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Piscicidal effects of plant seed extracts on predatory fish, *Channa punctatus* (Teleostei: Channidae) reared in aquarium

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Abstract
This study was conducted to develop an environmentally safe and effective phyto-piscicide (plant-origin). Piscicidal activity of four indigenous plant's seed powder such as, tea (*Camelia sinensis* Linn.), mahua (*Madhuca indica* Gmel.), kanta begun (*Solanum surattens* Buryn. f) and kunch (*Abrus precatorious* Linn.) against common freshwater air breathing predatory fish (*Channa punctatus*) and their behavioral changes were investigated. The Fishes were exposed to five different concentrations of seed powder (60, 90, 120, 150,180 ppm) for toxicity test and one remained a control. During exposure, fish exhibited erratic swimming, gulping for air, slow operculum movement and ultimately settling at the bottom motionless just before death. Among the four different seed powder kunch and kanta begun seed extract showed the remarkable piscicidal activities. At a concentration of 180 ppm, kunch and kanta begun seed powder separately took only 0.30 and 0.50 hr respectively for killing all the fish whereas 60 ppm solutions maximized the duration to 2.0 and 3.8 hrs respectively to perform the same. The various doses of these four seed powder of four different indigenous plants had strongly (p<0.001) positive and negative correlation with body weight of fish and time of killing of fishes. The results of the current study suggest that the seed powder of four indigenous plants may be used to eradicate predatory fishes from aquatic habitats.

Keywords: Piscicidal effects, toxicity test, seed extracts, predatory fish, *Channa punctatus*

1. Introduction
The predatory and weed fishes and many other aquatic animals such as snakes, frogs, turtles, etc. hamper fish culture in many ways and thus reduce the production. Eradication of these predatory fishes from aquaculture ponds is a good management practice to increase fish production. Hence, for total eradication of these unwanted aquatic animals a number of toxicants have been used all over the world. Due to lack of modern technique for preparation of plant materials as a fish poison and also their limited availability in the market the fish farmers in Bangladesh use insecticides and pesticides *viz*., DDT, Aldrin, Dieldrin etc. for cleaning the nursery and stocking ponds [9]. The increasing use and the toxicity of these synthetic insecticides and pesticides have a lot of adverse effects on the environment, *viz*., i) residual toxicity which may magnify biologically in the aquatic food chain, ii) most of them are highly toxic to mammals, and require precautions in handling and technical skill in operation [15]. Some of them are alleged to have caused disturbances in the normal physiological functioning of aquatic plants, hampering their growth and development and affecting future generations. Most of the pesticides used in the third world countries are imported from the developed countries [10]. Moreover these piscicide are bio hazardous. However, the most effective and safe method is the use of plant origin fish toxicants as these satisfy all the requirements of ideal fish poison [9]. The need for developing safe poison materials that would leave no residual toxicity is getting increased priority.
Piscicide of plant origin, *viz*., rotenone, pyrethroids, saponin etc. are preferable as they detoxicate within a few days of application leaving little or no toxic residues. At present, to eliminate undesirable species from the water bodies in our country, rotenone (extracted from root of *Derris* spp.) is being widely used in Bangladesh. However, price of rotenone is very high, its availability and quality are decreasing gradually due to adulteration. Therefore, it has become necessary to develop a piscicide from the locally available raw materials.
Tea is an important export item of Bangladesh. Although the tea plants are cultivated for their leaves, they yield a considerable amount of seeds which may be used as piscicide cum

fertilizer in fish ponds. In China, tea seeds are extensively used as piscicide cum Fertilizer in the nursery ponds. But the some other plant toxins such as seed of mahua (*Madhuc indica* Gmel.) kanta begun (*Solanum surattens* Buryan. f) and kunch (*Ahrus precatorius* Linn.) are abundantly available in the northern part of Bangladesh especially in Barind soils. Plant materials like derris powder or tea seed cake is commonly used in Japan to kill the fish selectively from the shrimp culture ponds reported by (Shigueno, 1975) [31]. Chowdhury *et al*., 2014 [10] observed that the dry seed extracts of *Albizza procera, Annona reticulata* and seed kernel extracts of *Achras zapota* are potentially good piscicide against *C. punctatus*. Chiayvareesajja *et al.,* 1997 [8] studies the toxicity of 221 plants in Thailand and found five plant species that are effective in killing predatory fishes. In Bangladesh piscicidal studies such aspects were carried out to some extents (Chowdhury *et al*., 2014; Latifa *et al*., 1997; Nasiruddin *et al*., 1998 [10, 23, 27].

Presently, to eliminate predatory and undesirable species from the water bodies, rotenone is being widely used by farmers in Bangladesh. Rotenone derived from derris root powder is proved to be hazardous to workers as inhalation may result in respiratory paralysis [14]. Consequently, use of rotenone is strictly controlled by many countries. Owing to the high price, quality and unavailability of the indigenous *Derris* root powder in the country, the need for finding out an alternative herbal piscicide are pressing. Keeping this view in mind, the present research work has been undertaken to prepare piscicide from plant seed powder. Findings of this research may be useful to develop some low cost plant origin herbal piscicide which may help to protect the fish fries from predators and thus to increase the fish production in commercial fish farms and aquatic habitats.

2. Materials and Methods

2.1Collection and preparation of seed powder

Tea seeds (Family-Theaceae) were collected through the courtesy of Mr. Shahedul Islam, Assistant Manager, Huglichara Tea Garden, Shreemongal, Moulavi Bazar. Mahua (Family-Sapotaceae), kanta begun (Family-Solanaceae) and kunch (Family-Leguminosae) seeds were collected from upazilla Nachole, Dist. Chapai-Nawabganj (Rajshahi). The seeds were pulverized into fine powder first in a hand mortar then ground sieved (mesh size = 0.37 mm), dried in the room temperature and kept in packets with number.

2.2 Collection and acclimatization of experimental fishes

Healthy and live fishes of *C. punctatus* (average length and weight of 14.3 ± 2.40 cm and 25.8 ± 4.5 g) were collected from the cooperative market of Bangladesh Agricultural University and used as test animal. The collected fishes were acclimatized for seven days in the laboratory condition before using for experiment. During that period, the aquarium water was aerated continuously and fishes were daily fed on artificial feed like fishmeal. Water was changed at every 24 h. The water temperature in aquaria is 28±1°C and the pH is 7.2±0.2.

2.3 Experimental design and procedures

Experiments were conducted in glass aquaria each measuring (25 x 45 x 25) cm^3 and containing 10 liters of water. At first the aquaria were cleaned and filled with water. The pH, temperature and dissolved oxygen values of experimental as well as control aquaria were recorded before the application to the toxicants into the water and at several time interval of the experiment and at the time when detoxication occurred completely.

Five concentrations (60, 90,120,150,180 ppm) of all the four different seed powder were used. Each treatment had three replicates. These concentrations were made on the basis of the results of initial experiments with arbitrary concentrations. Four fishes were kept in each aquaria. Room temperature was maintained throughout the study period. Specific quantity of powder was added in the test aquaria and gently mixed with a glass rod.

2.4 Effects of seed powder on Fish behavior

The fishes which became affected by the action of four different seed powder first expressed distressed symptoms. Gradually, they became inactive and started losing their balance. For this action, they went to the bottom of the aquaria; lie down on their sides till death. Here a fish was considered dead when its respiratory movements checked and became non-sensitive to mechanical stimuli. Experiments were also conducted to determine the detoxification time of the toxicants. The dead animals were removed as soon as possible from test container to prevent water fouling.

2.5 Statistical analysis

The design of experiment was set in such a way to make it possible to do analysis of variance in completely Randomized Design (CRD). LSD analysis was done to test mean difference. The linear correlation of various doses of piscicide with body weight of fishes and time of killing of fishes were analysed. All the statistical analyses were carried out by following key given by Gomez *et al*., 1987 [15].

3. Results

3.1 Behavioral responses of fishes to test concentrations

When fishes exposed to the different concentration of seed powder exhibited various abnormal behavioral patterns before death occurred. Erratic and agitated movement, trying to jump out of the test media, gulping for air and loss of equilibrium, restlessness, were frequently observed. Their rate of operculum movement also increased. Control group of fishes are devoid of such behavioral changes.

3.2 Toxicological effects of the seed powder

The results of toxicity test of different plant seed powder against *C. punctatus* are presented in Tables 1 and 2. Seed powder of tea, mahua, kanta begun and kunch killed the fishes effectively in prospect to doses and time and the effect were significant at 1% level of probability. Statistical analysis revealed that the kanta begun seed powder showed its highest efficiency in time and significant (P<0.01) as piscicide to kill fishes than the kunch powder followed by tea seed powder and mahua seed powder (Table 1).

Table 1: Efficiency of four seed powder applied to kill (*C. punctatus*).

Name of plant	Average time (h) to kill fishes
Kanta begun (*Solanum surrattens* Buryn. f)	1.42 (85 minutes and 12 seconds)
Kunch (*Abrus precatorious* Linn.)	1.44 (86 minutes and 24 seconds)
Tea (*Camelia sinensis* linn.)	6.16 (369 minutes and 36 seconds)
Mahua (*Madhua indica* Gmel.)	7.58 (454 minutes and 48 seconds)

In case of mahua seed powder, the dose of 180 ppm showed maximum efficiency and killed fishes within 3.9 hrs and significantly (p<0.01) followed by 120, 90 and 60 ppm which killed the fishes within 4.2, 6.4, 10.3 and 13.1 hrs, respectively (Table 2).There was no significant difference between the effect of the doses of 150 and 180 ppm. Moreover, in case of tea seed powder, the dose of 150 ppm and 180 ppm killed fishes within 2.0 hrs showed their highest efficiency and significantly (p<0.01) differed from 120, 90 and 60 ppm which killed fishes within 4.50, 9.80 and 12.50 hrs, respectively. Again, kunch seed powder showed their highest efficiency at the doses of 150 ppm and 180 ppm which killed fishes within 0.30 hr and significantly (p<0.01) followed by 120, 90 and 60 ppm which killed fishes within 0.50, 1.4 and 2.0 hours (Table 2). There was no significant difference between the effect of 60 and 90 ppm. The dose of 180 ppm of kanta begun seed effectively killed more fishes within 0.50 hr significantly (p<0.01) followed by 150, 120, 90 and 60 ppm which killed fishes within 0.60, 0.7,5.5 and 3.8 hrs. There was no significant difference between the effect of the doses of 120, 150 and 180 ppm during the current study.

Table 2: Toxicity test of different seed powder against fish *C. punctatus*

Name of Plant	Doses of Powder (ppm)	Time (hr) to kill fishes	Range of body weight of fish (g)
Kunch (*Abrus precatorius* linn.)	60	2.10a	
	90	1.40a	24-32.4
	120	0.50b	
	150	0.30b	
	180	0.30b	
Kanta begun (*Solanum surrattens* Buryn. f)	60	3.80a	
	90	5.50b	21-32
	120	0.70c	
	150	0.60c	
	180	0.50c	
Tea (*Camelia sinensis* linn.)	60	12.5a	
	90	9.8a	14.0-22.7
	120	4.5c	
	150	2.0c	
	180	2.0c	
Mahua (*Madhua indica* Gmel.)	60	13.1a	
	90	10.3b	13.5-22.4
	120	6.4c	
	150	4.2d	
	180	3.9d	

df= 20, 4

Table 3: Correlations between the doses of four piscicide with body weight and time to kill *C. punctatus*

Doses of powder (ppm)	Time (hour) to kill fishes	Range of body weight of fish (g)
60	0.641*	-0. 528*
90	0.631*	-0. 528*
120	0.598*	-0. 526*
150	0.574*	-0. 620*
180	0.645*	-0. 601*

df = 23, and *P<0.001

Various doses of seed powder had directly (positively) strong (p<0.001) correlations with body weight of fishes, and the same was inversely (negatively) and strongly (P<0.001) correlated with time of killing of fishes (Table 3). In the present observation, kunch and kanta begun seed powder had most effective and powerful piscicidal action at the doses of 120, 150 and 180 ppm. The efficiency of killing fishes and their recommendation depend on the time to kill fishes and expenditure of seed powder. So, in case of mahua and tea seed powder, the dose of 150 ppm will be better to recommend to eradicate predatory fishes from the ponds or aquatic habitats.

4. Discussion

Plant extracts are considered as promising agents because of their easy availability, high efficiency, rapid biodegradability and lower toxicity to non-targeted animals and safe for mankind and environment. All the four plant seed powder in the current study was more or less toxic against test species. Toxicities of plant seed extracts varied with different doses as well as with time. Due to the piscicidal action various abnormal behaviors showed by the fishes such as restlessness, loss of equilibrium, respiratory disorder, erratic swimming before death. The initial increase in opercular movement can be taken as index of the suffocation stress felt by the fishes exposed to various plant seed powder. The nearly dead fish has an opaque body color compared with normal fish and slow response or movement. Fishes of control group is free from any such type of behavioral changes so it is clear that only plant seed extract or powder were responsible for the altered behavior and mortality. The behavioral responses observed in the current studies are in tandem with the findings of (Ashraf *et al*., 2010; Chowdhury *et al*., 2014; Latifa *et al*., 1993,1997; Nasiruddin *et al*., 1998, 2002; Tiwari *et al*., 2003) [3, 10, 23, 24, 27, 28, 32] when they exposed fish to acute concentrations of different plant extracts. Mortality time was found to be directly related to concentration in all the cases. Bhuyan, 1967 [6] observed behavioural changes in weed fishes, *Ambossis nama; Barbus sophore; Amblypharyngodom mola* and *Esomus danricus* with plant *Mallitia pachycarpa*. They found that roots and fruit extract affected the fishes within 1-3 hrs. of exposure and higher doses proved lethal. This toxic compound did not show any adverse changes in physico-chemical condition of water. Several authors [1, 4, 26] also reported abnormal movement and high respiration rate in different fishes when exposed to plant products. Similarly, Nwani *et al*., 2010 [29] observed behavioral changes and high

respiration rate in tilapia exposed to glyphosate herbicide. Abnormal nervous behaviours are associated with the impacts of the toxicants on fishes [14]. This may be due to nervous system involvement or failure [33] or may be due to biochemical body derangement including hepatic compromise [19].

Statistical analysis revealed that the powder of kunch (1.44h) and kanta begun (1.42) had the best piscicidal action among the four piscicide. The ranking of toxicity of four plant seed powder was Kanta>Kunch>Tea>Mahua. The 96-h LC_{50} values from plant derivatives studied by several authors and Kavitha *et al*., 2012 [21] reported the value was 124.0 mg l-1 for *Moringa oleifera* seed extract against *Cyprinus carpio*. Tiwari and Singh, 2003 [32] observed the toxicity of *Nerium indicum* leaf extract to the fish *C. punctatus* and indicate that the toxicity depends on the solvent used for extraction; the LC50 value of diethyl ether, acetone, chloroform and methanol extract of *N. indicum* leaf extract were found to be 17.34 mg/L, 40.01 mg/L, 40.61 mg/L and 106.37 mg/L, respectively. The differences in the LC_{50} value of various parts of plant species to fish depend on the chemicals present in the plants and also the sensitivity of the fish used for the experiment. Comparatively in our study, 150 ppm kunch and Kanta begun seed powder most effective in controlling *C. punctatus* from aquatic environment.

In our current observation, mortality caused by the four different plant seed powder showed a clear significant positive correlation between dose and mortality. The positive correlation between dose and mortality in all cases was noted because increase concentration of piscicide in aquarium water resulted in more intake or entry of active moieties in the bodies of fishes. This result is in conformity with the findings of Tiwari and Singh, 2003 [32] who observed positive correlation between concentration and mortality of *C. punctatus* when exposed to ethanol extract of *Nerium indicum*.

Several researchers [11, 34] have also observed that tea seed cake is botanical pesticides could be extensively used in aquaculture to eliminate predatory fishes in fish and prawn ponds. The results obtained by Zhu *et al*., 1991 [34] indicated that tea saponin was strongly toxic to six species of harmful fish found in prawn ponds. Moreover, 7-8% saponin and low content of protein were identified in the defatted meal of tea seed [11, 18]. Saponin is reported to be haemolytic in action. It is possible that saponin dissolved from powder of seeds into water entered the blood stream of the fishes through semi-permeable gills and oral tissues, haemolysed the red blood corpuscles and thus caused death to the fishes. The results of the current study in case of tea seed cake also consistent with the findings of De *et al*., 1987 [11] who observed that the tea seed cake when added to water at a dose of 100 ppm resulted in the death of tilapia within 5 to 6 h.

In India, Mahua oil cake (oil cake of *Bassia latifolia*) is most commonly used and widely accepted fish poison because it has both manurial and toxic effect [30]. Bhatia, 1970 [5] reported that under laboratory conditions approximate threshold dose of mahua oil cake was found to be 60 ppm to kill *Cirrhinus mrigala*, *Puntius ticto*, *Cyprinus carpio*, *Colisa fasciata* and *Channa gachua*. It has also been reported that waters containing 100,200,300,400 and 500 ppm of mahua oil cake loose their toxicity to fishes after about 48, 72, 96, 144, and 196 h, respectively (Jhingran, 1977) [20]. In case of mahua seed powder, the findings of this study are in line with the findings of Bhatia, 1970 [5] who reported that mahua oil cake at a concentration of 100 ppm was sufficient to kill all the test fishes within 10 hours. Latifa *et al*., 1993 [25] also observed higher mortality rate of fishes at a dose of 75 to 100 ppm mahua oil cake. According to (Deb *et al*., 1987; Homechaudhuri *et al*., 1987) [12, 17] toxin saponin of mahua oil cake breaks the blood cells and degrades muscle protein. Moreover, it is evident from the analysis of variance that the differences in doses on the mortality of test fishes were significant at 1 percent level of probability which has the similarity with the observation made by Ameen *et al*; 1983 [2].

Detoxification time was found to vary from one seed powder to another in respect to different treatment in the present observation. It varied from 10-18 day is probably due to variation in concentration of the seed powder of four different plants. The findings of this study are in line with the results of (Chowdhury *et al*., 1981; Kholil, 1984) [9, 22] with regard to detoxification time. From the observation made by the several researchers and results obtained in the current research work conclude that under toxic condition fish tries to acclimate to the surroundings, if toxic dose is lethal then the death of the fishes is ultimate. Thus, it is believed that plant seed powder may be used in aquatic environment for controlling unwanted populations of *C. punctatus*. Although long term toxic effect has to be studied further to its commercial use.

5. Conclusion

Aquaculture is the fastest growing food producing sector in the world. For Successful or commercial aquaculture, control and eradication of unwanted fishes form the water bodies is prerequisite. Synthetic chemicals are very effective in killing unwanted fishes in a shorter period of time, but not environmental safety to use. In other cases, plant piscicide are chosen because of their eco-friendliness and have duel effects, killing fishes as well as act as manure after a certain period of time interval. To sum up, it can be concluded that locally available plant materials like kanta begun, kunch have the potential to eradicate unwanted fishes and can be used as piscicide in large scale which is biodegradable and environmental safety.

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