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Jagtap HS
Department of Zoology,
Shri Shivaji College, Parbhani-431 401, Dist.-Parbhani,
Maharashtra, India

Correspondence
Jagtap HS
Department of Zoology,
Shri Shivaji College, Parbhani-431 401, Dist.-Parbhani,
Maharashtra, India

Piscicidal activity of *Parthenium hysterophorus* L. on *Labeo rohita* - A herbal piscicides

Jagtap HS

Abstract
The present study was conducted to assess the piscicidal activity of locally available plant on *Labeo rohita*. The studies were focused on the laboratory determination of lethal concentrations (LC_{50} and LC_{100}) through a *static bioassay test*. Leaf extracts of *P. hysterophorus* L. (Family Apocynaceae) were used for these studies. Concentrations range from 0.5ml/L to 15ml/L were tried. Based on results it was found that in advanced fry, LC_{50} (1ml/L) and LC_{100} (1.5ml/L) and in semi fingerlings LC_{50} (2ml/L) and LC_{100} (3ml/L) were observed. Based on the 96 h lethal concentration study, *P. hysterophorus* showed the strongest piscicidal activity. During exposure, fish exhibited discoloration, gulping for air, erratic swimming, loss of reflexes, slow opercular movement and ultimately settling at the bottom motionless. Present results showed that locally available weed plants have the potential to be used as piscicides, which can be used for eradication of undesirable fish species present in fish ponds.

Keywords: Fish, piscicides, lethal concentration, *Labeo rohita*, fingerlings

Introduction
P. hysterophorus L. probably entered India before 1910 (through contaminated cereal grain) commonly known as gajar ghass, congress weed and feverfew, considered to be one of the world's seven most notorious weed and it is estimated that about 35 million hectares area in India has been invaded by it [6]. The chemical analysis has indicated that all plant parts including trichomes and pollen contains toxins from the chemical group of sesquiterpene lactones [11]. The major components of toxic parthenin and other phenolic acids such as caffeic acid, vanillic acid, anisic acid, p-anisic acid, chlorogenic acid, and parahydroxy benzoic acid are lethal to human beings and animals [8, 10]. It contains several important chemical constituents, mainly histamine, saponin, glucosides and triterpene (sesquiterpene). There are numerous bioassay studies on plant extract, first of such work involving some local plants was reported as early as 1965 [9]. Indian and Chinese major carps displayed abnormal behavior exposed to different concentrations of *Nerium oleander*, *Cannabium sativum* and *P. hysterophorus* leaf extracts respectively [2]. Very limited work has been done on the toxicity of *P. hysterophorus* on fishes.
There are numerous bioassay studies on plant extract. This study was conducted as part of the research to study the biological activity of leaves extract of *P. hysterophorus* L. on Rohu, L. *rohita* is one of the important Indian major carp cultured in freshwater throughout India. The aim of the present study was to assess the piscicidal activity of *P. hysterophorus* on non-target fish rohu, *L. rohita*.

Materials and Methods
Extract Preparation
P. hysterophorus leaves were collected from adjacent areas. Leaves cut into small pieces and crushed in mixture. Crushed materials pass into a porous cloth and then pressed the crushed materials, liquid extract releases. The liquid extracts collected in glass bottle and kept in refrigerator till use to avoid loss/evaporation. From 310gm leaves obtained 150 ml liquid extracts.

Experimental Animals
Laboratory reared *L. rohita* advanced fry (mean length 4.1±0.2cm and weight 3.26±0.3 gm) and semi fingerlings (mean length 8.12±0.3 cm weight 6.24±0.5 gm) were collected from the private fish farm, for the present study.

Toxicity Experiments

Toxicity experiment was performed according to the method of [13]. Experimental fishes were acclimatized in aquarium for 72 h containing well water at room temperature 25 ^{0}C – 27 ^{0}C. The liquid extract of *P. hysterophorus* different concentration tried on *L. rohita*, advanced fry and semi fingerlings were ranges from 0.5ml/L to 15 ml/L. Experiments were arranged in small size glass aquaria (30x30x30 cm) at room temperature. The fishes were exposed for 24h, 48h, 72h and 96h for different concentrations. Performance tests lasted for 96 h. The dead fish were removed continuously to avoid pollution related effect and were counted for determination of LC_{50} and LC_{100}. Water parameters like temperature, pH and dissolved oxygen were monitored throughout the exposure period to determine any tangible water quality deterioration effect on fish, which might confound treatment effects [1]. Three small aquaria were set up for each concentration and each aquarium contains ten fishes in 6 L of de-chlorinated tap water. Control animals were kept in similar condition without any treatment. Fishes were considered dead if they failed to respond to vigorous poking with glass rod.

Results

Behavioural Changes

L. rohita displayed abnormal behaviors when exposed to different concentrations of *Parthenium hysterophorus* leaf extracts like disturbed swimming, rapid opercular movements, incessant gulping of air, darkening of the whole body and fish settling at the bottom motionless, restlessness, hyperactivity, abrupt erratic and jerky swimming, increased mucus secretion, discolouration of skin, drooping of fins, loss of balance and finally the death. Reddish colour observed near the gill region and in stomach region in all sets. The skin color of the fishes became light grey. In control group there were no such changes observed.

Toxicity Tests

Advanced Fry

When exposed to leaves extract 0.5ml/L no mortality occurred throughout the experiment. The 96 h, LC_{50} value (1ml/L), and LC_{100} value (1.5ml/L) were observed in advanced fry. At higher dose 5ml/L within 15 min 100 % mortality were observed. At concentration 10 ml/L and 15 ml/L within 10 min all died in both the groups.

Semi fingerlings

No mortality occurred at the concentration 0.5ml/L, 1ml/L and 1.5ml/L respectively. The 96 h, LC_{50} value (2ml/L) and LC_{100} value (3 ml/L) were observed in semi fingerlings. At concentration 5 ml/L within 25 min all died and at dose 10 ml/L and 15 ml/L within 10 min all fishes died in both groups (Table 2 & 3).

Table 2: LC_{50} and LC_{100} of *P. hysterophorus* L. leaf extracts for L. *rohita*

Developing stage of *L. rohita*	LC_{50}	LC_{100}
Advanced fry	1 ml/L	1.5 ml/L
Semi fingerlings	2 ml/L	3 ml/L

There was no mortality in control groups. LC_{50} and LC_{100} at 96 h.

Table 3: 100 % mortality of *L. rohita* exposed to *P. hysterophorus* L. leaf extracts at higher doses

Developing stage of *L. rohita*	Dose (ml/L)	100 % mortality (time in min)
Advanced fry	5 ml/L	15
	10 ml/L	10
	15 ml/L	10
Semi fingerlings	5 ml/L	25
	10 ml/L	10
	15 ml/L	10

There was no mortality in control groups.

Water Parameters

After experiments different water parameters were analyzed. The data exhibit a decline in dissolved oxygen (DO) content of test solution at different concentrations of the toxicant, as compared to control. The DO declines significantly when extract concentration increases. There after due to fish mortality, the oxygen utilization decreased and the decline in DO content became insignificant (Table 1).

Table 1: Ranges of water quality parameters during toxicity experiments of *P. hysterophorus* L. leaf extracts on L. *rohita* at different concentration

Parameters	Control	Extract concentration (ml/L)							
		0.5	1	1.5	2	3	5	10	15
Dissolved oxygen (mg/L)	6.4-6.6 (6.2)	6.1-6.3 (6)	6-5.8 (5.8)	4.9-4.3 (4.7)	4.7-4.5 (4.6)	4.7-4.6 (4.6)	4.3-4.5 (4.2)	4.2-4 (4)	4.2-4 (4)
pH	7-8 (7.5)	7-7.8 (7.5)	6.9-7 (6.8)	6.6-6.8 (6.7)	6.3-6.6 (6.5)	6-5.9 (5.9)	5.9-6 (5.8)	5.8-5.9 (5.8)	5.7-5.9 (5.8)
Temp (^{0}C)	26-27 (26.5)	26-27 (26.5)	26-27 (26.5)	26-27 (26.5)	26-27 (26.5)	26-27 (26.5)	26-27 (26.5)	26-27 (26.5)	26-27 (26.5)

The figures in parentheses indicate the mean values.

After completion of experiments the required quantity of water is taken out for water parameters analysis and experimental setup including treated water kept as it is for further toxicant degradation study. After 8 days of first experiment same sets were used for toxicity tests on *L. rohita*, advanced fry and semi fingerlings. No mortality occurred at the above said concentrations except 15 ml/L.

Discussion

The mortality of the fish was chosen as the measurable effect to determine the toxicity of the crude liquid extract. It is clear from the results that *P. hysterophorus* L. crude liquid extract is acute toxic against freshwater fish rohu, L. *rohita*, thus, the plant is having piscicidal activity. The plant *P. hysterophorus* may be used as a potential source of piscicides since as a crude preparation of the leaves extract has efficient piscicidal activity. As mentioned in result, the type of behavior responses indicate that tested materials may be neurotoxic, which might be active at the neuromuscular system of fish *L. rohita.*

In the present work it was also found that, DO content of experimental water decreases when extract concentration

increases, due to may be increases activity of fishes, the higher metabolic rate results into more oxygen utilization**.** It was reported some toxins paralyse fish, others work by reducing oxygen content in water [3]. The stressful breathing behavior exhibited by fish may be as a result of respiratory impairment due to effect of toxicant on the gills. Mortality caused by tested materials showed a significant positive correlation between dose and mortality. It may be due to increase of extract concentration in water resulted in more intakes of their active moieties in fish body. The increase in mortality with increased exposure periods could be affected by several factors (loss of equilibrium, paralyse fish, respiratory impairment, irregular, erratic and sometimes jerky movement) [5].

Behavioral changes observed in the present study somewhat similar to toxic study reported in, Indian and Chinese major carps displayed abnormal behavior when exposed to *N. oleander*, *C. sativum* and *P. hysterophorus* leaf extracts respectively [2].

Present studies revealed that fish exposed to toxicants exhibit marked behavioral changes like swift opercular movement, sudden jerky swimming body movements, which demonstrated a sensitive indicator of physiological stress in fish. Davis, observed similar behavior when fish were subjected to sub-lethal concentrations of pollutants [4]. The behavioral responses observed in current studies can be favorably compared with those observed in formalin test on seabass, *Lates calcarifer* fry [12]. Similarly, reported abnormal movement and high respiration rate in hybrid Tilapia, *Oreochromis mossambicus* induced by ammonia, indicating neurological dysfunction and gill damage [7]. The erratic behaviour prior to death in the present and past studies can be conveniently associated with the impact of toxicants on fish. More efforts are required for chemical analysis and their toxicity on different fish species at biochemical and tissue level. Moreover, control of predatory or trash fishes is not selective.

In the present study it was also found that the toxicity of the *P. hysterophorus* liquid extract added in to the experimental water, reduced within 8-10 days that does not showed toxic effect on *L. rohita*, advanced fry and semi fingerlings.

Conclusion

Findings of the present study indicates that the *P. hysterophorus* have potent piscicidal activity on *L. rohita*. So these leaf extract liquid compound cannot be used directly in water bodies or for medicinal purpose, without knowing their structural activity relationship with non-target aquatic organisms. This study can be apply to control the predatory and trash fishes from fish pond but need more research work.

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