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PISCICIDAL ACTIVITY AGAINST FRESHWATER FISH IN LABORATORY AND CEMENT PLASTERING POND CONDITION

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study assesses piscicidal activity of (i.e., acetone, diethyl ether, ethyl alcohol, chloroform, and carbon tetrachloride) latex extracts of *Thevetia peruviana* (Pers.) L. Schum. (Family: Apocynaceae) against *Labeo rohita* (Hamilton) in both conditions. Toxicity of all the organic solvent *Thevetia peruviana* plant was time as well as dose-dependent against freshwater fish. There was a significant ($P<0.05$) negative correlation between LC values and exposure periods. *Thevetia peruviana* plant decrease from 3.62 mgL^{-1} (24h) > 2.69 mgL^{-1} (48h) in laboratory conditions and 7.86 mgL^{-1} (24h) > 7.24 mgL^{-1} (48h) in cement plastering pond condition against fish. The same trend of toxicity was also observed in the case of other solvents i.e., diethyl ether, ethyl alcohol, chloroform, and carbon tetrachloride latex extracts of *Thevetia peruviana* against *Labeo rohita* in both conditions. *Thevetia peruviana* medicinal plant is used as a better cathartic, febrifuge useful in different kinds of intermittent fever while the latex of this plant is used in teeth cavities for relief from toothache.

Keywords: *Thevetia peruviana*; *Labeo rohita*; Latex; different organic solvent; piscicides; toxicity; laboratory and pond condition; hours.

1. INTRODUCTION

The use of natural pesticides derived from plants has recently gained popularity as a way to reduce the dangers of organic and synthetic pesticides. It is possible to substitute organic pesticides with pesticides of plant origin. Due to their biodegradability, botanical pesticides are environmentally safe and do not leave any toxic residues in the aquatic environment. Botanicals are plant extracts that are poisonous to fish and are referred to as piscicides [1]. Alkaloids such as resin, tannins, saponins, nicotine, and diosgenin are found in piscicidal plants [2-6]. These alkaloids, however, are poisonous to fish at high quantities and wear off quickly [7-9]. *Thevetia peruviana* is also reported in piscicidal activity [10-12]. The *Labeo rohita* was chosen as the test animal because it is found in almost all freshwater reservoirs in India and can be used to monitor toxicity [13-15]. Extensive use of synthetic chemical pesticides has become an essential part of present-day agricultural practices. Indiscriminate use of synthetic pesticides by human activities causes a high risk to non-target organisms [1]. Excessive use

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of pesticides results in the inflow of toxicants, mainly into the aquatic bodies [16-18]. The pesticides that have received the most attention and carbamates since they are widely used and are continuously being contaminated by the toxic wastes of chemical pesticides [19-22] and potential direct threat to freshwater organisms, particularly to sensitive animals, such as fishes and prawns [23-26].

The present work is aimed to find out acute toxicity on freshwater fish *Labeo rohita* by determining the LC_{50} values at various exposure periods in both conditions. This type of study will help to estimate the safe level dose and strengthen the baseline data by which comparative sensitivity of plant pesticides could be analyzed.

2. MATERIALS AND METHODS

2.1 Experimental Animal

Labeo rohita (5.50±0.6 cm length and 362 mg weight) were collected from the Gorakhpur district, Uttar Pradesh, India. The fishes were stocked in cement plastering stocking cemented pond containing 1000 L de-chlorinated tap water for acclimatization.

2.2 Plant

Thevetia peruviana (Apocynaceae) was collected from the Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, where a voucher specimen is deposited.

Fig. 1. *Labeo rohita* fish

Fig. 2. *Thevetia peruviana* plant

2.3 Extraction of Active Compounds

Latex was collected and lyophilized at -40°C and the lyophilized powder was used for further study. Took one gm lyophilized latex in 50 mL different solvent mix well in each solvent and left for 48h then centrifuged at 2000 rpm for 20 min. The solvent evaporated at low temperature by vacuum pump to obtain the active moiety in dried form.

2.4 Toxicity Experiments

Toxicity experiments were performed by Singh and Agarwal [27] method. 50 animals like *Labeo rohita* cement plastering pond condition in 50 L de-chlorinated tap water for 24h, 48h, 72h, 96h, and 10 animals were kept in laboratory condition in 10 L de-chlorinated tap water for 24h, 48h, 72h, 96h. Different solvent *Thevetia peruviana* (1.5, 2.5, 3.5, 4.5 mgL^{-1} in laboratory condition and 6, 7, 8, 9 mgL^{-1} in cement plastering pond condition), respectively. During the 96-hour observation period, mortality was recorded every 24 hours. The probit log approach of Russel et al., [28], was used to calculate the LC values.

2.5 Experimental Conditions of Experimental Water

The laboratory, as well as cement plastering pond toxicity of the experiment, were determined by the methods of APHA/AWWA/WPCE [29].

Parameters	Laboratory condition	Cement plastering pond condition
Atmospheric presser	30.5-31.5°C	31.6-32.8°C
water temperature	27.0-28.0°C	28.0-29.0°C
pH	7.3-7.5	7.5-7.6
dissolved oxygen	6.8-7.6 mgL^{-1}	7.2-8.3 mgL^{-1}
free carbon dioxide	4.4-6.5 mgL^{-1}	5.4-7.5 mgL^{-1}
bicarbonate alkalinity	105.0-109.0 mgL^{-1}	108.0-113.0 mgL^{-1}

3. RESULTS AND DISCUSSION

Results obtained after toxicity evaluation of latex extracts of *Thevetia peruviana* plant were tested against freshwater fish *Labeo rohita* in both conditions. The $LC_{10,50,90}$ of the different solvents of this plant 24h to 48h of *Labeo rohita* is shown in Table 1 and 2. The calculated accuracy for the LC_{50} values is summarized in Table 1 and 2. The LC_{50} of acetone extract of *Thevetia peruviana* 3.62 mgL^{-1} (24h) > 2.69 mgL^{-1} (48h) in laboratory conditions, 7.86 mgL^{-1} (24h) > 7.24 mgL^{-1} (48h) in cement plastering pond conditions, respectively Table 1 and 2 against freshwater fish *Labeo rohita*. The same trend of toxicity was also observed in different solvents (i.e., diethyl ether, ethyl alcohol, chloroform, and carbon tetrachloride) latex extract against the freshwater fish *Labeo rohita* in both conditions, respectively Table 1 and 2.

Different solvent latex extracts of these plants are least effective in cement plastering pond conditions in comparison to laboratory conditions against the freshwater fish *Labeo rohita*. So, the cement plastering pond condition the solvent latex extracts of the above plant doses will be very high in comparison to the laboratory condition, respectively Table 1 and 2.

Results of the toxicity of different solvent latex extracts shown in both conditions. Toxicity of freshwater fish was time and dose-dependent. The active moiety of extracts could be time-dependent, leading to a progressive increase in the titer of the active ingredient and its effect in the *Labeo rohita*, or the active moiety of extracts could be converted into more toxic metabolites in the body of the *Labeo rohita* resulting in a time-dependent effect. Result of this study is similar to those of [7,30] who reported different tolerance limits of various aquatic organisms to various piscicides. Result was also found in the case of karanj, *Pongamia pennata* seed on different fishes i.e., *Gudusia giuris*, *Chanda nama*. *Oreochromis mossambicus* [31,32]; *Maesa ramentacea* and *Sapindus emarginatus* are the most effective plants against the *Moina* sp. *Oreochromis niloticus* and *Anabas testudineus* [30] and *Euphorbia heterophylla* plant are the most effective against the fingerlings of *Barbus occidentalis* [33]. Laboratory conditions, the LC_{50} values of above plant against *Labeo rohita* were 3.62 mgL^{-1} (24h) in acetone extracts. Cement plastering pond conditions, the toxicity was 7.86 mgL^{-1} (24h).

Obviously, under cement plastering pond conditions the toxicity of above plants was reduced. The reason for reduced toxicity could be sand particle adsorption or acceleration of the toxicant degradation process by temperature [34]. A same trend was reported by Perchbacher and Sarkar, [35] in which the toxicity persistence of *Masea ramentacea* and tea seed cake was short and fish could be stocked into ponds 4 days after applying the pesticides. The potential for using *Masea ramentacea* as a substitute for tea seed cake for killing predatory fish in freshwater has been shown; however, the effective concentration must be

determined against the predatory air-breathing fish, such as *Clarias* sp. *Ophicephalus striatus* and *Anabas testudineus* are generally more tolerant of toxicants than other fishes [35].

In the present investigation, the LC_{50} values given in the Table 1 and 2 were steep and the heterogeneity factor was less than 1.0 indicates that the result was found to be 95% confidence limits of LC_{50} values. The regression test ('t' ratio) was greater than 1.96 at all the probability levels. The dosage mortality graphs show steep values [36]. The steepness of the slope line implies that there is a significant rise in *Labeo rohita* mortality with a relatively little increase in toxicant concentration.

Table 1. Lethal dose and concentration of *Thevetia peruviana* to freshwater fish *Labeo rohita* in laboratory condition

Solvents	Exposure periods	Effective dose (mg/L)	Limits (mg/L)		Slope value	't' ratio	Heterogeneity
			LCL	UCL			
Acetone	24h	LC_{10}=1.22 **LC_{50}=3.62** LC_{90}=10.82	2.49	5.17	2.70±0.51	3.18	0.16
	48h	LC_{10}=0.91 **LC_{50}=2.69** LC_{90}=7.98	1.52	3.61	2.72±0.48	3.24	0.14
Diethyl ether	24h	LC_{10}=1.62 **LC_{50}=4.08** LC_{90}=10.25	3.09	5.66	3.20±0.57	3.48	0.11
	48h	LC_{10}=1.27 **LC_{50}=3.36** LC_{90}=8.93	2.35	4.47	3.02±0.51	3.50	0.30
Ethyl alcohol	24h	LC_{10}=1.58 **LC_{50}=5.18** LC_{90}=16.91	3.78	12.32	2.49±0.56	2.77	0.07
	48h	LC_{10}=1.21 **LC_{50}=3.62** LC_{90}=10.81	2.49	5.16	2.69±0.50	3.18	0.16
Chloroform	24h	LC_{10}=1.71 **LC_{50}=4.22** LC_{90}=10.41	3.24	5.91	3.26±0.58	3.50	0.22
	48h	LC_{10}=1.21 **LC_{50}=3.23** LC_{90}=8.64	2.22	4.28	3.00±0.50	3.49	0.15
Carbon tetrachloride	24h	LC_{10}=1.70 **LC_{50}=4.59** LC_{90}=12.35	3.49	7.25	2.98±0.58	3.22	0.08
	48h	LC_{10}=1.21 **LC_{50}=3.62** LC_{90}=10.81	2.49	5.16	2.70±0.51	3.18	0.16

- *Batches of 10 fishes were exposed to four different concentrations of Thevetia peruviana plant.*
- *Concentrations given are the final concentrations (w/v) in laboratory conditions.*
- *Regression coefficient showed that there was a significant (P<0.05) negative correlation between exposure time and different LC values.*
- *LCL=Lower confidence limit; UCL=Upper confidence limit.*

Table 2. Lethal dose and concentration of *Thevetia peruviana* to freshwater fish *Labeo rohita* in cement plastering pond condition

Solvents	Exposure periods	Effective dose (mg/L)	Limits (mg/L)		Slope value	't' ratio	Heterogeneity
			LCL	UCL			
Acetone	24h	LC_{10}=5.50 **LC_{50}=7.86** LC_{90}=11.24	6.89	8.71	8.25±1.96	3.86	0.05
	48h	LC_{10}=4.96 **LC_{50}=7.24**	6.00	8.04	7.81±1.94	3.64	0.12

Solvents	Exposure periods	Effective dose (mg/L)	Limits (mg/L) LCL	UCL	Slope value	't' ratio	Heterogeneity
		LC_{90}=10.56					
		LC_{10}=5.79					
	24h	**LC_{50}=8.49**	7.53	9.61	7.71±1.97	3.63	0.05
Diethyl ether		LC_{90}=12.44					
		LC_{10}=5.04					
	48h	**LC_{50}=7.80**	6.51	8.82	6.75±1.86	3.34	0.09
		LC_{90}=12.08					
		LC_{10}=5.91					
	24h	**LC_{50}=8.77**	7.80	10.10	7.47±1.98	3.53	0.15
Ethyl alcohol		LC_{90}=13.02					
		LC_{10}=5.29					
	48h	**LC_{50}=7.95**	6.83	8.94	7.23±1.89	3.53	0.11
		LC_{90}=11.96					
		LC_{10}=5.10					
	24h	**LC_{50}=8.50**	7.18	10.27	5.77±1.83	2.93	0.08
Chloroform		LC_{90}=14.18					
		LC_{10}=4.57					
	48h	**LC_{50}=7.58**	5.84	8.71	5.82±1.80	2.97	0.09
		LC_{90}=12.58					
		LC_{10}=6.07					
	24h	**LC_{50}=9.22**	8.21	11.15	7.05±1.99	3.32	0.02
Carbon tetrachloride		LC_{90}=14.02					
		LC_{10}=5.32					
	48h	**LC_{50}=8.34**	7.18	9.65	6.57±1.87	3.26	0.05
		LC_{90}=13.07					

- *Batches of 50 fishes were exposed to four different concentrations of Thevetia peruviana plant.*
- *Concentrations given are the final concentrations (w/v) in pond conditions.*
- *Regression coefficient showed that there was a significant (P<0.05) negative correlation between exposure time and different LC values.*
- *LCL=Lower confidence limit; UCL=Upper confidence limit.*

4. CONCLUSION

The test species, *Labeo rohita* has shown a differential toxicity level with the function of period in the current study. Toxicity assessment LC_{50} values and behavioural changes in fish are particularly sensitive markers of pesticide toxicity. The fish's overall health is affected by the behavioural changes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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