

Full Length Research Paper

Toxicity of pawpaw (*Carica papaya*) seed powder to sharptooth catfish *Clarias gariepinus* fingerlings and effects on haematological parameters

Ezekiel Olatunji Ayotunde, Benedict Obeten Offem*, Irom Bassey Okey, Gabriel Ujong Ikpi, Stephen Ncha Ochang, Nnanke Effiom Agbam and Dodeye Eno Omini

Department of Fisheries, Faculty of Agriculture and Forestry, Cross River University of Technology, P. M. B. 102, Obubra Campus, Calabar, Cross River State, Nigeria.

Accepted 08 October, 2019

The mesocarp of pawpaw fruits (*Carica papaya*) is a delicacy in the Tropics but the seeds are known to contain toxic substances (Carpine, Papain). However, there have been very few studies on the toxicity of these substances to organisms. First part of the present study determines the toxicity of an aqueous extract of pawpaw (*Carica papaya*) seed powder to catfish (*Clarias gariepinus*) fingerlings using static bioassay. The second part investigates the haematological effects. 400 healthy catfish (*Clarias gariepinus*) fingerlings 10.5 ± 3.2 cm (total length), 15.5 ± 6.4 g (body weight) were exposed to triplicate concentrations of 10, 11, 12, 13, 14 and 15 mg/l in eighteen 30 L glass tanks, each with 22 fish in 20 L aerated rain water. 24, 48, 72 and 96 h LC₅₀ values were 19.0 ± 3.3 , 13.0 ± 2.8 , 12.8 ± 1.6 and 12.9 ± 2.1 mg/L, respectively. There was significant reduction ($p < 0.05$) in the value of blood parameters [Blood cell count (erythrocytes and leucocytes) and basic erythrocyte indices (mean cell haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH)] of *C. gariepinus* fingerlings after exposure to 96 h in aqueous extract of *C. papaya* seed powder. Toxic reactions include; erratic movement, air gulping, loss of reflex, molting and discoloration. Maximum admissible toxicant concentrations ranged from 0.1 to 1.29 mg/L (confidence level: 22 - 78.8%). The results showed that concentrations of pawpaw seed powder in excess of 1.29 mg/L can be potentially harmful to *C. gariepinus* fingerlings.

Key words: Toxicity, pawpaw seed, catfish, fingerlings, haematology, water quality.

INTRODUCTION

Pawpaw (*Carica papaya*) is a common man's fruits available throughout the year in the Tropics. The fruits, leaves, seeds, and latex are used (Akah et al., 1997; Eno et al., 2000) as a cure for many tropical diseases hence the common name "medicine tree" or "melon of health." The major active ingredients (carpine, chymopapain, papain, bactericidal aglycone of glucotropaeolin benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosin, and carposamine) are in the black seeds (Akah et al., 1997;

Eno et al., 2000; Wilson et al., 2002). The fleshy part of the fruits (mesocarp) is a delicacy and nutrient-rich drinks of high demand are produced from them. However, some of the active substances (e.g carpine and papain) from pawpaw are toxic (Eno et al., 2000). Carpine are present in traces in the black seeds of papaya. In large quantities, it is said to lower the pulse rate and depress the nervous system. Externally the latex is irritant, dermatogenic and vesicant. Internally, it causes severe gastritis. Some people are allergic to the pollen, the fruit, and the latex. Papain can induce asthma and rhinitis. The acid fresh latex can cause severe conjunctivitis and vesication. Carpine and papain also have anti-fertility properties (Lohiya et al., 1999). Complete loss of fertility has been

*Corresponding author. E-mail: benbeff06@yahoo.com. Tel: 08055930219.

reported in male rabbits, rats and monkeys fed extracts of papaya seeds (Lohiya et al., 1999; 2002; Pathak et al., 2000), suggesting that ingestion of papaya seeds may adversely affect the fertility of human males or other male mammals.

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 has been reported (Lohiya et al., 2002) and the negative effects on aquatic life have been proven (Ayotunde and Offem, 2008). However, despite their widespread use, little is known about their toxicity to fish.

The sharptooth catfish, *Clarias gariepinus* is of ecologic importance and commercially valued fish in the Nigerian fishing industry (Ita, 1980). These sharp-tooth catfish are frequently and widely cultured in ponds, and occur freely in African natural fresh waters. The demand for this fish species by almost 75% of African population has necessitated the cropping of it in large number using poisons. 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 (LC₅₀) of the aqueous extracts of *C. papaya* seed powder to *C. gariepinus* fingerlings and their effects on haematology and water quality.

MATERIALS AND METHODS

Four hundred (400) healthy catfish, *C. gariepinus* fingerlings 10.5 ± 3.4 cm (total length), 15.5 ± 6.8 g (body weight) collected from Cross River University of Technology fish farm were acclimated for 2 weeks in the laboratory, in eighteen (18) 30 L glass tanks. Each test chamber contains equal volume of rain water (20 L) and equal number of fish (22). For nomenclature of the fish taxa conformed to www.fishbase.org and <http://www.catalogueoflife.org>. The fish were fed to satiation twice daily (0900 and 1600 h) with pelleted fish diet containing 35% crude protein during the acclimatization period. Feeding was discontinued 48 h before the commencement of the

experiment, to minimize the production of waste in the test container.

Preparation of pawpaw seed powder

Large quantities of ripe pawpaw seeds were purchased from the farmers in Obubra village, Cross River State, Nigeria. The seed powder was prepared by opening the mature pawpaw fruit, and the fresh seeds were extracted and sun dried to constant weight. The seeds were ground to a fine powder using the coffee mill attachment of a Moulinex domestic food bender. The powder was kept in desiccators to be used for the preparation of experimental stock solutions.

Acute toxicity test

Preliminary range finding test was conducted to determine the toxicity level of pawpaw seed powder following OECD (2001) Direction No. 203 and Methodical Manual ISO 7346/2. Triplicate six (6) test concentrations were used for this investigation: one control and five tests solutions of pawpaw seed powder, in triplicates. *C. gariepinus* fingerlings were batch-weighed with a top-loading mettler balance (Mettler Toledo (K)), and distributed randomly in triplicate per treatment. The glass tanks were covered with mosquito net to prevent fish from jumping out; there was no aeration, no water change nor feeding throughout the test. The toxicant was introduced at concentrations; 5, 10, 15, 20 and 25 mg/l with a control of 0 mg/l. The behavior and mortality of the test fishes in each tank was monitored for 24 h and recorded every 15 min for the first hour, once every hour for the next three hours and every four hours for the rest 24 h period.

Definitive test was conducted using triplicate concentrations of 10, 11, 12, 13, 14 and 15 mg/l of Pawpaw seed powder determined from the range finding test. This test comprised one sub-lethal toxicity test according to the OECD (2001). Fish mortality was monitored and recorded hourly for the first four hours, every 4 h for the next 24 h, and subsequently every 24 h, for the next 96 h. The inability of fish to respond to external stimuli was used as an index of death. Apart from monitoring and recording fish mortality, the fish behavior such as: erratic swimming, air gulping, loss of reflex, discoloration and molting was monitored. 96-LC₅₀ (concentration of pawpaw seed powder, estimated to be lethal to 50% of test organisms after exposure time of 96 h) was determined graphically using Probit transformation (Herwig, 1979; USEPA, 2000).

Haematological examination of fish

Blood samples (3 - 5 ml/fish) were collected from the fish after 96 h exposure period by use of disposable 2 ml hypodermic syringe and needles. The method of collection of the blood was through the vertebral caudal blood vessel. Blood samples were emptied into 10 ml heparinized blood sampling bottle treated with ethylene diamine tetra-acetic acid (EDTA) as an anticoagulant. 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 Neubauer haemocytometer using a modified Yokoyama diluting fluid. The basic erythrocyte 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 before the experiment, during

Table 1. Percentage cumulative mortality of *C. papaya* to catfish *C. gariepinus* fingerlings (Range finding test).

S/No.	Conc. (mg/l)	15 min	30 min	45 min	60 min	2 h	3 h	4 h	8 h	12 h	16 h	20 h	24 h
1	0	0	0	0	0	0	0	0	0	0	0	0	0
2	5	0	0	0	3.3	10	10	13.3	23.3	30	33.3	33.3	33.3
3	10	0	0	3.3	6.6	10	16.6	36.3	43.3	46.6	46.6	46.6	53.3
4	15	0	0	10	20	26.6	33.3	46.6	46.6	53.3	80.0	80.0	90.0
5	20	3.0	3.3	23.3	36.6	43.3	43.3	53.3	73.3	100	100	100	100
6	25	0	0	0	0	0	0	3.3	96.6	100	100	100	100

Table 2. Percentage cumulative mortality of *C. papaya* to catfish *C. gariepinus* fingerlings (Definitive test).

S/No.	Conc. (mg/L)	1h	2 h	3 h	4 h	8 h	12 h	16 h	20 h	24 h	48 h	72 h	96 h
1	10	0	0	0	0	0	0	0	10	10	13.3	13.3	16.6
2	11	0	0	0	0	0	0	6.6	10	10	36.6	40	40
3	12	0	0	0	0	3.3	3.3	10	10	16.6	23.3	23.3	30
4	13	0	0	0	0	0	0	16.6	16.6	23.3	53.3	53.3	53.3
5	15	0	0	0	0	0	0	23.3	26.6	26.6	66.6	70	80
6	16	0	0	0	0	0	0	16.6	23.3	23.3	43.3	43.3	83.3

Water quality analysis

Water quality monitoring was done before the experiment, during the experiment and after the experiment. pH was determined using a digital pH meter (Mettler Toledo 320). The electrode was inserted into the bottle containing the water sample after standardization in different buffer, after which the reading was taken. D₂O was measured using a digital, dissolved oxygen meter once in a day at 8.00 a.m. While temperature was measured using a mercury in-glass thermometer, which was placed in the medium inside the test container until reading was taken. 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 analyses (Lichfield and Wilcoxon, 1949). The median lethal concentration LC₅₀ at selected period of exposure, and an associated 95% confidence interval for each replicate toxicity test was subjected to logit and probit analyses (Finney, 1971) using Statistical Package for Social Sciences (SPSS) 11.0 for Windows XP on PC. Data were analyzed using descriptive statistics (mean, standard deviation, frequencies and percentage). Comparison of data on physico-chemical properties between range finding tests and the definitive tests was carried out using analysis of variance (ANOVA) (Steel and

Torrie, 1980).

RESULTS

Acute toxicity test

Mortality pattern of Catfish *C. gariepinus* fingerlings for 24, 48, 72 and 96 h are shown in Tables 1 and 2. Acute toxicity of aqueous extract of pawpaw seed to catfish

fingerlings 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 20.0 mg l⁻¹. A sigmoid dose-response curve described the 24 h-LC₅₀ exposure period of catfish fingerlings to pawpaw seed powder. It showed an initial steady percentage fish mortality of 10% in test concentrations of 10.0 - 11.0 mg l⁻¹, followed by sharp increase in percentage mortality (28%) in concentrations of 11.5 - 13.5 mg l⁻¹ and thereafter decreased percentage mortality (22%) at concentrations between 15.0 and 18.0 mg l⁻¹ (Figure 1). Variations in the response of catfish fingerlings during the 48 and 72 h-LC₅₀ values were not significant ($p > 0.05$). Figure 2 showed that the two response curves were biphasic with two sharp phases of increased fish mortality (18 - 38%, 22 - 70%) at concentrations of 10 - 11.0 and 12 - 14.0 mg/L, respectively followed by two phases of decreased mortality (38 - 22 and 70 - 43%) at concentrations of 11 - 12 and 15 - 16 mg l⁻¹, respectively. Dose-response curve for the 96 h-LC₅₀ showed two sharp phases of increased percentage mortality (18 - 40 and 28 - 80%) at test concentrations of 10.0 - 11.0 and 12.0 - 15.0 mg/L separated by a phase of declined fish mortality (40 - 28%) at concentrations of 11.0 - 12.0 mg l⁻¹. The curve assumed a gradual increase in fish mortality (80 - 85%) at higher concentrations of 14.0 - 15.0 mg l⁻¹. Percentage mortality of test animals also increased with exposure time from 24 to 96 h with more marked increase (83 - 100%) observed at 96 h exposure time. During the course of conducting the acute toxicity assays a series of symptoms were observed in the test organisms. The first symptom involved; erratic swimming movements, air gulping, strong restlessness and haemorrhage, loss of

reflex, discoloration and death. The intensity of these symptoms was directly related to the concentration of toxicant in water and duration of exposure. It was observed that Catfish *C. gariepinus* fingerlings showed variations in their tolerance to aqueous extracts of *C. papaya* (Tables 3 and 4).

Haematological studies

There was significant reduction ($p < 0.05$) in the value of blood parameters of *C. gariepinus* fingerlings after exposure to 96 h in aqueous extract of *C. papaya* seed powder (Table 5). Haemoglobin increased from $4.33 \pm 0.58\%$ in the control to $5.40.0 \pm 0.69\%$ in test concentration of 15.0 mg/l while red blood cell increased from $2.90 \pm 3.16\%$ in the control to $3.07.0 \pm 5.31\%$. There was an increase in MCV from 554.0 ± 465.8 to 2090 ± 409.21 . Erythrocyte sedimentation rate reduced from $7.57 \pm 1.21\%$ in control to $4.27 \pm 0.25\%$ while white blood cells reduced from $9.93 \pm 0.1 \times 10^4$ to $6.63 \pm 0.17 \text{ mm}^3$. Mean haemoglobin concentration reduced from 0.003 ± 0.0013 in control to 0.00033 ± 0.005 , mean cell haemoglobin decreased from $1.5 \pm 0.2 \text{ g/dl}$ in control to 0.67 ± 0.51 . No significant difference was noticed in the PCV.

Water quality

The results of water quality parameters (pH, temperature, and dissolved oxygen) obtained for the test solutions during all experiments were not significantly different ($p > 0.05$) from the control (Table 6).

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.

The present study represents, to our knowledge, the first toxicological test of pawpaw powder extracts to aquatic animals. Our observed values for catfish fingerlings compared well with those from literature (Oladimeji and Offem, 1989, Ayotunde and Offem, 2005, Ayotunde and Offem, 2008).

Acute toxicity occurred at concentrations comparable to those of diazinon (Olufemi et al., 2007), 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 (Kaniowska-Prus, 1982) and ammonia (Mount and Norberg, 1984).

For such comparison to be meaningful, species variability

and possible differences in test medium quality 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 (Barata et al., 1998; 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. In our experiments, the 96 h LC₅₀ value ($12.9 \pm 2.1 \text{ mg/L}$) of aqueous extracts of pawpaw seed powder to *C. gariepinus* fingerlings was higher than the value (1.8 mg/l) obtained by Ayotunde and Offem (2005) for Pawpaw seed powder to *Tilapia Oreochromis niloticus* fingerlings and 4.2 mg/L reported by Ayotunde and Offem (2008) for adult tilapia *O. niloticus*. Also, values of the total mortality for tilapia occurred at concentrations (6.5 and 8.9 mg/L) lower than those of catfish fingerlings (20 mg/l) within same exposure period.

The difference may be due to higher resistance of catfish to aqueous extract of pawpaw seed powder that is inter-specific differences rather than by size differences. In a similar experiment with organochlorine substances, Albaiges et al. (1987) revealed that the levels of chemicals in the gonads and liver of fish are similar in adults and young specimen, which seem to indicate that the age of a fish is not a significant factor in the accumulation of toxicants. 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 inter-specific difference in lethal level will allow the effective usage of pawpaw seed as anti-fertility agent in tilapia polyculture with catfish (Ayotunde and Offem, 2005). *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 LC₅₀ for the pawpaw seed powder concentration to *Clarias* fingerlings 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 fingerlings 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 (Olufemi et al., 2007). This report also agrees with the work of many

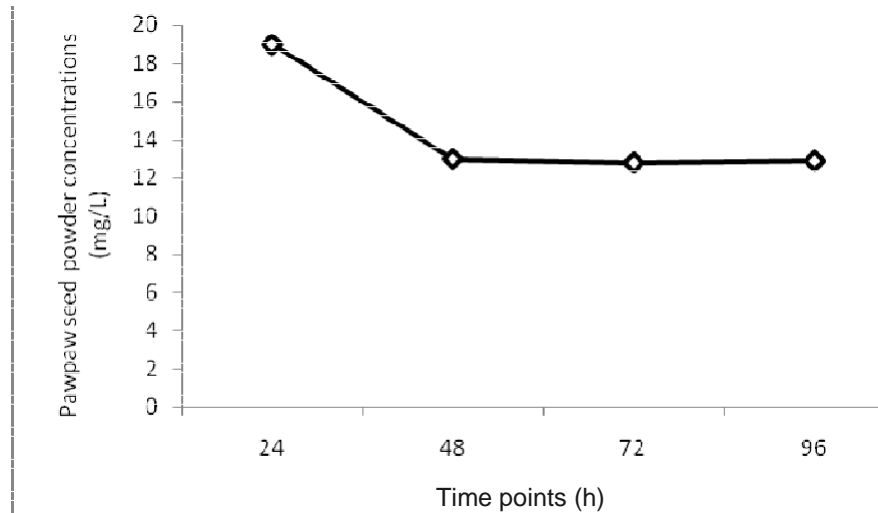


Figure 1. Median lethal concentration (LC₅₀) of pawpaw seed powder for fish fingerlings at different exposure periods.

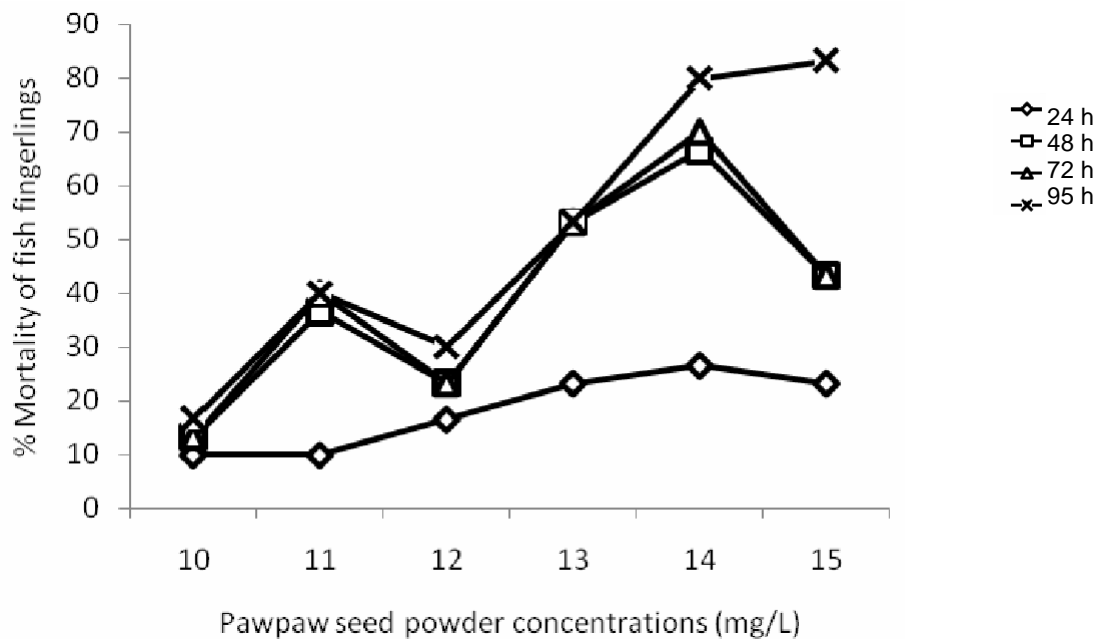


Figure 2. Relationship between percentage mortality of fish fingerlings and pawpaw seed powder concentration for exposures lasting 96 h.

authors (Muniyan and Veeraraghavan, 1999; Santhakumar and Balaji, 2000; Ayuba and Ofojekwu, 2002; Chung-Min 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 LC₅₀ value and the greater the toxicity. Results of this study showed similar pattern having lesser 96 h-LC₅₀ than 48 h-LC₅₀ and so on, with ratio of 24 h-LC₅₀ to 48 h-

LC₅₀ as 1.48 which showed the delayed acute toxic response. Kim et al. (2002) also reported comparable acute toxicity ratio (24 h-LC₅₀ to 48 h-LC₅₀) of 1.2 for plant toxins.

Dose-response approach in estimating the lethal effects of toxicants on organisms have been criticized for lacking real ecological meaning (Newman, 1995; Pemak et al., 2003). Nonetheless, regulatory norms have been built around LC₅₀ values that can be compared across toxicants and organisms (LeBlanc, 1980). Thus LC₅₀

Table 3. Behavioral monitoring for fingerlings catfish (Range finding test).

Behavior/exposure time	12 h						16 h						20 h						24 h					
Concentrations	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25	0	5	10	15	20	25
Loss of reflex	-	-	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+
Molting	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	+	+	-	-	-	-	+	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Air gulping	-	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	+
Erratic swimming	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	+
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

- = not present, + = Present.

Table 4. Behavioral monitoring for fingerlings catfish (Definitive test).

Behavior/exposure time	24 h						48 h						72 h						96 h					
Concentrations	10	11	12	13	14	15	10	11	12	13	14	15	10	11	12	13	14	15	10	11	12	13	14	15
Loss of reflex	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Molting	-	-	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Discoloration	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Air gulping	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Erratic swimming	-	-	+	+	+	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-	+	+
Haemorrhage	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

= not present, + = present.

Table 5. The summary of the effects of *Carica papaya* seed powder on haematological parameters of fingerlings catfish *Clarias gariepinus*.

Conc./parameters (mg/L)	Hb (g/dl)	PCV (%)	ESR (%)	WBC (10^4 mm^3)	RBC (10^6 mm^3)	MCH (pg)	MCHC (T/L)	MCV (μ^3)
T ₀ (0)	4.33 ± 0.58	16.1 ± 1.68	7.57 ± 1.21	9.93 ± 0.1	2.90 ± 3.16	1.5 ± 0.2	0.003 ± 0.0	0554.0 ± 465.8
T ₁ (10)	4.33 ± 0.58	13.8 ± 2.10	5.50 ± 1.87	2.91 ± 5.01	7.93 ± 4.04	0.57 ± 0.11	0.003 ± 0.0	5540 ± 356.82
T ₂ (11)	5.0 ± 1.93	16.47 ± 7.11	6.30 ± 2.95	7.00 ± 0.23	7.63 ± 5.57	0.63 ± 0.25	0.003.1 ± 0.0	2123 ± 917.60
T ₃ (12)	5.0 ± 0.20	16.00 ± 0.12	5.50 ± 1.32	3.36 ± 4.39	8.23 ± 8.32	0.63 ± 0.58	0.003 ± 0.0	1954.3 ± 206.78
T ₄ (13)	5.53 ± 1.86	17.13 ± 5.97	5.27 ± 1.70	4.31 ± 3.39	5.87 ± 4.24	0.37 ± 0.23	0.0003 ± 0.0	2060.0 ± 858.0
T ₅ (14)	5.07 ± 1.01	16.13 ± 3.32	6.07 ± 2.10	3.85 ± 3.41	8.10 ± 1.15	0.63 ± 0.21	0.003 ± 0.0	6314.14 ± 365.60
T ₆ (15)	5.40 ± 0.69	16.4 ± 2.06	4.27 ± 0.25	6.63 ± 0.17	3.07 ± 5.31	0.67 ± 0.51	0.0003 ± 0.0	2090 ± 409.21

Table 6. Summary result of physico-chemical parameter during the test.

Parameter	Range finding test			Definitive test		
	Before test	During test	After test	Before test	During test	After test
Temp. (°C)	25.5	26.5	27	26.2	24.5	25.4
DO ² (mg/L)	5.8	6.8	5.5	6.7	5.8	6.9
pH	6.2	7.9	6.8	6.3	6.1	7.0

values from dose-response bioassays have necessarily become starting points for ecologically relevant studies of toxicant effects on animal populations (Schindler, 1987).

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 pyrethroid insecticide, tetramethrin, on hematological and biochemical parameters in albino rats. Histopathological and biochemical alterations by plant toxins have been observed in *O. niloticus* (Zapata et al., 2000; Ayotunde and Offem, 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). The maximum admissible toxicant concentration of 0.129 - 1.29 mg/L with 95% confidence limit of 22.24 - 78.82 mg/L, established for catfish fingerlings was derived by multiplying a constant 0.01 - 0.1 by 96 h LC₅₀ (Koesomadinata, 2000).

Conclusion

The toxicity effect of pawpaw seed powder had a positive correlation with exposure time from 24 to 96 h, for the sharptooth catfish *C. gariepinus*. From the toxicity tests pawpaw seed powder concentration as low as 1.29 mgL⁻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.

ACKNOWLEDGEMENTS

Special thanks go to Department of Fisheries, Cross River University of Technology Calabar, Obubra campus for making facilities available for this research. Sincere thanks also go to the research assistant and Technologist Mr. Erinle and Mr. Adesida, respectively, for analysis of samples.

REFERENCES

- Albaiges J, Faran A, Soler M, Galiffa A, Marten P (1987). Accumulation and distribution of biogenic and pollutant hydrocarbon, PCB and DDT in tissues of Western Mediterranean fishes, *Marine Environ. Res.*, 22: 1-18.
- Akah PA, Oli AN, Enwerem NM, Gamaniel K (1997). Preliminary studies on purgative effect of *Carica papaya* root extract. *Fitoterapia*, 68 (4): 327-331.
- Ayotunde EO, Ofem BO (2005). Acute and Chronic Toxicity of Pawpaw (*Carica papaya*) Seed Powder to Nile Tilapia *Oreochromis niloticus* (Linne 1757), Fingerlings. *J. Agric. Technol. Environ.* 1: 1- 4.
- Ayotunde EO, Ofem BO (2008). Acute and Chronic Toxicity of Pawpaw (*Carica papaya*) Seed Powder to Nile Tilapia *Oreochromis niloticus* (Linne 1757), Adult. *Afr. J. Biotechnol.* 7 (13): 2265-2274.
- Ayuba VO, Ofojekwu PC (2002). Acute toxicity of the Jimson's weed (*Datura innoxia*) to the African catfish (*Clarias gariepinus*) Fingerlings *J. Aquatic Sci.*, 17: 2-12.
- Barata C, Baird DJ, Markich SJ (1998). Influence of genetic and environmental factors on the tolerance of *Daphnia magna* Straus to essential and non essential metals. *Aquatic Toxicology* 42: 115-136.
- Bianchini A, Grosell M, Gregory SM, Wood CH (2002). Acute silver toxicity in aquat. animals is a function of sodium uptake rate. *Environmental Science Technology*, 36:1763-1776.
- Bossuyt BAT, Janssen C (2005). Copper toxicity to different field-collected Cladoceran species: intra- and inter-specific sensitivity. *Environmental Pollution*, 136: 145-154.
- Canton JH, Adema DMM (1978). Reproducibility of short-term and reproduction toxicity experiments with *D. magna* and comparison of the sensitivity of *D. magna*, *D. pulex* and *D. cucullata* in short-term experiment. *Hydrobiologia*. 59:135-140.
- Chung-Min L, Bo-Ching C, Sher S, Ming-Chaalin, Chen-Wuing L, Bor-Cheng H (2003). Acute toxicity and bioaccumulation of arsenic in tilapia (*Oreochromis mossambicus*) from a blackfoot disease area of Taiwan. *Environmental Toxicology*. 18 (4): 252-259.
- Cowgill UM, Millazzo DP (1991). The sensitivity of *Ceriodaphnia dubia* and *Daphnia magna* to seven chemicals utilizing the three brood test. *Archives Environmental Contamination and Toxicology*, 20: 211-213.
- Dutta HM (1996). A composite approach for evaluation of the effects of pesticides on fish. In: Munshi, J.S.D., Dutta, H.M. (Eds.) *Fish morphology*. pp. 23-56. Science Publishers.
- Easley MF, Ross WG (Jr), Alan W, Klimek PE (2001). *Aquatic Toxicity Testing: Understanding and Implementing Your Testing Requirement*. Prepared by the Aquatic Toxicology Unit/Environmental Sciences Section Water Quality Section N.C. Division of Water Quality. <http://www.esb.enr.state.nc.us/ATUwww/Default.html>.
- Eno AE, Owo OI, Itam EH, Konya RS (2000). Blood pressure depression by the fruit juice of *Carica papaya* (L.) in renal and DOCA-induced hypertension in the rat. *Phytotherapy Res.*, 14: 235-239.
- Finney DJ (1971). *Statistical method in biological assay*, Hafner Pub. Co. New York. N.Y. p. 68.
- Goyer RA, Clarkson TW (2001). Toxic effects of heavy metals (In: Klaassen, C.D. (eds) *Casarett Doull's Toxicology*, (pp. 811-896) McGraw- Hill, NewYork).
- Guilhermino L, Diamantino T, Sylva MC, Soares AMVM (2000). Acute toxicity test with *Daphnia magna*: An alternative to mammals in the prescreening of chemical toxicity testing? *Ecotoxicology and Environmental Safety*, 46:357-359.

- Heijerick DG, Janssen CR, De Coen WM (2003). The combined effect of pH, hardness and dissolved organic carbon on the chronic toxicity of zinc to *D. magna*: Development of the surface response model. Archives Environmental Contamination and Toxicology, 44:210-300.
- Herwig N (1979). Handbook of drug and chemical used in the treatment of fish disease: A manual of fish farm and material Media. Charles Thomas Pub. Springfield Illinois U.S.A.
- ItaEO (1980). A Review of Recent Advance in Warm Water Aquaculture Resource and A Proposed Experiment Design for Maximising Fish Production in Nigeria. K.L.R.I. Rep Series, 51: 30.
- Kaniewska-Prus M (1982). The effect of ammonia, chlorine, chloramines toxicity on the mortality of *Daphnia magna* Straus. Hydrobiologia. 29:608-609.
- Koesomadinata S (2000). Acute toxicity of the insecticide formulation of endosulphan, chlorpyrifos, and chlorfluazuron to three freshwater fish species and freshwater giant prawn. J. Penelitian Perikan Indonesia. 4(3-4): 36-43.
- Koivisto S (1995). Is *Daphnia magna* an ecological representative zooplankton species toxicity test? Environ. Pollut., 90:263-289.
- LeBlanc GA (1980). Acute toxicity of priority pollutants to water fleas (*Daphnia magna*). Bull. Environ. Contam. Toxicol., 24: 684-689.
- Lohiya NK, Manivannan B, Mishra PK, Pathak N, Sriram S, Bhande SS, Panneerdoss S (2002). Chloroform extract of *Carica papaya* seeds induces long-term reversible azoospermia in langur monkey. Asian J. Androl., 4: 17-26
- Lohiya NK, Mishra PK, Pathak N, Manivannan B, Jain SC (1999). Reversible zoospermia by oral administration of the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* in rabbits. Adv. Contracept., 15: 141-161.
- Mount DI, Norberg TJ (1984). A seven day life-cycle cladoceran toxicity test. Environ. Toxicol. Chem., 3: 425-434.
- Muniyan M, Veeraraghavan K (1999). Acute toxicity of ethofenprox to the fresh water fish *Oreochromis mossambicus* (PETERS). J. Environ. Biol., 20: 153-155.
- Newman C (1995). Quantitative methods in aquatic toxicology. Lewis publishers, Florida. pp. 56-78.
- OECD (2001). Organisation of Economic Cooperation and Development. Guidance document on Aquatic Toxicity Testing of Difficult substances and mixtures, pp. 34-57.
- Oh HS, Lee SK, Kim YH, Rah JK (1991). Mechanism of selective toxicity of diazinon to killifish *Oryzias laticeps* and loach (*Misgurnus anguillicaudatus*). Aquat. Toxicol., 14:343-354.
- Oladimeji AA, Offem BO (1989). Toxicity of Lead to *Clarias lazra*, *Oreochromis niloticus*, *Chironomis tentans* and *Benacus* spp. Air, Water. Soil Pollution, 44:191-195.
- Olufemi BA, Adedeji AO, Adeyemo AK, Agbede SA (2008). Acute toxicity of diazinon to African catfish (*Clarias gariepinus*). Afr. J. Biotechnol. 7: 651-664.
- Pemak J, Sobaszekiewicz K, Misca I (2003). Antimicrobial activities of ionic liquids. Green Chem., 5:52-55.
- Pathak N, Mishra PK, Manivannan B, Lohiya NK (2000). Sterility due to inhibition of sperm motility by oral administration of benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* in rats. Phytomedicine, 7: 325-333.
- Reed W, Burchard J, Jonathan T, Ibrahim Y (1967). Fish and fisheries of Northern Nigeria. Min. of Agric. Northern Nigeria.
- Santhakumar M, Balaji M (2000). Acute toxicity of an organophosphorus insecticide monochrotophos and its effects on behaviour of an air breathing fish, *Anabas testudineus* (BLOCH). J. Environ. Biol., 21: 21-123.
- Saleh AT, Sakr SA, Al-Sahhaf ZY, Bahareth OM (1988). Toxicity of pyrethroid insecticide "Tetramethrin" in Albino Rat: Haematological and biochemical effect. J. Egyptian Soc. Zool., 25: 35-52.
- Schindler DW (1987). Detecting ecosystem responses to anthropogenic stress. Canadian J. Fisheries Aquat. Sci., 44: 6-17.
- Svobodova D, Ravds J, Palackova W (1991). Unified method of Haematological examination of fish. Research Inst. of Fish Culture and Hydrobiology. Vonnony Czechoslovakia.
- Tong Z, Huailin Z, Hongjun J (1996). Chronic toxicity of acrylonitrile and acetonitrile to *Daphnia magna* in 14-d and 21-d toxicity tests. Bull. Environ. Contam. Toxicol., 67:655-658.
- USEPA (2000). Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. 4th ed. Environmental Monitoring and support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio. EPA 600/4-85/013.
- Wade J W, Omoregie E, Ezenwaka (2002). Toxicity of cassava (*Manihot esculenta* CRANTS) effluent on the Nile Catfish *Clarias gariepinus* (l) under laboratory condition. J. Aquat. Sci., 17: 2-34.
- Wilson RK, Kwan TK, Kwan CY, Sorger GJ (2002). Effects of papaya seed extract and benzyl isothiocyanate on vascular contraction. Life Sci., 71: 497-507.
- Zapata - Perez O, Sima-Alvarez R, Norena -Baroso E, Guemes J (2000). Toxicity of sediments from Bahia de Chetumal, Mexico as assessed by hepatic EROD induction and histology in the Nile tilapia *Oreochromis niloticus*. Marine Environ. Res., 50: 345-356.