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THE TOXICITY OF LEAF AND BARK OF *Thevetia peruviana* PLANT TO FINGERLINGS OF *Labeo rohita* (HAMILTON) IN DIFFERENT CONDITIONS

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ABSTRACT

The piscicidal activity of different organic solvent (i.e. acetone, diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride) extracts from leaf and bark of *Thevetia peruviana* (Pers.) L. Schum. plant which is belong to Family: Apocynaceae against fingerlings of *Labeo rohita* (Hamilton) in laboratory and pond conditions was investigated. The LC_{50} values of acetone leaf extracts of *Thevetia peruviana* plant is 70.62 mg/L (24h) in laboratory condition and 380.49 mg/L (24h) in pond condition and bark extracts of *Thevetia peruviana* plant is 82.19 mg/L (24h) in laboratory condition and 424.09 mg/L (24h) in pond condition against fingerlings of *Labeo rohita*. Similar trend of toxicity was also observed in case of other solvents i.e. diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride leaf and bark extracts of *Thevetia peruviana* plant against the freshwater non-target fingerlings of *Labeo rohita* in laboratory and pond conditions. The toxicological actions of *Thevetia peruviana* plant may be due to the presence of apigenin-5-methyl ether (flavonoid) and triterpenoid glycosides. Although, at present very little literature is available on the control of fishes through plant origin pesticides, an attempt has been made in this paper to assemble all the known information on piscicidal properties of common medicinal plants of eastern Uttar Pradesh, India which might be useful for the control of harmful snails, mosquitoes and fishes.

Key words: *Thevetia peruviana*, *Labeo rohita*, solvents, fingerlings, piscicides

INTRODUCTION

A number of compounds (alkaloids, glycosides, saponins, tannins, alkenylphenols, di and tri-terpenoids etc.) present in several plants belonging to different families with piscicidal activities are used to control of fish (Singh & Singh, 2000; Tiwari & Singh, 2003 and Tiwari *et al.,* 2008). The toxicological actions of *Thevetia peruviana* plant may be due to the presence of apigenin-5-methyl ether (flavonoid) and triterpenoid glycosides (Voigtlander & Balsam, 1970; Graf 2000; Patel *et al.,* 2007). Plant extracts are referred to as botanicals and when poisonous to fish are called piscicides (Burkill 1985; Neuwinger 2004). Such piscicidal plants contain different active ingredients known as alkaloids such as resin, tannins, saponins, nicotine and diosgenin (Wang & Huffman, 1991). However, these alkaloids are toxic to fish at high concentrations and wear off within a short time (Kulakkattolickal 1987; Adewumi 1991). A large number of compounds of various classes that have insecticidal, piscicidal and molluscicidal properties (Mohaptra & Nayak, 1998; Mohaptra & Sovan Sahu, 2000; Srivastava *et al*., 2003; Singh & Singh 2003; Singh *et al*., 2004; Singh *et al*., 2005; Singh & Singh 2005; Tiwari & Singh 2004; Singh & Singh 2005 and Singh *et al.,* 2010).

Application of synthetic pesticides is one of the methods used to control of fish population. Due to their long-term persistence, slow degradability in the water, toxicity to other organisms (Arasta *et al.,* 1996) and accumulation inside the fish body, synthetic piscicides adversely affect the aquatic environment (Cullen & Connell, 1992 and Waliszewski *et al.,* 1999). The Indian major carp of *Labeo rohita* was used as the test animal because it is present in almost all freshwater reservoirs in India and is suitable for toxicity monitoring (Ashraf *et al*., 1992 and Sarvanan *et al.,* 2003).

In the present study the piscicidal activity of different organic solvents from leaf and bark extracts of *Thevetia peruviana* plant to test the toxic effects on freshwater non-target fingerlings of *Labeo rohita* in laboratory and pond conditions were investigated. Because non-target fish *Labeo rohita* is present in

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almost all freshwater reservoirs in India and is suitable for toxicity monitoring.

MATERIALS AND METHODS

Non-target animal: The freshwater non-target fingerlings of *Labeo rohita* (2.15±0.2 cm in total length and 145 mg wet weight) was collected from the Government Hatcheries Centre Chappia, district Gorakhpur (U.P.), India. The fishes were stocked in pond containing 1000 L de-chlorinated tap water for acclimatization. Care was taken to remove the dead animals as soon as possible in order to prevent the decomposition of the body in the pond. The stocking cemented ponds are large (5' x 10' x 6' feet), while, the experimental cemented ponds are 5' x 5' x 6' feet in size.

Plant: Plant *Thevetia peruviana* (Family: Apocynaceae) was collected from Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, India and identified at Plant taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, (U.P.), India, where a voucher specimen No. 1785 is deposited. The leaf and bark were obtained from this plant.

Extraction of active compounds: The fresh leaves and bark was dried 40ºC over night and pulverized in a mortar and pestle. The dried powder of leaf and bark was extracted in Soxhlet apparatus, using 200 mL of five different organic solvents (i.e. acetone, diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride) by the method of Jain (1965). The extracted material was dried through vacuum pump. The extracted dried powder was stored in airtight desiccators and used for further toxicity studies.

Toxicity experiments: Toxicity experiments were performed by the method of Singh and Agarwal (1988). Fifty experimental animals like fingerlings of *Labeo rohita* were kept in pond condition containing 50 L de-chlorinated tap water for 24h, 48h, 72h and 96h and ten experimental animals were kept in laboratory condition containing 10 L de-chlorinated tap water for 24h, 48h, 72 and 96h. These were exposed to four different concentrations of different solvent leaf extracts of *Thevetia peruviana* plant (50, 60, 70, 80 mg/L in laboratory condition and 200, 300, 400, 500 mg/L in pond condition), respectively and four different concentrations of different solvent bark extracts of *Thevetia peruviana* plant (60, 70, 80, 90 mg/L in laboratory condition and 250, 350, 450, 550 mg/L in pond condition) for the fingerlings of *Labeo rohita*. Control groups were kept in de-chlorinated tap water without any treatment. Each set of experiments was replicated six times. Mortality was recorded after every 24h during the observation period of 96h. The LC values were calculated by the probit log method of Robertson *et al.* (2007).

Experimental conditions of experimental water: Experimental conditions of water were determined in the beginning of the experiments by the method of APHA (2005). Atmospheric and water temperature was ranging from 30.5–31.5ºC and 27.0–28.0ºC, respectively, pH of water was 7.3–7.5, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were ranging from 6.8–7.6 mg/L, 4.4–6.5 mg/L and 105.0–109.0 mg/L, respectively, during the experiments in laboratory condition.

In case of cemented pond condition the atmospheric and water temperature was ranging from 31.6–32.8ºC and 28.0–29.0ºC, respectively, pH of water was 7.5–7.6, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were ranging from 7.2–8.3 mg/l, 5.4–7.5 mg/l and 108.0–113.0 mg/l, respectively, during the experiments.

RESULTS

In the present study different organic solvent of leaf and bark extracts of *Thevetia peruviana* plant was tested against freshwater non-target fingerlings of *Labeo rohita*. Fish mortality was used as a bioassay for monitoring the piscicidal activity. The LC values of the different organic solvent leaf and bark extracts of this plant for period 24h of fingerlings of *Labeo rohita* is shown in Table 1 to 4. The LC_{50} of acetone leaf extracts for fingerlings of *Labeo rohita* is 70.62 mg/L (24h) in laboratory condition, respectively (Table 1) and 380.49 mg/L (24h) in pond condition, respectively (Table 2). Similar trend of toxicity was also observed in case of other different organic solvent (i.e. diethyl ether, ethyl alcohol, chloroform and carbon tetrachloride) leaf extracts of *Thevetia peruviana* plant against the fingerlings of *Labeo rohita* in laboratory and pond condition, respectively (Table 1 and 2).

The LC_{50} of acetone bark extracts of *Thevetia peruviana* plant for fingerlings of *Labeo rohita* is 82.19 mg/L (24h) in laboratory condition, respectively (Table 3) and 424.09 mg/L (24h) in pond condition, respectively (Table 4). Similar trend of toxicity was also observed in case of other different organic solvent bark extracts of *Thevetia peruviana* plant against the fingerlings of *Labeo rohita* in laboratory and pond condition, respectively (Table 3 and 4).

Both the plant parts were effective in killing the freshwater non-target fingerlings of *Labeo rohita* at different concentrations. Bark extracts of *Thevetia peruviana* is the least effective plant parts against the fingerlings of *Labeo rohita* in laboratory and pond conditions, respectively (Table 3 to 4). While the leaf extracts of *Thevetia peruviana* plant is the most effective plant parts against fingerlings of *Labeo rohita* in both the conditions, respectively (Table 1 to 2).

Statistical analysis of the data on toxicity brings out several important points. The X^2 test for goodness of fit (Heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep values. The steepness of the slope line indicates that there is a large increase in the mortality of fingerlings of *Labeo* with relatively small increase in the concentration of the toxicant.

Table 1. Toxicity ($LC_{10,50,90}$) of different solvent leaf extracts of *Thevetia peruviana* plant against freshwater non-target fingerlings of *Labeo rohita* in laboratory condition after 24 hour exposure period

Solvents	Exposure period	Effective dose (mg/L)	Limits (mg/L) LCL	UCL	Slope value	't' ratio	Hetero-geneity
Acetone	24h	LC_{10}=44.59 **LC_{50}=70.62** LC_{90}=111.83	60.03	80.88	6.43±3.35	3.57	0.21
Diethyl ether	24h	LC_{10}=47.62 **LC_{50}=75.87** LC_{90}=120.91	65.94	89.21	6.33±3.38	3.51	0.33
Ethyl alcohol	24h	LC_{10}=52.08 **LC_{50}=77.11** LC_{90}=114.19	68.56	88.22	7.51±3.65	3.87	0.11
Chloroform	24h	LC_{10}=50.40 **LC_{50}=80.45** LC_{90}=128.42	70.51	97.67	6.31±3.51	3.39	0.17
Carbon tetrachloride	24h	LC_{10}=49.49 **LC_{50}=84.59** LC_{90}=144.58	73.20	114.72	5.50±3.47	3.01	0.09

- Batches of 10 fishes were exposed to four different concentrations of *Thevetia peruviana*.
- Concentrations given were the final concentrations (w/v) in laboratory conditions.
- LCL=Lower confidence limit; UCL=Upper confidence limit.

Table 2. Toxicity ($LC_{10,50,90}$) of different solvent leaf extracts of *Thevetia peruviana* plant against freshwater non-target fingerlings of *Labeo rohita* in pond condition after 24 hour exposure period

Solvents	Exposure period	Effective dose (mg/L)	Limits (mg/L) LCL	UCL	Slope value	't' ratio	Hetero-geneity
Acetone	24h	LC_{10}=175.09 **LC_{50}=380.49** LC_{90}=826.86	266.07	475.70	3.80±2.69	3.70	0.23
Diethyl ether	24h	LC_{10}=181.75 **LC_{50}=431.88** LC_{90}=1026.27	328.23	576.10	3.41±2.65	3.39	0.28
Ethyl alcohol	24h	LC_{10}=174.00 **LC_{50}=485.54** LC_{90}=1354.90	362.16	786.45	2.88±2.60	2.93	0.36
Chloroform	24h	LC_{10}=215.88 **LC_{50}=494.31** LC_{90}=1131.82	390.64	697.05	3.56±2.83	3.34	0.16
Carbon tetrachloride	24h	LC_{10}=209.43 **LC_{50}=539.40** LC_{90}=1389.30	417.55	925.99	3.12±2.78	2.98	0.08

- Batches of 50 fishes were exposed to four different concentrations of *Thevetia peruviana*.
- Concentrations given were the final concentrations (w/v) in pond conditions.
- LCL=Lower confidence limit; UCL=Upper confidence limit.

Table 3. Toxicity ($LC_{10,50,90}$) of different solvent bark extracts of *Thevetia peruviana* plant against freshwater non-target fingerlings of *Labeo rohita* in laboratory condition after 24 hour exposure period

Solvents	Exposure period	Effective dose (mg/L)	Limits (mg/L) LCL	UCL	Slope value	't' ratio	Hetero-geneity
Acetone	24h	LC_{10}=59.25 **LC_{50}=82.19** LC_{90}=114.05	73.89	90.85	9.02±4.21	4.12	0.58
Diethyl ether	24h	LC_{10}=57.32 **LC_{50}=86.09** LC_{90}=129.30	76.18	99.16	7.25±3.97	3.52	0.31
Ethyl alcohol	24h	LC_{10}=54.79 **LC_{50}=89.78** LC_{90}=147.12	78.04	112.60	5.98±3.85	2.99	0.27
Chloroform	24h	LC_{10}=60.12 **LC_{50}=90.63** LC_{90}=136.61	80.74	107.46	7.19±4.11	3.39	0.16
Carbon tetrachloride	24h	LC_{10}=60.65 **LC_{50}=92.20** LC_{90}=140.15	82.12	111.54	7.05±4.12	3.32	0.02

• Details as given in Table 1.

Table 4. Toxicity ($LC_{10,50,90}$) of different solvent bark extracts of *Thevetia peruviana* plant against freshwater non-target fingerlings of *Labeo rohita* in pond condition after 24 hour exposure period

Solvents	Exposure period	Effective dose (mg/L)	Limits (mg/L) LCL	UCL	Slope value	't' ratio	Hetero-geneity
Acetone	24h	LC_{10}=220.77 **LC_{50}=424.09** LC_{90}=814.67	331.66	510.69	4.52±3.15	3.83	0.12
Diethyl ether	24h	LC_{10}=232.40 **LC_{50}=459.55** LC_{90}=908.08	365.44	564.38	4.33±3.12	3.72	0.42
Ethyl alcohol	24h	LC_{10}=227.78 **LC_{50}=485.59** LC_{90}=1035.20	382.33	624.66	3.89±3.07	3.42	0.25
Chloroform	24h	LC_{10}=223.12 **LC_{50}=517.79** LC_{90}=1201.65	403.98	726.78	3.51±3.03	3.11	0.29
Carbon tetrachloride	24h	LC_{10}=266.96 **LC_{50}=564.10** LC_{90}=1191.99	458.33	793.99	3.94±3.25	3.27	0.04

• Details as given in Table 2.

The slope is, thus an index of the susceptibility of the non-target animal to the plant origin pesticides used.

DISCUSSION

The present study, indicate that the organic solvent of leaf and bark extracts of *Thevetia peruviana* plant has high piscicidal activity in laboratory and pond conditions. The uptake of 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 fingerlings tissues, or the active moiety of extracts could be converted into more toxic metabolites in the body of the fingerlings of *Labeo rohita* resulting in a time dependent effect. The results of this study are similar to those of (Kulakkattolickal, 1989 and Chiayvareesajja *et al*., 1997) who reported different tolerance limits of various aquatic organisms to various piscicides. Same result was also found in case of karanj, *Pongamia pennata* seed on different fishes i.e. *Gudusia giuris*, *Chanda nama*, *Oreochromis mossambicus* (Mohapatra & Nayak, 1998 and Mohapatra & Sovan Sahu, 2000); *Maesa ramentacea* and *Sapindus emarginatus* are the most effective plants against the *Moina* sp. *Oreochromis niloticus* and *Anabas testudineus* (Chiayvareesajja *et al*., 1997). The same result was also found in case of *Euphorbia royleana* against *Channa punctatus* (Singh & Singh, 2002; 2005) and *Euphorbia royleana* plant is most effective against *Channa punctatus* (Tiwari *et al.,* 2008).

In laboratory conditions, the LC_{50} values of the tested plant against fingerlings of *Labeo rohita* was 70.62 mg/L (24h) in acetone leaf extracts of *Thevetia peruviana* plant and 82.19 mg/L (24h) in acetone bark extracts of *Thevetia peruviana* plant. In a pond conditions, the toxicity of *Thevetia peruviana* plant acetone leaf extracts was 380.49 mg/L (24h) and the toxicity of *Thevetia peruviana* plant acetone bark extracts was 424.09 mg/L (24h) against the non-target fingerlings of *Labeo rohita*.

Obviously the toxicity of tested plants was reduced under pond conditions. The reason for reduced toxicity could be sand particle adsorption (Dawson *et al*., 1991) or acceleration of the toxicant degradation process by temperature. A similar trend was reported by Perchbacher & Sarkar (1992), 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* that are generally more tolerant of toxicants than other fishes (Perchbacher & Sarkar, 1992). The toxicological actions of *Thevetia peruviana* plant may be due to the presence of apigenin-5-methyl ether (flavonoid) and triterpenoid glycosides (Voigtlander & Balsam, 1970; Graf, 2000 and Patel *et al.,* 2007).

Statistical analysis of the data on toxicity brings out several important points. The X^2 test for goodness of fit (Heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep values (Rand & Petrocelli, 1988). The steepness of the slope line indicates that there is a large increase in the mortality of fingerlings of *Labeo* with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the non-target animal to the plant origin pesticides used.

CONCLUSION

In conclusion, the organic solvent of leaf and bark extracts of the tested plant may be used as potent source of piscicides. Because plant products are less expensive, easily available and easily soluble in water, they may be preferred over commercial pesticides.

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