Acute Toxicity Of Nile Tilapia (*Oreochromis niloticus*) Juveniles Exposed To Aqueous And Ethanolic Extracts Of *Ipomoea aquatica* Leaf

*****Simeon O. Ayoola[1], Kuton M.P[1], Idowu A.A[2] and Adelekun, A.B[1]

[1]Department of Marine Sciences, University of Lagos, Akoka, Yaba, Lagos State, Nigeria

[2]Department of Aquaculture and Fisheries Management, University of Agriculture, Abeokuta, Ogun State.

*****Email:soaayoola@yahoo.com, sayoola@unilag.edu.ng, Tel: +234(80)34650102

ABSTRACT: The differential acute toxicity of aqueous and ethanolic extracts of *Ipomoea aquatica* leaf on Nile Tilapia, *Oreochromis niloticus* were carried out under laboratory conditions. The LC_{50} after 96hr of exposure for aqueous and ethanolic extracts of *Ipomoea aquatica* were 2.659g/L and 0.196g/L respectively. These values showed that ethanolic extract of *Ipomoea aquatica* was more toxic than its aqueous extract. Signs of agitated behaviours, respiratory distress and abnormal nervous behaviors including eventual deaths were observed in exposed fish. Control fish neither died nor exhibited any unusual behaviour. The randomized analysis of variance (ANOVA) showed that there were significant differences ($P<0.05$) in the quantal response (mortality) of *O. niloticus* to aqueous and ethanolic extracts of *I. aquatica* at 24hrs, 48hrs, 72hrs and 96hrs of exposure period. It was investigated that leaf of *Ipomoea aquatica* has piscicidal property and can be put into use in the control and management of fish ponds to eradicate predators by farmers.

[Simeon O. Ayoola, Kuton M.P, Idowu A.A and Adelekun, A.B. **Acute Toxicity Of Nile Tilapia (*Oreochromis niloticus*) Juveniles Exposed To Aqueous And Ethanolic Extracts Of *Ipomoea aquatica* Leaf.** Nature and Science 2011;9(3):91-99]. (ISSN: 1545-0740). http://www.sciencepub.net.

Keywords: Acute toxicity, *Ipomoea aquatica, Oreochromis niloticus*

INTRODUCTION

Agrochemical, such as pesticides especially chlorinated hydrocarbons are routinely employed as part of the integrated farming practice to protect crops and animals from insects, weeds and diseases. Widespread use of pesticide on farm is now a worldwide phenomenon (Omitoyin *et al*., 2006). The use of chlorinated hydrocarbon such as DDT, dieldrin and Lindane as pesticides has been documented. Pesticides currently in use are biocides that have high mammalian toxicity and necessitate considerable precautions in their application.

The aquatic ecosystem as a greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Omitoyin *et al*., 2006) . Pesticides become readily available in the food chain and subsequent bioaccumualate in both aquatic and terrestrial flora and fauna, with possible unquantifiable disastrous consequences on the ecosystem (Odiete, 2009). Due to the residual effects of pesticides, important organ like kidney, liver, gills, stomach, brain, muscle and genital organs are damaged in fish exposed to pesticide (Odiete, 2009).

Many plants contain chemicals which have traditionally been used to harvest fish in almost all parts of the world. Fish farmers in Nigeria have persistently and indiscriminately abused these natural plant piscicides (*Derris elliptia*) by using much higher concentrations than necessary, causing mass mortality of fish in ponds, contaminating the freshwater bodies and affecting non target organisms [1]. The physical and chemical changes in aqueous environment often cause some physiological changes in fish, thus, the water quality of an aquatic body is very crucial because it determines the productivity and other parameters necessary for fish survival. Many countries including Nigeria have legislated against the use of chemical poisons in aquatic systems and instead have policies favouring the use of natural bio–degradable alternatives to remove unwanted fish species in aquatic systems (Olufayo, 2009).

Unwanted fishes may enter aquaculture farms through water supplies or along with seed brought into the fish farm. Occasional draining of the pond and fishing are usually inadequate to control and eradicate unwanted fishes. Screening is the standard

method, but it does not stop the entry of predatory fishes in larval form. In ponds where water enters through pipes, screens may significantly restrict the flow of water (Bardach *et al*., 1972). The best way of ensuring total eradication of unwanted fishes is through the use of fish toxicants (piscicide) in the pond water (Chakroff, 1976). The use of piscicides as a tool in pond management during pond preparation to get rid of predators before fish stocking is an important tool Harwood and sytsma(2003) . Ideally, ponds should be sundried and the pond bottom cracked dried to help get rid of fish predators. However, this practice is not always possible particularly during the wet season. Moreover, farmers who are always in a hurry to prepare their ponds always resort to the use of inorganic fish toxicants (Cagaman, 1995). In view of this, farmers resort to nonconventional and unregistered fish toxicants such as agro-pesticides and sodium cyanide because they are fast acting and readily available in the market. However, these chemicals may have negative effects on the environment and farmers and health (Ayoola and Ajani, 2007). Hence, there is a need to explore other environment and health-friendly fish toxicants such as botanical plants with piscicidal activity. Plants are virtually inexhaustible source of structurally diverse biologically active substances (Istvan, 2000). Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties. Unlike synthetic chemical pesticides which leave harmful residues in the aquatic environment (Koesoemadinata, 1980), botanical insecticides are believed to be more environments friendly because they are easily biodegraded and leave no residues in the environment. Since some of these pesticidal compounds present in plants were also toxic to fishes, botanical pesticides can be used as piscicide to eradicate unwanted fishes in the pond. Many plants from different families have been applied for catching fish all over the world. Examples of these plants are of the genera *Derris*, *Tephrosia* and *Lonchocarpus* of the family L*eguminosae*. The toxic parts of plants employed as fish poisons include roots, seeds, fruits, bark, latex or leaves(Olufayo,2009).

Ipomoea aquatica is a member of the Morning Glory Family, Convolvulaceae, which contains 500 species. *I. aquatica* and *I. fistula* are the only aquatic species in the genus, which also includes the sweet potato (*Ipomoea batatas* L.) (Cook, 1990). *I. aquatica* has been cultivated for its edible shoots and medicinal properties but sometimes, it is considered to be a serious weed.

Oreochromis niloticus is a member of the family cichlidae. It is considered as one of the organism suitable for toxicity test. This study intends to assess the piscicidal activity of extracts from *Ipomoea aquatica* on *Oreochromis niloticus* as indicator to eradicate predators in pond. This study is to investigate the acute toxicity of *Ipomoea aquatica* on juveniles of *Oreochromis niloticus* and to determine the lethal concentration (LC_{50}) of aqueous and ethanolic extracts of *Ipomoea aquatica* on Nile Tilapia (*Oreochromis niloticus).*

MATERIALS AND METHOD

Four hundred juveniles of *Oreochromis niloticus* were used as test organism for the toxicity test because of its suitability. The Tilapia juveniles were bought from a fish farm in Badagry, Lagos State. The juveniles were transported in two aerated polythene bags to the laboratory in the early hours of the morning (11:00 am). The water to be used for stocking of the juveniles was dechlorinated by exposing it to sun for a period of 48 hours. The Nile Tilapia juveniles were kept in a rectangular glass tank and allowed to acclimatize to laboratory conditions for a period of 14 days in an already dechlorinated tap water. The stock tank had cosmo 10,000 air pump with voltage 220-240v. The juveniles were fed twice daily using coppen commercial supplementary feed (42% protein content). The water was change daily to prevent accumulation of toxic waste metabolite. Experimentation was carried out under ambient laboratory conditions (temp.27 ± 3^0C), Feeding of the juveniles stop a day before the bioassay test.

The fresh leaves of *I. aquatica* were collected along the Oge creek, University of Lagos Akoka, Lagos State. The plant was identified to nearest taxonomical level. The extraction of *I. aquatica* was done using Ohaustriple 700-800 series weighing balance. The fresh leaves of (1 kg) were collected and washed well to remove any adhering foreign particles and soil materials. The washed leaves were oven dried at 48^0C for 36 hours to prevent enzyme action. After drying, it was coarsely powdered and later soaked in 1 litre of clean water for 72 hours. The solution was filtered through a muslin cloth to separate aqueous extract from residue. The aqueous solution was then kept in a black plastic container at room temperature, until the time of use.

1kg of coarsely powdered, well dried fresh leaves of *I. aquatica* was put in a soxhlet extractor with 250ml of 98% absolute ethanol as the extracting

solvent.the set up was placed on a heating mantle and heated for 3-4 hours.

The solvent extract was transferred to a rotary evaporator, Buchii, type 661 (with vacuum pump). The solvent was recovered with the concentration of the extract of the dried leaves reduced up to 25% volume. The extract was then transferred from the rotary evaporating flask to 100ml beaker and further concentrated in a hot-air oven at 80^0C. The extract was a dark-brown pasty substance. The extract was collected and stored at room temperature in a black plastic material that will not allow light penetration. Salinity was measured by using a hand refractometer. pH was measured by the use of Hanna instrument pH 211-micro processor pH meters. Dissolved oxygen (DO) was measured with DO meter (model EUTECH DO 600); water temperature was determined by simple mercury in glass thermometer, calibrated in centigrade (^{o}C).

BIOASSAY PROCEDURES

The preliminary tests were carried out at first to determine suitable range of concentration for the bioassay experiment. The concentration ranges chosen for the aqueous and ethanolic extract of *Ipomoea aquatica* after preliminary test were: 0.5g/L, 1.5gL, 2.5g/L, 3.5g/L, 4.5g/L and 0.11g/L, 0.21gL, 0.43g/L, 0.53g/L, 1.07g/L respectively. These concentrations were carefully measured out to make up 8 litres of solution in 5 boiassays containers. Another bioassay container with 8 litres of water, free of the extract, served as control. In each of the container, 10 juveniles (8.7±0.3) cm were introduced. Care was taken to minimize the stress on the fish by using a hand net to collect and drop the fish carefully into the rectangular plastic tanks. The Tilapia juveniles exposed to different concentration of aqueous and ethanolic extract of *Ipomoea aquatica* were monitored for mortality at 24, 48, 72 and 96 hours.

STATISTICAL ANALYSIS

The quantal response (mortality) was analysed by Probit analysis (Finney, 1980). The logdose values for LC_5, LC_{50} and LC_{95} were obtained and tabulated. Graph of Probit values were against logdose values were plotted using the line of best fit for a straight curve. The following indices of toxicity and their 95% confidence unit derived from a computer statistical programme SPSS 10.5 were:

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

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

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

One Way Analysis of Variance (ANOVA) and comparison of means by Student Newman (SNK) test were used to test for statistical differences in the result of 96-hrs toxicity tests.

RESULTS

The test organisms showed distress in behaviour on introduction into the bioassay tanks. There were changes in the frequency of movement of the fish subjected to different concentrations of *I. aquatica.* Behavioral changes such as uncoordinated movements, somersaulting, excess secretion of mucus, erratic swimming and increase in operculum ventilation, respiratory distress, strong spasm, paralysis, and prior to the death, paleness of fish were observed during the exposure of fish to *I. aquatica.* The colour of the skin of fish exposed to the toxicant changed from normal darkly pigmentation in the dorsal and the lateral part.

The death of the fish is confirmed by its floating on its side or failure to respond to stimulus even when touch with a forcep. The number of such fishes were recorded and removed from the test media to prevent contamination of the whole media. The numbers of dead fishes were computed as percentage (%) mortality per period of exposure. This process was replicated to eliminate bias that may result due to handling, differences in size and weight and other intrinsic physiological imbalance in the test organisms. The mean values obtained for the physico-chemical parameter of the test media throughout the period of the experiment are presented in tables 1 and 2 for aqueous and ethanolic extract respectively.

Table 1: Mean physico-chemical parameters of the test concentrations *(Ipomoea aquatica*) on *Oreochromis niloticus* using aqueous extracts

Concentration	Physico – chemical Parameters			
g/L	Do (mg/l)	Salinity($^0/_{00}$)	pH	Temp 0C
0.0	5.9±0.1	0	7.0	26.0±0.6
0.5	5.8±0.1	0	7.0	27.0±0.1
1.5	5.8±03	0	7.0±0.1	27.0±0.1
2.5	4.4±0.1	0	6.9±0.2	27.1±0.2
3.5	4.0±0.1	0	6.7±0.3	27.0±0.1
4.5	3.0±0.1	0	6.4±0.1	27.3±0.2

*Mean values followed by the same superscript in each column are not significant different (p>0.05)

Table 2: Mean physico-chemical parameters of the test concentrations *(Ipomoea aquatica*) on *Oreochromis niloticus* using ethanolic extracts

Concentration	Physico – chemical Parameters			
g/L	Do (mg/l)	Salinity($^0/_{00}$)	pH	Temp 0C
0.0	5.9±0.1	0	7.0	26.0±0.6
0.11	5.8±0.1	0	7.0	27.0±0.1
0.21	5.8±0.2	0	7.0	26.9±0.2
0.43	4.1±0.1	0	6.8±0.2	26.8±0.2
0.53	4.0±0.1	0	6.7±0.3	27.0±0.1
1.07	3.0±0.1	0	6.4±0.1	27.3±0.2

*Mean values followed by the same superscript in each column are not significant different (p>0.05)

EFFECT OF AQUEOUS EXTRACT OF *Ipomoea aquatica on Oreochromis niloticus*

The result of the acute toxicity test of aqueous extract of the leaf of *I. aquatica* against *Oreochromis niloticus* juveniles at 24hrs, 48hrs, 72hrs and 96hrs of exposure period is shown in Table 3.

Figure 1 shows the graph of probit response and log – dose drawn from the probit line equation tables, (Microsoft Excel, 2007). The LC_{50} values obtained at 24hrs, 48hrs, 72hrs and 96hrs for aqueous extract were 9.178g/L, 9.157g/L, 4.623g/L and 2.659g/L respectively.

The randomized analysis variance (ANOVA) showed there was significant difference ($P<0.05$) between all the concentrations at 24, 48, 72 and 96 hrs of exposure. Using the Student Newman – Keul's (SNK) test ($P<0.05$) shown. Table 4 shows the mean quantal response of 1.5g/L was significantly different from the control at 48, 72 and 96 hrs exposure. At 72 and 96 hrs exposure period, 0.5g/L and 1.5g/L showed no significant difference so also is the case for 2.5g/L and 3.5g/L at 96 hrs exposure period.

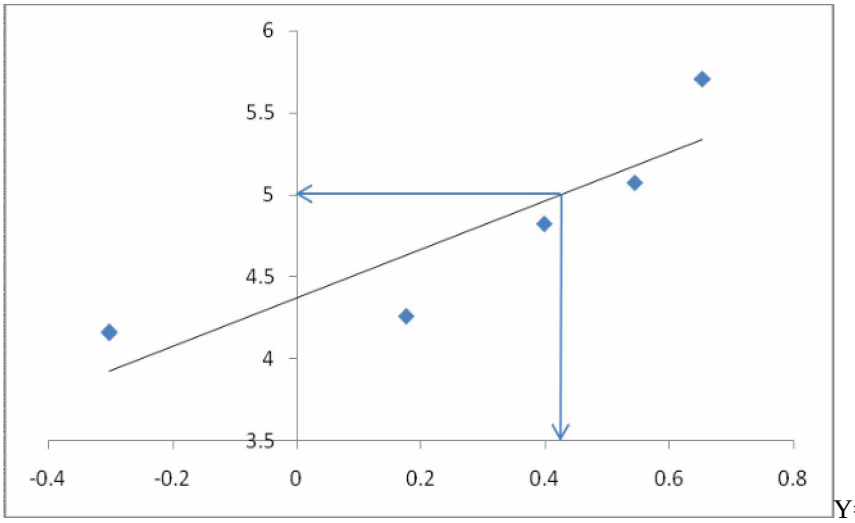
TABLE 3: ACUTE TOXICITY EFFECT OF AQUEOUS EXTRACT OF the leaf of *Ipomoea aquatica* AGAINST *Oreochromis niloticus* juvenile AT 24, 48, 72 AND 96 HOURS EXPOSURE.

Exposure time (hrs)	LC_{50} (95% C.L g/L)	LC_{95} (95% C.L g/L)	LC_5 (95% C.L g/L)	Slope ± S.E	T.F	D.F	Probit Line Equation
24	9.178	41.394	2.035	2.514±0.93	1.00	3	Y=2.579+2.514X
48	9.157	40.822	0.800	1.554±0.49	1.00	3	Y=3.506+1.554X
72	4.623	37.035	0.240	1.280±0.36	1.99	3	Y=4.149+1.280X
96	2.659	31.485	0.225	1.532±0.35	3.35	3	Y=4.349+1.532X

L.C = Lethal concentration; T.F = Toxicity factor; S.E = Standard Error; D.F = Degree of freedom;

C.L = Confidential Limit

$$T.F = \frac{LC_{50} \text{ at 24hrs}}{LC50 \text{ at any other period time}}$$



Y=4.349+1.532X

Fig.1: Linear relationship between probit response and log concentration of Aqueous Extract of *I. aquatica* on juveniles of *O. niloticus*

TABLE 4: MEAN MORTALITY RESPONSE OF *O. niloticus* EXPOSED TO DIFFERENT CONCENTRATION OF AQUEOUS EXTRACT OF THE LEAF OF *I. aquatica* FOR 96 HOURS

Concentration (g/L)	Total no. 0f Organisms	Percentage Mortality Response/Time Hours			
		24	48	72	96
Control	30	0^{a}	0^{a}	0^{a}	0^{a}
0.5	30	0^{a}	3^{ab}	17^{b}	20^{b}
1.5	30	1^{b}	10^{ab}	17^{b}	23^{b}
2.5	30	7^{b}	17^{bc}	30^{b}	43^{c}
3.5	30	13^{b}	27^{cd}	43^{c}	53^{c}
4.5	30	23^{c}	33^{d}	60^{d}	76^{d}

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

TABLE 6: ACUTE TOXICITY EFFECT OF ETHANOLIC EXTRACT OF the leaf of *Ipomoea aquatica* AGAINST *Oreochromis niloticus* juvenile AT 24, 48, 72 AND 96 HOURS EXPOSURE.

Exposure time (hrs)	LC_{50} (95% C.L g/L)	LC_{95} (95% C.L g/L)	LC_{5} (95% C.L g/L)	Slope ± S.E	T.F	D.F	Probit Line Equation
24	0.642	1.692	0.244	3.910±0.64	1.00	3	Y=5.751+3.910X
48	0.391	1.468	0.104	2.863±0.41	1.64	3	Y=6.168+2.863X
72	0.276	1.189	0.064	2.594±0.39	2.33	3	Y=6.450+2.594X
96	0.196	0.842	0.046	2.600±0.41	3.28	3	Y=6.839+2.600X

L.C = Lethal concentration; T.F = Toxicity factor; S.E = Standard Error; D.F = Degree of freedom

C.L = Confidential Limit

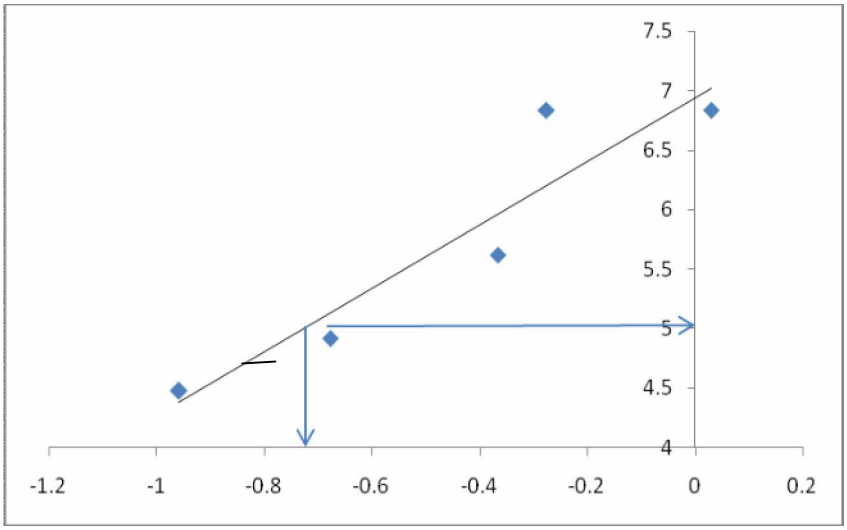
$$T.F = \frac{LC_{50} \text{ at 24hrs}}{LC50 \text{ at any other period time}}$$

EFFECT OF ETHANOLIC EXTRACT OF *Ipomoea aquatica on Oreochromis niloticus*

The result of the acute toxicity test of ethanolic extract of the leaf of *I. aquatica* against *Oreochromis niloticus* juveniles at 24hrs, 48hrs, 72hrs and 96hrs of exposure period is shown in Table 6.

Figure 2 shows the graph of probit response and log – dose drawn from the probit line equation tables (Microsoft Excel, 2007). The LC_{50} values obtained at 24hrs, 48hrs, 72hrs and 96hrs for aqueous extract were 0.642g/L, 0.391g/L, 0.276g/L and 0.196g/L respectively.

The randomized analysis variance (ANOVA) showed there was significant difference (P<0.05) between all the concentrations at 24, 48, 72 and 96 hrs of exposure. Using the Student Newman – Keul's (SNK) test (P<0.05) shown in Tables 7 and 8, the mean quantal response of 0.11g/L was significantly different from the control of 48, 72 and 96 hrs exposure. At 48 and 72 hrs exposure period, 0.11g/L and 0.21g/L showed no significant difference so also is the case for 0.53g/L and 1.07g/L at 96 hrs exposure period.



Y=6.839+2.600X

Fig.2: Linear relationship between probit response and log concentration of Ethanolic Extract of *I. aquatica* on juveniles of *O. niloticus*

TABLE 7: MEAN MORTALITY RESPONSE OF *O. niloticus* EXPOSED TO DIFFERENT CONCENTRATION OF ETHANOLIC EXTRACT OF THE LEAF OF *I. aquatica* FOR 96 HOURS

Concentration (g/L)	**Total no. 0f Organisms**	**Percentage Mortality Response/Time Hours**			
		24	48	72	96
Control	30	0^{a}	0^{a}	0^{a}	0^{a}
0.11	30	0^{a}	13^{b}	23^{b}	30^{b}
0.21	30	3^{a}	17^{b}	30^{b}	47^{c}
0.43	30	10^{a}	20^{b}	47^{c}	73^{d}
0.53	30	57^{b}	90^{c}	93^{d}	97^{e}
1.07	30	77^{c}	93^{c}	97^{d}	97^{e}

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

DISCUSSION

The result obtained from this study showed that both the aqueous and ethanolic extract of *Ipomoea aquatica* had toxic effect on the juveniles of *Oreochromis niolticus* and the effect of their toxicity increases with time of exposure. The LC_{50} values at 96hrs of exposure of *O. niloticus* to aqueous and ethanolic extracts were 2.659g/L and 0.196g/L respectively.

The difference in the level of toxicity of the extracts could be as a result of the method of extraction used. In the case of aqueous extract, the alkaloids was extracted but the presence of water dilute it, hence reducing it potency, while the alkaloids of ethanolic extract obtained using soxhlet extractor and ethanol as extracting medium remains almost undiluted .

Oreochromis niloticus exhibited erratic movement and aggressiveness (Abalaka and Auta, 2010) when placed in the bioassay tanks. Some attempted to jump out of the tanks. This behavior continued for a few hours after which their movement becomes normal and calm.

Increased physical activity, convulsion, excess secretions of mucus, incessant gulping of air, erratic swimming, respiratory distress, paralysis, sudden quick movement, increase in opercula ventilation and prior to death darkening of fish were associated with *I. aquatica* toxicity in this study. This agreed with the findings of Abalaka and Auta, 2010 on *Oreochromis niloticus* exposed to trichloroform. Omitoyin *et al*., 1999 reported similar observation in *Sarotherodon galilaeus* (Tilapia) fingerlings exposed to piscicidal plant extracts of *Tetrapleura tetraptera.*

The intensity of respiratory distress increased with increasing extracts concentrations but decreased with exposure period for both extracts while nervous abnormality increased with increasing extracts concentrations and exposure period for both extracts (Abalaka and Auta, 2010). Extracts of *I. aquatica* probably poisoned the fish leading to pathological alterations in their tissues and organs (Gabriel *et al*., 2007) which eventually lead to the direct death of the tested organism. Indirect death could also result from changes in the physicochemical conditions of their immediate external environment (Ayoola, (2008), Olufayo, (2009)). The observed respiratory distress may be due to decreased in the dissolved oxygen contents (Dede and Kaglo,2001).

Warren (1997) had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolve oxygen concentration, which will impair respiration leading to asphyxiation. Generally, one could deduce from this research work that the introduction of *Ipomoea aquatica* into water bodies would threaten the life and existence of fish. Therefore, this plant can actually be use as a biological control in eradicating predators and unwanted organisms in the ponds by farmer instead of using agrochemicals.

REFERENCES

Omitoyin, B.O. Ajani, E.K. Adesina, B.T. and Okuagu, C.N.F. (2006). Toxicity of Lindane (Gamma Hexachloro - CycloHexane) to *Clarias gariepinus* (Burchell 1822). *World Journal of Zoology*. **1**(1): 57-63.

Odiete, W.O. (1999). Environmental Physiology of animals and pollution Diversified resources Ltd Lagos. 261pp

Olufayo M.O (2009): Haematological characteristics of *Clarias gariepinus* (Burchell 1822) Juveniles exposed to *Derris elliptia* root powder. *African journal of food Agriculture, Nutrition and Development.*Vol 9, (3)920-932

Bardach, J. E., Ryther, J. H. and McLarney, W. O. (1972). *Aquaculture: The Farming and Husbandry of Freshwater and Marine Organisms.* John Wiley and Sons, Inc. USA: 868 pp.

Chakroff, M. (1976). Freshwater Fish Pond Culture and Management. Volunteers in Technical Assistance. Vita Publications, USA. pp. 171-172.

Harwood, E. and Sytsma, M. (2003). *Risk assessment for Chinese Water Spinach (Ipomoea aquatica) in Oregon*. Portland State University, Portland, OR. <www.oregon.gov/ ODA/PLANT/docs/pdf/oisc_ipaq_ra.pdf.> last visited on 24th July, 2010.

Cagauan, A. G. (1995). The impact of pesticides on ricefield vertebrates with emphasis on fish. pp. 203 - 248. *In*: P. L. Pingali and P. A. Roger (eds.). Impact of Pesticides on Farmer Health and the Rice Environment. International Rice Research Institute. Kluwer Academic Publishers. 664 pp.

Ayoola, S.O. and Ajani, E.K. **(2007):** Histopathological Effect of Cypermethrin on Juvenile Nile Tilapia (*Oreochromis niloticus*). *African Journal of Livestock Extension.* **5**; 1-13

Istvan, U. (2000). Semi-natural products and related substances as alleged botanical pesticides. Pest Management Science. **56**(8): 703-705.

Koesoemadinata, S. (1980). Pesticide as a major constraint in integrated aquaculture farming system. 45-51pp. *In*: R. S. V. Pullin and Z. H. Shehadeh (eds.). Integrated Agriculture-Aquaculture Farming Systems. ICLARM Conf. Proc. 4.

Cook, C.D.K. (1990). Origin, autecology, and spread of some of the world's most troublesome aquatic weeds. 31-38pp. In: A.H. Pieterse and K.J. Murphy (eds.), *Aquatic Weeds: The Ecology and Management of Nuisance Aquatic Vegetation.* Oxford University Press, New York.

Sharma, M. (1994). Taxonomic Notes on North Indian Plants – X. *Journal of Economic and Taxonomic Botany*. **18**(2):387-394.

McCann, J.A., L.N. Arkin and J.D. Williams. (1996). *Nonindigenous Aquatic and Selected Terrestrial Species of Florida: Status, Pathway and Time of Introduction, Present Distribution, and Significant Ecological and Economic Effects*. Gainesville: University of Florida, Institute of Food and Agricultural Sciences (IFAS). http://aquat1.ifas.ufl.edu/mctitle.html Accessed 5/1/03.

Patnaik, S. (1976). Autecology of *Ipomoea aquatica* Forsk. *Journal of the Inland Fisheries Society of India*. **8**: 77-82.

Arrignon, J.V.C. (1998). *The Tropical Agriculturist*: *Tilapia*. Macmillian Education: 73pp.

Finney, D.J. (1980). *Probit analysis*. Cambridge University Press, Cambridge. Greatm Britain. (3): 124pp.

Abalaka, S.E. and Auta, J. (2010). Toxic effects of Aqueous and Ethanolic extracts of *Parkia biglobosa* pods on *Clarias gariepinus* adults. *World Journal of Biological Research.* **3**(1): 9-17.

Alkahem, H.F., Ahmed, Z., Al-Akel, A.S. and Shamsi, M.J.K. (1998). Toxicity Bioasay and changes in haematological parameter of *Oreochromis niloticus* induced by trichlorfom. *Arab Gulf J.Scient. Res.* **16**: 581-593.

Omitoyin, B.O., Ogunsami, A.O. and Adesina, B.T. (1999). Studies on acute toxicity of Piscicidal plant extracts (*Tetrapleura tetraptera*) on tilapia (*Sarotherodon gailiaeus*) fingerlings. *Trop.J. Anim. Sci*. **2** (2): 191 – 197.

Gabriel, U.U., Ezeri, G.N.O. and Amakiri, E.U. (2007). Liver and kidney histopathology: Biomarkers of No. 1 fuel toxicosis in African catfish, *Clarias gariepinus*. *Journal of Animal and Veterinary Advances*. **6**(3): 379-384.

Ayoola, S.O. (2008a). Toxicity of Glyphosate herbicide on Nile tilapia (*Oreochromis nilotus*) juvenile. *African Journal of Agricultural Research*. **3**(12): 825-834.

Dede, E.B., Kaglo, H.D., 2001. Aqua-toxicological effects of water soluble fractions (WSF) of diesel fuel on *O. niloticus* fingerlings. *Journal of Applied Science and Environmental Management*. 5 (1): 93-96.

Warren, D. (1977). Biology and water pollution control. Saunder, W.B. company, Philadelphia, Fish Edtion: 24-39.

2/18/2011