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TOXIC RESPONSE OF *HETEROPNEUSTES FOSSILIS* (BLOCH) TO SOME INDIGENOUS PLANT SEED EXTRACTS

MUNIRA NASIRUDDIN[1], MONIKA RAHMAN AND ROZINA AKHTER
Department of Zoology, University of Chittagong, Chittagong- 4331, Bangladesh

ABSTRACT

Toxic responses of distilled water, 50% ethyl and absolute ethyl alcohol extracts of six indigenous plants, *Datura innoxia* (Mill), *Clerodendrum viscosum* (Vent), *Amoora rohituka* (Roxb) Wt. et. Arnott, *Acacia auriculaeformis* A. Cunn. ex. Benth., *Pongamia pinnata* (L) Pierre and *Acacia catechu* Willd were studied on a predatory fish *Heteropneustes fossilis* (Bloch) in laboratory conditions. Amongst the extracts the toxicity range varied, absolute ethyl alcohol extract of *A. rohituka* seeds being the most toxic (LC_{50} = 8.67 ppm) whilst distilled water extract of *D. innoxia* seeds was the least toxic (LC_{50} = 1950.03 ppm). On the basis of LC_{50} and relative potency values the relative toxicity of the six plants was in the order- *A. rohituka* > *C. viscosum* > *P. pinnata* > *A. auriculaeformis* > *A. catechu* > *D. innoxia* seed extracts. These six indigenous plant seed extracts assayed might be helpful as controlling agent of undesirable fishes in fish culture ponds.

Key words: Toxicity, Plant toxins, Behaviour, Mortality, *H. fossilis*

INTRODUCTION

Eradication of predatory fishes is an essential pre-requisite for scientific management of perennial nursery and stocking ponds. Indiscriminate use of synthetic pesticides pollute the aquatic environment affecting aquatic fauna, mainly fishes. Age old, traditionally screened botanical control agents have been used to remove undesirable fishes from the freshwater environments. For total or near total eradication of harmful fishes, a wide range of piscicides derived from plants is employed. Such plant poisons are the safe substitutes as they possess the requisite properties of high potency, easy decomposability and unabated supply.

The plant preparations such as the Derris root powder (Shirgur 1972), Nicotine (Konar 1970, 1977), Rotenone (Haque and Tilton 1970), and Antimycin (Lennon and Berger 1970) have potentiality as fish poison. Powder from the roots

Corresponding author email: maazadi@yahoo.com

of *Balanites roxburgii*, seeds of *Randia dumertorum*, twigs and roots of *Derris elliptica* and bark of *Albizzia lebbeck* also have piscicidal effects (Shirgur 1975).

In Bangladesh, a good numbers of herbs, shrubs and plants have piscicidal properties (Ameen and Shahjahan 1987, Ameen *et al*. 1987, Latifa *et al*. 1987, 1988, 1992, 1997, 2002, 2004, Latifa and Begum 1993 and Nasiruddin *et al*. 1997, 1998, 2006). There is need for further research to screen fish toxicants which are safe and useful in aquaculture. In the present study attempts were made to find out whether some of the local plants could be used as effective fish poison. The present study is also concerned with the comparative assaying of extracts of six indigenous plant seeds i.e. *Datura innoxia* (Mill), *Clerodendrum viscosum* (Vent), *Amoora rohituka* (Roxb) Wt. et. Arnott, *Acacia auriculaeformis* A. Cunn. ex Benth, *Pongamia pinnata* (L) Pierre and *Acacia catechu* Willd on a predatory fish, *Heteropneustes fossilis* (Bloch).

MATERIALS AND METHODS

Toxic effects of the plant extracts of six indigenous plant seeds of *D. innoxia* (Sada dhutra), *C. viscosum* (Vat), *A. rohituka* (Pitraj), *A. auricalaeformis* (Akashmoni), *P. pinnata* (Karinja) and *A. catechu* (Khair) were determined on *H. fossilis*. Every set of predatory fishes (n = 60) were collected from city markets of Chittagong city. In the laboratory the fishes were stocked in glass aquarium (60x30x30 cm) containing tap water and were acclimatized for 4-5 hours. Active and healthy fishes were used for the experiments. The average total length and weight of the fishes were 12.50±0.21 cm and 8.69±0.97 g respectively.

Dry seeds were weighed in a semi automatic table balance and finely grinded in a grinder. Ten g of seed powder was mixed in 100 ml of either of distilled water, 50% ethyl or absolute ethyl alcohol solvents in a conical flask which was shaken vigorously in a magnetic stirrer for 4-5 hours to allow maximum extraction of the toxic components. The extract was filtered through fine muslin cloth and the filtrate was the stock solution prepared on the day of the experiment. The different test concentrations recorded in terms of ppm were calculated from the stock solution by dilution (APHA 1976) such that the total volume in the aquarium was five liters in each of the replicates.

Each final experiment was performed in the laboratory to determine 10-90% mortality in the liquid seed extracts of the six plants against *H. fossilis*. Five concentrations of each extract were bioassayed, each concentration was replicated twice (Latifa *et al*. 2002) and the test fishes were kept for 24 h. test exposure

period. In each experiment a control set was similarly maintained. After the release of the fishes behaviour pattern in the highest concentrations was observed. Mortality was counted on those fishes which were killed within 24 h of exposure at various concentrations of the toxicants.

Mortality data were subjected to probit analysis (Finney 1971) which was used to determine LC_{50} values of each extract upon the fishes. The values of chi-square were compared with tables of statistics for n-1 degrees of freedom at 0.05 level of significance. Analysis of variance of percentage mortality of fishes was made to estimate the variation among treatments at 0.01 level of significance. Relative potency of the equitoxic toxicants was calculated by taking the highest LC_{50} value of a toxicant as unit and comparing the respective LC_{50} values of the other toxicants in the study.

RESULT AND DISCUSSION

Behaviour of control fishes

The fishes in the control sets showed normal behaviour throughout the experiment. They remained healthy and active with vigorous movement. The fishes swam gently by regular opening of their operculum, with normal barbels and soft fins. All the fishes were physically well-balanced. No control mortality was noticed.

Effects of the seed extracts on the behaviour of the fishes

When the fishes were exposed to *D. innoxia* seed extracts, they showed reaction in abnormality within half an hour. They showed up and down movements and regular surfacing for taking air. Gradually their movement slowed down and settled to the bottom. Dead fishes were slimy and pale in colour with stiffened body, straight fins and wavy barbels. Some of the dead fishes floated roughly at 45^{o} angle to the water surface but most laid at the bottom.

With *C. viscosum* seed extracts, the first visible reaction started within 5 minutes after exposure to the toxicant. The fishes moved vigorously and were very restless. Opercular movements increased and were occasionally surfacing. After an hour their movement became sluggish, lost equilibrium, settled to the bottom and died at different intervals. Profuse pale yellowish slime was secreted. Fins were broken and with droopy barbels. Some of the dead fishes were seen floating parallel to the water surface and some were seen floating at various angles.

On exposure to *A. rohituka* seed extracts, the fishes showed immediate reaction. They swam erratically with rapid opercular movement and were found gulping for air. As a result most of the fishes were seen near the surface. The fishes became paralyzed within an hour and finally dropped to the bottom. Most of the time they remained immutable. The dead fishes were found floating at the surface and some remained at an angle of 90^{o}. Skin was pale and slime secretion was thick and yellowish. A mucous layer was also seen on the water surface.

The first visible reaction started within 25-30 minutes after exposure to the extracts of *A. auriculaeformis* seeds. The fishes rapidly moved up and down. After sometimes their movement slowed down and settled to the bottom and died at different intervals. Dead fishes showed less slime secretion, with slight mouth and opercular openings and skin showed signs of scar. Some of the fishes were seen in half floating condition and the rest remained at the bottom of the aquaria.

After exposure to the seed extracts of *P. pinnata*, the fishes showed vigorous erratic movements. At times they moved to the surface to gulp air. Fishes were stupefied within 30 minutes of exposure and gradually settled down. Dead fishes were seen with large mouth opening, wavy barbels and straight fins. Some of the dead fishes remained at the bottom with their ventral side directing upwards and few fishes were found floating at 90^{o} angle.

After releasing the fishes into the seed extracts of *A. catechu*, the fishes began to move up and down rapidly and were repeatedly raising towards the surface for taking air. Such movement continued for a while. Then the fishes failed to keep their balance, as a result their movement slowed down. At last they settled to the bottom and ultimately died at intervals. Slime secretion after death was seen. At times dead fishes were seen floating at an angle of 45^{o} but in most cases fishes sank down to the bottom of the aquaria.

On exposure to the toxicants, the fishes became restless with quick surfacing, agitated swimming and rapid opercular movement. Gradually they became balanceless, stupefied and died at various intervals with characteristic changes in their fins, barbels, mouth and skin. The characteristics observed in the dead and stiffened fishes were mainly mucous secretion and the nature of death as some fishes laid flat at the bottom of the aquaria, some were seen floating at angles of 45^{o} or 90^{o} or floating above and parallel to the water surface. Such behavioural pattern was also recorded by Latifa *et al*. 1987, 1988 and Nasiruddin *et al*. 1997, 1998, 2006. Excited swimming behaviour in *Salmo gairdnerii* was observed by Wedmeyer (1970). Agitated swimming behaviour of

Pseudapocryptes dentatus, *Gambusia affinis* and *Aphinis mento* was also observed by Sharma *et al*. (1978). Bennet and Dooley (1982) regarded mucous secretion as defensive and excretory response whereas increased opercular movement is associated with loss of efficiency in oxygen uptake and decreasing physiological demand (James 1990). However, an increase in the toxic or concentration level caused a decrease in induction time of total equilibrium in the species and behavioural aspects were pronounced in more toxic extracts.

Toxicological effects of the seed extracts on mortality of the fishes

The result of percentage mortality of *H. fossilis* was recorded at an interval of 24 h of exposure to different concentrations of the seed extracts. A particular concentration of an extract caused a particular percentage of mortality and accordingly the experimental concentrations showed 10-90% mortality (Table 1). Concentrations of the extracts varied with the plant toxicity. Probit analysis was used to determine the LC_{50} values with their confidence limits and chi-square and F- tests were done to determine the results of toxicity for each type of extracts (Table 2).

The relative toxicities of the seed extracts

The relative potency values of the seed extracts for *H. fossilis* are given in Table 3. Amongst the extracts absolute ethyl alcohol extract of *A. rohituka* seeds was the most toxic having LC_{50} value of 8.67 ppm and high relative potency value of 224.92. The least toxic extract was the distilled water extract of *D. innoxia* with a LC_{50} value of 1950.03 ppm and relative potency value of 1.00. Fifty percent and absolute ethyl alcohol extracts of *C. viscosum* and *P. pinnata* and distilled water and 50% ethyl alcohol extracts of *A. rohituka* seeds were toxic with good LC_{50} values of 96.28, 36.04, 91.76, 54.97, 43.90 and 18.38 ppm respectively. Distilled water extracts of *C. viscosum* and *P. pinnata* and 50% and absolute ethyl alcohol extracts of *A. auriculaeformis* seeds were moderately toxic having LC_{50} values of 128.04, 217.67, 449.24 and 231.98 ppm respectively. Absolute ethyl alcohol extracts of *D. innoxia* and *A. catechu* and distilled water extract of *A. auriculaeformis* seeds were fairly toxic with LC_{50} values of 799.80, 757.74 and 668.87 ppm respectively. Fifty percent ethyl alcohol extracts of *D. innoxia* and *A. catechu* seeds were less toxic (LC_{50} = 1337.73 and 1341.99 ppm) whilst distilled water extracts of the same seeds were the least toxic (LC_{50} = 1950.03 and 1645.80 ppm) (Table 3). On the basis of LC_{50} and relative potency values the relative position of the extracts are as follows:

TABLE 1. PERCENTAGE MORTALITIES IN *HETEROPNEUSTES FOSSILIS* AT CONCENTRATIONS OF THE DIFFERENT EXTRACTS OF *DATURA INNOXIA*, *CLERODENDRUM VISCOSUM*, *AMOORA ROHITUKA*, *ACACIA AURICULAEFORMIS*, *PONGAMIA PINNATA* AND *ACACIA CATECHU* SEEDS AFTER 24 HOURS EXPOSURE.

Plant seed	Extract	Concen-tration (ppm)	Mortality (%) of *H. fossilis*	Plant	Extract	Concen -tration (ppm)	Mortality (%) of *H. fossilis*
Datura innoxia	Distilled water	1000	20	*Acacia auriculaeformis*	Distilled water	250	10
		1500	30			500	40
		2000	50			750	50
		2500	60			1000	70
		3000	80			1250	80
	50% ethyl alcohol	500	10		50% ethylalcohol	125	20
		1000	40			250	30
		1500	50			500	50
		2000	70			750	60
		2500	90			1000	80
	Absolute ethyl alcohol	250	10		Absolute ethyl alcohol	50	10
		500	30			125	30
		1000	50			250	50
		1500	80			500	70
		2000	90			750	90
Clerodendrum viscosum	Distilled water	50	10	*Pongamia pinnata*	Distilled water	50	20
		100	40			100	40
		150	50			250	50
		200	70			500	60
		250	90			750	80
	50% ethyl alcohol	25	10		50% ethyl alcohol	25	20
		50	30			50	40
		100	50			150	50
		150	60			200	70
		200	80			250	80
	Absolute ethyl alcohol	5	20		Absolute ethyl alcohol	10	10
		25	40			25	30
		50	50			50	50
		100	70			100	60
		150	80			150	80
Amoora rohituka	Distilled water	5	10	*Acaciacatechu*	Distilled water	1000	20
		25	30			1500	40
		50	50			2000	60
		75	60			2500	80
		100	80			3000	90
	50% ethyl alcohol	5	20		50% ethyl alcohol	500	10
		10	30			1000	30
		25	50			1500	50
		50	80			2000	70
		75	90			2500	90
	Absolute ethyl alcohol	1	10		Absolute ethyl alcohol	250	10
		5	30			500	30
		10	50			1000	60
		25	80			1500	80
		50	90			2000	90

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TABLE 2: TOXICITY PARAMETERS OF THE DIFFERENT EXTRACTS OF *DATURA INNOXIA*, *CLERODENDRUM VISCOSUM*, *AMOORA ROHITUKA*, *ACACIA AURICULAEFORMIS*, *PONGAMIA PINNATA* AND *ACACIA CATECHU* SEEDS ON *HETEROPNEUSTES FOSSILIS* AFTER 24 HOURS EXPOSURE.

Plant	Solvent	Dose range (ppm)	χ^2 value	P-value (χ^2) at 5%	F-value	P-value (F-test) at 1%	Estimated regression equation	LC_{50} (ppm)	Confidence limit	
									Lower	Upper
Datura innoxia	Distilled water	1000-3000	4.41	P > 0.05	14.25	P < 0.01	-5.86+3.30x	1950.03	1409.83	2781.36
	50% ethyl alcohol	500-2500	7.30	P > 0.05	15.83	P < 0.01	-5.18+3.27x	1337.73	904.85	1903.76
	Absolute ethyl alcohol	250-2000	7.10	P > 0.05	14.00	P < 0.01	-2.88+2.72x	799.80	526.98	1145.65
Clerodendrum viscosum	Distilled water	50-250	7.98	P > 0.05	10.00	P > 0.01	-1.68+3.16x	128.04	89.13	172.44
	50% ethyl alcohol	25-200	4.07	P > 0.05	12.17	P < 0.01	0.78+2.12x	96.28	59.73	165.33
	Absolute ethyl alcohol	5-150	6.92	P > 0.05	14.25	P < 0.01	3.62+0.88x	36.04	11.00	90.55
Amoora rohituka	Distilled water	5-100	6.93	P > 0.05	12.17	P < 0.01	2.5+1.51x	43.90	21.61	92.54
	50% ethyl alcohol	5-75	6.72	P > 0.05	15.50	P < 0.01	2.76+1.77x	18.38	09.59	31.66
	Absolute ethyl alcohol	1-50	4.88	P > 0.05	14.00	P < 0.01	3.69+1.41x	08.67	04.16	16.26
Acacia auriculaeformis	Distilled water	250-1250	4.47	P > 0.05	12.50	P < 0.01	-3.87+3.16x	668.87	452.42	951.88
	50% ethyl alcohol	125-1000	8.04	P > 0.05	14.25	P < 0.01	-0.09+1.92x	449.24	238.26	899.60
	Absolute ethyl alcohol	50-750	4.72	P > 0.05	10.00	P > 0.01	0.26+2.00x	231.98	135.22	384.33
Pongamia pinnata	Distilled water	50-750	6.92	P > 0.05	25.00	P < 0.01	1.92+1.31x	217.67	79.08	582.46
	50% ethyl alcohol	25-250	8.91	P > 0.05	24.24	P < 0.01	2.17+1.44x	91.76	37.08	189.26
	Absolute ethyl alcohol	10-150	10.13	P < 0.05	12.17	P < 0.01	2.64+1.41x	54.97	29.95	110.87
Acacia catechu	Distilled water	1000-3000	2.73	P > 0.05	4.55	P > 0.01	-8.97+4.35x	1645.80	1212.72	2029.34
	50% ethyl alcohol	500-2500	7.69	P > 0.05	10.00	P > 0.01	-5.63+3.40x	1341.99	963.05	1789.79
	Absolute ethyl alcohol	250-2000	1.39	P > 0.05	3.77	P > 0.01	-3.02+2.79x	757.74	497.37	1073.70

Absolute ethyl alcohol extract of *A. rohituka* seeds > 50% ethyl alcohol extract of *A. rohituka* seeds > Absolute ethyl alcohol extract of *C. viscosum* seeds > Distilled water extract of *A. rohituka* seeds > Absolute ethyl alcohol extract of *P. pinnata* seeds > 50% ethyl alcohol extract of *P. pinnata* seeds > 50% ethyl alcohol extract of *C. viscosum* seeds > Distilled water extract of *C. viscosum* seeds > Distilled water extract of *P. pinnata* seeds > Absolute ethyl alcohol extract of *A. auriculaeformis* seeds > 50% ethyl alcohol extract of *A. auriculaeformis* seeds > Distilled water extract of *A. auriculaeformis* seeds > Absolute ethyl alcohol extract of *A. catechu* seeds > Absolute ethyl alcohol extract of *D. innoxia* seeds > 50% ethyl alcohol extract of *D. innoxia* seeds > 50% ethyl alcohol extract of *A. caetchu* seeds > Distilled water extract of *A. catechu* seeds > Distilled water extract of *D. innoxia* seeds.

All the extracts were more or less toxic with variations in concentrations. Due to the effects of different concentrations mortality rate of the fishes varied. In the present study in terms of concentration, LC_{50} and relative potency values the toxic effects of the plants followed the order *A. rohituka* > *C. viscosum* > *P. pinnata* > *A. auriculaeformis* > *A. catechu* > *D. innoxia* seed extracts. Chi- square values showed most of the data to be insignificant excepting absolute ethyl alcohol extract of *P. pinnata* seeds indicating a good relationship between observed and expected mortalities. Analysis of data made between mortality and concentrations showed that most of the analyses were significant at 0.01 level which again indicated a good relationship between the concentrations used and mortalities obtained.

In the present study, The LC_{50} values of distilled water, 50% ethyl alcohol and absolute ethyl alcohol extracts of the six plant seeds on *H. fossilis* were 1950.03, 1337.73 and 799.80 ppm (*D. innoxia*), 128.04, 96.28 and 36.04 ppm (*C. viscosum*), 43.90, 18.38 and 8.67 ppm (*A. rohituka*), 668.87, 449.24 and 231.98 ppm (*A. auriculaeformis*), 217.67, 91.76 and 54.97 ppm (*P. pinnata*) and 1645.80, 1341.99 and 757.74 ppm (*A. catechu*) respectively. Latifa *et al*. (1992) obtained the LC_{50} values of 289.068 and 102.094 ppm for 50% ethyl alcohol and absolute ethyl alcohol extracts of *M. ferrea* seeds on *H. fossilis*. Nasiruddin *et al.* (1997) obtained the LC_{50} values of distilled water and 50% ethyl alcohol extracts of *A. indica* seed kernels on *H. fossilis* as 710.598 and 500.337 ppm respectively. The LC_{50} values of distilled water and 50% ethyl alcohol extracts on the same fish were 33.647 and 24.810 ppm (*A. procera* seed), 41.398 and 25.019 ppm (*S. mahagoni* seed kernel), 85.304 and 48.429 ppm (*S. multiflora* seed) and 805.061 and 381.646 ppm (*S. indicum* seed kernel), respectively (Nasiruddin *et al.* 1998).

TABLE 3: THE LC_{50}, RELATIVE POTENCY VALUES AND RANGE OF TOXICITIES OF THE DIFFERENT EXTRACTS OF *DATURA INNOXIA*, *CLERODENDRUM VISCOSUM*, *AMOORA ROHITUKA*, *ACACIA AURICULAEFORMIS*, *PONGAMIA PINNATA* AND *ACACIA CATECHU* SEEDS ON *HETEROPNEUSTES FOSSILIS* AFTER 24 HOURS EXPOSURE.

Plant seed	Extract	LC_{50} (ppm)	Relative potency	Range of toxicity
Datura innoxia	Distilled water	1950.03	1.00	Least toxic
	50% ethyl alcohol	1337.73	1.46	Less toxic
	Absolute ethyl alcohol	799.80	2.44	Fairly toxic
Clerodendrum viscosum	Distilled water	128.04	15.23	Moderately toxic
	50% ethyl alcohol	96.28	20.25	Toxic
	Absolute ethyl alcohol	36.04	54.11	Toxic
Amoora rohituka	Distilled water	43.90	44.42	Toxic
	50% ethyl alcohol	18.38	106.10	Toxic
	Absolute ethyl alcohol	8.67	224.92	Most toxic
Acacia auriculaeformis	Distilled water	668.87	2.92	Fairly toxic
	50% ethyl alcohol	449.24	4.34	Moderately toxic
	Absolute ethyl alcohol	231.98	8.41	Moderately toxic
Pongamia pinnata	Distilled water	217.67	8.96	Moderately toxic
	50% ethyl alcohol	91.76	21.25	Toxic
	Absolute ethyl alcohol	54.97	35.47	Toxic
Acacia catechu	Distilled water	1645.80	1.18	Least toxic
	50% ethyl alcohol	1341.99	1.45	Less toxic
	Absolute ethyl alcohol	757.74	2.57	Fairly toxic

Range of toxicity:

Most toxic	1 < 10 ppm	Toxic	10 < 100 ppm
Moderately toxic	100 < 500 ppm	Fairly toxic	500 < 1000 ppm
Less toxic	1000 < 1500 ppm	Least toxic	1500 < 2000 ppm

Whereas, the LC_{50} values of distilled water, 50% ethyl alcohol and absolute ethyl alcohol extracts were 1089.976, 642.632 and 566.565 ppm for *A. indica* seed and 99.549, 42.506 and 18.767 ppm for *B. accutangula* seed extracts (Nasiruddin *et al*. 2006). From the above findings it can be said that absolute ethyl alcohol extract of *A. rohituka* seed was the most toxic extract (LC_{50} = 8.67 ppm) and distilled water extract of *D. innoxia* seed was the least toxic (LC_{50} = 1950.03 ppm).

From the present study it is observed that the toxic effect of *A. rohituka* seed extract was highly effective on *H. fossilis*. The effectivity of the seed extracts of *C. viscosm, P. pinnata, A. auriculaeformis, A. catechu* and *D. innoxia* seemed to be promising at different dose levels. The six indigenous plant extracts analysed might be helpful as controlling agent of undesirable fish species in the nursery, rearing and stocking ponds of a fish culture farm. It is suggested that laboratory based toxicological studies of crude dry products can give optimal information about the effectivity of the plant toxins on the fish species.

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