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Acute toxicity of *mangifera indica* (mango seeds) extract on some biochemical, haematology and histopathology parmeters of *clarias gariepinus* juveniles

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Abstract
The acute toxicity of *Mangifera indica* seed extract was conducted on *Clarias gariepinus* juveniles under a static bioassay for 96 hours. Ten (10) juveniles of *C. gariepinus* were stocked in each of the six rectangular plastic tanks, each with a replicate. The fish were allowed to acclimatize for one (1) week. The water quality parameters, behavioural changes, haematology, biochemistry and histopathology of the test fish were studied on the concentrations of *M. indica* seeds extract at 4.00, 2.00, 1.00 and 0.5 g/L while 0.00 served as the control. The results of water quality parameters revealed that pH range between 5.86 and 6.79, free carbon dioxide ranged between 2.04 and 4.50 ppm, alkalinity ranged from 5.41-6.20. The biochemical parameters showed a significant difference ($P< 0.05$) as compared to the control. Also, the histopathology showed a clear variation in their appearance when compared with the control. All these alterations could be induced by the efficacy of the plant extract (*M. indica*). The 96 hours LC_{50} of *M. indica* extract on the juveniles of *C. gariepinus* was 0.7079 g/L with the upper and lower confidence limits of 0.5424 and 0.9239 respectively. This finding clearly suggests that the plant extract has deleterious effects on aquatic fauna especially fish. Therefore, concerted effort should be made to prevent the plant material (*M. indica)* seeds from reaching water bodies.

Keywords: *Mangifera indica*, suggests, concerted, material

1. Introduction
ish farming has taken a center stage in global discourse with aquaculture becoming one of the fast-growing industries as a consequence of upsurge in demand for fish and fish by-products. Fish plays a vital role, not only in human diets but also in animal and poultry rations. It is a palatable and easily digested food which is rich in vitamins, calcium, phosphorus and iodine (Mona, Shalaby, Nagwa & Abedelzaher, 2013) [74]. The flesh of healthy fish is considered as a biomarker for natural and good environment.

Aquatic pollution is still a problem in many fresh-water and marine environments. It is toxic to aquatic life (Fent, 2007) [53]. Mona *et al*. (2013) [74] reported that pollutants may affect immune system of fish either directly or by causing changes in water quality, which in turn may reduce the fish immunity. Also, water pollution may accelerate the life cycle of the external parasites and promote their spread (El-Saify *et al*., 2011) [49].

Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of the pollutants entering the water or the lethal effects on the aquatic organisms (Fegbenro, 2002).

Omeregie, Okunsebor & Onusiruika (2001) [88] reported that fish are extremely sensitive to aquatic pollution. In fresh water polluted conditions, each additional level of pollutants significantly affect the cost of production as a result of pollution induced morbidity (Akueshi *et al*., 2003) [9].

As a result of the ever-increasing growth of world population, man's activities resulting from industrialization is bound to cause more pollutants being passed into water bodies and this has deleterious effects on the aquatic environment and their biota (Omoregie & Ufodike, 2000) [87].

2. Materials and Methods
2.1 Materials
Mango seeds (*Mangifera indica Sp.*) were obtained from Sabon- Barki, Bukuru in Jos South Local Government Area of Plateau State, Nigeria. The seeds were peeled, sun dried for a week to a constant weight outside the postgraduate Hydrobiology Laboratory at the Department of Zoology, University of Jos, Jos, Nigeria. The dried seeds were pounded with mortar and pestle into powder. A sieve of 30 µm mesh size was used to obtain fine particles desired to enhance dissolution in water.

A total of 150 juveniles of *Clarias gariepinus* of mixed sex of the same brood stock (with an average weight of 32 g and an average length of 22 ± 2.0 cm) were obtain from CATFISH experts Global Ventures, beside NNPC filling station Zarmaganda, Jos, Plateau State, Nigeria and transported in oxygenated polythene bags to the laboratory and then transferred into plastic tanks of 20 liters capacity filled with well water and acclimatized for a period of fourteen (14) days. After acclimatization, they were transfer into rectangular experimental plastic tanks at ten (10) fish per ten liters tanks and fed twice daily with pelleted commercial feed while water was changed daily. 120

juveniles were used for the experiment.

2.2. Methods

Phytochemical Screening of *Mangifera indica Sp*

The plant seed extract was used for the phytochemical test for the following: Alkaloids, flavonoid, saponin, steroids, and terpenes, cardiac glycoside, carbohydrate, resin balsam, phenol and tannins using standard qualitative procedures. The procedures are those reported by Trease & Evans, 1984; Sofowora, 1982.

Tanks of twenty (20) liter capacity were maintained throughout the exposure period. Ten juveniles of mixed sex with an average weight of 32 g and an average length of 22 ± 2.0 cm were placed in each of the six (6) ten liters test tanks and that of the replicate as well during both acclimatization and the exposure periods. The fish were fed twice daily with pelleted commercial feed. After the acclimatization of fourteen (14) days, range finding test was carried out to determine the definit concentrations to be used for the acute evaluation test. This is in agreement with Rand, (2008) who reported that range finding test is conducted to estimated LC_{50} of chemical to which the organisms are exposed. The following concentrations of the toxicant were used in double replication: 4.00, 2.00, 1.00, 0.50, 0.25 and 0.00 g/L. The fish starved throughout the 96 hours toxicity test. In order to monitor the toxicant strength, level of dissolved oxygen (DO), free carbon (IV) oxide (CO_2), alkalinity, temperature, and pH. The water quality parameters were monitored using the method described by APHA, AWWA & WPCF, 1980. The research lasted for 96 hours (4 days) with a photoperiod of 12 hours darkness and 12 hours of light.

The behavioural responses, mortality rate and biochemical examination of the experimental fish were also observed and recorded with the aid of appropriate techniques. Furthermore, the mortality rate was transformed into percentage probit kill using a probit table. The probit mortality was plotted against log concentration in g/L. A linear line equation was used to fix the regression line between the points plotted on the graph paper. The 96 hrs LC_{50} was determined on the graph of which the lower and upper confidence limits were calculated using the method of Litchfield & Wilcoxon (1948) [70].

Collection of blood

The Fish from each of the test tank was sacrificed for collection of blood via the cardiac puncture with heparinized disposable sterile syringe of 2 mL and were emptied into 10 mL heparinized test tube immediately before analysis. The blood was examined for the following, Packed cell volume (PCV), White blood cells (WBC), Red blood cells (RBC), Haemoglobin (Hb) concentration, Mean cell hemoglobin (MCV), Mean cell volume (MCV), Mean cell hemoglobin concentration (MCHC), Neutrophil (N) and Lymphocyte (L) using Sysmex machine.

Packed Cell Volume (PVC), Haemoglobin Concentration (Hb), White Blood Cell (TWBC), Red Blood Cell (RBC) the procedure adopted is that described by Blaxhall and Daisley (1973) [29].

Biochemical Examination

Alkaline phosphatase

The Randox product AP542/AP307 enzyme kit manual of alkaline phosphatase was used in the study. According to the manual (Alkaline phosphatase manual Rx Monza) fresh distilled water was first used to perform a new gain calibration and a water blank were carried out. 0.1 mL of the sample and 0.5 mL of the reagent were pipetted into the test tube, mixed and transfered into a cuvette (1 cm path lenght) and in turn inserted into a photo spectrometer which determined the Alkaline phosphatase activity at 405 nm/min using – Aldrich Z37602-IEA (Spectronic Genesys spectrophotometer, USA) by reading the initial absorbance and the starting timer simultaneously. The values obtained were used to calculate ALP activity using the formula

$U/L = 2760 X \Delta A405nm/min.$

The data from the replicate calculated readings were averaged and the mean presented.

Histopathological Examination of *Clarias Gariepinus* Juveniles Exposed to Mango Seed Extract *Mangifera indica Sp.*

The fish (Live) was dissected and the gill and liver were carefully removed and then fixed in 10% formal saline solution. They were prepared for histological analysis using the routine histology methods and haemotoxylin-eosin staining techniques described by Drury & Wallington (1967) [46] and modified by Buck (1972). The automatic duplex processor, Standon and Southern (Model: C 35 H) was used to process the organs. Graded alcohol (70%, 90%, absolute I, II, III) and graded chloroform (I, II, III) were used in dehydrating and cleaning respectively and the infiltrated and embedded in molten paraffin wax. The Cambridge Rock Microtone (Model; M64) was used to section the organs to the desired thickness (0.2mm), and finally placed on a clear slide. During the staining procedure, the sections were de-waxed in xylene and hydrated in graded alcohol (95%, 80%, 70% and 50%) respectively. The sections were stained in haematoxylin for 5 minutes, differentiated in 1% acid alcohol (hydrochloric acid and 70% alcohol), washed with tap water and counter-stained in 5% aqueous eosin for 5 minutes and the section thoroughly washed and dehydrated in graded alcohol and chloroform and further de-paraffin in xylene. The section was finally mounted in Canada balsam and carefully covered with slide photo-microscopic camera.

2.2.1 Statistical Analysis

The results obtained were subjected to analyses of variance (ANOVA) single classification at 5% level of probability. This was used to test for significant difference between treatment means. The treatments were also subjected to standard deviation and standard error to determine the difference within treatment means. Regression analysis was used to find the line of best fit while the coefficient of regression was calculated to know the relationship between the corrected probit mortality and concentration and the confidence limit were determined to know the limit of the toxicant efficacy.

3. Result and Discussion

3.1 Result

Table 1: Phytochemical screening of *M. indica Sp*

Phytochemical	Quality	Color	Test
Alkaloids	++	Orange	Dragendof
Saponin	+++	Froth	Salkoki test
Flavonoid	+	Yellow	
Steroids and Terpens	+	Redish brown	
Resin	++	Violet	Burchard test
Phenol	+++	Blue-green	Ferric chloride
Cardiac glycoside	++	Brown	Keller-killani
Carbohydrate	+	Brick-red	Benedict
Balsam	+	Dark green	Ferric chloride

Key: +++ = High presence ++ = Moderate presence + = Weak presence

Table 2: Mean Values of Water Quality Parameters for Acute Bioassay of *C. gariepinus* juveniles Exposed to Mango seed extract *M. indica Sp*

	Concentrations (g/L)						
Parameters	**0.000**	**0.25**	**0.50**	**1.00**	**2.00**	**4.00**	**0.05 LSD**
Ph	6.79 (± 0.04)	6.32 (0.05)	6.46 (0.02)	6.39 (0.00)	6.35 (0.04)	5.86 (0.03)	0.08
FreeCO_2(ppm)	2.68 (±0.02)	2.43 (0.04)	3.74 (0.04)	4.57 (0.04)	2.84 (0.02)	2.04 (0.03)	0.08
Alkalinity	63.70 (±0.28)	59.01 (1.39)	69.08 (0.10)	72.42 (0.53)	79.00 (1.14)	90.00 (0.70)	2.19
Dissolved oxygen (O_2)g/L	3.56 (±0.05)	3.37 (0.04)	3.30 (0.07)	3.47 (0.04)	2.89 (0.09)	1.89 (0.06)	0.15
Temperature (ºC)	25.10 (±0.00)	25.01 (0.00)	25.30 (0.00)	25.20 (0.00)	25.04 (0.00)	25.50 (0.00)	NS

Footnote: NS= No Significant difference where P>0.05; Mean values in ± are Standard deviation.

Table 3: Mean mortality, percentage mortality and Probit mortality of *Clarias gariepinus* juveniles exposed to mango seeds extract of *M. indica Sp.* for 96 hours.

Conc. mg/L	Log concentration	Number Of fish Mean Mortalities/Hours								Total mortality	Mean% mortality	Probit mortality
				6	12	24	48	72	96			
4.00	0.6021		10	0	0	2	5.5	2.5	0	10	100	8.7190
2.00	0.3010		10	0	0	0	4.5	4.5	0	9	90	6.2816
1.00	0.000		10	0	0	0	1	4	2	7	70	5.5244
0.50	-0.3010		10	0	0	0	0	1	3	4	40	4.7467
0.25	-0.6021		10	0	0	0	0	0.5	1.5	2	20	4.1584
0.00	0.0000		10	0	0	0	0	0	0	0	0	0.0000

Table 4: Haematological Analysis of *Clarias gariepinus* Juveniles Exposed to Acute Concentrations of Mango Seed *Mangifera indica Sp.* for 96 hours

Conc. g/l	PCV (%)	WBC×10^9 /L	RBC×10^2g/L	MCV(fL)	MCH(pg)	MCHC(g/L)	HB(g/dL)	N (%)	L (%)
0.00	15.50(±6.30)	5.00(0.42)	3.40(0.99)	44.75(5.72)	15.00(1.85)	33.55(0.07)	5.20(2.12)	27.00(18.38)	73.00(18.38)
0.25	14.50(±0.71)	5.90(1.13)	2.75(0.212)	52.75(1.48)	17.65(0.64)	33.45(0.21)	4.85(0.21)	34.50(0.70)	65.50(0.71)
0.50	16.00(±1.41)	5.05 (0.78)	2.50(0.0)	64.00(5.65)	21.40(1.98)	33.40(0.14)	5.35(0.49)	29.00(4.24)	71.00(4.24)
1.00	13.50(±4.95)	6.60(0.99)	2.70(0.57)	49.15(7.99)	16.35(2.89)	33.25(0.35)	4.50(1.69)	26.506.36)	73.50(6.30)
2.00	20.00(±1.41)	6.500.42)	2.35(0.35)	86.55(19.02)	28.90(6.22)	25.03(0.07)	6.65(0.49)	38.00(8.48)	62.00(8.48)
4.00	17.0(±9.19)	4.75(1.34)	5.75(2.62)	38.00(33.23)	12.70(11.03)	33.45(0.21)	5.85(3.04)	27.00(2.83)	78.00(4.24)
NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Footnote: NS= No Significant difference where P>0.05; Mean values ± Standard Deviation

Table 5: Mean Biochemical Activities of the sera of *Clarias garcepinus* juveniles Exposed to Acute Concentrations of Mango Seed Extracts (*Mangifera indica Sp*)

Conc.g/l	ALP(µ/L)	AST(µ/L)	ALT(µ/L)	TP(g/L)	ALB(g/L)	LDH(µ/L)	Chol(mmol/L)	HDL(mmol/L)	LDL(mmol/L)
0.00	74.22 (±1.2)	228.82(1.42)	38.17(0.36)	79.79(5.77)	40.52(3.55)	3.59(1.44)	5.52(0.07)	2.42(0.25)	3.09(0.18)
0.25	246.02(±7.60)	269.60(0.70)	46.21(1.29)	89.32(11.32)	41.63(1.45)	12.72(0.59)	6.43(0.87)	2.41(0.33)	4.01(0.54)
0.50	26.38(±0.97)	147.76(42.76)	63.16(0.36)	54.00(4.72)	27.66(0.63)	5.00(0.49.)	4.71(0.66)	66.98(0.22)	2.74(0.43)
1.00	26.08(±2.15)	127.61(0.55)	65.09(0.40)	43.24(2.82)	15.17(0.45)	2.07(0.66)	3.98(0.02)	2.04(0.35)	1.95(0.37)
2.00	38.04(±3.58)	164.75(1.69)	50.34(1.08)	52.53(2.55)	24.71(4.18)	6.33(0.57)	4.61(0.87)	1.94(0.28)	2.68(0.59)
4.00	112.40(±6.93)	262.43(2.14)	63.97(0.04)	75.89(1.34)	32.56(3.6)	10.10(2.26)	6.08(0.82)	0.83(0.12)	5.25(0.94)
LSD(0.05)	10.07	38.51	1.62	12.71	6.07	2.62	NS	0.59	1.23

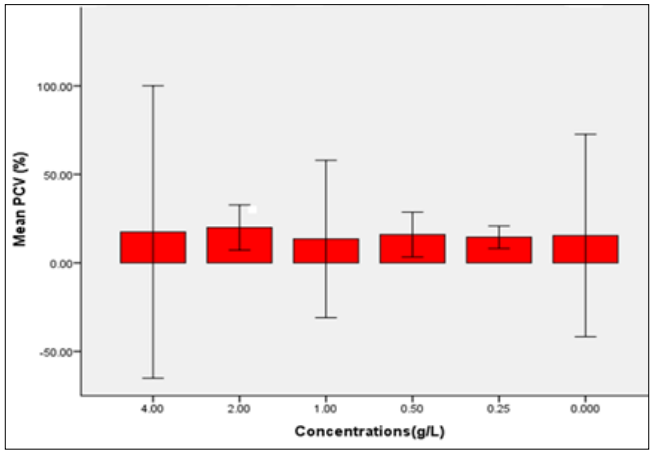

Fig 1: Parked Cell Volume (PCV) of Juveniles of *C. gariepinus* Exposed to Acute Concentrations of Mango Seed Extract *M. indica Sp.* for 96 hours LC_{50}.

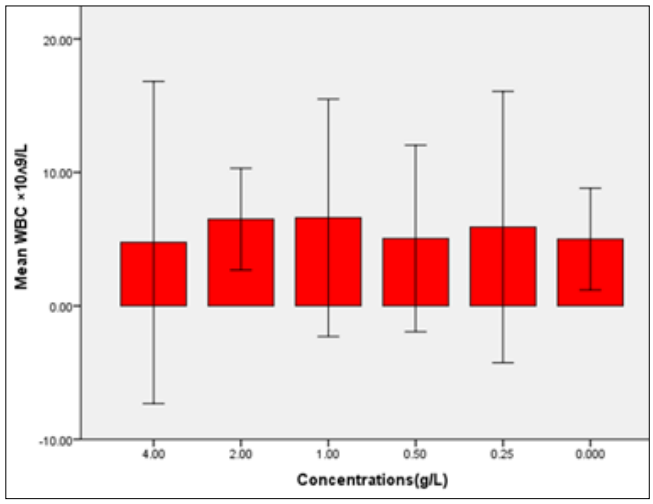

Fig 2: White Blood Cell (WBC) of *C. gariepinus* Exposed to Acute Concentrations of Mango Seed Extract *M. indica Sp.* for 96 hours LC_{50}.

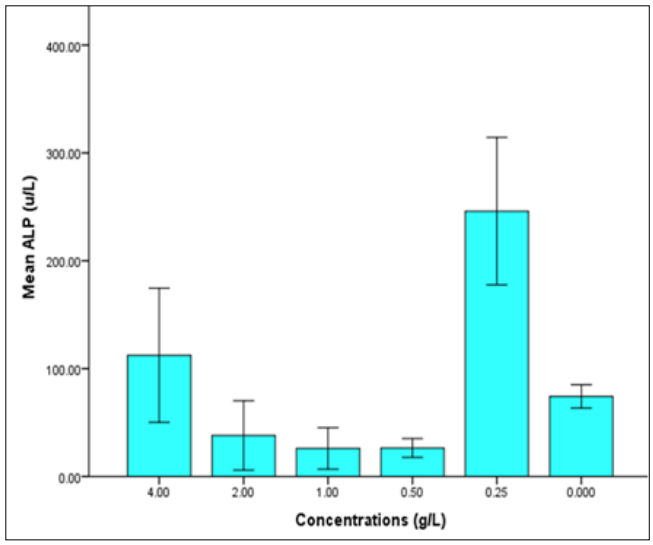

Fig 3: Alkaline Phosphatase (ALP) Activities of *C. gariepinus* Exposed to acute Concentrations of Mango Seed extract *M. indica Sp.*

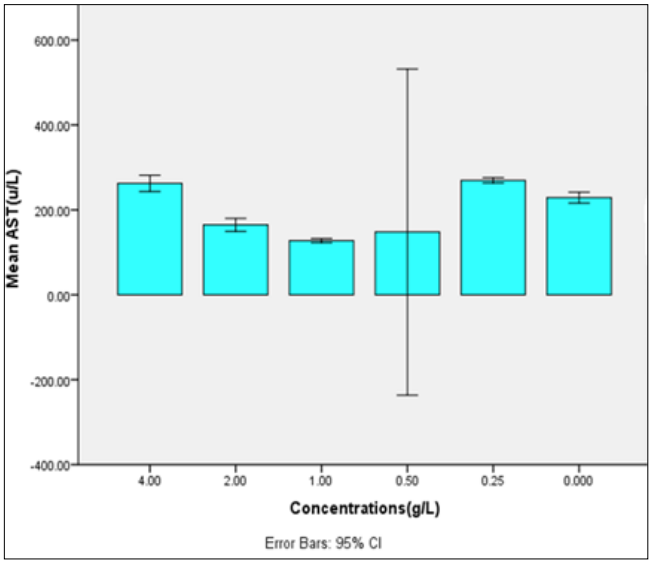

Fig 4: Aspartate aminotransferase Activities of *C. gariepinus* Exposed to acute Concentrations of Mango Seed extract *M. indica Sp.*

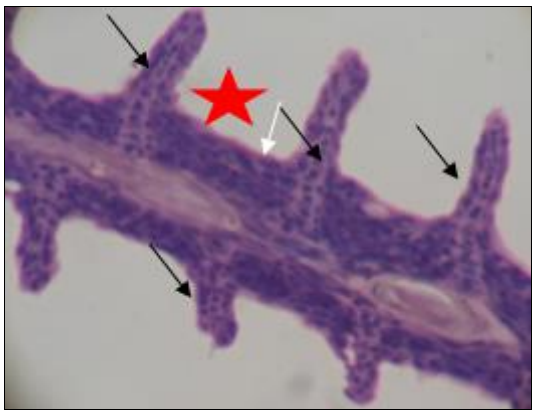

Fig 5A

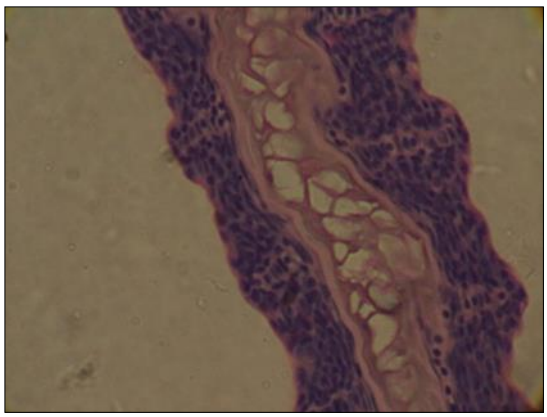

Fig 5B

3.2 Discussion

The phytochemical analysis of *M. indica Sp.*seeds revealed the presence of flavonoids and alkaloids, steroids and terpens, saponin, cardiac glycoside, resin, balsam, phenol and carbohydrate (table 1) as reported earlier by Oluwole & Bolarinwa (1995) [83], the plant extract contain several bio

agents that impaired physiology of the test organisms. As reported by Redmond (2007) [95], even small of alkaloid produce strong physiological effects in an animal because they all contain nitrogen atoms that are structurally related to those of ammonia. One of the alkaloids, nicotine is a potent insecticide. Alkaloids affect the nervous system by blocking adenosine receptors thereby slowing down nerve cell activity and stimulate cardiac activity and blood pressure (Francis, Lesaux, Kieffer & Rivera, 2006) [55]. This could have caused problems with blood circulation of the fish exposed to the crude seed extract of *M. indica Sp*. Phenols are acidic compounds that can stop all functions of living cells by altering or binding to proteins. Cardiac glycosides affect the central nervous system and nerve mechanism of the heart (Acevedo-Rodriguez, 1990) [4]. Considering the mode of action of these phytochemicals, it is evident that some of the behavioral patterns observed in the test fish were as a result of the effect of these substances on the test fish.

Throughout the exposure period, the physio-chemical parameters with the exception of temperature, all the others (Alkalinity, Dissolved, Free carbon (iv) oxide and pH) varied significantly ($P<0.05$) within the various test tanks. The result for alkalinity test showed some variation. This does not agree with the findings of Kunwun (2005) who reported that there was no significant difference between alkalinity of the treatments. The dissolved oxygen (DO) values decreases as the toxicant concentration increases. This report is in agreement with that of Mohammed (2005) [71] which says that dissolved oxygen showed inverse proportionality with the toxicant concentrations, this may be due to anti-oxidant property of the toxicant. Warren (1977) [121] observed that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration which will impair respiration leading to asphyxiation. The free carbon (iv) oxide shows that the treatment with 1.00g/L of mango seed *M. indica Sp.* had the highest value of 4.57g/L followed by those with 0.50g/L. The tank with the highest concentration (4.00g/L) had the least free carbon dioxide (3.74g/L). The statistical analysis showed that there is a strong significant difference ($P<0.05$). The pH values decrease with increase in toxicant concentration. This might be due to the acidic properties of the test material (*M. indica Sp*.). This opposes the report of Mohammed (2005) [71] who observed that the introduction of ammonia as a toxicant into water increases the water's pH. This is in agreement with the findings of Swilum (2006) [109] who reported that generally, sublethal concentrations of two pesticides in aquatic areas led to degradation of water quality and fish production in ponds.

The result of acute bioassay of mango seed extract of *M. indica Sp.* showed increased mortality of *Clarias gariepinus* with increase in concentrations of the test materials. At the highest concentration (4.00g/L), 100% mortality was recorded, 90% mortality was recorded at 2.00g/L. At 1.00g/L, 70% mortality was recorded. 40% mortality was recorded in the test tank exposed to 0.50g/L while only 20% was recorded at 0.25g/L. The test tank carrying the control (0.00g/L) experienced no mortality. This is a clear indication that the toxicity of *M. indica Sp.* increases with increase in concentration. This observation was similarly reported by Kunwun (2005) who showed that the mortality in higher concentration took place earlier than those of lower concentrations. This is further supported by Sprague (1973) [104] who said that increase in concentration of toxicant used for bioassays results in increase mortality rate. Often water has been considered quite adequate for fish as long as there has been no obvious mortality, which can be ascribed to known pollutants.

The 96 hours LC_{50} of *M. indica Sp*. extract was determine to be 0.7079g/L. This showed that the extract of *M. indica Sp*. is less toxic compared to juice of *Lermaireocereus laetus* which has 96 hours LC_{50} of 1.36mg/L (Kunwun, 2005) but is more toxic compared to inorganic fertilizer of the Nitrogen Phosphorus and Potassium (NPK) tested on *Clarias gariepinus* and was reported to have the 96 hours LC_{50} of 83.6mg/L (Ufodike & Onusiriuka, 1990) [113]. From the LC_{50} obtained, the extract of *M. indica Sp.* is toxic to the test fish and probably to other organisms. The washing of farm produce (especially toxic ones) in water bodies as a means of processing them could be deleterious to the ecosystem.

The test fish exhibited various kinds of behavioral patterns in the course of the 96 hours exposure period before death occurred. Increased in opercula ventilation and tail beat frequency were observed at the initial exposure of the fish to the toxicant which indicate hyperventilation during the first 24 hours of exposure, which thereafter dropped. The values however decreased below the status-quo at the 96 hours of exposure. Opercula ventilation has been reported to be an index of stress when fish is in an unfavorable environment (Sprague 1990). The results of the tail beat and opercula ventilation rate suggest that the exposed fish to *M. indica Sp.* seeds toxicant tend to exhibit avoidance syndrome as earlier observed by Ufodike (1990) [113]. The tail fin beat and opercula ventilation rates increased as the fish was trying to avoid the toxic area in the test medium. Since the whole medium was toxic to the fish, the attempt to escape made the fish became fatigue, hence subsequent drop of tail fin and opercula ventilation rates. These various signs indicate damages caused by the toxicant to the fish.

Conclusion

The results of the exposure of *Clarias gariepinus* juveniles to acute concentrations of mango seed extract *Mangifera indica Sp*. showed visible changes in behavioural pattern of the fish and led to mortality of most of the fish. The exposure also revealed significance changes in the haematological, biochemistry and histopathology variables. These parameters showed significant changes which is capable of altering the metabolic functions of the fish. This indicate sthat there is need for fish farmers to be cautions of seed of this plant that falls and decomposed into ponds, rivers water ways since this research work has been proven to be toxic to fish and other aquatic fauna.

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