

TOXICOLOGICAL EFFECT AND BEHAVIORAL RESPONSE OF A PREDATORY STINGING CATFISH, *HETEROPNEUSTES FOSSILIS* EXPOSED TO THREE INDIGENOUS PLANT SEED EXTRACTS

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Abstract: For the removal of undesired fish species from aquaculture ponds, an alternative to synthetic toxins is the use of botanical toxicants which are biodegradable and environmentally safer. Toxicological effects 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 studied on the predatory fish, *Heteropneustes fossilis* (Bloch) under normal laboratory conditions. Depending upon the type of plants, extracts and concentrations, percentage mortality varied. Behavioral activities increased with increasing concentrations. During exposure, fish exhibited discolouration, gulping for air, erratic swimming, loss of reflex, slow movement and ultimately became motionless before death. The extent of toxicity of extracts for *J. carcus*, *H. wightianus* and *A. moluccana* on *H. fossilis* could be ranked in the order: Acetone > methanol > 50% ethyl alcohol > distilled water. On the basis of LC₅₀ values the most toxic was acetone extract of *J. carcus* seeds and least toxic was distilled water extract of *A. moluccana* seeds. It is suggested that these plant seed extracts would be helpful in aquaculture to remove unwanted fish species from culture ponds.

Key words: Toxicological effect, *Heteropneustes fossilis*, plant extracts, mortality, lethal concentrations.

INTRODUCTION

Predatory fish are those that prey upon other fish or aquatic animals. Frequently introduced, these fish can ruin a pond or lake environment. Problems associated with the stocking of undesirable fishes may include under

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population of desirable fish species, habitat destruction, competition for food and nesting space, and the small size attained by the introduced fish. The use of piscicide as a tool in pond management during pond preparation to get rid of predators before fish stocking is important. Ideally, ponds should be sundried and the pond bottom crack dried to help to get rid of fish predators. Moreover, farmers who are always in a hurry to prepare their ponds always resort to the use of inorganic/chemical fish toxicants which are deleterious for the environment. Derris root, tea seed cake, camellia seed cake and powdered croton seeds are used as botanical piscicides. The toxic parts of plants employed as fish poisons can include roots, seeds, fruits, bark, latex or leaves (Nasiruddin *et al.* 2006, 2009a, 2010a, 2012a).

The piscicidal potential and phytotoxic properties of different parts of plant extracts have been reported by several researchers namely Ayotunde and Benedict (2008), Ayoola (2011), Akinduyite and Oyedapo (2011), Ayoola *et al.* (2011), Ayotunde *et al.* (2011), Fafioye (2012), Kumar *et al.* (2012), Adesina *et al.* (2013), Okey *et al.* (2013), Orji *et al.* (2014), Oshimagye *et al.* (2014), Ablaka and Auta (2015), Kishore *et al.* (2016) and Suely *et al.* (2016). In Bangladesh, Latifa *et al.* (2002, 2004), Latifa and Parvin (2006), Nasiruddin and Sultana (2007), Nasiruddin *et al.* (2006, 2009a, b, 2010a, b, 2011, 2012a, b, 2014), Ara *et al.* (2012) and Chowdhury *et al.* (2014) have reported the toxic effect of different plant part extracts on such fish.

The test organism is an Asian stinging catfish, *Heteropneustes fossilis*, is a species of air breathing catfish. It is found mainly in ponds, ditches, swamps, and marshes, but sometimes occurs in muddy rivers. It can tolerate slightly brackish water habitat. It is omnivorous. This species breeds in confined waters during the monsoon months, but can breed in ponds, derelict ponds, and ditches when sufficient rain water accumulates. It has a great demand due to its medicinal value.

To overcome the hazardous effects of organic pesticides, recent emphasis is on the use of natural pesticides, which are usually of plant origin. Some plants contain compounds of various classes that have insecticidal and piscicidal properties (Wang and Huffmann 1991). Unlike synthetic chemical pesticides, which leave harmful residues in the aquatic environment (Koesomadinata 1980), botanical pesticides are said to be more environmentally friendly because they are easily biodegraded and leave no residues in the environment.

In view of these considerations, the present study was undertaken to study the toxicological effect of three plant seed extracts, *Jatropha carcus*, *Hydnocarpus wightianus* and *Aleurites moluccana* on predatory fish, *Heteropneustes fossilis*. Their behavioral response was also observed in the present study during

bioassay to assess the toxicity of plant seed extracts. The aim was to develop an effective anesthetic from indigenous plant material that will be available at low cost to aqua culturists and which would be non-toxic to the fish and consumers.

MATERIAL AND METHODS

Sample collection and preparation: The toxicants were obtained from the dry seeds (Fig.1) of the plant *Jatropha carcus* (Family: Euphorbiaceae), *Hydnocarpus wightianus* (Family: Achariaceae) and *Aleurites moluccana* (Family: Euphorbiaceae) to carry out the experimental study. These seeds collected from the local area and local seed markets of Chittagong, K.C. Dey Road (22.337965, 91.835605) and Narsingdi Big Bazar (22.4780772, 91.7846100) were cleaned and dried under diffused sunlight for 10-15 days prior to stocking. The seeds were kept in airtight glass bottles in a cool place in the laboratory, so that these seeds remained dry and could be easily grinded for making extracts for experimental purpose.



Seeds of *Jatropha carcus*

Seeds of *Hydnocarpus wightianus*

Seeds of *Aleurites moluccana*

Fig. 1: Dry seeds of *Jatropha carcus*, *Hydnocarpus wightianus* and *Aleurites moluccana*.

The experimental healthy and live fish, *Heteropneustes fossilis* were collected from local fish markets of Chittagong city on the day of the experiment. Then they were acclimatized in a glass aquarium (Fig. 2) for 3-4 hours in laboratory condition. For the final experiment, only the healthy and mobile fishes were selected. During the experiment, the seeds were crushed into fine powder firstly in a mortar with a pestle and then grinded in an electric blender.



Fig. 2: Acclimatization of *Heteropneustes fossilis* in the laboratory.

The experimental seed powder was sieved through a sieve of 0.0025 cm² mesh size and was extracted with distilled water, 50% ethyl alcohol, methanol and acetone solvents separately. For experimental purposes the required amount of seed powder was weighed and mixed with relevant amount of the solvent in a glass Stoppard conical flask and kept on a magnetic stirrer for 3-4 hours. After extraction, the resultant liquid was filtered through filter paper (Whatman No. 1) and the different test doses were prepared from this stock solution. Desired concentrations of different test solutions were obtained by appropriate dilution of the stock solution (APHA 1995) which was prepared on the day of the experiment.

Procedure of bioassays: Experiments (Fig. 3) were done to determine 1-99% mortality of the liquid extracts obtained from the dry seed powder of *J. carcus*, *H. wightianus* and *A. moluccana* against the test fish *H. fossilis*. A set of glass aquarium (30 x 23 x 23 cm³) were used for the bioassays. Each of the aquarium contained 5L of tap water, toxicant and five test fish. The different proportions of seed extracts were added to obtain the required concentrations, so as to determine the LC₅₀ values of the seed extracts for the experimental fish. Five concentrations of each extract in triplicates were used in the final experiments after preliminary screening. A set of five healthy and active fishes were then released in each concentration randomly. The test fishes were kept in exposure to the toxicated water for 24±2 hours at room temperature (29±2°C) and in diffused light. A control set was maintained in each experiment which contained 5L of water and same number of fish. 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 was recorded only of those fishes which were killed within 24 hours after exposure to the various concentrations of the toxicants.

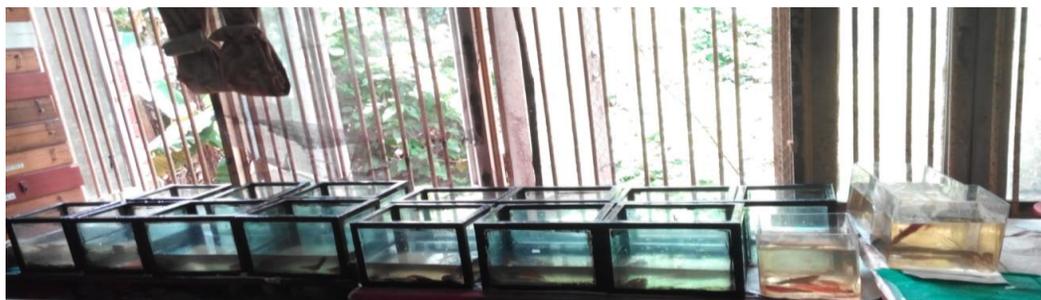


Fig. 3: Experimental set of test of seed extracts.

Statistical analysis: To determine the LC_{50} values of the dry seed extracts on the experimental fishes, probit analysis was done. The values of empirical probit, working probit and weighting probit were taken from the tables of Finney (1971). The regression equation was calculated from empirical probit, working probit and weighting probit. Values of LC_{50} with 95% confidence intervals were analyzed in a computer based probit analysis programme. The value of chi-square (χ^2) test was determined (Fisher and Yates 1963) and compared with tables of the statistics for (n-1) degrees of freedom at 1% and 5% level of significance. Analysis of variance of percentage mortality of fishes was made to estimate the variation among treatments and replicates at 0.01 and 0.05 levels of significance. Toxicity values or relative potency values were calculated on the basis of potency which is a reciprocal of the highest concentration. The relative potency of the toxicants was obtained by taking the highest LC_{50} values as unit and dividing the highest LC_{50} value of a toxicant with the respective LC_{50} value of other toxicants.

RESULTS AND DISCUSSION

Effect of the three seed extracts on behavior of H. fossilis: The differences in the behavioral changes with the seed extracts of *J. carcus*, *H. wightianus* and *A. moluccana* were observed in *H. fossilis*. With the acetone extract the affected fishes showed restlessness with erratic swimming from the very beginning with frequent gulping of air. Reddish colour was seen in the opercular and gill region in the dead fishes, indicating internal hemorrhage in the gills, as the acetone extract was the highly toxic extract. With the methanol extract huge slime secretion was seen. Most of the dead fishes floated upwards, lying parallel to the water surface of the aquaria. The characteristics seen in the fishes with other less toxic extracts were mainly that they showed erratic and abnormal behavior after a long time. The dead fishes floated at different angles as 45°, 60° and 90° or lying at the bottom of the aquarium with large and round mouth opening, damaged barbules, straight opercular fins, pale

skin and bulging eyes (Fig. 4). Hence with respect to behavior the toxicity of the four extracts could be ranked as: Acetone > Methanol > 50% ethyl alcohol > distilled water. According to toxicity of seeds, *J. carcus* seed extracts were the most toxic extract showing abnormalities, such as irregular swimming, moving up and down throughout the water, settling down at the corner or bottom of the aquaria, slowly gulping for air, inactivity, slow upward movement, becoming balance less, inactive and finally died within 24 hours.



Fig. 4: Behavioral responses of *H. fossilis* treated with experimental plant seed extracts.

Effects of extracts on mortality: The dose ranges, mortality ranges, regression equations of the slope lines, chi-square and ANOVA values and their significance at 0.01 and 0.05 levels, and LC₅₀ values of the three experimental plant seed extracts on *H. fossilis* are given in Table 1. Doses with *J. carcus* seed extracts ranged from 3-85 ppm, with *H. wightianus* seed extracts from 50-2000 ppm and with *A. moluccana* seed extracts from 550-2500 ppm. Mortality of the experimental fishes ranged from 6.67-93.33%. Chi-square values at 0.01 and 0.05 levels of significance showed that most of the extracts had insignificant values except 50% ethyl alcohol extract of *J. carcus* and *A. moluccana* seeds, and methanol extracts of *H. wightianus* and *A. moluccana* seeds. ANOVA test showed

that the treatment values were significant with all the extracts at 0.01 and 0.05 levels excepting methanol extract of *H. wightianus* seed at 0.01 level of significance. The replicate values were insignificant with all the extracts at 0.01 and 0.05 levels. The LC₅₀ values showed that amongst all the experimental seed extracts, acetone extract of *J. carcus* seeds was the most toxic extract and distilled water extract of *A. moluccana* seeds was the least toxic extract. From Table 2 it is observed that the lowest LC₅₀ value was found with acetone extract of *J. carcus* seeds and it was the most toxic extract having the LC₅₀ value 6.865 ppm and high relative potency value 217.05. The highest LC₅₀ value was observed with distilled water extract of *A. moluccana* seeds. Its LC₅₀ value was 1490.056 ppm and relative potency value was 1.00. Hence, it was the least toxic extract. Distilled water, 50% ethyl alcohol and methanol extracts of *J. carcus* seeds with LC₅₀ values of 60.084 ppm, 23.627 ppm, 16.015 ppm and with good relative potency values of 24.80, 63.07 and 93.04 respectively were also highly toxic; acetone and methanol extracts of *H. wightianus* seeds with LC₅₀ values of 291.749 ppm and 439.860 ppm and with relative potency values 5.11 and 3.39 respectively were toxic; fifty percent ethyl alcohol extract of *H. wightianus*, and acetone extract of *A. moluccana* seeds with LC₅₀ values of 895.912 ppm, and 906.998 ppm with relative potency values of 1.66 and 1.64 were medium toxic extracts; methanol extract of *A. moluccana* seeds with LC₅₀ of 1018.185 and relative potency of 1.46 was fairly toxic; fifty percent ethyl alcohol extract of *A. moluccana* seeds and distilled water extract of *H. wightianus* seeds with LC₅₀ of 1274.335 ppm, and 1396.726 ppm and with relative potency values of 1.17 and 1.07 were less toxic extracts. On the basis of their relative potency values, i.e. toxicities, the relative position of the extracts for *H. fossilis* were as follows:

Acetone extract of *J. carcus* seeds > Methanol extract of *J. carcus* seeds > 50% ethyl alcohol extract of *J. carcus* seeds > Distilled water extract of *J. carcus* seeds > Acetone extract of *H. wightianus* seeds > Acetone extract of *A. moluccana* seeds > Methanol extract of *H. wightianus* seeds > Methanol extract of *A. moluccana* seeds > 50% ethyl alcohol extract of *H. wightianus* seeds > 50% ethyl alcohol extract of *A. moluccana* seeds > Distilled water extract of *H. wightianus* seeds > Distilled water extract of *A. moluccana* seeds.

There are a good number of herbs, shrubs and indigenous plants in Bangladesh which have piscicidal property. The present investigation on indigenous plants is to explore the piscicidal activities of seeds of three indigenous plants *Jatropha carcus* (Bengali Jamal gota), *Hydnocarpus wightianus* (Bengali Chalmogra) and *Aleurites moluccana* (Bengali Kukui nut)

Table 1: Values of toxicity parameters of dry seed extracts of *Jatropha curcas*, *Hydnocarpus wightianus* and *Aleurites moluccana* on *H. fossilis*

Plant seed	Solvent	Dose range	Mortality range	Regression equation	χ^2 value	P-value (χ^2)	ANOVA value (Treat.)	P value ANOVA	ANOVA Value (Replicate)	P value ANOVA (Replicate)	LC ₅₀ (ppm)
<i>J. Curcas</i>	Distilled Water	45-85	13.33-86.67	7.116x-0.630	4.34	P>0.01 P>0.05	20.94	P<0.01 P<0.05	0.706	P>0.01 P>0.05	60.084
	50% ethyl alcohol	10-50	20.00-93.33	3.06x+0.791	16.57	P<0.01 P<0.05	20.74	P<0.01 P<0.05	0.211	P>0.01 P>0.05	23.627
	Methanol	10-30	20.00-93.33	4.670x-0.630	2.58	P>0.01 P>0.05	57.14	P<0.01 P<0.05	1.714	P>0.01 P>0.05	16.015
	Acetone	3-15	13.33-93.33	3.77x+1.830	4.69	P>0.01 P>0.05	30.25	P<0.01 P<0.05	2.250	P>0.01 P>0.05	6.865
	Distilled Water	750-2000	6.67-93.33	5.055x-0.86	4.83	P>0.01 P>0.05	29.41	P<0.01 P<0.05	0.706	P>0.01 P>0.05	1396.726
<i>H. wightianus</i>	50% ethyl alcohol	500-1500	13.33-93.33	5.121x-10.14	5.52	P>0.01 P>0.05	18.75	P<0.01 P<0.05	0.167	P>0.01 P>0.05	895.912
	Methanol	100-1000	6.67-93.33	2.670x-2.028	13.19	P>0.01 P<0.05	6.15	P<0.01 P<0.05	0.431	P>0.01 P>0.05	439.860
	Acetone	50-750	6.67-93.33	2.372x-0.823	7.83	P>0.01 P>0.05	40.43	P<0.01 P<0.05	0.286	P>0.01 P>0.05	291.749
<i>A. moluccana</i>	Distilled Water	750-2500	6.67-93.33	4.169x-8.129	8.92	P>0.01 P>0.05	43.00	P<0.01 P<0.05	4.33	P>0.01 P>0.05	1490.056
	50% ethyl alcohol	700-2000	13.33-93.33	4.870x-10.11	20.12	P<0.01 P<0.05	20.64	P<0.01 P<0.05	0.545	P>0.01 P>0.05	1274.335
	Methanol	650-1500	6.67-93.33	5.044x-10.17	19.01	P<0.01 P<0.05	45.50	P<0.01 P<0.05	4.33	P>0.01 P>0.05	1018.185
	Acetone	550-1250	6.67-93.33	6.857x-15.27	8.79	P>0.01 P>0.05	23.36	P<0.01 P<0.05	0.286	P>0.01 P>0.05	906.998

Table 2: LC₅₀ and Relative potency values with categories of distilled water, 50% ethyl alcohol, methanol and acetone extracts of *J. curcas*, *H. wightianus* and *A. moluccana* seeds on *H. fossilis*

Plant seed	Extract	LC ₅₀ (ppm)	Relative potency	Category
<i>Jatropha curcas</i>	Distilled water	60.084	24.80	Highly toxic
	50% ethyl alcohol	23.627	63.07	Highly toxic
	Methanol	16.015	93.04	Highly toxic
	Acetone	6.865	217.05	Most toxic
<i>Hydnocarpus wightianus</i>	Distilled water	1396.726	1.07	Less toxic
	50% ethyl alcohol	895.912	1.66	Medium toxic
	Methanol	439.860	3.39	Toxic
	Acetone	291.749	5.11	Toxic
<i>Aleurites moluccana</i>	Distilled water	1490.056	1.00	Least toxic
	50% ethyl alcohol	1274.335	1.17	Less toxic
	Methanol	1018.185	1.46	Fairly toxic
	Acetone	906.998	1.64	Medium toxic

with different solvents such as distilled water, 50% ethyl alcohol, methanol and acetone extracts were determined against predatory fish *Heteropneustes fossilis* as the test fish.

All the extracts were more or less toxic against the dose concentrations. The solubilizing capacity of the solvents for the three experimental seeds on *H. fossilis* on the basis of experimental analysis could be ranked in the order: Acetone > Methanol > 50% ethyl alcohol > Distilled water extracts. On the basis of ranking, it can be explained as that the plant components were mostly soluble in acetone, followed by methanol, then 50% ethyl alcohol and less soluble in distilled water which agrees with strong solubility in absolute ethyl alcohol,

slight solubility in 50% ethyl alcohol and almost insoluble in distilled water of finding of Ameen *et al.* (1983). All the seed extracts showed to be more or less toxic with variations of concentrations.

Various reactions were shown by the test fishes with special regards to their movement. Mortality of fishes was dependant on concentrations in all the extracts with all the three seed extracts. The fishes showed various abnormalities in their behavior within about 24 hours exposure to the concentrations. They displayed restlessness, frequent surfacing, irregular swimming, jerky movements, gulping air and extensions of fins and barbels. Erratic movements were also shown by the affected fish with gradual loss of balance and slime secretion, followed by death. Ultimately they died with large and wide mouth opening and with straight and spreaded fins. Faded colour and damaged gills, fins and barbels were also observed in the dead fishes. Some dead fishes were found floating at the surface of the aquarium with ventral side upward and some other fishes were floating at different angles. In some cases blood was seen around from the mouth of the fish. These findings are in agreement with the findings of Latifa *et al.* (1997), Latifa and Begum (1993) and Nasiruddin *et al.* (2006, 2011, 2012a, b, 2014). With respect to behavior in *H. fossilis*, toxicity of the four extracts was in the order: Acetone > Methanol > 50% ethyl alcohol > Distilled water extracts. With respect to seeds, the toxicity of the three seeds was in the order: *J. carcus* > *H. wightianus* > *A. moluccana* seeds. The LC₅₀ values also showed the trend of toxicity in the three seed extracts being ranked in the order: Acetone > Methanol > 50% ethyl alcohol > Distilled water.

From the present study it was observed that amongst all the seed extracts the highest toxic extract was acetone extract of *J. carcus* seeds with concentration ranges between 3-15 ppm and with LC₅₀ value 6.865 ppm whereas the least toxic extract was the distilled water extract of *A. moluccana* seeds at concentration ranges 750-2500 ppm with LC₅₀ value 1490.056 ppm.

On the basis of LC₅₀ values of distilled water extracts, the LC₅₀ value of *J. carcus* seeds (60.084 ppm) showed close similarity with LC₅₀ value of *Albizia procera* seeds (74.79 ppm) of Chowdhury *et al.* (2014), whilst LC₅₀ values of *H. wightianus* seeds (1396.726 ppm) and *A. moluccana* seeds (1490.056 ppm) showed similarity with LC₅₀ value of *Phyllanthus emblica* seeds (1488.60 ppm) of Nasiruddin *et al.* (2014).

On the basis of LC₅₀ values of 50% ethyl alcohol extracts, the LC₅₀ value of *J. carcus* seeds (23.627 ppm) was very close to the LC₅₀ of *Albizia procera* seeds (24.81 ppm) of Nasiruddin *et al.* (1998); LC₅₀ value of *H. wightianus* seeds (895.912 ppm) was almost similar to LC₅₀ value of *P. emblica* seeds (890.98 ppm) of Nasiruddin *et al.* (2014); and LC₅₀ values of *A. moluccana* seeds (1274.335

ppm) was more or less close to the LC₅₀ value of *Terminalia bellerica* seeds (1171.07 ppm) of Nasiruddin *et al.* (2014), *Datura innoxia* seeds (1337.73 ppm) and *Acacia catechue* seeds (1341.99 ppm) of Nasiruddin *et al.* (2014).

On the basis of LC₅₀ values of methanol extracts, the LC₅₀ value of *H. wightianus* seeds (439.860 ppm) was close to that of *P. emblica* seed extract (489.37 ppm) of Nasiruddin *et al.* (2014) and LC₅₀ value of *A. moluccana* seeds (906.998 ppm) was somewhat near to LC₅₀ value of *T. bellerica* seeds (777.43 ppm) of Nasiruddin *et al.* (2014). Whilst LC₅₀ value of acetone extract of *H. wightianus* seeds (291.749 ppm) was somewhat close to the LC₅₀ value of acetone extract of *Terminalia chebula* (247.749 ppm) seeds of Nasiruddin *et al.* (2014).

Chi-square values at 0.05 and 0.01 levels of significance showed that most of the extracts had insignificant values except 50% ethyl alcohol extract of *J. carcus* and *A. moluccana* seeds, and methanol extracts of *H. wightianus* and *A. moluccana* seeds which indicated a good relationship between the observed and expected mortalities. ANOVA test showed that the treatment values were significant with all the extracts at 0.01 and 0.05 levels except methanol extract of *H. wightianus* seeds at 0.0 level of significance. The replicate values were insignificant with all the seed extracts at 0.01 and 0.05 levels, hence, giving an indication of a good relationship between the concentrations applied and mortalities obtained.

It was apparent from the present study that the potentiality of different extracts like distilled water, 50% ethyl alcohol, methanol and acetone extracts of *J. carcus*, *H. wightianus* and *A. moluccana* seeds appeared to be promising in different concentration levels, and as such, they might be effective for controlling and preventing the undesirable or unwanted predatory fish species in different ponds such as nursery, rearing and stocking ponds of commercially valuable fish species. It is revealed that, various information about the efficiency of the plant toxins can be obtained from the laboratory based toxicity studies of crude dry powders. Therefore, for developing the effects of these plant seed powder on ecological systems, toxicity data of the present study is essential.

LITERATURE CITED

- ABALAKA, S.E. and AUTA, J. 2015. Toxic effect of aqueous and ethanolic extracts of *Parkia biglobosa* pods on *Clarias gariepinus* adult. *World Journal of Biological Research* **3**(1): 9-17.
- ADESINA, B.T., OMITOYIN, B.O., AJANI, E.K. and ADESINA, O.A. 2013. Acute-lethal toxicity (LC₅₀) 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.

- AKINDUYITE, I. and OYEDAPO, F. 2011. Acute toxicity of aqueous *Moringa lucida* leaf extracts to Nile Tilapia, *O. niloticus* (Linnaeus 1857). Proceedings of the Ninth International Symposium on Tilapia in Aquaculture, Shanghai, China. April 22nd-24th: 52-59.
- AMEEN, M., SHIREEN, K.F., RAHMAN, P.K.M. and AHMED, M.U. 1983. Insecticidal properties of *D. elliptica* (Wall) roots against the larva of *Culex fatigans* (Diptera: Culicidae). *Dhaka University Studies Part E* **2**(2): 71-77.
- APHA (American Public Health Association). 1995. *Standard methods for the examination of water and waste water*. 16th edition American Public Health Association Inc. New York.
- ARA, S.I., NASIRUDDIN, M. and HOSSAIN, A. 2012. Toxicity of leaf, bark and fruit extracts of *Tabernaemontana divaricata* (L.) against *Heteropneustes fossilis* (Bloch). *Bangladesh J. Environ. Sci.* **23**: 39-44.
- 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.
- AYOTUNDE, E.O., OFFEM, B.O. and BEKEH, A.F. 2011. Toxicity of *Carica papaya* L: Haematological and Piscicidal effects on adult catfish (*Clarias gariepinus*). *J. Fish. Aquat. Sci.* **6**: 291-308.
- CHOWDHURY, M.R., NASIRUDDIN, M. and AZADI, M.A. 2014. Piscicidal effects of three plant seed extracts on two predatory fish, *Heteropneustes fossilis* (Bloch) and *Channa punctatus* (Bloch). *Int. J. Int. Sci. Ins. Tech. Sec. B* **3**: 1-07.
- 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. 333pp.
- 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.
- KISHORE, D., BISWAS, S.J., and KARMAKAR, S.R. 2016. Study on the toxicity of neem (*Azadirachta indica* A. Juss) leaf extracts as phytopiscicide 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 RSV. 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.
- KUMAR, A., PRASAD, M., MISHRA, D., SRIVASTAV, S.K. and SRIVASTAV, A.K. 2012. Acute toxicity of Azadirachtin to a teleost, *Heteropneustes fossilis*. *Maringa* **34**(2): 213-216.
- LATIFA, G.A. and PARVIN, S.I. 2006. Piscicidal activity of dry root of *Nerium oleander* (Linn.) on *Heteropneustes fossilis* (Bloch). *Bangladesh J. Zool.* **34**(1): 155-158.
- LATIFA, G.A., BEGUM, S., AKHTER, A. and AHMED, M.S. 1997. Piscicidal properties of the dry barks of *Azadirachta indica* (A. juss) on *Heteropneustes fossilis* (Bloch). *Bang. J. Life. Sci.* **9**(2): 31-36.
- LATIFA, G.A., HAMID, A. and SHARMA, G. 2002. Piscicidal activity of dry bark of *Dyospyros ebenum* (Koen) on *Heteropneustes fossilis* (Bloch) and *Anabas testudineus* (Bloch). *Bang. J. Life. Sci.* **14**(1&2): 107-118.
- LATIFA, G.A., BEGUM, M.T. and BASCHAR, S.C. 2004. Piscicidal property of dry bark of *Leucaena leucaena* (Lam. De Wit) on *Channa punctatus* (Bloch) and *Channa striatus* (Bloch). *Bangladesh J. Zool.* **32**(2): 247-251.
- NASIRUDDIN, M. and SULTANA, N. 2007. Toxicological effect of seed extracts of four medicinal plants on *Heteropneustes fossilis* (Bloch)). *The Chittagong Univ. J. Biol. Sci.* **2**(1&2): 43-54.
- NASIRUDDIN, M., AZADI, M.A. and CHOWDHURY, R. 1998. Piscicidal effects of seed and seed kernel extracts of four indigenous plants on *Heteropneustes fossilis* (Bloch) and *Anabas testudineus* (Bloch). *Chittagong university Journal of Science* **22**(11): 1-10.
- NASIRUDDIN, M., AZADI, M.A., CHOWDHURY, R. and SULTANA, M.N. 2006. Studies on the piscicidal properties of *Azadirachta indica* (A. Juss) and *Barringtonia acutangula* (Gaertn) plant parts on *Heteropneustes fossilis* (Bloch). *Bangladesh J. Zool.* **34**(1): 95-104.
- NASIRUDDIN, M., AZADI, M.A. and RAHMAN, I.A.S. 2009a. Toxicological effects of *Accacia auriculaeformis* (A. Cunn. Ex. Benth.) and *Mesua ferrea* (Linn.) plant parts on *Heteropneustes fossilis* (Bloch). *Bangladesh J. Zool.* **37**(1): 103-112.
- NASIRUDDIN, M., AZADI, M.A. and AKHTER, R. 2009b. Toxic response of *Heteropneustes fossilis* (Bloch) to some indigenous plant seed extracts. *The Chittagong Univ. J. Biol. Sci.* **4**(1 & 2): 01-12.
- NASIRUDDIN, M., AZADI, M.A. and ASHRAFUNNESA. 2010a. Toxicity of *Madhuca indica* Gmel plant part extracts and its effects on haematological parameters of *Heteropneustes fossilis* (Bloch). *Bangladesh J. Zool.* **38**(2): 231-242.
- NASIRUDDIN, M., AZADI, M.A. and PARVIN, F.A.N. 2010b. Toxicological and Haemolytic effects of some plant seed extracts on *Heteropneustes fossilis* (Bloch). *J. Asiatic Soc. Bangladesh Sci.* **36**(1): 25-35.
- NASIRUDDIN, M., AZADI, M.A., RAHMAN, T. and ASHRAFUNNESA. 2011. Toxic response of three predatory fishes to four indigenous plant seed extracts. *The Chittagong Univ. J. Biol. Sci.* **6**(1&2): 27-39.

- NASIRUDDIN, M., AZADI, M.A. and CHAKMA, D. 2012a. Toxicological properties of *Achras zopota* (Linn.) plant parts on the predatory fishes *Heteropneustes fossilis* (Bloch) and *Channa punctatus* (Bloch). *Bangladesh J. Zool.* **40**(1): 109-119.
- NASIRUDDIN, M., AZADI, M.A. and NELY, M.S. 2012b. Piscicidal effects of extracts of karnja plant *Pongamia pinnata* (L.) Pierre and vat plant *Clerodendrum viscosum* (Vent.) on singhi fish *Heteropneustes fossilis* (Bloch). *The Chittagong Univ. J. B. Sci.* **7**(1&2): 65-78.
- 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 *reochromis niloticus* (L.). *Bangladesh J. Environ. Sci.* **26**: 103-110.
- OKEY, I.B., KEREMAH, R.I., IKPI, G.U. and EBENIRO, L.A. 2013. Toxicity of leaf powder of *Lepidagathas alopecuroides* to Nile tilapia, *Oreochromis niloticus* juveniles. *African Journal of Food Science* **7**(6): 128-133.
- OSHIMAGYE, M.I., AYUBA, V.O. and ANNUNE, P.A. 2014. Toxicity of aqueous extracts of *Parkia biglobosa* pods on *Clarias gariepinus* (Burchell 1822) juveniles. *Nigerian Journal of Fisheries Aquaculture* **2**(2): 24-29.
- ORJI, O.U., IBIAM, U.A. and AJA, P.M. 2014. Acute toxicity studies of the lyophilized aqueous extract of *Psychotria microphylla* leaf on *Clarias gariepinus* juveniles. *International Journal of Biology and Biological Sciences* **3**(4): 38-44.
- SUELY, A., ZABED, H., AHMED, A.B.A., MOHAMMAD, J., NASIRUDDIN, M., SAHU, S.N. and GANESAN, P. 2016. Toxicological and haematological effect of *Terminalia arjuna* bark extract on a freshwater catfish, *Heteropneustes fossilis*. *Fish physiol. Biochem* **42**: 431-444.
- WANG, S. and HUFFMAN, J.B. 1991. Botanochemicals: Supplement to petrochemicals. *Econ. Bot.* **35**: 369-382.

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