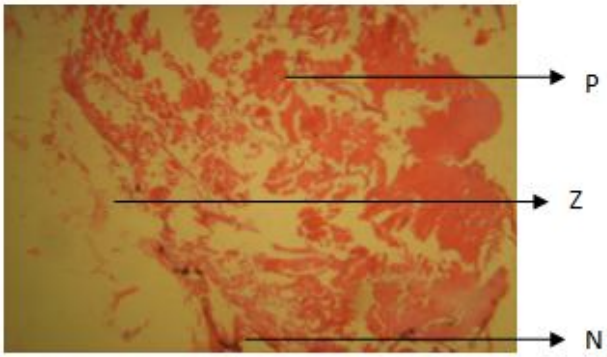


Plate 2e: Muscle of *O. niloticus*. The 96h exposed to 10.0mg/L (ethanolic extract of *E.poissonii*) showing necrosis, cellular degeneration and congestion.

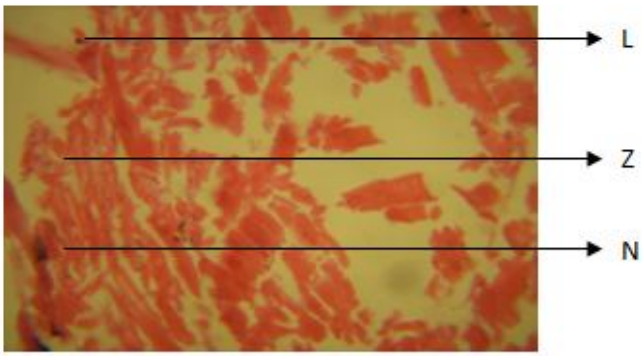


Plate 2f : Muscle of *O.niloticus*.The 96h exposed to 100mg/L(ethanolic extract of *E.poissonii*)showing severe cellular degeneration, necrosis, lesions.

Kek

Key: L= lesion; N= necrosis; Z= cellular degeneration; I= infiltration; H= haemorrhage; P= congestion

**Table 6: Mean physico-chemical parameters of the test concentrations (*Euphorbia poissonii*) on *Oreochromis niloticus***

Ethanolic extracts

| Concentration mg/L | Physico chemical Parameters | | | |
|---|---|---|---|---|
| | Dissolved Oxygen (mg/L) | Salinity ($^0/_{00}$) | $_pH$ | Temp.$^0$C |
| 0 | 5.8±0.1 | 0 | 7.0 | 26.0±0.2 |
| 20.0 | 5.7±0.1 | 0 | 6.9±0.1 | 26±0.1 |
| 40.0 | 5.4±0.1 | 0 | 6.9±0.1 | 27±0.2 |
| 80.0 | 5.0±0.1 | 0 | 6.7±0.2 | 27±0.2 |
| 160.0 | 5.0±0.1 | 0 | 6.7±0.2 | 27±0.1 |
| 320.0 | 5.0±0.1 | 0 | 6.5±0.1 | 27±0.1 |

## 5. DISCUSSION

In this study, the acute toxicity level based on the 96hrs LC50 value of aqueous extract of *Euphorbia poissonii* was found to be 7.13g/L when tested against the fingerlings of *Oreochromis niloticus*. The 96hrs LC50 value of the ethanolic extracts of *Euphorbia poissonii* was found to be 0.031g/L. This shows that the ethanolic extract is more toxic than the aqueous extract. This is due to the extraction process, because, for the aqueous extraction, the grounded leaves was simply soaked in water and filtered and by so doing the alkaloids, though extracted, had been diluted with water, thereby reducing the potency of the alkaloids. But for the ethanolic extract the soxhlet extractor extracted only the alkaloids almost undiluted using ethanol as extraction medium. 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 *I. aquatica* toxicity in this study. This agreed with the findings of [13] on *Oreochromis niloticus* exposed to trichloroform. [14]reported similar observation in *Sarotherodon galilaeus* (Tilapia) fingerlings exposed to piscicidal plant extracts of *Tetrapleura tetraptera.*

In this study, the stressful behaviour exhibited by fingerlings exposed to acute concentrations of aqueous and ethanolic extracts of *Euphorbia poissonii was* characterized by erratic movements, gulping of air and death. These abnormal behaviours correspond to both the contact and exertion phases of fish's respond to toxicants absorbing these offending extracts. *Oreochromis niloticus* exhibited erratic movement and aggressiveness [13].

The observed respiratory distress may be due to ensuing hypoxic states in exposed fishes brought about by both decreasing dissolved oxygen contents of reconstituted extracts vis-á-vis decreasing ability of exposed fishes to respire. This agreed with the finding of [15, 5, 16]. Decreasing dissolved oxygen of reconstituted extracts may be due to the continuous oxidative bio-degradation of the constituents of both extracts which may cause oxygen tension in such reconstituted extracts by diverting the much needed dissolved oxygen for this biodegradation process .However, the extent of such depletion is directly dependent upon the concentration of the toxicant [17, 18]. Gills and muscles pathological alterations have been reported in fishes exposed to toxicants. The histopathological effects of the aqueous extracts of Euphorbia poissonii indicated accumulation of mucus on the body surfaces and gill filament of dead fish. According to [9, 19], mucus accumulation results from increase in the activity of mucus cells subsequent to exposure to toxicants. The accumulation of mucus on gill filaments might have caused impairment in the branchial ion regulatory mechanism and gaseous exchange leading to suffocation of fish [23, 18, 21] observed similar effects when different fish species were exposed to heavy metals and pesticides.

In this study, the gill treated with aqueous extracts showed degenerated lamella cartilage with more damaging effects in the higher concentration (320mg/L). The gill treated with ethanolic extracts shows submucosal congestion and stunting of the secondary lamella in the higher concentration (100mg/L). Similar results was shown by[22] in the gill morphology of *Clarias gariepinus* juveniles exposed to extracts of *Parkia biglogbosa and Raphia vinifera* in which the gill morphology showed microthrombi in capillaries, degeneration of lamella cartilage, oedema of the submucosa and focal lamellae epithelial hyperplasia. The damages were more

severe in higher concentrations. Similar effects of aqueous and ethanolic extracts on fingerlings were also reported by [23] using Dipterex on fresh water fisheries.

## 6. CONCLUSION

This study showed that the extracts of *Euphorbia poissonii* is toxic to fingerlings of *Oreochromis niloticus* with the ethanolic extract being more potent than the aqueous extract because the alkaloids in the aqueous extract has been diluted with water during extraction. It is also seen that aqueous and ethanolic extracts of *Euphorbia poissonii* have varying histopathological effects on *Oreochromis niloticus* fingerlings with increasing effects in higher concentration. From the findings of this research work the direct use of *Euphorbia poissonii* should be discouraged in a pond system except as a biological control to disinfect the pond from predators and it is to be used in low concentrations.

## 7. References


1. Olufayo M.O (2009): Haematological characteristics of *Clarias gariepinus* (Burchell1822) Juveniles exposed to *Derris elliptia* root powder. *African journal of food Agriculture, Nutrition and Development.*Vol 9, (3)920-932
2. Baird, F. (1994). Pest control in tropical aquaculture. An ecological hazard assessment of natural Synthetic control agents. Mitteilungen international Verein Limnologie **24**:285-292.
3. **Simeon O. Ayoola,** Kuton M.P, Idowu A.A and Adelekun, A.B **(2011):** Acute Toxicity of Nile Tilapia (*Oreochromis niloticus*) Juveniles Exposed to Aqueous and Ethanolic Extracts of *Ipomoea aquatica* Leaf. *Nature and Science* **9** (3):91-99
4. Koesoemadinata, S. (1980). Pesticide as a major constraint in integrated agriculture aquaculture farming system. 45-51pp.
5. Fafioye, O.O. (2001). Lethal and sub lethal effects of extract of *Parkia biglobos*a and *Raphia vinifera* some freshwater fauna. Ph. D. thesis, University of Ibadan, Ibadan, Nigeria. 216pp.
6. Ernest Hodgson (2004): A textbook of modern toxicology (3rd ed). John Wiley & Sons. Inc., Hoboken, NewJersey. ISBN 0-471-26508-x, pp 557.
7. Rao, D.M.R. and Murty, A. S. (1982). Toxicity and metabolism of sulfan in three freshwater catfishes. *Environ. Pollut.* **27:** 223-231.
8. Odiete W.O. (1999): Environmental physiology of Animal and pollution. 1st Ed. Diversified Resources Ltd. ISBN 978-028-957-7; Lagos. Pp 360.
9. Solbe J .F. (1995): Fresh water In: *Handbook of Ecotoxicology* (Edited by peter calins) Black well Science ltd. Osneymeed ox 20EL. Pp 683.
10. Burkill, H.M. (1985). The useful plants of West Tropical Africa. 2nd Edition. Volume 2. Royal Botanic Gardens, Kew, Richmond, United Kingdom. 19pp.
11. Lorke, D. (1983). A new approach to acute toxicity testing. *Arc. Toxicol.* **54:** 275-287.
12. Finney, D.J. (1980). *Probit analysis.* Cambridge University Press, Cambridge. Greatm Britain. (3): 124pp.
13. Abalaka, S.E. and Auta, J. (2010). Toxic effects of Aqueous and Ethanolic extracts of *parkia biglobosa* pods on *Clarias gariepinus* adults. *World Journal of Biological Research.***3** (1):9-17.
14. Omitoyin, B.O., Ogunsanmi, A.O. and Adesina, B.T. (1999). Studies on acute toxicity of Piscicidal Plant extracts (*Tetrapleura tetraptera*) on tilapia (*Sarotherodon* galilaeus) fingerlings. *Trop. J. Anim. Sci.* **2**: 191-197.
15. Omoeregie, E., Ufodike, E. and Keke, I. (1990). Tissue chemistry of *Oreochromis niloticus* exposed to sublethal concentrations of gammalin 20 and acetillic 25. *Ec. J. Aq. Sci.* **5**: 33-36.
16. Omitoyin, B.O. Ajani, E.K. Adesina, B.T. and Okuagu, C.N.F. (2006). Toxicity of Lindane (Gamma Hexachloro - CycloHexane) to *Clarias gariepinus* (Burchell 1822). *World Journal of Zoology*. **1**(1): 57-63.
17. **Ayoola, S.O.** and Ajani, E.K. **(2007):** Histopathological Effect of Cypermethrin on Juvenile Nile Tilapia (*Oreochromis niloticus*). *African Journal of Livestock Extension.* **5**; 1-13
18. **Ayoola, S.O. (2008b):** Toxicity of Herbicide, Glyphosate on Nile tilapia (*Oreochromis niloticus)* Juvenile. *African Journal Agricultural Research.* 3(12), pp.825-834
19. Annune, P.A. and Ajike, S.U. (1999). Acute toxicity and Gill morphology of *Oreochromis niloticus* (Trewavas).Exposed to Rigor. J*ournal of Aquatic science*, **14**: 1-4
20. Chukwu, L.O and Odunzeh, C.C (2004). Toxic and sublethal effects of sport lubricant oil on the Gill morphology of the Tropical Teleost *Oreochromis niloticus*: *Journal of Nigerian Environment Society* **1**(3): 334-449.
21. Ebele, S., Oladimeji, A.A. and Daramola, J.A. (1990). Molluscidal and Piscicidal properties of Copper (ii) tetraoxosulphate ( vi) on *Bulinus globus* (Morelat) and *Clarias anguillarias* (L). *Aquatic Toxicology*. **17**:231-238.
22. Fafioye O.O.and Adebisi A.A (2002): Effects of sub lethal concentrations of *parkia biglobosa*

on heamatological parameters of the African catfish, *Clarias gariepinus J. Bio Sci. Res Comm. 12 (4).*

23. Chinabut, S. and Limsuwan. C. (1978). Toxic and sublethal effects of Dipterex on freshwater fisheries network of aquaculture centers in Asia NACA/. 20pp.