TOXICITY OF AQUEOUS EXTRACTS OF DRUMSTICK, *Moringa oleifera*, SEEDS TO NILE TILAPIA, *Oreochromis niloticus*, FINGERLINGS AND ADULTS

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Abstract

The 96-h bioassay was conducted to determine the median lethal concentration (LC50) for Nile tilapia, *Oreochromis niloticus*, fingerlings and adults to aqueous extracts of drumstick, *Moringa oleifera* seeds. The 24-h, 48-h, 72-h and 96-h LC50 of *M. oleifera* applied to *O. niloticus* fingerlings were 252, 251, 242 and 242 mg/l, respectively; while those applied to *O. niloticus* adults were 351, 349, 334, and 332 mg/l, respectively. Toxic reaction exhibited by the fish includes erratic movement, air gulping, loss of reflex, discolouration, molting, loss of scale, and haemorhhage. Haematological examination of the fingerlings showed an increase in RBC, WBC, PCV, Hb, MCH and MCHC values, while there was a reduction in the ESR, MCH and MCV values; wheareas in the adults, the RBC, PCV, Hb and MCH values increased and there was a reduction in the ESR, WBC and MCV, while there was no change ($P > 0.05$) in MCHC values. Mortality increased with increase in concentration of *M. oleifera* and time of exposure in both *O. niloticus* fingerlings and adults.

Introduction

Moringa oleifera (Family: Moringaceae) is cultivated across the tropics and used for a variety of purposes (Jahn 1986). Its seed powder is a good water purifier; and contains polyelectrolytes, which constitute active ingredients in water treatment. Aqueous extract of mature seeds from trees and shrubs of Moringacae family are effective in clarifying turbid and waste water in tropical countries (Jahn *et al*., 1986), especially during rainy season. Muyibi and Evison (1995) noted that *M. oleifera* seeds have been used in the treatment of hard water, and proved that hardness removal efficiency of *M. oleifera* increased with increasing dosage. *Moringa* seed powder is a natural alternative to imported alum (aluminum sulphate, the conventional synthetic coagulant) used in purifying turbid water in fish culture enclosures (earthen ponds, farm dams and irrigation canals). It is obtainable locally at a fraction of the cost of alum in many countries, simple to use and cheap to maintain (Jahn, 1986; Ndabigengesere and Nasarasiah, 1998).

During rainy seasons, water in fish culture enclosures in Nigeria exhibit turbidity levels up to 400 NTU in which Nile tilapia, *Oreochromis niloticus*, are stocked being the most widely cultivated African freshwater fish. *Moringa* seed extracts dose of 75-250 mg/l is

used to clear water to turbidity <5 NTU. Recently, persistent and indiscriminate use of *Moringa* seeds as natural coagulant caused mass mortality of fish culture enclosure in Nigeria. Grabow *et al*. (1985) reported the toxic effect of *M. oleifera* seed powder to guppies (*Poecillia reticulata*), protozoa (*Tetrahymens pyriformis*) and bacteria (*Escherichia coli*). The toxicity of *M. oleifera* seed extracts to tropical fishes has not been examined. In this study, acute and chronic toxicity tests were conducted using aqueous extracts of *M. oleifera* seeds on *O. niloticus* fingerlings and adults in order to provide baseline information and establish limit of using aqueous extracts of *M. oleifera* seeds in freshwater fish ponds. This study also determined the safe limits and effects of aqueous extracts of *M. oleifera* on water quality.

Materials and methods

Large quantities of freshly mature seeds of *M. oleifera* were obtained and seed powder was prepared according to the method described by Price (2000). The seeds were sun-dried, the testa and wings were manually removed and the white kernel was ground to fine powder, using the coffee mill attachment of a Moulinex domestic food blender. The powder was kept in a dessicator for later use in stock solutions. Apparently healthy *O. niloticus* fingerlings (7.5 - 8.3 cm total length; 11.6 - 17.6 g) and adults (15.8 - 17.9 cm total length; 89.6 - 105.6g) were collected and acclimated for one week to laboratory conditions, inside rectangular glass tanks (75 x 45 x 45cm) of 121.5 litres capacity, filled with 50 litres dechlorinated water. The fish was fed with a 35% crude protein pelleted diet during the acclimation period. Feeding was discontinued 48 hours before the commencement of the experiment to minimize the production of waste in the test container.

Two 24 hour range finding test was conducted using *O. niloticus* fingerlings and adults following static bioassay procedures described by Parrish (1985). The fingerlings and adults were batch weighed and distributed into rectangular glass tanks (75 x 45 x 45 cm) each filled with 50 litres of dechlorinated water; and allowing one hour for acclimation to laboratory conditions. The water was filtered and aerated to saturation prior to use. A set of six stock solutions each of aqueous extracts of *M. oleifera* was prepared by dissolving 150, 200, 250, 300, or 350 mg/l with a control of 0 mg/l in water and used for *O. niloticus* fingerlings. Another set of six stock solutions each of aqueous extracts of *M. oleifera* was prepared by dissolving 250, 300, 350, 400 or 450 mg/l with a control of 0 mg/l in distilled water for adult *O. niloticus*. Each of the stock solutions was introduced directly into the tanks in a single dose, in triplicate treatments. The behavior and mortality of the test fishes in each tank was monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours and every four hours for the rest of the 24 hour 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 Parrish (1985). Batches of ten *O. niloticus* fingerlings and adults were distributed into rectangular glass tanks each filled with 50 litres of dechlorinated water, the water was filtered and aerated to saturation prior to use. A set of six stock solutions of aqueous extracts of *M. oleifera* was prepared by dissolving 200, 210, 220, 230, 240 or 250 mg/l, earlier determined

in the range finding test for *O. niloticus* fingerlings. Another set of six stock solutions of aqueous extracts of *M. oleifera* was prepared and by dissolving 300, 310, 320, 330, 340 or 350mg/l, earlier determined in the range finding test for adult *O. niloticus*. Each of the stock solutions was introduced in a single dose directly into the glass tanks. Continuous aeration was provided to prevent hyperconcentration in certain areas of the tanks and to maintain dissolved oxygen (DO) near saturation throughout the test. The tilapia fingerlings and adults were unfed throughout the 96 hour tests.

The behaviour and mortality of the fingerlings in each tank were monitored and recorded every hour for the first four hours; once every four hours for the rest of the 24 hours; and once every 24 hours until 96 hours. Dead fish was removed immediately with a scoop net to avoid contamination due to rotting. Temperature was determined using mercury-in-glass thermometer, dissolved oxygen (DO) concentration was determined using a digital DO meter (Jenway 1971), pH was determined using a digital pH meter (Mettler Toledo 320). Blood collection and standard haematological methods described by Svobodova *et al*., (1991) were used to estimate the blood parameters.

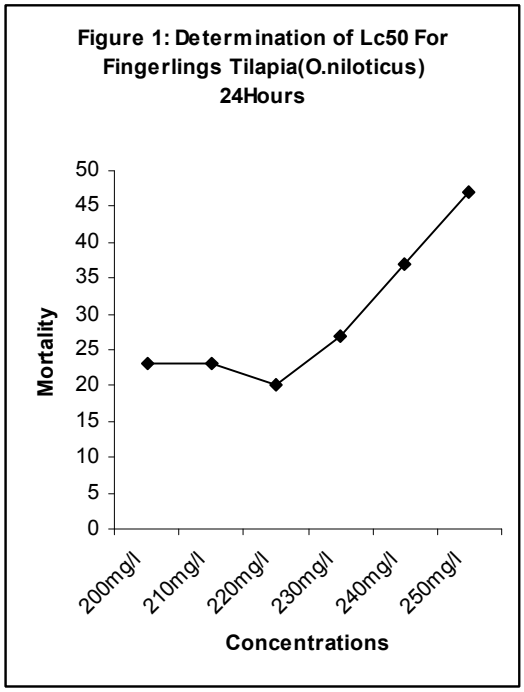
All results were collated and analysed using probit and logit analysis. The median lethal concentration LC_{50} at selected period of exposure, and an associated 95% confidence interval for each replicate toxicity test were subjected to logit and probit analysis (Finney 1971) using SPSS 11.0 for Windows XP on PC.

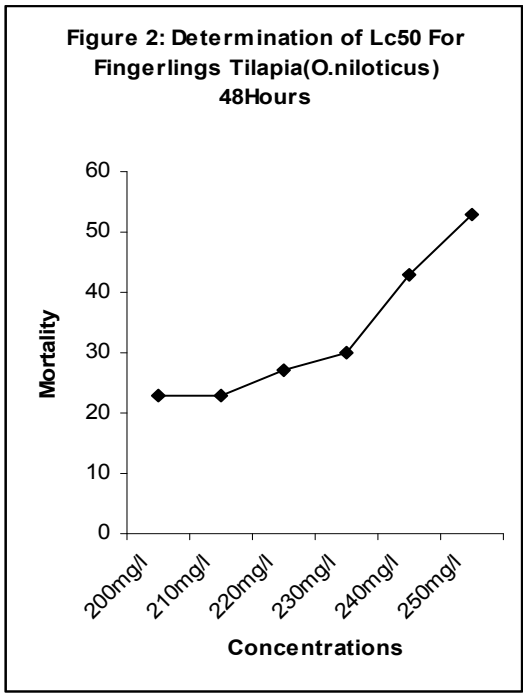
Results and discussion

The 96-h LC_{50} of an aqueous *M. oleifera* to *O. niloticus* fingerlings and adults are presented in Table 1 and Figures 1-8. Acute toxicity of *M. oleifera* decreased with time for both fingerlings and adult *O. niloticus*. Total mortality resulted at concentration of 350 mg/l of *M. oleifera* to *O. niloticus* fingerlings tilapia while total mortality resulted at 450 mg/l to adult *O. niloticus*. The maximum admissible toxicant concentration of 2.42-24.2 mg/l and 3.32-33.2 mg/l established for *O. niloticus* fingerlings and adults, respectively, was derived by multiplied a constant 0.01 - 0.1 by 96-h LC_{50} (Koesomadinata, 2000). *O. niloticus* fingerlings swam erratically; they exhibited loss of reflex, moulting, discouloration, air gulping, and loss of scales. Increases of loss of scales and haemorrhage were directly proportional to increase *M. oleifera* concentration and duration of exposure. At higher concentrations (340 – 350 mg/l) adult *O. niloticus* showed, erratic swimming, air gulping, loss of reflex, loss of scale, haemorrhage and molting. They finally settled at the bottom motionless with slow opercular movement. Lower concentrations of *M. oleifera* did not produce any serious change in the fish behaviour.

Table 1. The LC_{50} value for *O. niloticus* fingerlings and adults.

Time (hours)	LC50 (mg/l)	
	O. niloticus fingerlings	Adult *O. niloticus*
24	252	351
48	251	349
72	242	334
96	242	332





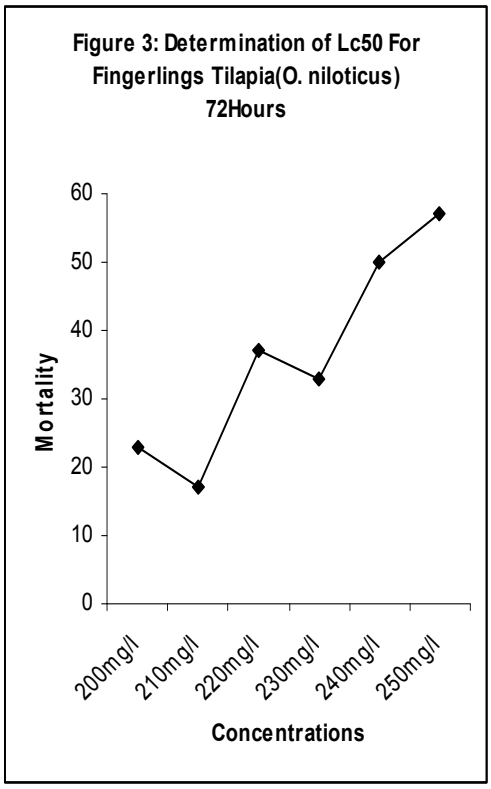


Figure 3: Determination of Lc50 For Fingerlings Tilapia(O. niloticus) 72Hours
Mortality
60
50
40
30
20
10
0
200mg/l
210mg/l
220mg/l
230mg/l
240mg/l
250mg/l
Concentrations

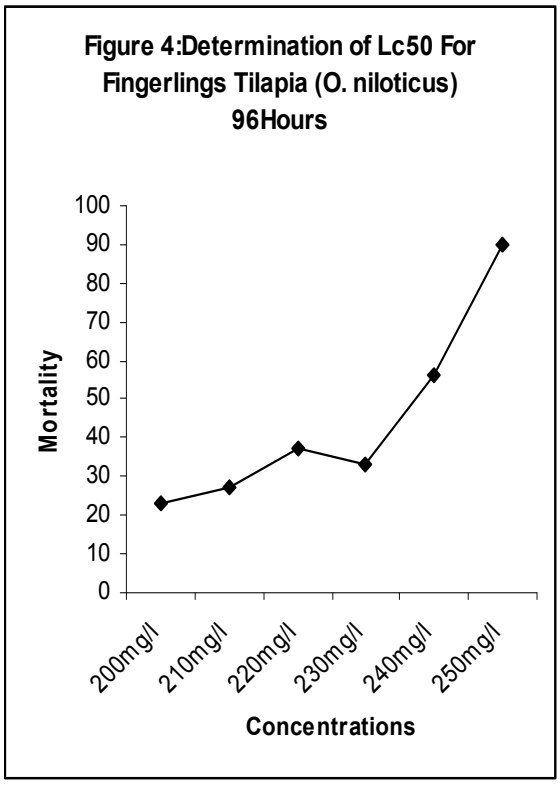


Figure 4:Determination of Lc50 For Fingerlings Tilapia (O. niloticus) 96Hours
Mortality
100
90
80
70
60
50
40
30
20
10
0
200mg/l
210mg/l
220mg/l
230mg/l
240mg/l
250mg/l
Concentrations

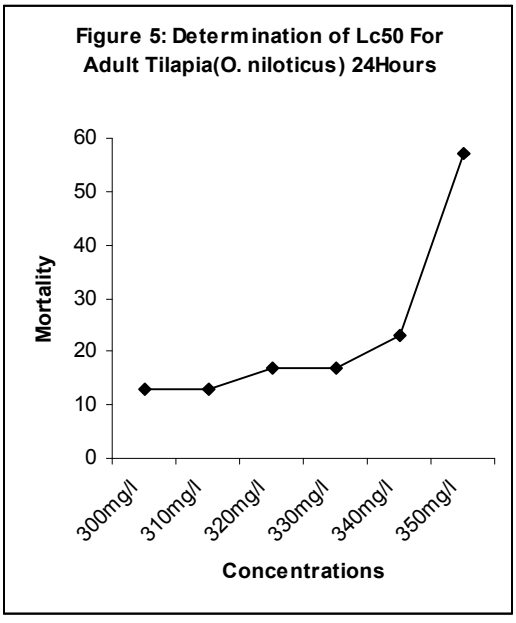


Figure 5: Determination of Lc50 For Adult Tilapia(O. niloticus) 24Hours
Mortality
60
50
40
30
20
10
0
300mg/l
310mg/l
320mg/l
330mg/l
340mg/l
350mg/l
Concentrations

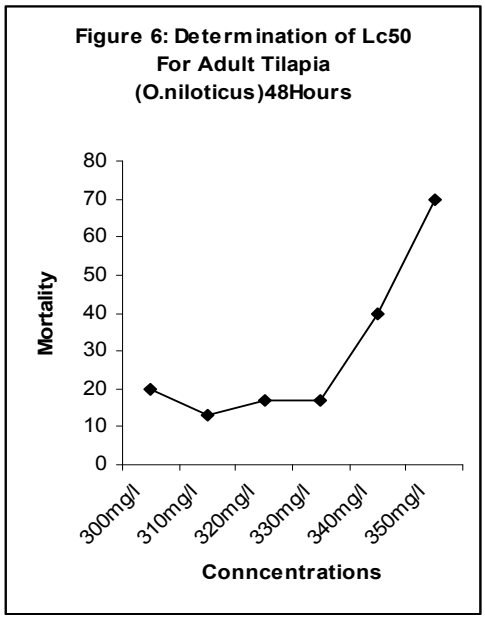
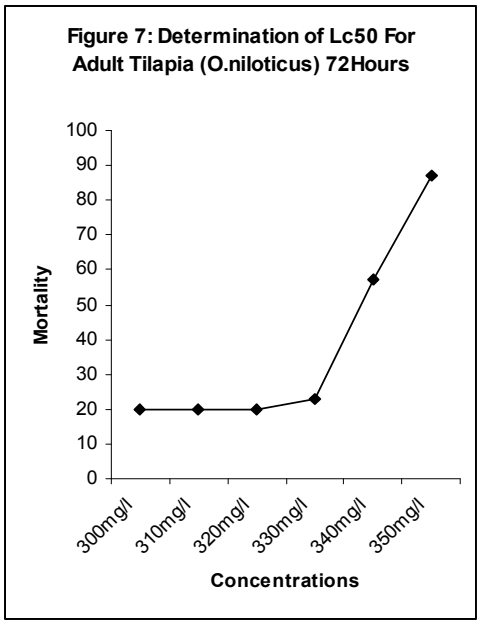
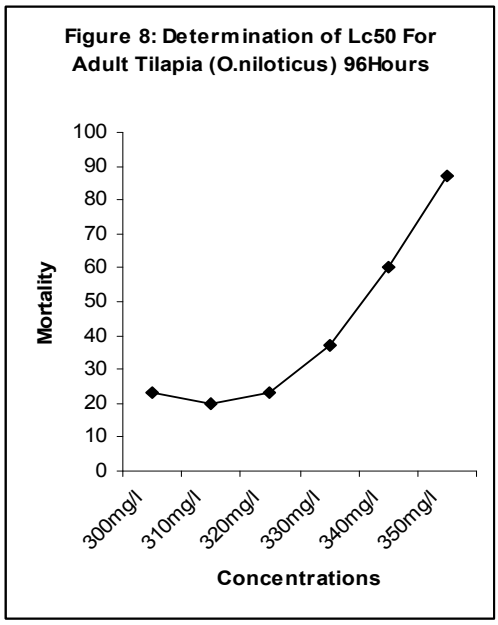


Figure 6: Determination of Lc50 For Adult Tilapia (O.niloticus)48Hours
Mortality
80
70
60
50
40
30
20
10
0
300mg/l
310mg/l
320mg/l
330mg/l
340mg/l
350mg/l
Conncentrations





The effects of *M. oleifera* on blood parameters of *O. niloticus* fingerlings and adults are presented in Table 2. Haematological examination showed an increase in red blood cell (RBC) count, white blood cell count (WBC), packed cell volume (PCV), haemoglobin concentration (Hb) and mean cell haemoglobin concentration (MCHC), while there was reduction in erythrocyte sedimentation rate (ESR), mean cell haemoglobin (MCH) and mean cell volume (MCV) values for the fingerlings while in adult *O. niloticus*, increase in RBC, PCV, Hb and MCH, while MCV, WBC and ESR values decreased and no significant difference ($P < 0.05$) was found in MCHC values, when compared with the control fish.

Table 2. Toxicity of aquaeous extracts of *M. oleifera* on some blood parameters of *O. niloticus* fingerlings and adults.

O. niloticus fingerlings								
(mg/l)	PCV	Hb	ESR	WBC	RBC	MCH	MCHC	MCV
T_0 (0)	17.5±0.7	5.7±0.3	12.0±1.4	3.7±0.1	1.1±0.1	5.0±0.9	31.2±1.7	1603±191.8
T_1 (200)	15.5±0.7	5.4±0.1	12.5±0.7	4.4±0.1	1.2±0.1	4.3±0.3	33.7±2.1	1277±150.8
T_2 (210)	19.5±0.7	6.4±0.2	11.5±0.7	3.4±0.2	1.4±0.1	5.4±1.0	33.6±10.0	1733±808.3
T_3 (220)	20.0±8.5	6.7±2.8	11.5±3.5	4.6±0.8	1.8±1.4	6.5±4.9	38.6±18.4	1623±522
T_4 (230)	17.5±6.4	5.9±2.1	11.5±3.5	4.6±1.1	1.7±1.3	3.6±1.7	40.8±10.5	891±210
T_5 (240)	14.5±0.7	5.0±0.4	11.0±0.0	4.7±0.1	1.1±0.3	4.7±2.0	37.5±6.0	1244±299
T_6 (250)	23.0±4.2	7.7±1.5	9.0±1.4	3.9±0.1	2.6±0.3	3.4±0.9	34.1±1.4	857±63.9
Adult *O. niloticus*								
T_0 (0)	13.5±0.8	4.7±0.8	12.0±1.4	4.3±0.1	0.9±0.1	5.2±0.1	33.0±2.5	1500±0.0
T_1 (300)	32.5±0.7	11.1±0.1	8.5±0.7	3.3±0.8	2.9±0.1	11.2±0.3	34.4±0.5	1087±79.2
T_2 (310)	12.5±3.5	4.3±1.2	13.5±2.1	4.3±0.5	1.0±0.1	12.4±1.8	34.2±0.3	1237.5±179
T_3 (320)	16.5±0.7	5.6±0.2	12.0±1.4	4.2±0.1	1.2±0.0	13.8±0.6	33.7±0.2	1375±59.4
T_4 (330)	13.5±0.7	4.6±0.3	13.0±1.4	4.6±0.9	1.1±0.1	12.5±2.1	34.1±0.4	1291±154.1
T_5 (340)	19.0±2.8	6.4±1.0	10.0±0.0	3.8±0.1	1.7±0.6	11.6±2.2	33.7±0.2	1154±217.8
T_6 (350)	28.0±2.8	9.5±1.1	10.0±1.4	2.8±0.3	2.5±0.2	11.0±0.4	33.8±0.4	1102±39.6

The 96-h LC_{50} of *M. oleifera* to *O. niloticus* fingerlings was 242 mg/l. Annune *et al.* (2002) reported lower concentration of ringworm plant, *Senna alata*, used in poisoning water bodies for fish capture in Benue State, Nigeria; the 96-h LC_{50} for juvenile *O. niloticus* was 13.93 mg/l, thus indicating that the extract caused sub-acute effect. The toxicity of *M. oleifera* to *O. niloticus* fingerlings and adults in this study is higher than the results of Wade *et al.* (2002) who reported that the 96-h LC50 of 0.19 mg/l, for *O. niloticus*, exposed to the toxicity of cassava (*Manihot esculenta*). The reason may be due to higher toxicant concentration in cassava effluent. Santhakumar and Balaji (2000) stated that the acute toxicity of an organophosphorus insecticide monocrotophos to the freshwater fish *Anabas testudineus* after 24-h, 48-h, 72-h, and 96-h LC50 were 22.65, 21.2, 9.75 and 19 ppm, respectively. The safe concentration of monocrotophos was 0.19 ppm.

Muniyan and Veeraraghavan (1999) reported the effect of insecticide, ethofenprox to Mozambique tilapia, *O. mossambicus*, using a static bioassay method, the median lethal concentration for 3, 6, 12, 24, 48, and 96-h were 2.03, 1.95, 1.90, 1.85, 1.79, 1.76 and 1.74 ppm, respectively. *O. niloticus* fingerlings and adults showed variations in their tolerance to aqueous extracts of *M. oleifera*. Upon addition of the toxicant, they showed various toxic reactions such as erratic movements, air gulping, molting, while increase in concentration and exposure time resulted in loss of scale and haemorrhage. This report agrees with the work of many authors (Muniyan and Veeraraghavan, 1999; Santhakumar and Balaji, 2000; Ayuba and Ofojekwu, 2002; Chung-Min Liao *et al*., 2003), who work on the toxicity of different freshwater fishes. Particularly, this is similar to the report of Okoli-Anunobi *et al* (2002) who investigated the lethal effect of the detergent, Elephant Blue® on *O. niloticus* and found 96-h LC50 as 9.77 mg/l, changes in behavioral pattern before death, opercular ventilation rate and pectoral fin beats were observed.

There were significant differences in values of some of the blood parameters of *O. niloticus* fingerlings and adults after exposure to 96 hours of *M. oleifera* (Table 2), particularly in higher concentrations. In *O. niloticus* fingerlings, PCV increased from 17.5±0.7 in the control to 23.0±4.2 in 250 mg/l *Moringa* seed powder/l of water; and there was an increase in the values of WBC from 3.7±0.3 to 3.9±0.1 ($10^4/mm^3$), and RBC from 1.1±0.1 to 2.6±0.3 ($10^6/mm^3$). In adult *O. niloticus*, PCV increased from 13.5±0.8 in the control to 28.0±1.1, Hb increased from 4.7±0.8 in the control to 9.5±1.1, RBC increased from 0.9±0.1($10^6/mm^3$) in the control to 2.5±0.2 ($10^6/mm^3$), and MCH increased from 5.2±0.1 in the control to 11.0±0.4 in 300-350 mg/l. WBC decreased from 4.3±0.1 to 2.8±0.3 from control to 350mg/l, while ESR and MCV decreased from 12±1.4 to 10.0±1.4, 1500±0.0 to 1102±39.6 from control to higher concentration of 350 mg/l, respectively. There was no significant difference ($P>0.05$) in MCHC value. This was similarly reported by Saleh *et al*. (1998) who studied the effect of inhalation of the pyrethroid insecticide, tetramethrin on blood and biochemical parameters in albino rats and recorded no significant changes in RBC, PCV, Hb, MCHC, MCV and MCH. On the other hand, WBC and lymphocytes percentage showed significant increase whereas the blood platelets decreased significantly in the treated rats. Serum triglycerides were significantly raised after 3, 6 and 9 days of treatment while cholesterol values showed significant increase after 15 days. Total proteins and albumin were not changed significantly in tetramethrin treated animals during the experiment.

The 96 hrs LC50 of *M. oleifera* to *O. niloticus* adults was 332 mg/l and the maximum toxicant admissible concentrations were 3.32 mg/l - 33.2 mg/l. This is similar to the work of Agbon *et al*. (2002) who studied the acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on *O. niloticus*. The extract was found to be toxic with a 48-h LC_{50} value of 109.6 mg/l. He established a maximum acceptable toxicant concentration of 5mg/l while safety level of 10.9 mg/l was established. The result in the present study is dissimilar to that of Okoli-Anunobi *et al*. (2002), who investigated the lethal effect of the detergent, Elephant Blue® on *O. niloticus.* They reported that 96h LC_{50} of 9.77 mg/l increased water quality, dissolved oxygen increases from 6.50 - 8.30 mg/l in tanks containing 120 mg detergent/l, pH shift slightly from 6.30 to the alkaline death point of 10.75.

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