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Toxicity of *Carica papaya* Linn: Haematological and Piscicidal Effect on Adult Catfish (*Clarias gariepinus*)

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ABSTRACT

Seeds of the *Carica papaya* (Linn.) are a potential piscicidal and antifertility agent used for catching and controlling excessive breeding of fish from the wild and cultured environment by native people in Southern Nigeria. In the present study, laboratory evaluation of the acute toxicity of the seeds of *Carica papaya* was done at various time intervals on the most widely culture adult Catfish *Clarias gariepinus*. Toxicity bioassays are often used in aquatic ecotoxicology, the main objective of such test is to determine the critical amount of toxicants or their mixtures for aquatic organisms and to predict a toxicant influence and fate. In the present study the LC_{50} value for adult catfish *Clarias gariepinus* at 10, 12, 16, 20, 24, 36, 48, 72, 96 h were 6.0. 5.4, 5.0, 4.8, 4.4, 3.9, 3.6, and 3.3 mg L^{-1}, respectively. Total mortality was recorded at the concentration of 25.5mg L^{-1} within 12 h. The maximum toxicant admissible concentration for 96 h-LC_{50} ranges between 0.033- 0.33 mg L^{-1} with 95% confidence interval of 7.2805% lower and 65.9529% upper. There was significant reduction ($p<0.05$) in the value of blood parameters of *C. gariepinus* adults after exposure to 96 h in aqueous extract of *C. papaya* seed powder; Pack cell volume reduces from 35.0±0.7% in the control to 32.00±4.4% in highest concentration of 17.50 mg L^{-1}; heamoglobin reduces from 11.7±0.2 g mm^{-1} in the control to 10.8±1.05 g mm^{-1} in highest concentration of 17.5mg L^{-1}, the red blood cell reduces from 3.6±0.1(10^4 mm^{-3}) in the control to 3.23±0.3(10^4 mm^{-3}) in the highest concentration, mean cell haemoglobin decreased from 3.3±0.1 g dL^{-1} in control to 3.23±0.3 in highest concentration There was an increase in erythrocyte sedimentation rate from 9.5±0.7 in the control to 10.33±1.5% in the highest concentration of 17.50 mg L^{-1}. Mean cell volume increase from 958.7±18.8(μ^3) in control to 961.63±82.2 (μ^3) in highest concentration of 17.50 mg L^{-1}. No significant difference was noticed in the mean cell haemoglobin concentration ($p<0.001$). The present study suggest that these seeds may be used as a potent aquaculture management tool to eradicate unwanted wild fish from culture ponds before stocking.

Key words: *Carica papaya, Clarias gariepinus*, toxicity, haematology, Piscicide, Antifertility

INTRODUCTION

Papaya seed contained piscicidal and antifertility properties which have been wildly used as fish poison and in controlling the excessive breeding of tilapia in aquaculture in Nigeria. The major active ingredients (carpine, chymopapain, papain, bactericidal a glycone of lucotropaeolin benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosin and carpasemine) are in the black seeds (Akah *et al.*, 1997). A piscicidal substance called carpine is present in traces in black seeds of papaya. Carpine in large quantities is said to lower the pulse rate and depress the nervous system. Papaya seed contains antifertility properties (Lohiya *et al.*, 1999). A complete loss of fertility has been reported in male Nile tilapia (Ekanemm and Okronko, 2003) and female Nile Tilapia (Ekanemm and Bassey (2003), rats and monkeys fed an extract of papaya seeds Pathak *et al.*, 2000; Lohiya *et al.*,1999, 2002).

The use of toxic plants for catching fish is a common practice world wide. The ichtyotoxic characteristics of some of these plants make them potent tools for catching or stupefying fish all over the world. Above some forty years ago, local fishermen in Nigeria have reportedly used specific biocides derived from plants for fishing (Reed *et al.*, 1967). Fisher folks of various African countries extensively use many plants and plant products for capturing fish (Neuwinger, 2004; Fafioye *et al.*, 2004). Barks of ethanobotanical origin and their application in capturing fish have also been reported from other regions of the world such as South America (Schultes and Raffauf, 1990), Nepal (Kulakkatolickal and Kramer,1988) and India (Singh and Singh, 2002). In addition to their use as traditional piscicidal agents for catching wild fish, plant derived fish toxicants are also used in aquaculture management for controlling the predatory and wild fishes.

The eradication of wild fishes in the culture ponds before the stocking of desired species is an important step in pond management as the former compete and/or prey upon the latter. In this aspect, the air-breathing predatory fish species are of particular importance as they are highly resistant to toxicants (Kulakkatolickal and Kramer, 1988) and may survive in moist borrows and mud even when ponds are drained. The use of plant origin ichthyotoxicant as a fisheries management tool has been practiced in at least 30 countries (Murphy and Willis, 1996; Sanger and Koehn, 1997; Lintermans, 2000). As the control and eradication of unwanted fishes in the pond require effective piscicides which are usually not easily accessible, farmers even use synthetic compounds including malachite green, sodium cyanide, antimycin, etc. and even pesticides (Chakraborty *et al.*, 1972; Terazaki *et al.*, 1980; Marking, 1992; Gribgratok, 1981).

Different species of plants employed as piscicides have different effects, depending on the species of fish targeted (Van Andel, 2002). The active principles in the plant part used (leaves, seeds, kernel and bark) have varying potencies and modes of action depending on whether it is applied directly and the forms of extracts, aqueous or alcohol used (Sambasivam *et al.*, 2003). However, there is no report on the effect of *Carica papaya* on the various aspects of the physiology and biochemistry of the clariids C. gariepinus adult. Exposure of fish to these biocides may cause stress in fish without necessarily leading to death. Stress response is characterized by biochemical and physiological changes which may be manifest in both acute and chronic toxicity tests (Singh and Singh, 2002; Tiwari and Singh, 2005). The disruption of biochemical and physiological integrity is assessable by the changes in the haematological and histopathological in functional organs (De La Torre *et al.*, 2000; Van der Oost *et al.*, 2000).

These toxic seeds find their way into the aquatic environment through effluents from industries that use pawpaw fruits as raw materials for the production of juice and drinks (Akah *et al.*, 1997). The acute toxicity of a chemical can easily be evaluated in a short term test and death determines the end point. From an ecological point of view, survival, growth, reproduction, spawning and hatching success provide reactions and adoption to environmental parameters regardless of whether they are natural or man-made. Macroscopically, overt signals of toxicity are almost always preceded by changes at the organs, tissues, cellular and molecular levels (Dutta, 1996; Lohiya *et al.*, 2002; Pathak *et al.*, 2000; Easley *et al.*, 2001). Although the aquatic environment is not the ultimate sink and the aquatic invertebrates and fish are not the target organisms, as a toxicant the agent can produce an adverse effect in any biological system, seriously damaging its structure or function or causing death (Easley *et al.*, 2001). Adverse response may be defined in terms of a measurement that is outside the "normal" range for healthy organisms, such as abnormal mortality, reproduction or growth. The presence of pawpaw seeds in water bodies has been reported (Lohiya *et al.,* 2002) and the negative effects on aquatic life have been proven (Ayotunde and Ofem, 2008). However, despite their widespread use, little is known about their toxicity to fish. In constructing laboratory, dose-response relationship, for aquatic species, one of the most important testing end points has become the determination of the maximum acceptable toxicant concentrations (MATC) (Maki, 1979). Maximum acceptable toxicant concentration can be defined as the highest test concentration at which no effect is observed on survival, growth or any reproductive parameters examined during the life cycle exposure.

Clarias gariepinus are freshwater fish found in the tropical regions of West Africa. They are widely distributed in Asia and Africa and they inhabit most lakes, ponds and rivers where they feed mainly on plankton, insects' larvae, snails, crustaceans, worms and small fishes. *Clarias* species of the family Clariidae is commonly called the "Mudfish" and apart from Tilapia, Clarias is the most cultured fish species in Nigeria and are generally strong fish, they possess an accessory respiratory organ, composed of a paired pear shaped air chamber containing two arborescent structures located on the fourth branchial arcs, that are supported by cartilage and covered by highly vascularised tissue which can absorb oxygen directly from the atmosphere. Since the air chamber communicates directly with the pharynx and the gill- chamber, this accessory air breathing organ enables them tolerate adverse aquatic conditions where other cultured fish species can not survive. They grow faster with additional supplementary feed; the demand for the fish is very high due to its oily flesh. They spawn naturally in captivity and their induction is easy (Olufayo, 2009). Based on the piscicidal properties of *C. papaya* seed, there is the need to evaluate the aqueous extracts of these plants for their potential as fish poisons. The objective of this study was therefore to determine the acute toxicity (LC_{50}) of the aqueous extracts of *C. papaya* seed powder to adult *C. gariepinus* and their effects on haematology and water quality.

MATERIALS AND METHODS

Apparently healthy adult catfish *C. gariepinus* (32.9-46.4 cm length and 251.9-298.7 g weight) were collected at Cross River University of Technology Calabar at Obubra Campus, Fish Farm, The fishes were acclimated to the laboratory condition for two weeks. The fish was fed with pelleted fish diet containing 35% crude protein, during the acclimation period. Feeding was discontinued 48 h before the commencement of the experiment to minimize the production of waste in the test container. The dried seeds of *Carica papaya* were ground to a fine powder, using the coffle mill attachment of a moulinex domestic food bender and the powder was kept in desiccators for later use in stock solutions. A stock solution of *Carica papaya* seed powder (1.0 g L^{-1}) was prepared by adding 1.0g of *C. papaya* in 1litre of distilled water, according to APHA, AWWA and WPCF (1989), APHA 1998 and Olaifa *et al.* (2003).

Bioassay Preliminary 24 h range finding test was conducted for adults *C. gariepinus* following static bioassay procedures described by Parish (1985), to determine the toxic range of aqueous extracts of *Carica papaya* seeds to adults Cat fish *C. gariepinus*. Ten adults *Clarias gariepinus* was batches weight and distributed into a set of 18 rectangular glass tanks (75×45×45 cm) each filled with 50 Lof well water and allowing two weeks for acclimation to laboratory conditions. The water was filtered and aerated to saturation prior to use. Six test solutions of aqueous extracts of *Carica papaya* (250, 300, 350, 400 and 450 mg L^{-1}) were prepared. Distilled water was used as a control.

Each of the test solutions was introduced directly into the glass tanks in a single dose, representing six triplicates treatments per fish. The behavior and mortality of the test fishes in each tank was monitored and recorded every 15 min for the first hour, once every hour for the next three hours and every four hours for the rest of the 24 h period.

Based on the results from the range finding (Lethal toxicity) test described above, 96-h definitive (sub-lethal toxicity) tests following static bioassay procedures described by Parish (1985) was carried out using adult Cat fish *C. gariepinus*. Batches of ten adult Cat fish *C. gariepinus* was distributed into a set of 18 rectangular glass tanks (75×45×45 cm) each filled with 50 L of well water, the water was filtered and aerated to saturation prior to test. Six test solutions each of aqueous extracts of *Carica papaya* was prepared by dissolving 400, 410, 420, 430, 440, 450 mg L^{-1}, earlier determined for the range finding test. Each of the test solution was introduced in a single dose directly into the glass tanks. Continuous aeration was provided to prevent hyper concentration in certain area of the tanks and to maintain dissolved oxygen near saturation throughout the test and the test fishes was not fed throughout these 96 h test.

The behavioral pattern and mortality of the test in each tank was monitored and recorded every hour for the first 4 h once every four hours for the rest of the 24 h and once every 24 h until 96 h. Dead fish was removed immediately with scoop net to avoid contamination due to rotting.

Calculation of lethal concentrations and statistics: The mortality rates observed during the stipulated exposure periods were recorded and using this data various LC_{50} were calculated by Finney's (Finney 1971; USEPA 2000) method along with the slope values. The heterogeneity of the data (if any) in each set was also checked using t-test.

Haematological analysis: Blood (3-5 mL $fish^{-1}$) was collected from the fish after 96 h exposure. Collection of blood was done through the vertebral caudal blood vessel with the help of disposable hypodermic syringe and needle. Blood sample was emptied into 10ml sample bottle treated with anticoagulant Ethylene Diamine Tetracetic Acid (EDTA). Haematological analysis of fish followed the method described by Svobodova *et al.* (1991). Blood cell count (erythrocytes and leucocytes) was carried out in an improved Neubaeur haemocytometer using a modified Yokoyama diluting fluid. The basic eryhrocyte indices, Mean Cell Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were computed from haemoglobin values and erythrocyte count.

Water quality analysis: Water quality monitoring was done prior, during, and after the experiment. pH was determined using a digital pH meter (Mettle Toledo, 320). Dissolved Oxygen (DO) was measured using a digital dissolved oxygen meter (Jenway 9071). While, temperature was measured using a mercury glass thermometer. The reading was taken at 10.00 a.m. on each day of the experiment.

Statistical analysis: All results were collated and analysed using computerized probit and logit analysis (Lichtfield and Wilcoxon, 1949). The median lethal concentration $LC_{50,}$ at selected period of exposurand an associated 95% confidence interval for each replicate toxicity test, was subjected to logit and probit analysis (Finney, 1971) using Statistical Package for Social Sciences (SPSS, v. 11.0).

RESULTS

Lethal concentrations The respective LC_{50} and the Maximum Admissible Toxicant Concentration (MATC) of *C. papaya* at various stages of administration to adult *C. gariepinus* is given in Table 1 and Fig. 1a and b. The estimated LC_{50} lie within the 95% confidence limits and are given along with the slope and F-values. LC_{50} are found to decrease with increasing exposure periods.

Mortality rate: The average percentages of mortality of adult catfish *C. gariepinus* at various stages of toxicant administration in both the modes are given in Fig. 1a and b. Using these mortality rates, the LC_{50} and MATC were calculated, The 10, 12, 16, 20, 24, 36, 48, 72 and 96 h LC_{50} for adult catfish were 6.0, 5.6, 5.4, 5.0, 4.8, 4.4, 3.9, 3.6 and 3.3 mg L^{-1} (Table 1) the maximum admissible concentration ranges between, 0.060-0.60; 0.056-0.56; 0.054-0.54; 0.050-0.50; 0.048-0.48; 0.044-0.44; 0.039-0.39; 0.036-0.36 and 0.033 0.33, respectively. Mortality pattern of Catfish *C. gariepinus* adult are shown in Table 2 and 3. Acute toxicity of aqueous extract of pawpaw seed to catfish adult increased with increasing concentration of the toxicant and time of exposure. Percentage mortality for the test organisms increased with concentration, total mortality occurring in test concentration of 25.5 mg L^{-1}. A sigmoid dose-response curve described the 24 h-LC_{50} exposure period of catfish adults to pawpaw seed powder. It showed an initial steady percentage fish mortality of 20% in test concentrations of 2.92-5.84 mg L^{-1}, followed by steady increase in percentage mortality (40%) in concentrations of 11.66-14.58 mg L^{-1} and thereafter sharp percentage mortality (40.5%) at concentrations between 14.58 and 17.50 mg L^{-1} (Fig. 1).

Variations in the response of adult catfish during the 48, 72 and 96h-LC_{50} values were not significant ($p>0.05$). Figure 1 and 2 showed that the two response curves were biphasic with two sharp phases of increased fish mortality (20-40%, 50-70%) at concentrations of 8.74-14.58 and 11.66-17.50 mg L^{-1}, respectively followed by two phases of decreased mortality (25-15 and 80- 53%) at concentrations of 11-12 and 15-16 mg L^{-1}, respectively. Dose-response curve for the 96 h-LC_{50} showed one sharp phases of decrease percentage mortality (30-40) at test concentrations of 5.84-8.74 mg L^{-1} followed by a phase of increased fish mortality (55-80%) at concentrations of

Table 1: The LC_{50} value for adult catfish *C. gariepinus* and 95% confidence intervals

			95% Confidence Interval	
Time (h)	LC_{50}(mg L^{-1})	MATC(mg L^{-1})	Lower	Upper
10	6.0	0.060-0.60	7.2805	65.9529
12	5.6	0.056-0.56	9.5161	68.3839
16	5.4	0.054-0.54	12.6350	78.4983
20	5.0	0.05.0-0.50	12.7224	81.7109
24	4.8	0.048-0.48	14.4992	91.0674
36	4.4	0.044-0.44	14.7815	96.3185
48	3.9	0.039-0.39	16.0463	97.2870
72	3.6	0.036-0.36	17.4692	99.0641
96	3.3	0.033-0.33	7.2805	65.9529

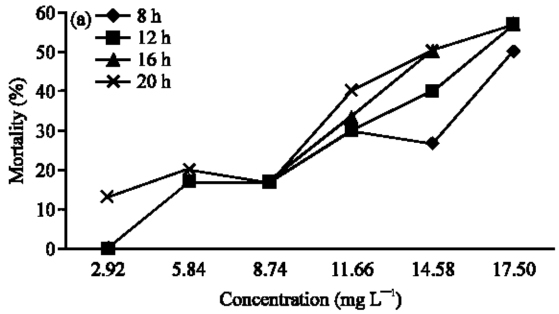

Fig. 1a: Determination of LC_{50} using probit method (Finney 1971; USEPA, 2000)

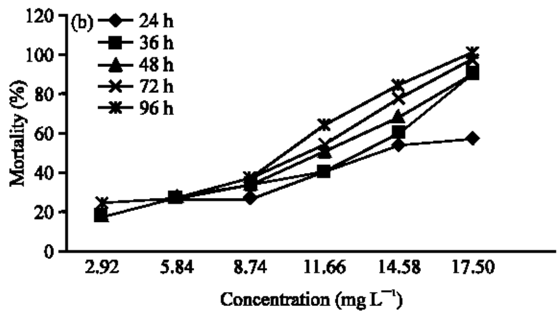

Fig. 1b: Determination of LC_{50} using probit method (Finney 1971; USEPA, 2000)

Table 2: Percentage cumulative mortality of catfish *Clarias gariepinus* Adult exposed to *Carica papaya* seed powder (range finding test)

	Treatment													
Concentration(mg L^{-1})	15 min	30 min	45 min	60 min	2 h	3 h	4 h	6 h	8 h	10 h	12 h	16 h	20 h	24 h
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15.5	0	0	0	0	0	13	13	17	27	27	27	27	30	30
17.5	0	0	13	17	17	23	30	37	37	37	43	43	43	50
20.5	0	23	30	40	40	50	50	50	63	63	70	73	76	76
22.5	13	17	36	43	43	43	50	50	57	63	76	90	90	93
25.5	0	13	37	37	53	60	76	80	90	93	100	100	100	100

Table 3: Percentage cumulative mortality of catfish *Clarias gariepinus* Adult exposed to *Carica papaya* seed powder (Definitive test)

	Treatment													
Concentration(mg L^{-1})	1 h	2 h	3 h	4 h	6 h	8 h	12 h	16 h	20 h	24 h	36 h	48 h	72 h	96 h
2.92	0	0	0	0	0	0	0	0	13	17	17	17	17	23
5.84	0	13	13	13	13	17	17	17	20	17	27	27	27	27
8.74	0	0	0	17	17	17	17	17	17	27	33	33	37	37
11.66	0	0	0	0	30	30	30	33	40	40	40	50	53	63
14.58	0	0	13	13	13	27	40	50	50	53	60	67	77	83
17.50	0	13	20	30	43	50	50	57	57	57	90	90	97	97

11.66-14.58 mg L^{-1}. The curve assumed a gradual increase in fish mortality (80-95%) at higher concentrations of 14.58-17.50 mg L^{-1}. Percentage mortality of test fish also increased with exposure time from 24 to 96 h with more marked increase (60-100%) observed at 96 h exposure time.

Water quality: The results of water quality parameters (pH, temperature, total solid, conductivity, hardness, total suspended solid, alkalinity, nitrite, nitrate, sulphate, chloride and dissolved oxygen) obtained for the test solutions during all experiments were not significantly different from the control (Table4).

Behavioral changes: In both the modes of administration of *Carica papaya* seeds, the acutely intoxicated fish exhibited violent swimming activities. They often came to the water surface and exhibited increased gulping activity. They also expressed highly increased opercular movements indicating respiratory distress and tried many times to jump out of the aquaria. Excessive amounts of mucus were also seen all over the body of the exposed fish. None of the control fish showed any of these behavioral changes. The dying ones in the experimental aquaria exhibited vertical positioning with head above the water surface. They also showed muscular twitching and tetany before death. Finally they lost balance, settled at the bottom of the aquaria and died. No mortality was observed in the control groups (Table 5, 6).

Haematological studies: There was significant reduction ($p<0.05$) in the value of blood parameters of *C. gariepinus* after exposure to 96 h in aqueous extract of *C. papaya* seed powder (Table 7 and Fig. 3-7). Pack cell volume reduces from 35.0±0.7% in the control to 32.00±4.4% in highest concentration of 17.50 mg L^{-1}, heamoglobin reduces from 11.7±0.2 g mm^{-1} in the control to 10.8±1.05 g mm^{-1} in highest concentration of 17.5 mg L^{-1}, the red blood cell reduces from 3.6±0.1(10^4 mm^{-3}) in the control to 3.23±0.3(10^4 mm^{-3}) in the highest concentration, mean cell haemoglobin decreased from 3.3±0.1 g dL^{-1} in control to 3.23±0.3 in highest concentration There was an increase in Erythrocyte sedimentation rate from 9.5±0.7 in the control to 10.33±1.5% in the highest concentration of 17.50 mg L^{-1}. Mean cell volume increase from 958.7±18.8(μ^3) in control to 961.63± 82.2 (μ^3) in highest concentration of 17.50 mg L^{-1}. No significant difference was noticed in the Mean cell haemoglobin concentration ($p<0.001$).

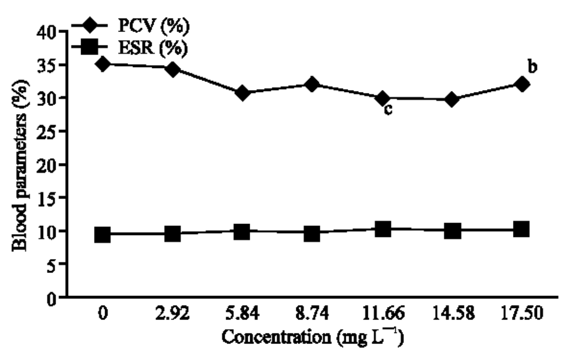

Fig. 2: Spatial variations in pack cell volume and erythrocyte sedimentation rate of *Clarias gariepinus* adult after 96 h exposure to aqueous extract of *Carica papaya*. Values with different letter show non-significal difference

Table 4: Physico-Chemical Parameter of Experimental Water.

Conc. (mg L^{-1})	Total solid (mg L^{-1})	pH	Conductivity (X104)	Hardness (mg L^{-1})	TSS	Alkalinity (mg L^{-1})	Nitrite (mg L^{-1})	Nitrate (mg L^{-1})	Sulphate (Mg L^{-1})	Chloride (mg L^{-1})	Temperature(°C)	D.O (mg L^{-1})
Control	0.29 ±0.1	6.3±0.4	3.1×100 ±1.8	42.3±5.0	0.2±0.1	114.7±64.3	0.4±0.3	0.7±0.1	1.17±0.38	18.67±2.3	26.00±2.8	5.00±0.20
2.92	0.19±0.25	6.6±0.1	3.2×100±1.0	5.3±14.1	0.1±0.1	152.0±63.5	0.5±0.3	0.8±0.01	1.6±0.92	17.33±1.15	23.25±0.96	5.97±1.12
5.84	0.30±0.12	6.1±0.5	5.3×100±3.9	42.0±3.5	0.2±0.1	328±36.7	0.3±0.4	0.6±0.1	1.60±0.35	35.33±5.03	24.50±0.58	6.67±0.46
8.74	0.30±0.12	6.4±0.1	8.1×100±2.5	40.0±4.0	0.1±0.0	322.7±104.0	0.8±0.7	0.7±0.2	1.27±0.42	26.67±11.55	25.00±1.83	5.70±0.87
11.66	0.4 ±0.3	6.4±0.1	9.0×100±0.5	45.3±24.8	0.16±0.1	332.0±22.3	0.34±0.3	0.99±0.74	1.33±0.50	38.0±10.39	25.00±1.85	5.53±0.45
14.58	0.7 ±0.5	6.4±0.1	7.0×100±0.1	44.7±8.1	0.5±0.4	370.7±79.4	0.80±0.7	0.47±0.12	1.15±0.27	38.0±2.0	25.00±1.15	6.47±0.21
17.5	0.4 ±0.3	6.4±0.1	6.4×100±0.1	41.0±4.4	0.18±0.2	262.0±48.5	0.1±0.02	0.61±0.19	1.37±0.45	17.33±15.53	24.75±0.95	6.30±1.25

Table 5: Behavioral of adult catfish (range finding test)

	Concentrations (mg L^{-1}) exposure time																							
	12 h						16 h						20 h						24 h					
Behavior	0	15.5	17.5	20.5	22.5	25.5	0	15.5	17.5	20.5	22.5	25.5	0	15.5	17.5	20.5	22.5	25.5	0	15.5	17.5	20.5	22.5	25.5
Loss of reflex	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
Molting	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Air gulping	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+
Erratic swimming	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+
Barbel deformation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Excessive mucus secretion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-: Absent, +: Present

Table 6: Behavioral response of adult catfish (Definitive test)

	Concentrations (mg L^{-1}) exposure time																							
	24 h						48 h						72 h						96 h					
Behavior	2.95	5.84	8.74	11.66	14.58	17.50	2.95	5.84	8.74	11.66	14.58	17.50	2.95	5.84	8.74	11.66	14.58	17.50	2.95	5.84	8.74	11.66	14.58	17.50
Loss of reflex	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+
Molting	-	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Air gulping	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Erratic swimming	+	+	-	-	+	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	-	+	-	+
Barbel deformation	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Excessive mucus secretion	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+

Key - = Absent, + = Present

Table 7: The Summary of effects of Carica papaya on haematological parameters of adult Catfish Clarias gariepinus

	Parameters							
Conc./ (mg L^{-1})	PCV (%)	Hb (g mm^{-3})	ESR (%)	WBC (10^4 mm^{-3})	RBC (106 mm^{-3})	MCH (pg)	MCHC (T L^{-1})	MCV (μ^3)
Control	35.0±0.7^{a}	11.7±0.2^{a}	9.5±0.7^{a}	5.6±0.4^{a}	3.6±0.1^{a}	3.3±0.1^{a}	35.9±3.0^{a}	958.7±18.0^{ab}
2.92	34.33±0.6^{a}	11.60±0.2^{a}	9.67±0.6^{a}	5.73±0.4^{a}	3.67±0.2^{a}	3.17±0.2^{a}	35.17±2.5^{a}	937.4±39.1^{a}
5.84	30.67±3.8^{ab}	10.26±1.3^{ac}	10.00±1.0^{a}	5.13±0.6 ^{a}	3.27±0.6^{a}	3.17±0.3^{a}	33.47±0.1^{ac}	947.2±64.7^{a}
8.74	32.00±3.5^{a}	10.89±1.2^{ab}	9.67±1.2^{a}	5.40±0.6^{ab}	3.50±0.7^{a}	3.13±0.3^{a}	33.73±0.3^{ab}	921.67±87.1^{a}
11.66	30.00±2.0^{ab}	10.33±0.7^{a}	10.33±0.6^{a}	5.17±0.7^{a}	3.30±0.4^{a}	3.10±0.3^{a}	33.67±0.3^{ab}	915.9±83.4^{a}
14.58	29.67±5.7^{a}	9.93±1.9^{ac}	10.00±1.7^{a}	4.70±1.5^{a}	3.10±1.0^{a}	3.33±0.4^{a}	33.50±0.2^{ac}	895.0±172.3^{ab}
17.5	32.00±4.4^{a}	10.8±1.5^{ab}	10.33±1.5^{a}	4.97±1.0^{ab}	3.23±0.3^{a}	3.23±0.3^{a}	35.03±2.6^{a}	961.63±82.2^{a}

Values are Mean±standard error. Means with same superscript along the same column within sampling day boxes are not significantly different, $p<0.05$ = Level of statistical significance

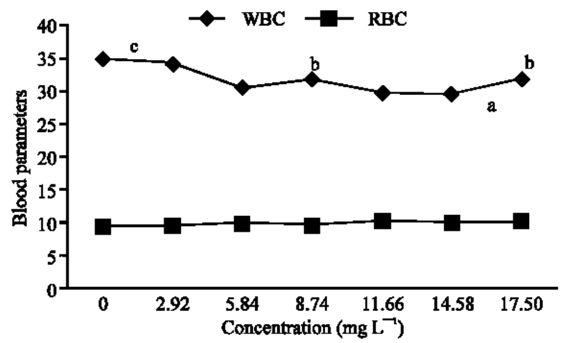

Fig. 3: Spatial variations in white blood cell and red blood cell of *Clarias gariepinus* adult after 96 h exposure to aqueous extract of *Carica papaya*. Values with different letter show non-significal difference

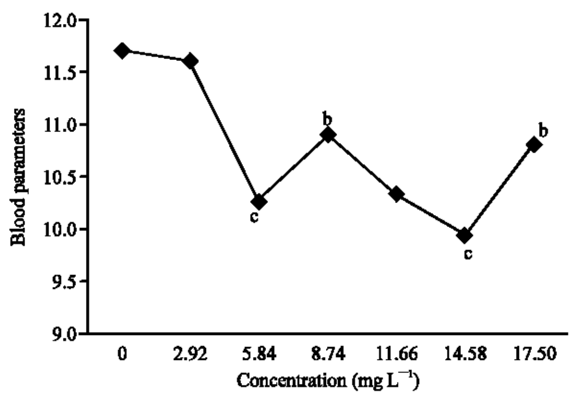

Fig. 4: Spatial variations in haemoglobin of *Clarias gariepinus* adult after 96 h exposure to aqueous extract of *Carica papaya*. Values with different letter show non-significal difference

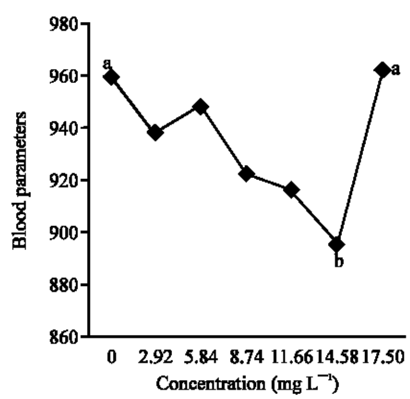

Fig. 5: Spatial variations in mean cell volume of *Clarias gariepinus* adult after 96 h exposure to aqueous extract of *Carica papaya*. Values with different letter show non-significal difference

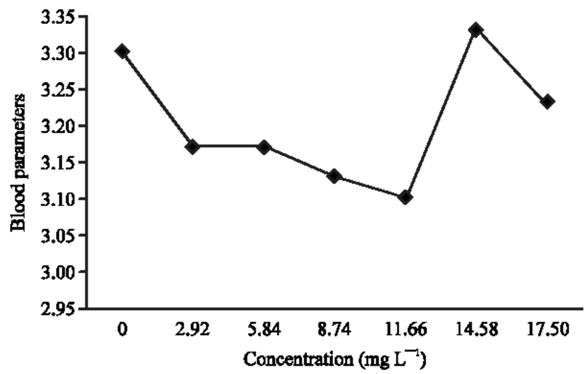

Fig. 6: Spatial varaations in mean cell haemoglobin of *Clarias gariepinus* adult after 96 h exposure to aqueous extract of *Carica papaya*

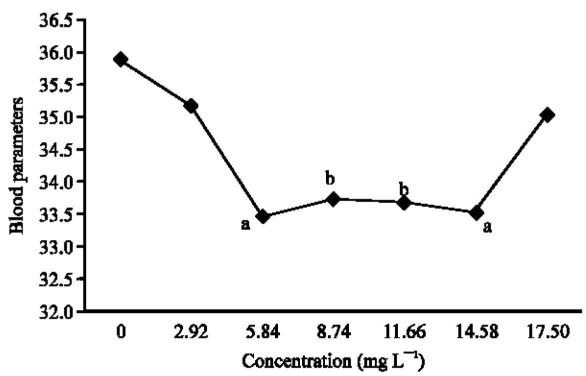

Fig. 7: Spatial varaations in mean cell haemoglobinConcentration of *Clarias gariepinus* adult after 96 h exposure to aqueous extract of *Carica papaya*. Values with different letter show non-significal difference

DISCUSSION

Toxicity bioassays are often used in aquatic ecotoxicology, the main objective of such test is to determine the critical amount of toxicants or their mixtures for aquatic organisms and to predict a toxicant influence and fate. In the present study the LC_{50} value for adult catfish *C. gariepinus* at 10, 12, 16, 20, 24, 36, 48, 72, 96 were 6.0. 5.4, 5.0, 4.8, 4.4, 3.9, 3.6 and 3.3 mg L^{-1}, respectively. The maximum toxicant admissible concentration for 96 h LC_{50} ranges between 0.033-0.33 mg L^{-1} with 95% confidence interval of 7.2805% lower and 65.9529%, established for catfish adults was derived by multiplying a constant 0.01-0.1 by 96 h LC_{50} (Koesoemadinata, 2000). These values for adult catfish compared well with those from literature (Olamdimeji and Offem, 1989; Ayotunde and Ofem, 2005, 2008; Ayotunde *et al*., 2010). Acute toxicity (100% death) occurred at 25.5 mg L^{-1} at 12 h exposure period. According to Cagauan *et al*. (2004), concentration causing 100% mortality forms the basis of calculating the piscicidal activity of the test plants. The concentrations comparable to those of diazinon (Adedeji *et al*., 2008), phenol (Cowgill and Milazzo, 1991) and tetrachloromethane (LeBlanc, 1980) but at lower concentrations than those of benzene (Canton and Adema, 1978), methanol (Tong *et al*., 1996) and acetonitrile (Guilhermino *et al*., 2000).

However, pawpaw powder was less toxic than chlorine (Kaniewska-Prus, 1982) and ammonia (Mount and Norberg, 1984). Dose-response approach in estimating the lethal effects of toxicants on organisms have been criticized for lacking real ecological meaning (Pernak *et al.*, 2003; Adedeji *et al.*, 2008). Nonetheless, regulatory norms have been built around LC_{50} values that can be compared across toxicants and organisms (LeBlanc, 1980). Thus LC_{50} values from dose-response bioassays have necessarily become starting points for ecologically relevant studies of toxicant effects on animal populations (Schindler, 1987).

In our experiments, the 96 h LC_{50} value of 3.3 mg L^{-1} of aqueous extracts of pawpaw seed powder to *C. gariepinus* adult was higher than the value of 1.8 mg L^{-1}) obtained by Ayotunde and Ofem (2005) for pawpaw seed powder to Tilapia *Oreochromis niloticus* fingerlings and lower than the value of 4.2 mg L^{-1} reported by Ayotunde and Ofem (2008) for adult tilapia *O. niloticus*. The difference may be due to higher resistance of catfish or Tilapia to aqueous extract of pawpaw seed powder that is inter-specific differences rather than by size differences. However, these results disagree with the size-specific sensitivity to acute chemical toxicity observed in some aquatic animals with the smallest individuals showing the highest sensitivity (Goyer and Clarkson, 2001; Bianchini *et al.*, 2002; Bossuyt and Janssen, 2005). The size-specific and interspecific difference in lethal level will allow the effective usage of pawpaw seed as anti-fertility agent in tilapia polyculture with catfish (Ayotunde and Ofem, 2005).

Banerjee (1993) is of the view that determination of LC_{50} is essential for acute toxicity testing and also for routine bioassay experiments. In view of these reports, the present study provides a range of LC_{50} of *Carica papaya* at various durations. As a fishing poison, the knowledge of LC_{50} at various exposure periods would provide more provisions to the farmers in case they want to kill/eradicate the predatory and weed fishes within a convenient duration from culture ponds before stocking. Further, the mortality rates observed in the present study suggests a clear relationship between dose, mortality and exposure period. The concentration of the toxicant is directly proportional to the mortality rate. On the other hand, as the duration of exposure increases the lethal concentration decreases. As the calculated F- test values (representing heterogeneity) are less than the respective table values, the mortality counts are not significantly heterogeneous indicating variables such as individual resistance, do not significantly affect the lethal concentrations as they lie within 95% confidence limits. The steepness of the slope is also an indication of large increase in mortality rate with relatively small increase in the concentration of the piscicidal agent.

Clarias sp. is ecologically adapted to muddy environments in which temporary changes in water chemistry are more rapid and the contaminant concentration are usually higher (Koivisto, 1995). Such an environmental stress may facilitate tolerance to increased concentrations of contaminants. This view is supported by our observation, which revealed that 96 h seem not to be sufficient length of time to determine the asymptotic LC_{50} for the pawpaw seed powder concentration to *Clarias* adults since mortality would have continued if exposure time was extended. Oh *et al.* (1991) gave three factors for the selective toxicity of toxicants for various fish species as: different inhibition of acetylcholinesterase, different detoxification and absorption. The above factors were probably responsible for the different toxic reactions showed in this experiment by catfish adults to varying concentration of aqueous extracts of *C. papaya*.

The reactions were more pronounced at higher concentration due to increased inhibition of acetylcholinesterase which eventually results in the death of the fish (Adedeji *et al.*, 2008). This report also agrees with the work of many authors (Muniyan and Veeraraghavan, 1999; Santhakumar and Balaji, 2000; Ayuba and Ofojekwu, 2002; Liao *et al.*, 2003), who work on the toxicity of different plant chemicals to freshwater fishes. In toxicological experiments, the time of exposure has large effect on biological response. The general rule of thumb is that the longer the exposure time, the lesser the LC_{50} value and the greater the toxicity. Results of this study showed similar pattern having lesser 96 h-LC_{50} than 48 h-LC_{50} and so on, with ratio of 24 h-LC_{50} to 48 h-LC_{50} as 1.48 which showed the delayed acute toxic response.

Studies revealed that organisms exposed to toxicants usually exhibit changes in opercular rate, erratic, sudden jerky swimming movements and different behavioral activities as shown in Table 5, 6) which demonstrated to be a sensitive indicator of physiological stress in fish subjected to sub-lethal concentration of pollutants (Maikai *et al.*, 2008). The behavioral responses obtained from the study compared favorably with the observation of Pascual *et al.* (1994) when formalin at different concentrations were used on sea bass (*Lates calcarifer*) fry. The observations in this study agreed with Lin and Liu (1990) who reported that clinical signs such as abnormal movement and high respiration rate in hybrid tilapia (*O.mossambicus*) induced by ammonia suggested neurological dysfunction and gill damage.

The results of water quality parameters (pH, temperature, Total Solid (mg L^{-1}), Conductivity (X104), Hardness (mg L^{-1}) TSS, Alkalinity (mg L^{-1}), Nitrite (mg L^{-1}), Nitrate (mg L^{-1}), Sulphate (mg L^{-1}) Chloride (mg L^{-1}) and dissolved oxygen) obtained for the test solutions during all experiments were not significantly different ($p>0.05$) from the control (Table 4). For such comparison to be meaningful, species variability and possible differences in test medium quality table needs to be accounted for. The latter is important, since hardness, alkalinity and pH of a medium can all influence the speciation of toxicants and the extent of toxicity (Heijerick *et al.*, 2003). However, because a standard test medium was used with a standard pH, we did not expect any changes in the effective toxicant concentration due to possible interaction with the medium.

The change in the value of blood parameters of *C. gariepinus* fingerlings after exposure to 96 h in aqueous extract of *C. papaya* seed powder in this experiment is similar to the results obtained from the work of Saleh *et al.* (1998) who studied the effect of inhalation of the pyrethroidinsecticide, tetramethrin, on hematological and biochemical parameters in albino rats. Histopathological and biochemical alterations by plant toxins have been observed in *O. niloticus* (Zapata-Perez *et al.*, 2000; Ayotunde and Ofem, 2008). The absence of significant changes in RBC count, haematocrit value, hemoglobin content and the blood indices (MCHC, MCV and MCH) and the significant increase of the WBC counts and lymphocytes percentage with the decrease in blood platelets agree with findings in treated rats specie (Saleh *et al.*, 1998).

Haematological value such as RBC, PCV, Hb (Table 4) were significantly affected by the treatment ($p>0.05$). There were no significant differences ($p>0.05$) in the WBC, Leucocyte and Neutrophil values in response to dietary DPS. Inclusion of DPS in the diets of broilers tended to improve their haematological competence especially at 5% dietary level. Haematological values are indirect pointers to the health of live stocks (Kecceci *et al.*, 1998; Jain, 1986) and are further modified by several other factors including diets (Talebi *et al.*, 2005). Most of these values recorded for DPS fed broilers are within the normal range for poultry (Nworgu *et al.*, 1999). The value of PCV at 5% inclusion level corresponds with the findings of Nworgu *et al.* (1999). The Hb values falls within (6.0-13%) reported by Iheukwumere *et al.* (2002) but lower than the normal value (9-13) reported by (Nworgu *et al.*, 1999). Birds fed 15 % had the lowest WBC of (10.53×109/1). This may be due to high levels of anti nutritional factor.

CONCLUSION

The toxicity effect of pawpaw seed powder had a positive correlation with exposure time from 24 to 96 h, for the adult catfish *C. gariepinus*. From the toxicity tests pawpaw seed powder concentration value of 3.3 mg L^{-1} in the medium can be potentially hazardous to some fish species in freshwater. Therefore, acute toxicity data of the present study provide baseline information needed to develop models of pawpaw seed powder effects on ecological systems. More information is needed to assess their potential impacts on aquatic environment.

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