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Toxic effect of *Zanthoxylum rhetsa* seed extracts on stinging catfish, *Heteropneustes fossilis* (Bloch, 1794)

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Abstract
Ninety six hours of toxicity test was conducted to observe the lethal concentration (LC_{50}) value of *Zanthoxylum rhetsa* seed extracts on a stinging catfish, *Heteropneustes fossilis*. The fishes were exposed to seven different concentrations of the extract (25 mg l^{-1}, 35 mg l^{-1}, 45 mg l^{-1}, 55 mg l^{-1}, 65 mg l^{-1}, 75 mg $l^{-1}$and 85 mg l^{-1}) for toxicity test and one remained as control. The LC_{50} value of *Z. rhetsa* in *H. fossilis* was found to be 70.1 mg l^{-1} for 96 hours exposure periods. Analysis of variance for the effect of the extract on the percentage mortality of fish showed a significant relation between both the factors ($p<0.05$, $F=16.29$). The correlation coefficient between concentration and mortality of fish was calculated ($R = 0.976$) and showed a strong positive correlation between different concentrations of the seed extracts and mortality percentage of fish. The present finding established that *Z. rhesta* has potential pisicidal effect on fish and could be use widely to control unwanted fishes.

Keywords: Fish toxicity, *Zanthoxylum rhetsa*, LC_{50} value, *Heteropneustes fossilis*

1. Introduction
For successful aquaculture production control of predatory, weed and unwanted fishes are prerequisite. Predatory fishes not only reduced the fish productivity by consuming the targeted fishes, side by side they also destroy the natural habitat of the ecosystem. Synthetic pesticide use widely to kill predatory fishes, but due to their long time residual effects, less biodegradable nature and harmful nature to other organisms, their used is restricted in certain limits. Moreover extensive and indiscriminate use of synthetic pesticides caused serious threat to aquatic ecosystem and their respective biodiversity. In this context the use of biodegradable natural botanical compounds gaining popularity and importance day after day. Moreover, the toxic effects of plant derivatives degraded within 7-12 days, they are safer to use and the fishes killed from such toxicants can be consumed by humans [1].
There are several botanical biodegradable compounds which are now widely used to control fishes [2-8]. Tribes of Arunachal Pradesh use various plants and their products as fish poison to easy catches fish, as arrow poison for hunting and as adhesive for bird catching [7]. A verity of plant and their products being used in Arunachal Pradesh to as fish poison and *Zanthoxylum rhetsa* one of them. The crude pounded fruits of *Z. rhetsa* of the family Rutaceae locally known as 'Onger' used by the Adi tribes of East Siang district of Arunachal Pradesh as piscicides in community fishing activities [7].
The stinging catfish (*Heteropneustes fossilis*) belongs to the family Heteropneustidae is an air-breathing hardy carnivore fish. It is found in almost all fresh water bodies including muddy, marshy and derelict ponds having low levels of water and dissolved oxygen or even in contaminated water [9]. *Heteropneustes fossilis* has been reported to use as a model fish in aquatic toxicology [9, 10]. The degree of toxicity as well as piscicidal activity of any plant extract can be assessed by exposing fishes to it and subsequent estimation of the median lethal concentration (LC_{50}) [3]. No works has been reported on piscicidal effects of *Zanthoxylum rhetsa* against *Heteropneustes fossilis*. In this context an attempt has been made to see piscidal effects of *Zanthoxylum rhetsa* extracts on stinging catfish.

2. Materials and methods
2.1 Experimental site and collection of *Zanthoxylum rhetsa* seed
The present study was carried out in the laboratory of Department of Fisheries Resource Management, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Panchasayar, Kolkata, India. The seed of *Zanthoxylum rhetsa* was collected from the forest of Tarak village, located in Siang district of Arunachal Pradesh.

The collected seed was then immediately sun dried after collection and the dried seed was brought to the laboratory. The seed, pericarp & seed with pericarp were segregated separately and also grinded by using an electric grinder.

2.2 Phytochemical analysis

The phytochemical analysis of the seed, pericarp and seed with pericarp of plant *Zanthoxylum rhetsa* was done by using different extraction process as well as by using different solvents. Two solvents were used i.e. 70% ethanol and double distilled water. By using aqueous extract methods, the extract from seed, pericarp and seed with pericarp were prepared and than phyotochemical analysis was done.

2.3 Collection and acclimatization of experimental fish sample

Healthy fingerlings of *H. fossilis* (average length and weight of 12.16±0.78 mm and 10 ±0.97 gm) was purchased from the local fish market and maintained in cemented cistern for 3 weeks, prior to the experiment for acclimatization. The fishes were feed with pelleted feed and maintained with optimum level of water quality. Fish tanks were well aerated and the water was exchanged with fresh water as and when required.

2.4 Toxicity test of on *Heteropneustes fossilis*

For 96 hour median lethal concentration value (LC_{50}), the experiments were conducted in glass aquariums filled with 15 litre of chlorine free tap water. Taking into account the moisture content of the extract, different concentrations of the extract (25 mg l^{-1}, 35 mg l^{-1}, 45 mg l^{-1}, 55 mg l^{-1}, 65 mg l^{-1}, 75 mg $l^{-1}$and 85 mg l^{-1}) were made by adding the extracts proportionately to the water of the aquarium. In each aquarium, 20 fishes were kept and exposed to different concentrations as above with replications. In control, no extract was added and the fishes were maintained in the water without extract concentration. The stinging catfish, *H. fossilis* was exposed to aqueous extract of seed of *Z. rhetsa* for 96 hours by following standard procedure used for toxicity test [11]. Feeding of fishes was stopped during the experiment period. Hypoxic condition of water was avoided by adequate aeration. The tested fishes were kept under continuous observation during the experimental period. The behaviour of the fish were observed and recorded from time to time. The mortality rate was recorded periodically in each aquarium. The dead fishes were removed and preserved for further investigation. The LC_{50} value of the fish species was calculated by using Probit analysis method (Finney, 1971).

2.5 Analysis of water quality parameter

Some important physicochemical parameter of water such as Dissolved Oxygen (DO), free carbon dioxide (CO_2), total alkalinity and the ammonia content were studied. Water quality parameter during median lethal test for 96 hour experiment were analysed at the beginning and end of the experiment by using the methods described in APHA [11].

2.6 Statistical analysis

The LC_{50} value of *Z. rhetsa* for *H. fossilis* was calculated using Probit analysis method (Finney's, 1971). One way ANOVA were performed using SPSS software to assess the effect on concentration on the mortality of fish. The regression analysis were done by Microsoft excel to assess the relation between mortality and exposure period in different concentration of aqueous extract of Z. *rhetsa* seed.

3. Results and discussion

3.1 Toxicity test of on *Heteropneustes fossilis*

The LC_{50} value of *H. fossilis* was found 70.1 mg l^{-1} and the relationship between the seed extract of *Z. rhetsa* concentrations and the mortality rate was shown in Fig 1. Analysis of variance for the effect of the extract on the percentage mortality of fish showed a significant relation between both the factors ($p<0.05$). The correlation coefficient between concentration and mortality of fish was calculated ($R = 0.976$); and showed a strong positive correlation between different concentrations of the seed extracts and mortality percentage of fish. Similarly, the correlation regression analysis between different mortality of fish and exposure period also show a strong positive relation with correlation coefficient (R value of 0.949, 0.7746, 0.447, 0.9486, 0.9439, 0.9439 and 0.9438) for different concentrations (25 mg l^{-1}, 35 mg l^{-1}, 45 mg l^{-1}, 55 mg l^{-1}, 65 mg l^{-1}, 75 mg $l^{-1}$and 85 mg l^{-1}) (Fig 2-8). No mortality was reported in control.

[2] Reported LC_{50} values for aqueous extract of *Euphorbia tirucalli* latex at various exposure periods of catfish *Heteropnuestes fossilis* were 3.450 ml l^{-1} for 24 h, 2.516 mg l^{-1} for 48 h, 1.623 mg l^{-1} for 72 h and 1.315 mg l^{-1} for 96 h. [3] Found that the LC_{50} values of dimethoate on freshwater catfish, *Heteropnuestes fossilis* was 3.38, 3.23, 3.08 and 2.98 mg l^{-1} for 24, 48, 72 and 96 hours respectively.

The LC_{50} values of *Z. rhetsa* seed extract against *H. fossilis* was found 70.1 mg l^{-1} which is comparable with those of the findings as reported and conducted with various other botanical extracts. Increase in fish mortality over time could be due to activities of some factors that may act individually or synergistically [4]. The report of the present study was similar with the report of many works who have reported on tolerance limit of various plant extract with different cat fish. The 96 hour LC_{50} extracts *Nicotiana tobaccum* values have been reported as 626.0 mg l^{-1} against *Clarius garipenus* [12, 6]. Reported that LC_{50} value of *Terminalia arjuna* berk extract on a freshwater catfish *Heteropnuestes fossilis* were found to be 12.7, 8.94, 5.63 and 4.71 mg l^{-1} for 24, 48, 72 and 96 h, respectively at 96 hours exposure.

The 96-h LC_{50} values from plant derivatives studied by several authors and reported the value was 124.0 mg l^{-1} for *Moringa oleifera* seed extract against *Cyprinus carpio* [13], 4.8 g l^{-1} against of neem leaf extract against *Prochildu lineatus* [14], 56.8 mg l^{-1} for alcoholic extract of *Euphorbia royleana* [15] and 54.65 mg l^{-1} for alcoholic extract of *Nerium indicum* leaf against *Channa punctatus* [15-17]. Studied the 96-h LC_{50} values by using synthetic chemicals such as malachite green and cypermethrinn and found the value as 5.6 and 7.2 mg l^{-1} respectively against *H. fossilis*. While dimethoate was used as fish toxicant the 96-h LC_{50} value was reported 65 mg l^{-1} for *Clarias batrachus* [18], 47 mg l^{-1} (96 hr) for *Channa punctatus* [19] and 17.9 mg l^{-1} (24 hrs) for *C. punctatus* [20].

[21] Reported a sub-lethal dose of *Mohua* extract as 100 mg l^{-1} against *Clarius batracus.* [22] Also reported different pisicidal plant from Nepal against catfish like *Ophiocephalus punctatus*, *Clarias batrachus* and *Heteropneustes fossilis* with LC_{50} value of 90 mg l^{-1}, 102.4 mg l^{-1} and 109.1 mg l^{-1} respectively. The LC_{50} value of plant based pisicide for catfish are on an average higher than the LC_{50} value for carps, the reason may be due to the hardy nature of catfish. This comparison have revealed higher potential of *Zanthoxylum rhetsa* extract as piscicide due to its lower LC_{50} values than those of other plant extracts. However, a lower 96 hour LC_{50} (12.7 mg l^{-1}) has been reported for *Terminalia arjuna* [6], the reason may be a better extraction of phytochemical using

more sophisticated analytical tool for extraction.

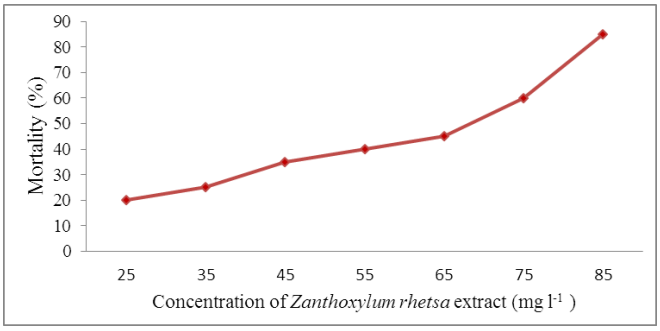

Fig 1: Percentage mortality of *Heteropneustes fossilis* observed at 96 hour exposure period exposed to various concentration of *Zanthoxylum rhetsa* extract.

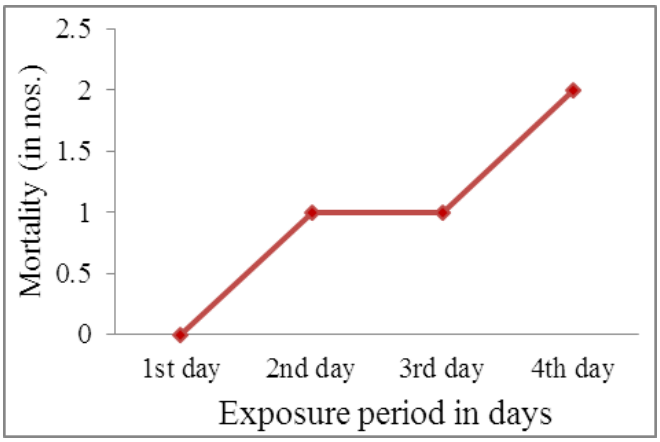

Fig 2: Mortality rate of *H. fossilis* at 25 mg l^{-1} conc.

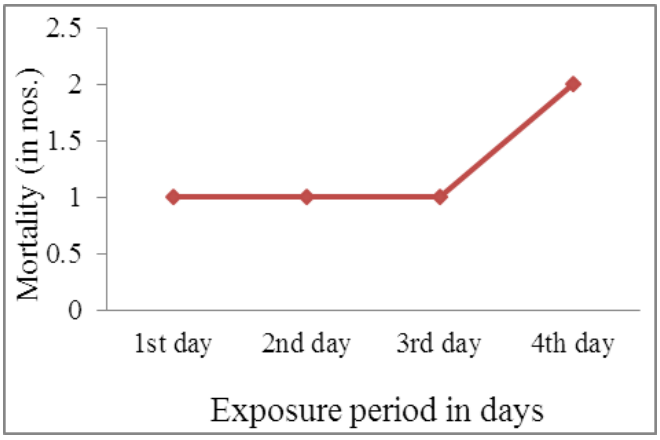

Fig 3: Mortality rate of *H. fossilis* at 35 mg $^{-1}$ conc.

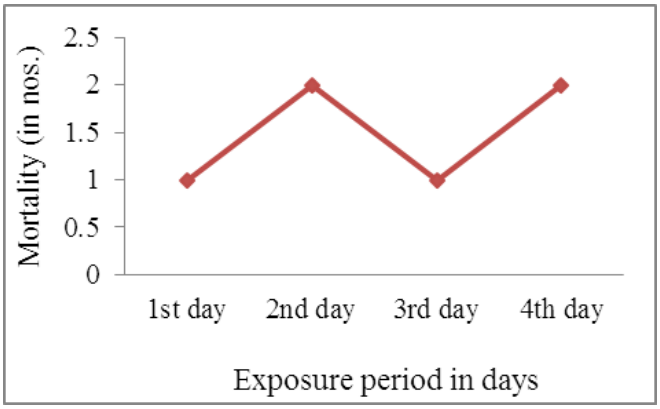

Fig 4: Mortality rate of *H. fossilis* at 45 mg $^{-1}$ conc.

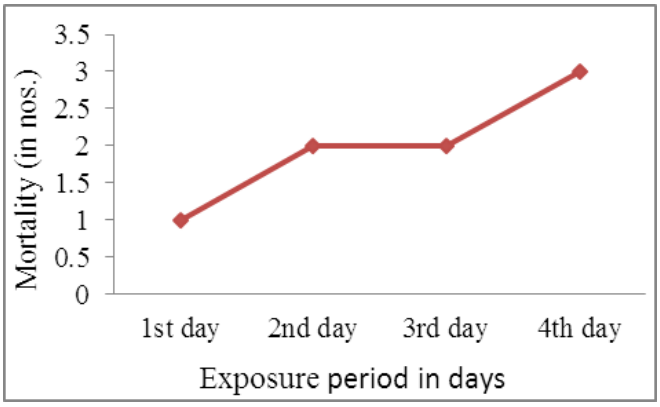

Fig 5: Mortality rate of *H. fossilis* at 55mg $^{-1}$ conc.

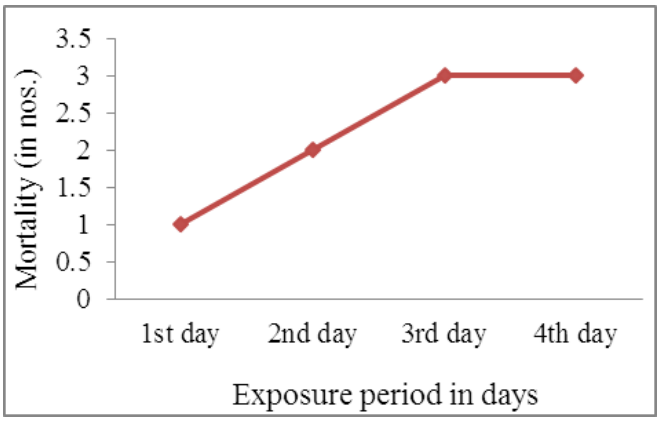

Fig 6: Mortality rate of *H. fossilis* at 65 mg $^{-1}$ conc.

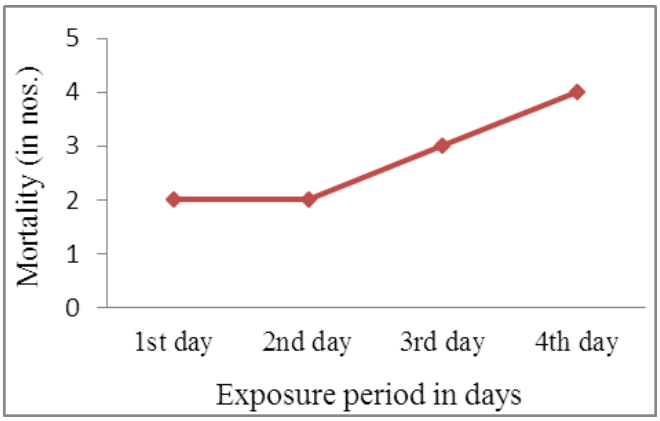

Fig 7: Mortality rate of *H. fossilis* at 75mg $^{-1}$ conc.

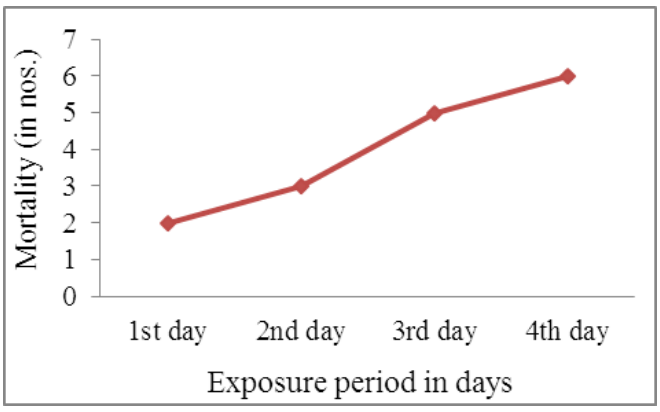

Fig 8: Mortality rate of *H. fossilis* at 85 mg l^{-1} conc.

3.2 Behavioural changes observed in *Heteropneustes fossilis*

At different exposure periods (24, 48, 72 and 96 hour), the

behavioural alteration in *Heteropneustes fossilis* was observed carefully. Behavioural changes observed during 96 hours exposure period depicted in the Table 1.

Table 1: Behavioral change of *Heteropneustes fossilis* observed during 96 hour exposed period

Exposure periods (hour)	Behavioural changes
1-12	Fishes were jumping and trying to move away from the extract.
13-24	Trying to jump out from water.
25-36	Discolouration of skin was observed in comparison to control fish.
37-48	Fish exhibited more opercula movement, increased mucous secretion and progressively became sluggish and lethargic
49-96	Mucus secretion in skin, settled down in aquarium bottom.

The changes observed in the treated group after exposure were not observed in fish in control which demonstrate that the effect was due to exposure into *Zanthoxylum rhetsa*. The fish show swimming and jumping out of the extract medium which can be correlated with adaptive mechanism of fish. Changes in breathing rate and or jumping frequencies are the general symptoms noticed in the fish after exposure to the toxicant and these activities help the fish to avoid contact with poison and fight against stress.

With increase in time of exposure the energy content get drained out gradually leading to lethargic state in final stage of exposure. Excessive mucus secretion and accumulation in the fishes exposed to toxicant was observed in the treated fish which may be an adoptive response providing additional protection against corrosive nature of the extract to the sensitive dermal layer of skin and they avoid the absorptions of the toxicant by the general body surface. This agrees with the findings earlier authors [23].

[24] Mentioned that, restlessness and hyperactivity in fish may occur due to accumulation of acetylcholine at synaptic junctions which increased metabolic activities. [25] Stated that, consumption of more oxygen indicates higher metabolic rate. An initial increase in operculum movement frequency in chlorpyrifos exposed *Tilapia* reported by [26]. [26] Mentioned that, the toxins exposure in fishes increases the operculum movement and was well established.

4. Conclusion

Plant based piscicide have been address as best alternative of chemical piscicide in aquaculture to control fish fry predators and unwanted fishes. Plant extracts are considered as desirable due to their properties of eco-friendliness, ease of availability, high efficiency, reduced toxicity to non-targeted animals and rapid biodegradability. The result of this research provides the knowledge about an indigenous plant species (*Zanthoxylum rhetsa*) as potential piscicide.

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