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Research Article
Ichthyotoxic Effect of *Draceana arborea* Back and root Extract on *Clarias gariepinus* Post Fingerlings

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Abstract

Background and Objective: *Draceana arborea* as a piscicidal plant that is used in villages to kill fish in streams and rivers around Akwa Ibom state. The objective of the study was to test the maximum admissible level of the plant *D. arborea* to freshwater fish. Therefore, the ichthyotoxic properties of the plant was tested on *Clarias gariepinus* post fingerlings with a static bioassay method. **Materials and Methods:** The test fish were introduced to the following concentrations; 0.8, 1.0, 1.2, 1.4 and 1.6 mg L^{-1} of the bark and root extracts of *D. arborea*, each of 5 treatment tanks in were in replicated 3 times for accurate data generation. **Results:** The LC_{50} for the back extract was observed at 1.4 mg L^{-1} while for the root extract, LC_{50} was recorded at 0.8 mg L^{-1}. Gulping, discolouration, erratic swimming, molting and mucus secretion were observed from the 1st day on both extracts solutions. There were significant changes ($p<0.05$) in both test solutions as compared to the control. **Conclusion:** Therefore, based on the LC_{50} figures, it is adjudged that any introduction of *D. arborea* into any aquatic environment at above 1.4 mg L^{-1} for back extract and above 0.8 mg L^{-1} for root extract is highly toxic and unsafe.

Key words: Ichthyotoxic, draceana, arborea, clarias, fingerlings, extract

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The African catfish *Clarias gariepinus* is common in Nigeria, West African countries and in the Middle East. The species has a native range from White Nile specifically the Jebel and Ghazal systems of Sudan[1], Gambela region of Ethiopia[2], Lake Chad basin, Zaire (Congo), Senegal River basins[3]. *Clarias gariepinus* has been reported to have a preference for marginal vegetation and flood plain habitat in the Sudan[1] and occupies a wide variety of habitats including streams, rivers, lakes, lagoons and marshes[3], which of course are seen to be among the major receptors of agricultural waste, toxicants and waste water from sewage which include pesticides, herbicides and detergents. In flowing water, the species occupies calm areas. The species has also been indicated to be 'widely distributed' in marshy habitats and can also be found in bank vegetation of river channels[4].

The genus *Dracaena* is well known as an indoor ornamental plant. Some of the *Dracaena* species possess several medicinal properties and are used in curing a number of diseases[5]. *Dracaena* produces several steroidal saponins, which showed *cystostatic* activity on leukemia cells[6]. Commonly called Dragon's blood, a red resin exuding from the stem of *D. draco* has been used frequently as a herbal remedy in traditional medicine. Despite their medicinal and ornamental importance not much work has been done in *Dracaena* species *in vitro* conditions[7] and mostly grows vegetatively. The vegetatively propagated plants are sensitive to several bacterial, fungal, viral and mycoplasmal diseases acquired from the air, soil and insect vectors as a result of which their productivity declines, even mass propagation through seeds has many limitations like seed dormancy, low rate of germination and progeny variation in other plant species.

More than 700 plants species are recognized as being potentially dangerous in the world and a large number (around 500 species) of these are frequently used as ornamentals[8]. Dangerous plants are classified in two large groups according to the type of interaction with organisms: those that cause injuries by external contact and those that do so after ingestion or aspiration. Poisoning by ingestion or aspiration is caused from the direct consumption of poisonous plant species, by the use of excessive doses of medicinal plants, for the ingestion of honey from toxic plants, smoking, inhaling smoke, eating barbecued meat supported in branches of toxic plants[9]. Plants from different families have been applied for catching fish all over the world. The toxic parts of plants employed as fish poisons include the roots, seeds, fruits, barks or leaves. Plant extracts used as piscicides in fisheries are considered advantageous when viewed against the backdrop of using persistent chemicals.

Dracaena plants are frequently used by fisher folks to catch fishes because they are readily available and highly toxic to fish as reported by Tiwari and Singh[10]. Frequent applications of high concentrations of these ichthyotoxins in water may have adverse effects not only on fish species but also on other aquatic fauna. Ichthyotoxic plants used for baiting or stupefying fish are often crushed and cast into stagnant, slow moving water or spread on mud flats in order to poison the target fish. Several studies have shown that these plant toxins at low concentrations are very toxic to all other groups of aquatic fauna[11-13] the leaves of the plant are indiscriminately used for catching fish in many water bodies in several parts of the country, yet there are non documented effects of the plant materials on important fish species such as the clariids[14].

A poisonous plant is generally defined as that which contains toxic compounds that might be harmful if consumed by humans or other animals[8,15,16]. However plants can have special structures (spines, hairs, crystals, pollen) that cause mechanical or contact injuries without being ingested and which may then result in a systematic reaction due to the chemicals liberated in the contact[17].

There are various hypothesis to explain the origin of toxic plant substances. They may be considered as waste products, intermediate products in metabolic processes or as secondary metabolites that fulfill non-essential functions in plants but intervene in plants/environmental interactions. Some are pigments that give colour to flowers and fruits playing a fundamental role in reproduction by attracting insect pollinators or animals that contribute to seed dispersal. Others protect the plant from predators, acting as repellants due to their bitter taste or poisonous characteristics[18].

Freshwater fishes show dissimilar pattern of responses when exposed to toxicants[19]. The extent of damage varies with body parts, nature of the toxicants, medium and duration of exposure[20]. To understand the impact of toxicants in aquatic ecosystems, it is necessary to study the behavioural pattern and most importantly, the histopathological effects of poisons on different fish organs.

The *D. arborea* was observed to be a commonly used piscicidal plant in the stunning and thereafter harvesting of fish from freshwater and ponds amongst the people of Itu in Akwa Ibom state, Nigeria. The method might seem simple to them but they might not know the environmental and health effects of the act of obnoxious fishing. Research has it that these plants will affect also the non-target population thereby causing a serious ecological balance and depletion in these water bodies which does not encourage sustainable fishing.

It is as a result of this that the researchers decided to carry out the experiment on *D. arborea* bark and root extracts to ascertain the safe level of the plant of freshwater fish species. At the end of the experiment, the researchers will be able to advise on the Maximum Admissible Toxicant Concentration (MATC) of the plant.

MATERIALS AND METHODS

Seven hundred and Twenty healthy post fingerlings of *C. gariepinus* of mixed sex and of the same brood stock of weight ranging from 13.57-5.6 g and length ranging from 12.5-8.4 cm were obtained from Umana's Farm in Akwa Ibom state, Nigeria. The fishes were acclimatized in the Department of Fisheries and Aquatic Environmental Management, University of Uyo Hatchery for 2 weeks in transparent rectangular plastic containers with a dimension of 35×25×25 cm each with a total capacity of 30 L and filled with 20 L of clean water. The experiment took place in the place of acclimatization.

The fishes were fed twice daily with commercial fish feed pellets containing 35% crude protein during the acclimatization period. Feeding was discontinued 24 h before the commencement of the experiment, to minimize the production of waste in the test containers[21]. The experiment being a static assay test, lasted for 96 h. This is the standard for any static bio assay experiment as recommended by APHA[22].

Collection and preparation of aqueous extract of *D. arborea*: About 100 kg of back and roots of *D. arborea* were collected from Idu Uruan in Uruan Local Government in Akwa Ibom state, Nigeria.

The back and roots of the assay plant were extracted using sterilized distilled water as a medium by maceration for 24 h. It was filtered and concentrated using freeze dryer model ultra dry 1800, NS 5H in the Pharmacognosy and Natural Medicine Laboratory of the Department of Pharmacy, University of Uyo. The yield was 30 g at the end of extraction. The extracts was stored in the refrigerator at 40°C to avoid degradation[23].

A range finding test was conducted to determine the toxicity level of the plant extracts[24]. Direction No. 203 and Methodical Manual ISO 7346/2. Triplicate six test concentrations were used for this investigation: One control and five tests solutions of *Dracaena arborea* back and roots extract in triplicates. The *C. gariepinus* post 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 to prevent fish from jumping out, there was no aeration, no water change nor feeding throughout the test. The back and roots extracts of the test plant were introduced at concentrations of 1, 1.5, 2, 2.5 and 3 mg L^{-1} with a control of 0 mg L^{-1}. The response (reaction) of the test fishes in each tank were monitored for 24 h and recorded every 15 min for the first hour, once every h for the next 3 h and every 4 h for the rest of the 24 h.

Based on the results of the range finding test, a definitive test was conducted using triplicate concentrations of the test extracts with concentrations of 0.8, 1.0, 1.2, 1.4 and 1.6 mg L^{-1} for the bark and roots extracts. The tests comprised of one sub-lethal toxicity test for each extract according to the OECD[24]. Fish mortality was monitored and recorded hourly for the first 4 h and once in every 4 h for the next 24 h. Subsequently, it was conducted every 24 h for the next 96 h. The inability of fish to respond to external stimuli was used as an index of death[24]. Apart from monitoring and recording mortality, the fish reaction such as: Erratic swimming, air gulping, loss of reflex, discoloration and molting were monitored. About 96-LC_{50} (concentration of *Dracaena arborea*, estimated to be lethal to 50% of test organisms after exposure time of 96 h) was determined graphically using probit transformation[25,26].

Water quality characteristics: Water quality monitoring was done before during and after the experiment. The 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, dissolved oxygen was measured using a digital dissolved oxygen meter once in a day at 8.00 a.m. Temperature was measured using a mercury in glass thermometer, which was placed in the medium inside the test container until readings were taken. The readings were taken at 8.00 a.m. on each day of the experiment[21] (Table 1).

All results were collected and analyzed using computerized, probit analysis[27]. 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 probit analysis[28] using Statistical Package for Social Sciences (SPSS) 20.0 for Windows. Data were analyzed using descriptive statistics (mean, standard deviation, frequencies and percentages). Comparison of data on physico-chemical properties between range finding tests and the definitive tests were carried out using analysis of variance (ANOVA).

Statistical analysis: Toxicological dose responses (fish mortality) were subjected to probit analysis using Statistical Package for Social Sciences[27] (SPSS) 22.0. The median lethal concentration (LC_{50}) at selected period of exposures and an associated 95% confidence interval for each replicate toxicity test were subjected to probit analysis[28] using Statistical Package for Social Sciences (SPSS) 20.0 for Windows. Data were analyzed using descriptive statistics (mean, standard deviation, frequencies and percentages). Comparison of data and physico-chemical properties between the control and other treatments were carried out using one-way analysis of variance (ANOVA)[22].

RESULTS

Mortality: Mortality for back and root of *D. arborea* extract in the lowest concentration of 0.8 mg L^{-1} was observed at the 20th h and in the highest concentration of 1.6 mg L^{-1} mortality was observed at the 8th h. The LC_{50} for the concentration of 0.8 mg L^{-1} was recorded at the 96th h, in the concentration of 1.0 mg L^{-1} LC_{50} was observed at the 72nd h, in the concentration of 1.2 mg L^{-1} LC_{50} was recorded at the 48th h and in the concentration of 1.4 mg L^{-1} LC_{50} was observed at the 24th h. The highest concentration of 1.6 mg L^{-1} mortality of 100% was recorded at the 72nd and 96th h (Fig. 1, 2).

Behavioral/physiological changes: Air gulping, discolouration, erratic swimming, molting and mucus secretion were observed in the experiment as shown in Table 2. Apart from erratic swimming, all other behavioural responses were observed in the 48th h, through to the last day of the experiment for bark extract (Table 2).

Also in the root extract, erratic swimming and loss of reflex were observed in the 24th h, while, air gulping, discoloration and mucus secretion were absent. Air gulping, discoloration, loss of reflex and mucus secretion started showing up in the 48th h, through to 96th h as shown in Table 3, 4.

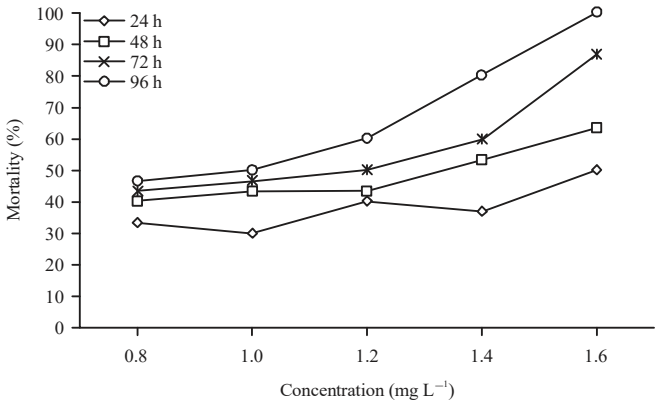

Fig. 1: Percentage mortality of *C. gariepinus* exposed to *D. arborea* bark extract

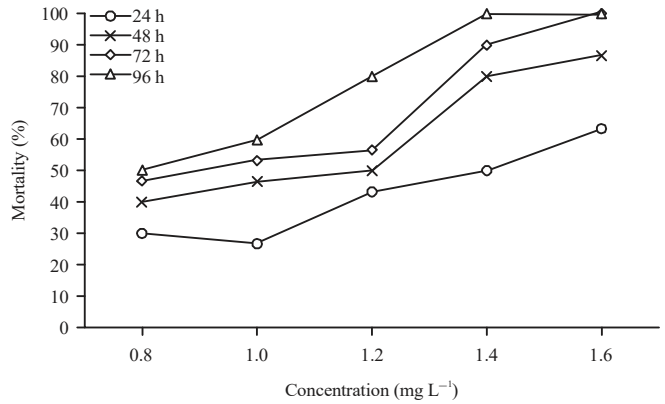

Fig. 2: Percentage mortality of *C. gariepinus* exposed to *D. arborea* root extract

Table 1: Summary of water quality parameters of *D. arborea* leaf, bark and root extract

Parameters	Control	Before	During	After
Temperature	27.41±0.04[a]	27.16±0.05[a]	26.81±0.06[b]	26.88±0.05[b]
Dissolved oxygen	6.50±0.11[a]	6.46±0.07[a]	5.58±0.04[b]	4.31±0.01[c]
pH	6.55±0.10[a]	6.46±0.05[a]	6.40±0.03[b]	6.04±0.05[c]

Same letter means not significantly different (p>0.05), different letter means not significantly different (p<0.05)

Table 2: Behavioral response/changes of *C. gariepinus* exposed to *D. arborea* bark extracts

Duration/h	24 h	48 h	72 h	96 h
Concentration (mg L^{-1})	0.0, 0.8, 1.0, 1.2, 1.4, 1.6	0.0, 0.8, 1.0, 1.2, 1.4, 1.6	0.0, 0.8, 1.0, 1.2, 1.4, 1.6	0.0, 0.8, 1.0, 1.2, 1.4, 1.6
Air gulping	---+++	-+++++	-+++++	--++++
Discoloration	----++	---+++	---+++	---+++
Erratic Swimming	---+++	------	------	------
Molting	---+++	-+++++	-+++++	--++++
Loss of reflex	------	--++++	--++++	--++++
Mucus secretion	--++++	--++++	--++++	--++++

-: Not present, +: Present

Table 3: Behavioral response/changes of *C. gariepinus* exposed to *D. arborea* root extracts

Duration/h	24 h	48 h	72 h	96 h
Concentration (mg L^{-1})	0.0, 0.8, 1.0, 1.2, 1.4, 1.6	0.0, 0.8, 1.0, 1.2, 1.4, 1.6	0.0, 0.8, 1.0, 1.2, 1.4, 1.6	0.0, 0.8, 1.0, 1.2, 1.4. 1.6
Air gulping	------	---+++	-+++++	--++++
Discoloration	------	----++	---+++	---+++
Erratic Swimming	--++++	------	------	------
Loss of reflex	--++++	--++++	--++++	--++++
Mucus secretion	------	---+++	--++++	--++++

-: Not present, +: Present

Table 4: Percentage cumulative mortality of *C. gariepinus* exposed to *D. arborea* root extract (Definitive test)

Concentration (mg L^{-1})	1 h	2 h	3 h	4 h	8 h	12 h	16 h	20 h	24 h	48 h	72 h	96 h
0.00	-	-	-	-	-	-	-	-	-	-	-	-
0.8	-	-	-	-	-	-	-	16.7	30.0	40.0	46.7	50
1.0	-	-	-	-	-	-	-	10.0	26.7	46.7	53.3	60
1.2	-	-	-	-	-	-	30.0	36.7	43.3	50.0	56.7	80
1.4	-	-	-	-	-	10.0	43.3	43.3	50.0	80.0	90.0	100
1.6	-	-	-	-	13.3	26.7	43.3	46.7	63.3	86.7	100.0	100

DISCUSSION

The aquatic environment, where fish and other aquatic organisms live, is subjected to different types of pollutants, which enter water bodies through industrial, domestic and agricultural discharge systems thereby introducing stress to living creatures. Stress is a general and non-specific response to any factors disturbing homeostasis. Stress reaction involves various physiological changes including alteration in the histological composition and immune mechanisms[29]. It has also been linked as one major factor of disease outbreaks, low productivity and mortality in aquaculture. Other toxic endpoints include decreased growth, mobility and reproductive effects[30]. Stress in fish may be induced by various abiotic environmental factors change in water temperature, pH, dissolved oxygen, concentration and pollution.

Behavioural responses of fish to most toxicants and differences in reaction times have been observed to be due to the effect of chemicals, their concentration, species, size and specific environmental conditions. The recorded responses for the fishes in this study is in accordance with earlier reports of other authors for clariid under various stress conditions which identified four main phases in the responses of fish to toxicants: The contact phase (brief period of excitability), exertion, (visible avoidance characterized by fast swimming, leaping and attempts to jump out of the toxicant), loss of equilibrium and lethal (death) phase when opercula movement and response to tactile stimuli ceased completely. Difference in mortality of *Clarias gariepinus* may be due to the differential toxicity of the plant extracts.

The values of 96 h LC_{50} of 1.0 mg L^{-1} for bark and 0.8 mg L^{-1} for root extract reported in this study are far lower than those earlier reported by Oti and Ukpabi[31] and Fafioye *et al.*[32] for some clariid species exposed to *Thevetia peruviana*, *Parkia biglobossa* and *Raphia vinifera* plant extracts and higher than that reported by Gabriel and Okey[33] on hybrid catfish exposed to *Lepidagatis alopecuriodes*. This observation implies that *D. aborea* is more toxic to African

catfish, *C. gariepinus* than *T. peruviana*, *P. biglobossa* and *R. vinifera*. The lower value obtained by Gabriel and Okey[33] might be as a result of the genetically modified state of the hybrid catfish which of course might have made it a bit weaker than the hardy *C. gariepinus.* Mucus production and accumulation on the gills may have caused a blockade in the gills there by contributing immensely to the increase in opercular beat and mortalities recorded in this study. Konar[34] reported that accumulation of mucus on the gills reduces respiratory activity in fishes. This might be due to inability of the gills surface to actively carry out gaseous exchange. The observed restlessness and mortalities of the test fish might be due to the effect of flavonoids, alkaloids and saponins present in the extracts[35].

Saponins are ichthyotoxins which destroy the erythrocytes and are assimilated directly through the gills[36]. Alkaloids on the other hand inhibit oxidative phosphorylation, blocks the mitochondrial enzymes, Nicotinamide Adenine Dinucleotide (NADH) ubiquinone reductase, hence impairing their oxygen consumption[37,11]. Olaifa *et al.*[38] and Omitoyin *et al.*[39] reported a 96 h LC_{50} of copper as 0.67 mg L^{-1} and lindane 0.38 mg L^{-1} for *C. gariepinus*, respectively stating that they are highly toxic.

Studies have revealed that fish exposed to toxicants usually exhibits some behavioral changes such as increased opercula beat rate, erratic swimming, mucus secretion and air gulping before death[40,41]. The pattern of behavioural changes observed in this study compared favorably with the report of Fafioye *et al.*[32] when African catfish (*Clarias gariepinus*) was exposed to *Parkia biglobossa* and *Raphia vinefera* extracts and hybrid catfish finger lings treated with cassava mill effluents[42]. Increased concentrations of *D. arborea* bark and roots extract led to increased opercula beat frequency, molting, discoloration and mortality as was also observed in *C. gariepinus* exposed to aqueous extracts of *Blighia sapida* and *Kigelia africana*[43], hybrid catfish exposed to *Thevetia peruviana*[44] and 2-4 dichlorophenoxyacetic acid[45] and quickly killed mudskippers (*Periophthalmus p apilio*) exposed to *L. alopecuroides*[46]. The marked deviation in the rate of opercula beat frequency, discoloration molting and air gulping especially in the bark and root extracts from reference (control) suggests an adjustment in physical fitness as a result of the stress condition[47,48]. The bark and root extracts were found to be more toxic than the leaf extract in this experiment. It was also observed that on the last day of the experiment, survived fishes were found swimming with normalcy. This suggests that the toxicant may not be a bio-accumulating plant and might have degraded. Thus suggesting the plant as a means of weed eliminator in closed aquaculture systems prior to stocking.

CONCLUSION

Results obtained from the study revealed that the bark and roots extracts of *D. arborea* are highly toxic than most ichthyotoxic plants reported and therefore is not safe. The study also evaluated the effects of *D. arborea* bark and roots extract, at acute lethal doses on survival, morphology, behavior of the test organism (*C. gariepinus*) post fingerlings. Mean values of the water temperature were not significantly ($p>0.05$) affected by the concentrations of *D. arborea*. On the other hand, pH and dissolved oxygen significantly ($p<0.05$) decreased as the concentrations of *D. arborea* bark and roots extracts as compared to the control. It is therefore, advisable to discontinue the use of *D. arborea* by local fisher folks since they would not be able to measure the safe amount.

SIGNIFICANCE STATEMENT

This study is the first to be conducted on the test plant (*Dracaena arborea*) plant extract. The study discovered the toxicant admissible level of the test plant, which will serve as benchmark information to researchers who intend to carry out an inept experiment on the effect of *D. arborea* on the tissues and blood of other test organisms. The study will therefore help researchers discover the medicinal effect of *D. arborea*, which other researches have not explored yet. Thus, a new theory on the dose response of organisms to the plant maybe arrived at.

REFERENCES

1. Bailey, R.G., 1994. Guide to the fishes of the River Nile in the republic of the Sudan. J. Nat. Hist., 28: 937-970.
2. Golubstov, A.S., A.A. Darkov, Y. Dgebuadze and M.V. Mina, 1995. An Artificial Key to Fish Species of the Gambella Region (The White Nile basin in the limits of Ethiopia). Joint Ethio-Russian Biological expedition, Addis Ababa, Ethiopia, Pages: 84.
3. Bonou, C.A. and G.G. Teugels, 1985. Revision systematique du genre Parachanna Teugels et Daget 1984 (Pisces: Channidae). Rev. Hydrobiol. Trop., 18: 267-280.
4. Lowe-McConnell, R.H., 1988. Broad Characteristics of the Ichthyofauna. In: Biology and Ecology of African Freshwater Fishes: Collection Travauxet Documents 216, Leveque, C., M.N. Bruton and G.W. Ssentongo (Eds.). Institut Francais de Recherche Scientifique Pour le Developpementen Cooperation, Paris, France, pp: 93-105.

5. Peterson, M.S., W.T. Slack, N.J. Brown-Peterson and J.L. McDonald, 2004. Reproduction in nonnative environments: Establishment of Nile tilapia, *Oreochromis niloticus*, in Coastal Mississippi watersheds. Copeia, 2004: 842-849.
6. Yokosuka, A., Y. Mimaki and Y. Sashida, 2000. Steroidal saponins from *Dracaena surculosa*. J. Nat. Prod., 63: 1239-1243.
7. Junaid, A., A. Mujib, M.A. Bhat, M.P. Sharma and J. Samaj, 2007. Somatic embryogenesis and plant regeneration in *Catharanthus roseus*. Biol. Planta., 51: 641-646.
8. Iramain, M.S., M.A. Herrero, S.M. Volpe and S.E. Toro, 2008. Plantas Ornamentales Toxicas. 1st Edn., BM Press, Buenos Aires, Pages: 152.
9. Najera, M.T., 1993. Aportes al conocimiento de las plantas toxicas. I parte. Reojasiana, 1: 43-48.
10. Tiwari, S. and A. Singh, 2003. Metabolic changes in the Snake head fish *Channa punctatus*, due to lattices of *Euphorbia royleana*. Asian Fish. Sci., 16: 147-155.
11. Tiwari, S. and A. Singh, 2003. Control of common freshwater predatory fish, *Channa punctatus*, through *Nerium indicum* leaf extracts. Chemosphere, 53: 865-875.
12. Goktepe, I., R. Portier and M. Ahmedna, 2004. Economical assessment of neem based pesticides. J. Environ. Sci. Health, 39: 311-320.
13. Kreutzweiser, D.P., R.C. Back, T.M. Sutton, K.L. Pangle and D.G. Thompson, 2004. Aquatic mesocosm assessments of a neem (Azadirachtin) insecticide at environmentally realistic concentrations-2: Zooplankton community responses and recovery. Ecotoxicol. Environ. Saf., 59: 194-204.
14. Obomanu, F.G., O.K. Ogbalu, U.U. Gabriel, G.K. Fekarurhobo and B.I. Adediran, 2006. Larvicidal properties of *Lepidagathis alopecuroides* and *Azadirachta indica* on *Anopheles gambiae* and *Culex quinquefasciatus*. Afr. J. Biotechnol., 5: 761-765.
15. Turner, N.J. and A.F. Szcawinski, 1991. Common Poisonous Plants and Mushrooms of North America. Timber Press, Portland, Oregon.
16. Wagstaff, D.J., 2008. International Poisonous Plants Checklist: An Evidence-Based Reference. CRC Press, Boca Raton, Florida, pp: 24-46.
17. Cambi, V. and V.P. Cuadra, 2010. Conozcamos las Plantas: Galeria Fotografica de Ornamentales Presentes en los Jardines de Infantes del Partido de Bahia Blanca (Arg.). Lugar, Bahia Blanca, Argentina.
18. Olsnes, S., 2004. The history of ricin, abrin and related toxins. Toxicon, 44: 361-370.
19. Mount, D.I., 1968. Chronic toxicity of copper to fathead minnows (*Pimephales promelas*, rafinesque). Water Res., 2: 215-223.
20. Vijayamadhavan, K.T. and T. Iwai, 1975. Histochemical observations on the permeation of heavy metals into taste buds of goldfish. Bull. Jap. Soc. Sci. Fish, 41: 631-639.
21. APHA., 1979. Standard Methods for Examination of Water and Waste Water. 18th Edn., American Public Health Association, Washington DC., USA.
22. APHA., 2005. Standard Methods for Examination of Water and Waste Water. 25th Edn., American Public Health Association, Washington DC., USA.
23. CTA., 1986. African Pharmacopoeia. CTA., Wageningen, The Netherlands.
24. OECD., 2001. Guidance document on aquatic toxicity testing of difficult substances and mixtures. Organisation of Economic Cooperation and Development, Paris, France, pp: 34-57.
25. 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, Springfield Illinois, USA., pp: 122.
26. USEPA., 2000. Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms. 4th Edn., Environmental Monitouring and Support Laboratory, US. Environmental Protection Agency, Cincinati, Ohio.
27. Newman, C., 1995. Quantitative Methods in Aquatic Toxicology. Lewis Publishers, Florida, pp: 56-78.
28. Finney, D.J., 1971. Statistical Method in Biological Assay. 2nd Edn., Cambridge University Press, London, pp: 668.
29. Witeska, M., 2003. The effect of metals (Pb, Cu, Cd and Zn) on hematological parameters and morphology of blood cells in common carp. Akademia Podlaska Publishers, Siedlce, Monograph No. 72, pp: 113.
30. Allen, P., 1995. Accumulation profiles of lead and cadmium in the edible tissues of *Oreochromis aureus* during acute exposure. J. Fish Biol., 47: 559-568.
31. Oti, E.E. and U.H. Ukpabi, 2000. Acute toxicity of milk extracts *Thevetia peruviana* (Persoon) to African freshwater catfish, *Clarias gariepinus* (Tengels). Afr. J. Sci. Technol., 1: 132-138.
32. Fafioye, O.O., A.A. Adebisi and S.O. Fagade, 2004. Toxicity of *Parkia biglobosa* and *Raphia vinifera* extracts on *Clarias gariepinus* juveniles. Afr. J. Biotechnol., 3: 627-630.
33. Gabriel, U.U. and I.B. Okey, 2009. Effects of aqueous leaf extracts of *Lepidagathis alopercuroides* on the behaviours and mortality of hybrid catfish (*Heterobranchus bidorsalis* ♂ and *Clarias gariepinus* ♀) fingerlings. Res. J. Applied Sci. Eng. Technol., 1: 116-120.
34. Konar, S.K., 1970. Nicotine as a fish poison. Progr. Fish-Cult., 32: 103-104.
35. Obomanu, F.G., G.K. Fekarurhobo and I.C. Howard, 2005. Antimicrobial activity of extracts of leaves of *Lepidagathis alopecuroides* (Vahl). J. Chem. Soc. Nig., 30: 33-35.
36. Fry, D.M., 2004. Toxic effects of lead on condors and other wildlife. Presentation at Condors and Lead Action Summit, October 15, 2004, Stratus Consulting, Inc., Boulder, Co., San Francisco, CA.

37. Haffor, S.A. and M.I. Al-Ayed, 2003. The effect of lead bioaccumulation on haem biosynthetic enzymes in fish. J. Environ. Biol., 24: 271-280.
38. Olaifa, F.E., A.K. Olaifa and O.O. lewis, 2003. Toxic stress of lead on *Clarias gariepinus* (African catfish) fingerlings. Afr. J. Biomed. Res., 6: 101-104.
39. Omitoyin, B.O., E.K. Ajani and A.O. Fajimi, 2006. Toxicity gramoxone (Paraquat) to juvenile African catfish, *Clarias gariepinus* (Burchell, 1822). Am. Euras. J. Agric. Environ. Sci., 1: 26-30.
40. Davis, J.C., 1973. Sublethal effects of bleached Kraft pulp mill effluent on respiration and circulation in Sockeye Salmon (*Oncorhynchus nerka*). J. Fish Res. Board Can., 30: 369-377.
41. Nwanna, L.C., O.A. Fagbenro and E.T. Ogunlowo, 2000. Toxicity of Textile Effluent to *Clarias gariepinus* and *Heterobranchus bidorsalis* and Hybrid Fingerlings. In: Responsible Aquaculture in the New Millennium (Abstract), Fos, C.R. and L. Cesswell (Eds.)., European Aqua Solution Special-Publication, Belgium, pp: 510.
42. Oti, E.E., 2000. Acute toxicity of water extracts of bark of the *Thevetia peruviana* to the African freshwater catfish heteroclarias hybrid fingerling. J. Fish. Technol., 2: 124-130.
43. Onusiriuka, B.C. and E.B.C. Ufodike, 1994. Acute toxicity of water extracts of sausage plant *Kigelia Africana* and *Akee apple*, *Blighia sapida* on African catfish, *Clarias gariepinus* (Teugals). J. Aquat. Sci., 9: 35-41.
44. Oti, E.E. and U.H. Ukpabi, 2000. Acute toxicity of milk extracts *Thevetia peruviana* (Persoon) to African catfish, *Clarias gariepinus* (Tengels). Afr. J. Sci. Technol., 1: 132-138.
45. Koffi, M.B., 2005. Acute toxicity of dichlorophenoxyacetic acid to postfingerlings of *Clarias gariepinus* and hybrid. PGD Project, Rivers State University of Science and Technology, Port Harcourt, Nigeria, pp: 44.
46. Obomanu, F.G., O.K. Ogbalu, U.U. Gabriel, S.G.K. Fekarurhobo and S.U. Abadi, 2007. Piscicidal effects of *Lepidagathis alopecuroides* on mudskipper, *Periophthalmus papillio* from the Niger delta. Res. J. Applied Sci., 2: 382-387.
47. Edwards, C.A. and S.W. Fusher, 1991. The Use of Chlorinesterases Measurements in Assessing the Impacts of Pesticides on Terrestrial and Aquatic Invertebrates. In: Chlorines Terases Inhibiting Insecticides: Their Impact on Wild and the Environment, Mineau, P. (Ed.). Elsevier, The Netherlands, pp: 255-275.
48. Heath, A.G., 1995. Water Pollution and Fish Physiology. CRC Press, Florida, USA., pp: 245.