Fishery Technology 57 (2020) : 17 - 21

Median Lethal Concentration of *Zanthoxylum rhetsa* seed extracts on Grass carp Fingerlings (*Ctenopharyngodon idella*) (Valenciennes, 1844) in Captive Condition

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Abstract

The bark extracts of the plant *Zanthoxylum rhetsa* have been widely used by people of Arunachal Pradesh as a mean of catching fish. Therefore, the median lethal concentration (LC_{50}) value of *Zanthoxylum rhetsa* bark extracts on grass carp (*Ctenopharyngodon idella*) (Valenciennes, 1844) has been determined in the present study. The seeds of *Z. rhetsa* were collected from the forest of Tarak village, located in the Siang district of Arunachal Pradesh and the toxicity test was conducted in the control condition. Five different concentrations of the extract *viz.*, 25 mg l^{-1}, 35 mg l^{-1}, 45 mg l^{-1}, 55 mg l^{-1} and 65 mg l^{-1} were prepared by adding the extracts proportionately to the water. The LC_{50} value of *Z. rhetsa* against *C. idella* was hosted as 45 mg l^{-1} for 96 h exposure period. Toxicity of aqueous solution of *Z. rhetsa* against fingerlings of grass carp was found to be time and dose-dependent. It was found that there was a significant ($p<0.05$) relationship between the mortality of the treated fish with the concentration of seed extract of *Z. rhetsa.* A very strong correlation coefficient was found between the mortality of test fish and exposure of seed extract of *Z. rhetsa* ($r=0.998$) at different concentrations. The finding established that *Z. rhesta* has a potential piscicidal effect on fish and could be used widely to control unwanted fishes in aquaculture systems.

Keywords: Acute toxicity, LC_{50} values, *Zanthoxylum rhetsa, Ctenopharyngodon idella*

Received 07 September 2018; Revised 31 October 2019; Accepted 16 December 2019

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Introduction

Botanical extracts are of much interest in aquaculture to control fish fry predators and unwanted fishes from fish ponds and reservoirs as an attempt to replace chemical pesticides and piscicides (Kumar et al., 2010; Das, 2013). Extensive and indiscriminate use of non-biodegradable synthetic chemicals results in harmful impacts on non-targeted aquatic organisms. Plant extracts are eco-friendly; easily available, rapidly biodegradable and has reduced toxicity to non-targeted organisms (Yunis et al., 2014). Several compounds like saponin, tannins, alkaloids, alkenylphenols, di and tri-phenoids etc. present in plants belonging to different families have piscicidal activities that are used to control weed fish (Ramanujam & Dominic, 2012). Many plants have been investigated to evaluate their piscicidal properties.

Tribes of Arunachal Pradesh use various plants and their products as fish poison to catch fish. They observed that the bark extract of the *Zanthoxylum rhetsa* plant has some piscicidal properties that can be used in the pond management system of aquaculture. The bark extracts of this plant have been also widely used by them as a mean of catching fish. *Ctenopharyngodon idella* commonly known as grass carp is a large herbivorous freshwater fish native to eastern Asia. Grass carp is a fast-growing fish and therefore preferred for aquaculture practice and has a high growth rate in pond system. During the present study, the grass carp was chosen as it is one of the common species grown in the polyculture system, where fish species do not contest with each other for food and habitat. *C. idella* a surface-dwelling feeder is selected so that an efficient piscicidal activity of fish in the whole water column can be suggested.

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Determination of effective doses using the dose-response experiment is essential not only for a new toxicant either chemical or botanical but also for the fish specimen being exposed to this toxicant. The present study was carried out to study the toxicity of *Z. rhetsa* as a potential eco-friendly chemical for the eradication of weed fish. The crude pounded fruits of *Z. rhetsa are* used by Adi tribes of East Siang district of Arunachal Pradesh as piscicides in community fishing practices (Jomang et al., 2017). There has been no report of *Z. rhetsa* seed extract against *C. idella* and hence, fishes were exposed to different concentrations of the aqueous extract to determine LC_{50} values for this carp in captive condition.

Materials and Methods

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 *Z. rhetsa* was collected from the forest of Tarak village, located in the Siang district of Arunachal Pradesh. Three aqueous extraction methods were followed for the phytochemical analysis as per Davis (1956), Kalavani et al. (2012) and Redfern et al. (2014). Among the three extraction procedures, the aqueous extract method by Kalavani et al. (2012) was considered as best due to its higher yield and enriched phytochemical contents. Healthy fingerlings of *C. idella* (7.4±0.8 mm of total length and 3.82±0.75 g weight) were purchased from the local fish market and maintained in a cemented cistern for 3 weeks, before the experiment for acclimatization. The fishes were fed with artificial pelleted feed at the rate of 2 to 3% of their body weight daily i.e. during the morning and the evening hours. Fish tanks were well-aerated and water was exchanged with fresh water once in every week. For 96 h median lethal concentration value (LC_{50}), the experiments were conducted in glass aquaria filled with 15 l of chlorine-free tap water. Different concentrations of dry *Z. rhetsa* seed powder (25 mg l^{-1}, 35 mg l^{-1}, 45 mg l^{-1}, 55 mg l^{-1} and 65 mg l^{-1}) were proportionately added to the water in the aquarium.

In each aquarium, 10 fishes were kept and exposed to different concentrations as above with two replications. Control tanks without the extract was also used. The animals were exposed to the treatment for 96 h by following the standard procedure used for other toxicity tests (APHA, 2005). Feeding of fishes was stopped during the experiment period. The tested fishes were kept under continuous observation during the experimental period. The behavior of the experimental fish samples was observed and recorded from time to time. The mortality rate was recorded periodically (at 12 h interval) in each aquarium. The dead fish were removed and preserved for further investigation.

Some important physicochemical parameters of water such as Dissolved Oxygen (DO), free carbon dioxide (CO_2), total alkalinity and the ammonia content were studied. The water quality parameter during the median lethal test for a 96 h experiment was analyzed at the beginning and end of the experiment by using the methods described in APHA (2005).

The LC_{50} value of *Z. rhetsa* for *C. idella* was calculated using the Probit analysis method (Finney's, 1971). One way ANOVA was performed using SPSS^R software to assess the effect on the concentration of the extract on the mortality of fish. Regression analysis was carried out to assess the relationship between mortality and exposure period in different concentrations of aqueous extract of *Z. rhetsa* seed.

Results and Discussion

Throughout the experimental period, the water quality parameters mainly dissolved oxygen (DO) and free carbon dioxide (CO_2) were found in suitable condition for carps (Table 2). The median lethal concentration or 96 h LC_{50} value of *Z. rhetsa* was found to be 45 mg l^{-1} for *C. idella*. The relationship between the aqueous extract of *Z. rhetsa* concentrations and the mortality rate of the tested fish is presented in Table 1. The percentage of mortality of fishes being exposed to seed extract increased with the increase in the concentration of extract and also the time of exposure. Analysis of variance (ANOVA) for effect of concentration of extract on the percentage of mortality of fish was found significant ($F=8.637$, $p<0.005$), showing a significant relationship between the concentration of extract and the mortality of fish. The correlation coefficient (r) value between the concentration of the extract and the fish mortality was 0.998. Mortality of treated fish showed a strong positive linear relationship with correlation coefficient (R) values of 0.891, 0.833, 1.00, 0.891 and 0.980 for concentrations (25 mg l^{-1}, 35 mg l^{-1}, 45 mg l^{-1}, 55 mg l^{-1} and 65 mg l^{-1}), respectively. No mortality was recorded in control.

The 96 h median lethal concentration (LC_{50}) value of *Z. rhetsa* seed extract against *C. idella* was found to be 45 mg l^{-1} in the present study, which were compared with the finding of earlier reports done for other Cypriniforms (Adesina et al., 2005) in which the mean value of 96 h LC_{50} has reported as 97.61 mg l^{-1} against *Oreochromis niloticus*. The 96 h LC_{50} value of 44.92 mg l^{-1} for *Jatropha gossypifolia* plant extract and 43.62 mg l^{-1} for *Codiaeum variegatum* plant extract for *Cirrhinus mrigala* as also was reported (Pratap & Singh, 2015). Similarly, 96 h LC_{50} values of *Moringa oleifera* seed extract had been reported to be 124.0 mg l^{-1} for *Cyprinus carpio* (Kavitha et al., 2012), which was much higher than 45 mg l^{-1} as obtained in the present study.

The strong positive correlation of mortality with the concentration is in agreement with Singh & Singh (2009) and Kucukgil et al., 2012 which have shown that increasing concentration of extracts has strong positive results of mortality. The result revealed the fact that a higher dose of the extract and exposure time could exert higher piscicidal activity, possibly for causing an enhanced intake of the active components into the fish body (Kucukgil et al., 2012). Jomang et al. (2017) report found the LC_{50} value of *Z. rhetsa* in *Heteropneustes fossilis* as 70.1 mg l^{-1} for 96 h exposure periods. They also observed a high correlation coefficient between the concentration and mortality of fish. Saha et al. (2017) could found a higher LC_{50} value of *Luffa cylindrical* fruit extract on tilapia fingerlings, *Oreochromis mossambicus* as 10.54 g l^{-1} for 96 h exposure periods from the same environmental areas.

Various abnormal behavioral changes were observed in *C. idella* during 96 h exposure in extract for median lethal toxicity. These changes in behaviour were observed from 12 to 96 h and recorded in Table 3.

In the laboratory, fish behaviour can be a sensitive marker of toxicant-induced stress (Prashanth & David, 2010). Therefore the behavioural changes were carefully observed and recorded. During the

Table 1. The LC_{50} value of *Zanthoxylum rhetsa* on *C. idella* for 96 h calculated by Probit analysis method (Finney's, 1971)

Concentration (mg l^{-1})	Total number	Total death	% Mortality	Probit analysis	Log_{10} concentration (±SD)
Control	20	0	0	-	-
25	20	5	25	4.33	1.40±0.16
35	20	8	40	4.64	1.54±0.16
45	20	11	55	4.87	1.65±0.16
55	20	13	65	5.13	1.74±0.16
65	20	16	80	5.38	1.81±0.16

Table 2. Variation of Dissolved oxygen (DO) and free carbon dioxide (CO_2) of water during 96 h toxicity test of *Zanthoxylum rhetsa*

Concentration (mg l^{-1})	Free carbon dioxide (CO_2) (mg l^{-1})		Dissolved oxygen (DO) (mg l^{-1})	
	Initial	Final	Initial	Final
Control	9.66±1.53	9.67±0.57	6.83±0.05	6.56±0.20
25	9.00±1.53	10.66±0.53	6.77±0.35	6.16±0.15
35	9.27±1.53	11.00±1.00	6.40±0.36	6.36±0.10
45	9.15±1.30	11.67±0.57	6.92±0.58	6.70±0.10
55	10.66±1.2	11.33±0.53	6.00±0.50	6.70±0.10
65	9.84±1.30	12.07±0.57	6.50±0.70	6.45±0.15

Table 3. Behavioural changes of *C. idella* observed during 96 h exposure of the aqueous seed extract of *Z.rhetsa*

Exposure period	Behavioural changes
1-12 h	Fish become alert, disrupt schooling behaviour, change in swimming patterns in experimental fish were observed.
13-24 h	Other changes involved jumping, loss of equilibrium, increased air gulping, hyperactivity and increased aggression after 13^{th} h till 24 h of exposure.
25-36 h	Fishes went to the side of the aquarium. Fish became sluggish and operculum beats decreased. Excessive mucus secretion in skin observed.
37-48 h	Operculum movement was restricted indicating lethargies.
49-96 h	Mucus secretion in gill was disrupted with red patches and wounds. Operculum breathing decrease. At the end of the exposure period fish gathered at the aquarium corner and become motionless with taking a vertical position sometime before dying.

experiments, fish showed abnormal behavioural changes due to exposure to extract. The behavioural change increased with an increase in the concentration of extract and exposure period. Gulping of air and jumping was observed due to decrease in dissolved oxygen level and irritation was observed in extract-treated water (Table 2).

Morphological changes have also been observed in *C. idella* exposed to a higher concentration of *Z. rhetsa* extract and which was observed by seeing the colour changes of the skin with swollen and reddish eyes, heavy secretion of mucus from all over the body. The experimental specimens showed excessive mucus secretion and hyper-excitability at the higher concentrations during 96 h of exposure time. The change in fish treated with *Z. rhetsa* extract was not seen in the control which demonstrates that *Z. rhetsa* exposure is responsible for the changes. Edafe et al. (2018) observed similar behaviour changes in *Clarias gariepinus* exposed to *Luffa cylindrica* fruit extracts. The present findings also agree with the works of Jomang et al. (2017) in *Heteropneustes fossilis* when exposed to *Z. rhetsa* extracts and Saha et al. (2017) in *Oreochromis mossambicus* when exposed to *Luffa cylindrical* fruit extracts.

The result generated from the present study contribute largely to the introduction of a new plant-based piscicide in aquaculture management systems for fish farmers and fishermen. Due to its dual effects, which initially act as a fish toxicant and later on as manure, the plant-based piscicides are the best alternatives to chemical piscicides to eradicate unwanted fishes from aquaculture systems. On the other hand plant-based, piscicides are not hazardous to the environment and are biodegradable. The present findings show that *Z. rhetsa* extracts were found to be toxic for the fingerlings of grass carp. The percentage of mortality of fishes was increased with the increase in the concentration of extract and also the time of exposure. Thus, *Z. rhetsa* could be used as an organic piscicide in aquaculture pond management, to wipe out predatory fishes from the culture water bodies before stocking.

Acknowledgements

The first author is thankful to the Head of the Department and Dean, Faculty of Fishery Sciences, Panchasayar, Chakgaria, Kolkata, West Bengal for providing necessary facilities to conduct the study.

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