Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(1): 749 – 760 (2021)
www.ejabf.journals.ekb.eg

Piscicidal action and behavioral responses of Cichlid fish Tilapia, *Oreochromis niloticus* (Linn) exposed to three indigenous plant seed extracts

Sadia Sultana[1,*], Munira Nasiruddin[2] and Mohammad Ali Azadi [3]
[1]Department of Zoology, Noakhali Science and Technology University, Bangladesh
[2]Department of Zoology, University of Chittagong, Chittagong, Bangladesh
[3]International Islamic University Chittagong, Chittagong, Bangladesh

***Corresponding author: sadia12cu@gmail.com**

ARTICLE INFO

Article History:
Received: Jan. 19, 2021
Accepted: Feb.11, 2021
Online: Feb. 18, 2021

Keywords:
Toxicological effects,
Botanical piscicides,
Lethal concentrations,
Oreochromis niloticus,
plant seed extracts

ABSTRACT

Piscicidal action of distilled water, 50% ethyl alcohol, methanol and acetone extracts of three indigenous plant seeds *Jatropha carcus* (Linn.), *Hydnocarpus wightianus* (Blume) and *Aleurites moluccana* (Linn. Willd) were tested for the first time upon the live Cichlid fish, Tilapia *Oreochromis niloticus* (Linn.) collected from a local fish hatchery. The fishes exhibited different types of behavior in different concentrations of the seed extracts, such as hyperactivity, erratic swimming, frequent gulping of air, loss of movement, ultimately leading to death. Fish mortality percentage varied in accordance with plant seeds, solvents and concentrations. According to mortalities %, the extent of toxicity of the solvents for *J. carcus*, *H. wightianus* and *A. moluccana* seeds on *O. niloticus* was ranked in the following order: Acetone > methanol > 50% ethyl alcohol > distilled water. To determine the LC_{50} of the seed extracts, relationship between observed and expected mortalities of the fish and dose-mortality relationships, probit calculation, chi-square and ANOVA tests were done. Based on the LC_{50} values, the relative toxicity of the seed extracts was recorded in order: *J. carcus*>*H. wightianus*>*A. moluccana*. The most toxic was the acetone extract of *J. carcus* seed, whereas the least toxic was distilled water extract of *A. moluccana* seed. These plant seed extracts may be useful to remove the unwanted weed fish species from the fish culture ponds.

INTRODUCTION

Destructive influence of human beings on the aquatic environment is due to sub-lethal pollution, which is caused by the use of chemical pesticides. Persistent use of such pesticides has led to hazardous effects on aquatic lives, which has resulted in the need for the use of alternative biodegradable chemicals; the botanical pesticides. Extracts of botanical piscicides are toxic or poisonous to fish **(Murthy, 1986)**. Botanical piscicides are often used to control competing species in fish production, especially in small water bodies, eradicate predator or unwanted fish species or restore native species. The usage of such pesticides is encouraged due to their minor toxicity to aquatic organisms and nonhazardousness to the environment **(Olufayo, 2009)**. Sometimes ponds may become over populated with unwanted fish species. Problems related to stocking of undesirable fishes include: overpopulation, habitat destruction, competition for food and nesting space and the small size attained by the introduced fish.

Thus, to overcome the hazardous effects of chemical pesticides, emphasis is given on the use of natural pesticides with plant origin. Some plants contain compounds of various classes that have insecticidal, piscicidal and molluscicidal properties **(Wang & Huffman, 1991).** Plant poisons, in other name botanicals, are extracted from flowers, bark, pulp, seeds, roots, leaves and sometimes the entire plant **(Sirivam *et al*., 2004)**. Powder of root of Derris **(Shirgur, 1972)**, Nicotine **(Konar, 1970, 1977)** and antimycin **(Lennon & Berger, 1970)** are preparations from plants that have a fish poison potentiality. Tobacco leaf dust has been used as an effective pesticide and treatment of predators/pest in water (pond) as it is completely biodegradable **(Omoniyi *et al.*, 2002)**.The piscicidal potential and phytotoxic properties of plant extracts on *Oreochromis niloticus* have been reported by workers at home **(Nasiruddin *et al*., 2009, 2014; Chowdhury, 2011; Ali, 2013)** and abroad **(Ibrahim, 2000; Agbon *et al*., 2002; Ayotunde & Benedict, 2008; Mousa *et al*., 2008; Ayoola, 2011; Ayoola *et al*., 2011; Akinduyite & Oyedapo, 2011; Fafioye, 2012; Okey *et al*., 2013; Adesina *et al*., 2013; Kishore *et al*., 2016).**

The test fish, an African freshwater cichlid called the Nile Tilapia, *Oreochromis niloticus* has been regarded as one of the world's important food fishes. Owing to the hardy nature and the wide range of trophic and ecological adaptations, Nile Tilapia has been widely introduced for aquaculture, augmentation of capture fisheries and sport fishing **(Trewavas, 1983; Welcomme, 1988),** and in the meanwhile it is found in every country in the tropics.

Apart from synthetic chemical pesticides, which leave harmful residues in the aquatic environment **(Koesomadinata, 1980).** Pesticides from plant origin are environmentally friendlier because of their biodegradable nature, leaving no toxic residues in the environment. In context, the study was undertaken to determine the piscicidal effects of seed extracts of three indigenous plants, *Jatropha carcus* (Linn.), *Hydnocarpus wightianus* (Blume) and *Aleurites moluccana* (Linn. Willd), in addition to the toxic effects of which have not been studied earlier on an omnivore fish, the Nile Tilapia, *Oreochromis niloticus* (Linn.). Behavioral responses and mortality rates were observed during the bioassay, to assess the extent of toxicity of the extracts of the three plant seeds.

MATERIALS AND METHODS

1. Collection and preparation of samples

The toxicants were obtained from the dry seeds of the plants *Jatropha carcus* (Family: Euphorbiaceae), *Hydnocarpus wightianus* (Family**:** Achariaceae) and *Aleurites* *moluccana* (Family: Euphorbiaceae). These experimental seeds were collected from the local area and local seed markets of Chittagong and Narsingdi in Bangladesh. The experimental healthy and live fish Tilapia, *Oreochromis niloticus*, were collected from Thai Bangla Fish Hatchery, Hathazari, Chattagram on the day of the experiment and brought to the laboratory of Department of Zoology, University of Chittagong. The fishes were acclimatized in a broad glass aquarium (60x30x30 cm^3) for 2-3 hours under normal laboratory condition (29±2^0C). Average length and weight of the experimental fish were 3.50±0.5 cm and 2.60±0.1 gm, respectively.

After collection, the dry seeds were macerated in a mortar using a pestle, and blended into fine powder using an electric blender, and then sieved through a sieve of mesh 0.0025 cm^2. Ten grams of each seed powder was extracted with 100 ml of distilled water, 50% ethyl alcohol, methanol and acetone solvents separately in 500 ml glass stopper conical flasks, and then, the mixture was vigorously stirred on a magnetic stirrer for 3-4 hours at room temperature (29±2^0C) for maximum extraction of toxic substances of the seeds. The obtained extracted solvent was filtered through Whatman filter paper, and the obtained filtrate was the 'stock solution'. Appropriate dilution of the stock solution was done to obtain the test concentrations (ppm) of different test solutions following **APHA (2012).**

2. Procedure of bioassays

Several preliminary screenings were carried out to determine the final doses (ppm) resulting 1-99% mortalities before the final experiments. A set of glass aquarium (30 x 23 x 23 cm^3) was used for the bioassays, each aquarium contained five litres of tap water and the toxicant. According to **APHA (2012)** to obtain the required concentration (ppm), different proportions of stock solutions were added to the aquarium water, so as to obtain the LC_{50} values of the experimental seed extracts for the experimental fish. During the final experiments, for each extract, five concentrations were used after preliminary screening. A total of five healthy and active fishes were then released randomly in each of the concentrations. The test fishes were kept in exposure to the test concentrations for 24±1 hours. All the experiments were conducted in the departmental laboratory at normal room temperature (29±2°C) and under diffused light. A control set in water was maintained in each experiment which contained same number of fishes. The behavioral pattern of control fishes were also observed and noted at that time for comparison with the affected fishes by the toxicants. Mortality of fishes, within 24 hours after exposure to the different concentrations of the seed extracts, was recorded.

3. Statistical analysis

The mortality data obtained from the experiments were analyzed following the methods of **Finney (1971).** Probit analysis was undertaken to calculate the LC_{50} values of the effect of seed extracts on the experimental fishes. The values of empirical probit, working probit and weighting probit were taken from the tables of **Finney (1971)**. The regression equation was calculated from respective empirical probit, working probit and weighting probit. The LC_{50}, with 95% confidence intervals, were analyzed in a computer based probit analysis program. Chi-square (χ^2) analysis was done **(Fisher & Yates, 1963)** on the basis of the experimental data using the following formula:

$$\chi^2 = \sum \frac{(O-E)^2}{E(1-n)}$$

Where,

O=Observed number killed,

E=Expected number killed,

n=Expected proportion killed.

and compared with tables of the statistics for n-1 degrees of freedom at 0.01 and 0.05 level of significance. Analysis of Variance (ANOVA) on mortality (%) of fishes was recorded to notify the significance among treatments and replicates at 0.01 and 0.05 levels of significance. Toxicity values or relative potency values were calculated taking the highest concentration value as the unit and dividing the highest LC_{50} value of a toxicant with the respective LC_{50} values of other toxicants.

RESULTS

1. Piscicidal effects of the three seed extract on behavior of *Oreochromis niloticus*

In the present study, different behavioral activities were observed in *O. niloticus* when exposed to the three seed extracts of *J. carcus*, *H. wightianus* and *A. moluccana.* The fishes of the control sets exhibited regular swimming movements all through the experiments without any mortality. The treated fishes showed their aggressive behavior within thirty minutes after being exposed to the toxic media. These fishes showed erratic swimming, hyperactivity, gulping for air at the surface, losing balance and dying at different intervals with the advancement of time of exposure and concentrations. Discoloration with reddish tinge in the body, bulged eyes, with few scales being shed off and damaged fins and tails were also observed. For sometimes, they swam throughout the water in the aquaria, but afterwards, they became inactive and imbalanced and hanged in the water with head upward and tail downward. Finally, mortality occurred and some of the dead fishes floated parallel or vertical or at an angle with the surface water or remained flat at the aquarium bottom. With respect to behavior, the toxicity of the experimental solvents was ranked as: Acetone > Methanol > 50% ethyl alcohol > Distilled water and *J. carcus* seed extract was the most toxic showing maximum abnormalities followed by *H. wightianus* and *A. moluccana* seed extracts.

2. Piscicidal effects of the three seed extract on mortality of *Oreochromis niloticus*

For *O. niloticus* (Table 1), doses with *J. carcus* seed extracts ranged from 1.5-10 ppm, with *H. wightianus* seed extracts from 25-1500 ppm and with *A. moluccana* seed extracts from 50-2000 ppm.

Table 1. Values of toxicity parameters of seed extracts of *Jatropha carcus*, *Hydnocarpus wightianus* and *Aleurites moluccana* on *Oreochromis niloticus.*

Seed	Solvent	Dose range (ppm)	Mortality Range (%)	Regression equation	χ^2 value	ANOVA Value of treatment	ANOVA value of replicate	LC_{50} (ppm)	Confidence limit (LC_{50})
Jatropha carcus	Distilled water	2-10	20.00-93.33	3.029x+3.13	9.12	31.27**	6.91*	4.265	2.968-5.500
	50% ethyl alcohol	2-8	13.33-93.33	4.287x+2.52	0.49	30.25**	1.00	3.749	3.057-4.509
	Methanol	2-5	26.67-93.33	4.475x+2.87	8.70	9.063**	0.375	3.018	2.293-3.569
	Acetone	1.5-5	13.33-93.33	5.230x+3.07	12.97*	22.95**	0.211	2.335	1.931-2.708
Hydnocarpus wightianus	Distilled water	500-1500	6.67-93.33	5.642x-11.83	6.52	28.67**	1.556	958.748	824.728-1102.767
	50% ethyl alcohol	300-900	13.33-93.33	4.829x-8.216	9.23	32.61**	3.69	547.885	457.027-643.504
	Methanol	50-400	6.67-86.67	3.667x-3.253	18.08**	22.00**	0.545	180.611	133.399-241.786
	Acetone	25-300	6.67-86.67	2.276x+0.38	3.07	30.25**	1.00	113.544	80.397-163.762
Aleurites moluccana	Distilled water	1000-2000	13.33-86.67	5.864x-13.63	8.74	25.00**	1.00	1512.716	1356.166-1697.783
	50% ethyl alcohol	500-1000	6.67-93.33	9.568x-22.53	8.67	27.70**	0.001	748.393	686.997-812.479
	Methanol	100-500	13.33-93.33	3.585x-3.79	33.63**	18.57**	1.00	278.776	208.698-346.507
	Acetone	50-350	13.33-86.67	2.774x-1.10	6.97	21.05**	0.211	161.911	119.387-223.289

χ^2 value =* significant at 0.05 level, **= significant at 0.01 level
ANOVA (Treatment) value = ** significant at 0.01 level
ANOVA (Replicate) value = * significant at 0.05 level

Mortality of the experimental fishes ranged from 6.67-93.33%. Results of Chi-square values showed that almost all the seed extracts were insignificant at 0.01 and 0.05 level of significance, excepting acetone extract of *J. carcus* seeds at 0.05 level and methanol extracts of *H. wightianus* and *A. moluccana* seeds for at 0.01 and 0.05 levels of significance. The $LC_{50,}$ values with confidence limits, indicated the extent of toxicity of the seed extracts.

ANOVA test estimated the treatment values of all the seed extracts as significant at 0.01 and 0.05 levels, and all replicate values as insignificant at 0.01 and 0.05 levels of significance, except for the distilled water extract of *J. carcus* seed at 0.05 level. The LC_{50} values showed that amongst all the seed extracts, acetone extract of *J. carcus* seed was the most toxic,while the distilled water extract of *A. moluccana* seed was the least toxic for the test fish.

As shown in Table 2, it was observed that the lowest LC_{50} value was found with acetone extract of *J.carcus* seed . It was the most toxic extract with LC_{50} of 2.335 ppm with a high relative potency value of 647.84. Highest LC_{50} value was observed with distilled water extract of *A. moluccana* seed with LC_{50} value of 1512.716 ppm and a relative potency value of 1.00. Hence, it was the least toxic extract. Distilled water, 50% ethyl alcohol and methanol extracts of *J. carcus* seeds with LC_{50} values 4.265 ppm, 3.749 ppm and 3.018 ppm and with good relative potency values 354.68, 403.50 and 501.23 respectively were also the most toxic extracts. Methanol and acetone exracts of *H. wightianus* and *A. moluccana* seeds with LC_{50} of 180.611, 113.544, 278.776 and 161.911 ppm and with relative potency values 8.38, 13.32, 5.43 and 9.34 respectively were toxic extracts. 50% ethyl alcohol extract of *H. wightianus* seed, with LC_{50} of 547.885 and relative potency of 2.76, was the medium toxic extract. Distilled water extract of *H. wightianus* and 50% ethyl alcohol extract of *A. moluccana* seeds with LC_{50} 958.748 and 748.393 ppm with relative potency of 1.58 and 2.02 were fairly toxic extracts. Whilst distilled water extract of *A. moluccana* seed with LC_{50} of 1512.716 ppm and relative potency of 1.00 was the least toxic extract.

With regards to relative potency values, i.e. toxicities, the order of toxicity of the extracts for *O. niloticus* was: Acetone extract of *J. carcus* seed > methanol extract of *J. carcus* seed > 50% ethyl alcohol extract of *J. carcus* seed > distilled water extract of *J. carcus* seed > acetone extract of *H. wightianus* seed > acetone extract of *A. moluccana* seed > methanol extract of *H. wightianus* seed > methanol extract of *A. moluccana* seed > 50% ethyl alcohol extract of *H. wightianus* seed > 50% ethyl alcohol extract of *A. moluccana* seed > distilled water extract of *H. wightianus* seed > distilled water extract of *A. moluccana* seed.

Table 2. The LC_{50} and Relative potency values with categories of Distilled Water, 50% Ethyl Alcohol, Methanol and Acetone extracts *of Jatropha carcus, Hydnocarpus wightianus* and *Aleurites moluccana* seeds on *Oreochromis niloticus.*

Seed	Solvent	LC_{50} (ppm)	Relative potency	Category
Jatropha carcus	Distilled water	4.265	354.68	Most toxic
	50% ethyl alcohol	3.749	403.50	Most toxic
	Methanol	3.018	501.23	Most toxic
	Acetone	2.335	647.84	Most toxic
Hydnocarpus wightianus	Distilled water	958.748	1.58	Fairly toxic
	50%ethyl alcohol	547.885	2.76	Medium toxic
	Methanol	180.611	8.38	Toxic
	Acetone	113.544	13.32	Toxic
Aleurites moluccana	Distilled water	1512.716	1.00	Least toxic
	50% ethyl alcohol	748.393	2.02	Fairly toxic
	Methanol	278.776	5.43	Toxic
	Acetone	161.911	9.34	Toxic

Ranking of categories (on the basis of LC_{50} values)

Most toxic → < 100 ppm

Toxic → 101-300 ppm

Medium toxic → 301-600 ppm

Fairly toxic → 601-1000 ppm

Less toxic → 1001-1500 ppm

Least toxic → > 1500

DISCUSSION

In the present investigation, piscicidal activity of seeds of three indigenous plants *Jatropha carcus*, *Hydnocarpus wightianus* and *Aleurites moluccana* with four solvents i.e. distilled water, 50% ethyl alcohol, methanol and acetone were determined against the omnivore fish *Oreochromis niloticus* as the test fish. All the extracts were more or less toxic against the applied dose concentrations. The solubilizing capacity of the solvents for the three experimental seeds on *O. niloticus* on the basis of experimental analysis was ranked in the order: acetone > methanol > 50% ethyl alcohol extract > distilled water, showing greatest solubility in acetone and least solubility in distilled water.

Abnormal behaviors were observed by the test fish during exposure period to the concentrations of the plant seed extracts. Upon introduction to the extracts, they showed hyperactivity and tried to escape from the water as time increased, moved towards the surface for gulping air. Initial hyperactivity, in the exposed fish, was probably an early indication of their avoidance of the toxicant which might be related to narcotic effects or to the change in sensitivity of chemo receptors **(Suterlin, 1974).** Pale colour of the body, bulged eyes and reddish tinge on the body were also noticed in the dead fishes. A few of the dead fishes were found floating with damaged fins and tails. Some of the dead fishes were seen floating horizontally or vertically or at different angles with the surface water, while others remained flat at the bottom of the aquarium. Most of these findings are in agreement with the observations of **Nasiruddin *et al*. (2009, 2014), Chowdhury (2011)** and **Ali (2013).**

With respect to seeds, the toxicity of the three seeds in case of *O. niloticus* was in the following order*: J. carcus* > *H. wightianus* > *A. moluccana* seeds. The LC_{50} values showed the trend of toxicity of the solvents in *J. carcus, H. wightianus* and *A. moluccana* seed extracts on *O. niloticus*, being ranked in the order: acetone > methanol > 50% ethyl alcohol > distilled water extracts. Amongst all the seed extracts, the highest toxic extract was acetone extract of *J. carcus* seed, with concentration ranges between 1.5-10 ppm and with LC_{50} values, ranging between 2.335-4.265 ppm, whereas the lowest toxic extract was the distilled water extract of *A. moluccana* seed at concentration ranges of 50-2000 ppm and with LC_{50} values ranging between 161.911-1512.716 ppm.

The Chi-square values at 0.01 and 0.05 levels of significance denoted that almost all of the experimental seed extracts had insignificant values except acetone extract of *J. carcus* seed at 0.05 level and methanol extracts of *H. wightianus* and *A. moluccana* seeds at 0.01 and 0.05 levels of significance, an indication of good relationship obtained between the observed and expected mortalities of the fish. ANOVA values denoted that with all the seed extracts, the treatment values were significant at 0.01 and 0.05 levels, and all the replicate values were insignificant at 0.01 and 0.05 levels, excepting distilled water extract of *J. carcus* seed, indicating a close relationship between the concentrations applied and mortalities obtained. Amongst all the experimental seed extracts, acetone extract of *J. carcus* seed showed the maximum toxic effect whilst distilled water extract of *A. moluccana* seed showed minimum toxic effect on the experimental fish as indicated by LC_{50} and relative potency values.

On the basis of LC_{50} values of distilled water extract, LC_{50} of *J. carcus* seed (4.625 ppm) was somewhat close to the LC_{50} of *Moringa lucida* leaf (1.869 ppm) **(Akinduyite & Oyedapo, 2011)** and LC_{50} of *Albizzia procera* seed (14.28 ppm) **(Nasiruddin *et al*., 2009);** LC_{50} of *H. wightianus* seed (958.748 ppm) was close to the LC_{50} of *Phyllanthus emblica* seed (1024.68ppm) **(Nasiruddin *et al*., 2014);** and LC_{50} of *A. moluccana* seed (1512.716 ppm) was somewhat close to LC_{50} of *Terminalia bellerica* seed(1253.73 ppm) **(Nasiruddin *et al*., 2014).**

Based on LC_{50} values of 50% ethyl alcohol extracts, LC_{50} of *J. carcus* seed (3.749 ppm) was somewhat near to LC_{50} of *A. procera* seed (13.40 ppm) **(Nasiruddin *et al*., 2009);** LC_{50} of *H. wightianus* seed (547.885 ppm) was close to LC_{50} of *T. bellerica* seed

(593.34 ppm) **(Nasiruddin *et al*., 2014)** and almost close to LC_{50} of *Tectona grandis* seed (505.93 ppm) **(Chowdhury, 2011);** and LC_{50} of *A. moluccana* seed (748.393 ppm) was somewhat close to LC_{50} of *T. bellerica* seed (593.34 ppm) **(Nasiruddin *et al*., 2014).**

On the basis of LC_{50}values of methanol extracts, LC_{50} of *J. carcus* seed (3.018 ppm) was somewhat related to LC_{50} of *Polygonum hydropiper* leaf (27.525 ppm) (Ali, 2013); LC_{50} of *H. wightianus* seed (180.611 ppm) was almost close to LC_{50} of *Terminalia chebula* seed (171.81 ppm) and close to LC_{50} of *P. emblica* seed (212.93 ppm) **(Nasiruddin *et al*., 2014);** and LC_{50} of *A. moluccana* seed (278.776 ppm) was almost close to LC_{50} of *T. bellerica* seed (283.14 ppm) and quite close to LC_{50} of *P. emblica* seed (212.93 ppm) **(Nasiruddin *et al*., 2014).**

Based on LC_{50} values of acetone extracts, LC_{50} of *J. carcus* seed (2.335 ppm) was somewhat close to LC_{50} of *P. hydropiper* leaf (11.404 ppm) (Ali, 2013); LC_{50} of *H. wightianus* seed (113.544 ppm) was somewhat close to LC_{50} of *T. chebula* seed (150.10 ppm) and LC_{50} of *P. emblica* seed (178.55 ppm) **(Nasiruddin *et al*., 2014);** and LC_{50} of *A. moluccana* seed (161.911 ppm) was almost close to LC_{50} of *T. chebula* seed (150.10 ppm) and LC_{50} of *P. emblica* seed (178.55 ppm) **(Nasiruddin *et al*., 2014).**

The potentiality of distilled water, 50% ethyl alcohol, methanol and acetone extracts of *J. carcus*, *H. wightianus* and *A. moluccana* seeds appeared promising in different concentration levels. It is revealed that information on the efficacy of the plant toxins can be obtained from the laboratory based toxicity studies on dry powders. Botanicals are environmentally friendly due their biodegradable nature and the non toxic residues effect on the environment. Toxicity data obtained in this study may be helpful to remove unwanted fish species from nursery, stocking and rearing ponds, in which commercially valuable fish species are cultured.

ACKNOWLEDGEMENTS

We are grateful to Chairman, Department of Zoology, University of Chittagong for providing laboratory facilities and Manager, Thai Bangla Fish hatchery for supplying the live fish samples.

REFERENCES

Adesina, B. T.; Omitoyin, B. O.; Ajani, E. K. and Adesina, O. A. (2013). Acute-lethal toxicity (LC_{50}) effect of *Moringa oleifera* (Lam.) fresh root bark extract on *Oreochromis niloticus* juveniles under renewal toxicity exposure. Journal of Applied Agricultural and Apicultural Research., 9: 182-188.

Agbon, A. O.; Omniyi, I. T. and Tecko, A. A. (2002). Acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on *Oreochromis niloticus* and haematological change resulting from sublethal exposure. Journal of Aquaculture Science., 17: 5-8.

Akinduyite, I. and Oyedapo, F. (2011). Acute toxicity of aqueous *Moringa lucida* leaf extracts to Nile Tilapia, *Oreochromis niloticus* (Linnaeus 1857). Proceedings of the Ninth International Symposium on Tilapia in Aquaculture, Shanghai, China. April 22nd-24th: 52-59.

Ali, M. K. (2013). Toxicity and behavioral response of *Heteropneustes fossilis* (Bloch) and *Oreochromis niloticus* (Linn.) exposed to *Polygonum hydropiper* (L.), *Lantana camara* (L.) and *Datura metel* (L.) leaf extracts. MS thesis, Department of Zoology, University of Chittagong., 176 pp.

APHA (2012). Standard methods for the examination of water and waste water. 22nd edition.Edited by E. W. Rice, R. B. Baird, A. D. Eaton & L. S. Clesceri. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington, D.C., USA.

Ayoola, S. O. (2011). Acute toxicity and histopathology of Nile Tilapia (*Oreochromis niloticus*) fingerlings exposed to aqueous and ethanolic extract of *Euphorbia poissonii* leaves. New clues in science.,1: 55-68.

Ayoola, S. O.; Kuton, M. P.; Idowk, A. A. and Adelekun, A. B. (2011). Acute toxicity of Nile Tilapia (*Oreochromis niloticus*) juveniles exposed to aqueous and ethanolic extract of *Ipomoea aquatica* leaves. Nature and Science., *9*(3): 91-99.

Ayotunde, E. O. and Benedict, O. O. (2008). Acute and chronic toxicity of *Carica papaya* seed powder to adult Nile tilapia (*Oreochromis niloticus* Linn.). African Journal of Biotechnology., 7: 2265-2274.

Chowdhury, S. (2011). Toxicity and haematological effects of *Heteropneustes fossilis* (Bloch) and toxicological effect on *Oreochromis niloticus* (Linn.) of *Samanea saman* (Jacq) Merr, *Tectona grandis* (L.) and *Dipterocarpus turbinatus* (Gaertn) seed extracts. MS Thesis, Department of Zoology, University of Chittagong., 175 pp.

Fafioye, O. O. (2012). Acute and sub-acute toxicities of five plant extracts on white tilapia, *Oreochromis niloticus* (Trewavas). International Research Journal of Agricultural Science and Soil Science., 2(13): 525-530.

Finney, D. J. (1971). Probit analysis.3rd ed. Cambridge Univ. Press. London.,333 pp.

Fisher, R. A. and Yates, F. (1963). Statistical tables for Biological Agriculture and Medicinal Research.6th ed. Oliver and Boyd. Ltd. Edinburgh., pp. 47-50.

Ibrahim, B.; Mibatchi, B.; Mounzeo, H.; Bourobou, H. P. and Posso, P. (2000). Effect of *Tephrosia vogelii* and *Justicia extensa* on *Tilapia nilotica* in vivo. J. Ethnopharmacol., 69: 99-104.

Kishore, D.; Biswas, S. J. and Karmakar, S. R. (2016). Study on the toxicity of neem (*Azadirachta indica* A. Juss) leaf extracts as phyto-piscicide on three life stages of Mozambique tilapia (*Oreochromis mossambicus* peters) with special reference to their ethological responses. International Journal of Experimental Research and Review., 3: 7-13.

Koesomadinata, S. (1980). Pesticides as a major constraint to integrated agriculture aquaculture farming systems, p. 45-51. In R.S.V. Pullin and Z.H. Shehadeh (eds). Proc. of the ICLARM-SEARCA conf. on Integrated Agriculture-Aquaculture Farming Systems, 6-9 August 1969, Manila, Phillipines., 258pp.

Konar, S. K. (1970). Nicotine as a fish poison. Progr. Fish. Cult., 32: 103-104.

Konar, S. K. (1977). Toxicity of nicotine to aquatic life. Indian J. Fish., 24(1&2); 124-128.

Lennon, R. E. and Berger, B. L. (1970). A resume on field application of Antimycin A to control fish. In: Investigation in fish control Report. US Dept. Inst. Fish and Wildl Serv. Bur. Report. Fish and Wildl., 40: 19 pp.

Mousa, M. A. A.; El-Ashram, A. M. M. and Hamed, M. (2008). Effects of neem leaf extract on freshwater fishes and zooplankton community. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, Central Laboratory for Aquaculture Research, October 12-14, 2008, Cairo, Egypt., 307-318.

Murthy A. S. (1986). Toxicity of pesticides to fish. CRC Press Inc. Boca Raton, FL., USA., 143 pp.

Nasiruddin, M.; Azadi, M. A. and Begum, N. (2009). Piscicidal effects of *Albizzia procera* (Benth) seeds, *Swietenia mahagoni* (Jacq) and *Azadirachta indica* (A. Juss) seed kernels and *Camellia sinensis* (Wild) seed cake extracts on *Oreochromis mossambicus* (Peter) and *Channa punctatus* (Bloch). The Chittagong Univ. J. Biol. Sci., 4(1& 2) :153-163.

Nasiruddin, M.; Azadi, M. A.; Rashid, T. S. and Chowdhury, R. (2014). Efficacy of medicinal plant seed extracts against fish species of *Heteropneustes fossilis* (Bloch) and *Oreochromis niloticus* (L.). Bangladesh J. Envron. Sci., 26: 103-110.

Okey, I. B.; Keremah, R. I.; Ikpi, G. U. and Ebeniro, L. A. (2013).Toxicity of leaf powder of *Lepidagatha salopecuroides* to Nile Tilapia, *Oreochromis niloticus* juveniles. African Journal of Food Science., 7(6): 128-133.

Olufayo, M. O. (2009). Heamatological characteristics of *Clarias gariepinus* juveniles exposed to *Derris elliptica* root powder. Afr. J. food, Agric. Nut. Dev., 9(3): 920-932.

Omoniyi, I. A.; Agbon, O. and Sodunke, S. A. (2002). Effects of lethal and sub-lethal concentrations of Tobacco (*Nicotiana tobaccum*), leaf dust extraction on weight and haematologicalchanges in *Clarias gariepinus* (Burchell). J. Appl. Sci. Environ. Manage., 6 : 37-41.

Shirgur, G. A. (1972). Development of indigenous Derris powder. Indian Fish. Assoc., 2: 35-39.

Sirivam, V.; Babu, M., Immanuel; G., Murugdass, S.; Citarasu, T. and Marian, M. P. (2004). Growth and immune response of juvenile greasy grouper (*Epinephelus tauvina*) fed with herbal antibacterial active ingredient supplemented diets against Vibrio infections. Aqua., 237 : 9–20.

Suterlin, A. M. (1974). Pollutants and chemicals of aquatic animals'perspective.Chem. Senses. Flavour., 1:167-178.

Trewavas, E. (1983). Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia*. Publication No. 898. British Museum of Natural History, London, UK., 583pp.

Wang, S. and Huffman, J. B. (1991). Botanochemicals: Supplements to petrochemicals. Eco. Bota., 35(4) : 369-382.

Welcomme, R. L. (1988). International introductions of inland aquatic species. FAO Fisheries Technical Paper., 294. 318 pp.