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Toxic Effect of *Euphorbia pulcherima* Plant to Fingerlings of *Labeo rohita* (Hamilton) in Different Culturing Conditions

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Abstract: The piscicidal activity of aqueous and acetone latex extracts of *Euphorbia pulcherima* plant which is belong to Family: Euphorbiaceae against fingerlings of *Labeo rohita* (Hamliton) in laboratory and cemented pond conditions was investigated. Toxicity of aqueous and acetone latex extracts of this plant was time as well as dose dependent against fingerlings of *Labeo rohita*. The aqueous latex extracts of this plant is least effective in comparison to acetone latex extracts of *Euphorbia pulcherima* plant against the fingerlings of *Labeo rohita*. There was significant ($P<0.05$) negative correlation between LC values and exposure periods. Thus, the LC_{50} values of acetone latex extracts of *Euphorbia pulcherima* plant decrease from 1.35 mg/L (24h) > 1.10 mg/L (48h) in laboratory conditions and 5.25 mg/L (24h) > 4.94 mg/L (48h) in cemented pond condition against fingerlings of *Labeo rohita*. In case of aqueous latex extracts of *Euphorbia pulcherima* plant the LC_{50} values decrease from 5.58 mg/L (24h) > 4.94 mg/L (48h) in laboratory conditions and 18.05 mg/L (24h) > 17.17 mg/L (48h) in cemented pond condition against fingerlings of *Labeo rohita*. The doses of aqueous extract were so high. So its purification is necessary for developing a new and effective herbal piscicides. The acetone latex extracts of *Euphorbia pulcherima* plant is 4-5 time more toxic than the aqueous latex extracts of this plant against the fingerlings of *Labeo rohita*. In conclusion, the aqueous and acetone latex 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.

Key words: *Euphorbia pulcherima* % *Labeo rohita* % Latex % Aqueous % Acetone % Fingerlings

INTRODUCTION

A number of compounds (saponins, tannins, alkaloids, alkenylphenols, di and tri-terpenoids etc.) present in several plants belonging to different families with piscicidal activities are used to control of fish [1-3]. Within the family Euphorbiaceae, the sixth largest among flowering plants, the genus *Euphorbia* L. accounts for almost a sixth of the whole group [4]. The genus *Euphorbia* is composed essentially of latex bearing species [5]. Many of them have been the objects of chemical and pharmacological investigations because of the irritant and medicinal properties of their latexes [6]. Plant extracts are referred to as botanicals and when poisonous to fish are called piscicides [7, 8]. Such piscicidal plants contain different active ingredients known as alkaloids such as resin, tannins, saponins, nicotine and diosgenin [9]. However, these alkaloids are toxic to fish at high concentrations and wear off within a short time [10, 11]. Several plant materials have shown to be toxic to zooplanktons [12], shrimps [13] and commercial fish species both in the laboratory and field studies [14 - 17].

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 [18] and accumulation inside the fish body, synthetic piscicides adversely affect the aquatic environment [19, 20]. To solve this problem, studies have been carried out on the possibility of using local plants as pescicides [21, 22] and they are safe for users. A large number of compounds of various classes that, have insecticidal, piscicidal and molluscicidal properties have been tested [23 - 32]. The Indian major carp *Labeo rohita* (Ham.) was used as the test animal because it is present in almost all freshwater reservoirs in India and is suitable for toxicity monitoring [33 - 35].

The current study deals with the piscicidal activity of aqueous and acetone latex extracts of *Euphorbia pulcherima* plant to test the toxic effects on fingerlings of *Labeo rohita* in laboratory and cemented pond conditions.

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MATERIALS AND METHODS

Fish: The freshwater 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 cemented pond containing 1000 L de-chlorinated tap water for acclimatization. Care was taken to remove the dead fish 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 *Euphorbia pulcherima* (Family: Euphorbiaceae) was collected from Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur, (U. P.), India and identified at Plant taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, (U. P.), India, where a voucher specimen is deposited. The latex was obtained in this plant.

Extraction of Active Compounds

For Aqueous Extracts: One ml of latex was obtained from both the plants and initially diluted in 5 ml of distilled water and centrifuged at 2000 g for 15 min. The water soluble supernatant was lyophilized at -40°C and lyophilized powder was stored for further toxicity experiments in both the conditions.

For Solvent Extracts: The latex of this plant is collected in test tube by cutting stem apices and lyophilized at -40°C and the lyophilized powder was used for further study. Took one g lyophilized latex in 50 ml acetone mix well and left for 48h then centrifuged at 2000 rpm for 20 min. Solvent was evaporated at low temperature by vacuum pump to obtain the active moiety in dried form. This dried powder was used for toxicity experiments in both conditions.

Toxicity Experiments: Toxicity experiments were performed by the method of Singh and Agarwal [36]. Fifty experimental fish like fingerlings of *Labeo rohita* were kept in cemented pond condition containing 50 L de-chlorinated tap water for 24, 48, 72 and 96h and ten experimental fish were kept in laboratory condition containing 10 L de-chlorinated tap water for 24, 48, 72 and 96h. These were exposed to four different concentrations of aqueous latex extracts of *Euphorbia pulcherima* plant (04, 05, 06, 07 mg/l in laboratory condition and 16, 17, 18, 19 mg/l in cemented pond condition) against the fingerlings of *Labeo rohita*. The acetone solvent latex extracts of *Euphorbia pulcherima* plant (0.75, 1.25, 1.75, 2.25 mg/l in laboratory condition and 4.50, 5.00, 5.50, 6.00 mg/l in cemented pond condition), against 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, upper and lower confidence limits, slope value, 't' ratio and heterogeneity were calculated by the probit log method (POLO computer programme of Robertson *et al*., [37].

Experimental Conditions of Experimental Water: Experimental conditions of water were determined in the beginning of the experiments [38]. Atmospheric and water temperature was ranging from 30.5-31.5°C and 27.0-28.0°C, 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 conditions.

In case of cemented pond condition the atmospheric a nd water temperature was ranging from 31.6-32.8°C and 28.0-29.0°C, 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

The LC values of the aqueous and acetone latex extracts of *Euphorbia pulcherima* for periods ranging from 24 to 48h of fingerlings of *Labeo rohita* is shown in Table 1 and 2. The toxicity was time as well as dose dependent, as there was a significant negative correlation ($P<0.05$) between LC_{50} values and exposure periods. Thus, the LC_{50} of aqueous latex extracts of *Euphorbia pulcherima* plant decrease from 5.58 mg/L (24h) > 4.94 mg/L (48h) in laboratory conditions, respectively (Table 1) and 18.05 mg/L (24h) > 17.17 mg/L (48h) in cemented pond conditions against the fingerlings of *Labeo rohita*, respectively (Table 2). The LC_{50} of acetone latex extracts of *Euphorbia pulcherima* plant for fingerlings of *Labeo rohita* decreased from 1.35 mg/L (24h) > 1.10 mg/L (48h) in laboratory conditions, respectively (Table 1) and 5.25 mg/L (24h) > 4.94 mg/L (48h) in cemented pond conditions, respectively (Table 2).

Table 1: Toxicity ($LC_{10, 50, 90}$) of aqueous and acetone latex extracts of *Euphorbia pulcherima* plant against freshwater fingerlings of *Labeo rohita* in laboratory condition at different time intervals

			Limits (mg/L)				
Solvents	Exposure periods	Effective dose (mg/L)	LCL	UCL	Slope value	't' ratio	Hetero-geneity
Aqueous extract	24h	LC_{10}=3.32					
		LC_{50}=5.58	4.46	6.41	5.74±1.25	3.65	0.02
		LC_{90}=9.29					
	48h	LC_{10}=3.17					
		LC_{50}=4.94	3.89	5.64	6.63±1.34	3.80	0.17
		LC_{90}=7.72					
Acetone extract	24h	LC_{10}=0.56					
		LC_{50}=1.35	0.91	1.72	3.38±0.27	3.78	0.49
		LC_{90}=3.22					
	48h	LC_{10}=0.46					
		LC_{50}=1.10	0.65	1.42	3.42±0.25	3.66	0.42
		LC_{90}=2.61					

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

Table 2. Toxicity ($LC_{10, 50, 90}$) of aqueous and acetone latex extracts of *Euphorbia pulcherima* plant against freshwater fingerlings of *Labeo rohita* in pond condition at different time intervals.

			Limits (mg/L)				
Solvents	Exposure periods	Effective dose (mg/L)	LCL	UCL	Slope value	't' ratio	Hetero-geneity
Aqueous extract	24h	LC_{10}=15.31					
		LC_{50}=18.05	17.03	18.95	17.88±5.99	3.77	0.06
		LC_{90}=21.29					
	48h	LC_{10}=14.96					
		LC_{50}=17.17	16.07	17.88	21.50±6.87	3.92	0.19
		LC_{90}=19.69					
Acetone extract	24h	LC_{10}=4.01					
		LC_{50}=5.25	4.67	5.66	10.94±2.22	3.67	0.43
		LC_{90}=6.88					
	48h	LC_{10}=3.92					
		LC_{50}=4.94	4.33	5.29	12.75±2.51	3.70	0.62
		LC_{90}=6.22					

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

The aqueous latex extracts of this plant was least effective in comparison to acetone latex extracts of this plant against fingerlings of *Labeo rohita* in both the conditions. So, the aqueous latex extracts the doses will be very high in comparison to the acetone latex extracts of this plant. While the acetone extract is the most effective against fingerlings of *Labeo rohita* in both conditions (Tables 1 and 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. did not significantly affect the LC_{50} values, as these were found to lie within the 95% confidence limits. The steepness of the slope line indicated that there was a large increase in the mortality of

fingerlings of *Labeo rohita* with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the fish to the plant origin pesticides used.

DISCUSSION

The present study indicated that the aqueous and acetone latex extracts of *Euphorbia pulcherima* had high piscicidal activity in laboratory and cemented pond conditions. The toxicity against the fingerlings of *Labeo rohita* was time as well as dose dependent. There was a significant negative correlation between LC_{50} values and exposure periods, thus, increasing the exposure time, the LC_{50} values were decreased. The increase in mortality with increase in exposure periods could be due to several factors, which may be acting separately or conjointly. For example, 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 were similar to those of several workers [39-41] who reported different tolerance limits of various aquatic organisms to various pesticides. In case of karanj, *Pongamia pennata* seed on different fishes i.e. *Gudusia giuris*, *Chanda nama*. *Oreochromis mossambicus* [23, 24]; *Maesa ramentacea* and *Sapindus emarginatus* are the most effective plants against the *Moina* sp. *Oreochromis niloticus* and *Anabas testudineus* [21]. The same result was also found in case of *Euphorbia royleana* against *Channa punctatus* [14, 22] and *Euphorbia royleana* plant is most effective against *Channa punctatus* [3].

Different species of plants employed as piscicides have different effects, depending on the species of fish targeted [42]. The active principles in the plant part used (leaves, seeds, kernals and bark) have varying potencies and mode of action depending on whether it is applied directly and the forms of extracts (aqueous and alcohol) used [15]. *Euphorbia pulcherima* (Family: Euphorbiaceae) is a common medicinal plant of India, which has a variety of use, such as in the treatment of rheumatism, snakebite, asthma, obstipation and skin-diseases [43].

In laboratory conditions, the LC_{50} values of the tested plant against fingerlings of *Labeo rohita* was 1.35 mg/L (24h) > 1.10 mg/L (48h) in acetone latex extracts of *Euphorbia pulcherima* plant. In a cemented pond conditions, the toxicity of *Euphorbia pulcherima* plant acetone extracts was 5.25 mg/L (24h) > 4.94 mg/L (48h) against the fingerlings of *Labeo rohita*. In pond condition LC_{50} values is very high in comparison to laboratory condition.

Obviously under pond conditions the toxicity of tested plant was reduced. The reason for reduced toxicity could be sand adsorption [44] or acceleration of the toxicant degradation process by temperature. A similar trend was reported by Perchbacher and Sarkar, [45] 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. *Ophiocephalus striatus* and *Anabas testudineus* that are generally more tolerant of toxicants than other [45]. Also, Yadav and Singh [46] also reported that the *Euphorbia pulcherima* plant is toxic to snail *Lymnaea acuminata* in pond condition.

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 [47]. 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 fish to the plant origin pesticides used.

In conclusion, the aqueous and acetone latex 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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