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Original article

Toxic effect of euphorbia hirta plant to fingerlings of labeo rohita (hamilton) in different culturing conditions

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The piscicidal activity of aqueous and acetone latex extracts of Euphorbia hirta 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 hirta plant against the fingerlings of Labeo rohita. There was significant ($P<0.05$) negative correlation between LC values and exposure periods. Thus, the LC50 values of acetone latex extracts of Euphorbia hirta plant decrease from 2.11 mg/L (24h) >1.47 mg/L (48h) in laboratory conditions and 6.38 mg/L (24h) >5.75 mg/L (48h) in cemented pond condition against fingerlings of Labeo rohita. In case of aqueous latex extracts of Euphorbia hirta plant the LC50 values decrease from 6.37 mg/L (24h) >5.59 mg/L (48h) in laboratory conditions and 26.29 mg/L (24h) > 25.52 mg/L (48h) in cemented pond condition against fingerlings of Labeo rohita. The dose of aqueous extract is so high, that its purification is necessary to develop a new and effective herbal piscicide. The acetone latex extracts of Euphorbia hirta plant is 4-5 time more toxic than the aqueous latex extracts of this plant against the fingerlings of Labeo rohita.

1. Introduction

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 (Obomanuet al., 2005). However, these alkaloids are toxic to fish at high concentrations and wear off within a short time (Adewumi, 1991). Several plant materials have shown to be toxic to zooplanktons (Kreutzweiser et al., 2004) and commercial fish species both in the laboratory and field studies (Sambasivan et al., 2003; Obomanu et al., 2007). Euphorbia hirta L. is a medicinal, rhizomatous herb distributed in Southern Western parts of India (Abdul Rahuman et al., 2007). The plant is also widely used in diarrhea and dysentery, especially amoebic dysentery. The extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Chika et al., 2007; Patil et al., 2009). The plant E. hirta is present compounds flavonoids, polyphenols, tannins, triterpenes and phytosterols and alkanes (Gnecco, 1996; Martinz, 1999) 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 fish (Singh and Singh, 2000; Tiwari and Singh, 2003; Tiwari et al., 2008; Singh and Singh, 2009). Within the family Euphorbiaceae, the sixth largest among flowering plants, the genus Euphorbia L. accounts for almost a sixth of the whole group (Webster, 1994). The genus Euphorbia is composed essentially of latex bearing species (Lynn and Radford, 1987). Many of them have been the objects of chemical and pharmacological investigations because of the irritant and medicinal properties of their latexes (Alberto et al., 1997).

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 et al., 1992; Waliszewski et al., 1999). To solve this problem, studies have been carried out on the possibility of using local plants as pescicides (Chiayuareesajja et al., 1997; Singh and Singh, 2005) that are considered safe for users. A large number of compounds of various classes that have, piscicidal, molluscicidal and larvicidal properties have been tested (Mohaptra and Nayak, 1998; Singh et al., 2004; Singh and Singh, 2009; Singh et al., 2010; Srivastava et al., 2003). The Indian major carp L. rohita (Hamilton) was used as the test animal because it is common species that is present in almost all freshwater reservoirs in India a suitable for toxicity monitoring (Ashraf et al., 1992; Mohaptra and Sovan Sahu, 2000; Sarvanan et al., 2003).

The current study deals with the piscicidal activity of aqueous and acetone latex extracts from E. hirta plant on fingerlings of L. rohita in laboratory and cemented pond conditions.

2. Materials and methods

2.1. 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.

2.2. Plant

Plant Euphorbia hirta (Family: Euphorbiaceae) was collected from the Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur, (U. P.), India and identified by the Plant taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, (U. P.), India, where a voucher specimen is deposited. The latex was obtained from this plant.

2.3. Extraction of active compounds

2.3.1. Latex 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 the lyophilized powder was stored for further toxicity experiments in laboratory and cement tank conditions.

2.3.2. Latex solvent extracts

The latex of this plant is collected in a test tube by cutting the stem apices and lyophilized at -40°C the lyophilized powder was then 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. The latex solvent was evaporated at low temperature with a vacuum pump to obtain an active moiety in dried form. This dried powder was used for both the toxicity experiments.

2.4. Toxicity experiments

Toxicity experiments were performed by the method of (Singh and Agarwal, 1988). Fifty experimental fingerlings of L. rohita were kept in cemented ponds containing 50 L of de-chlorinated tap water for 24 to 48h and ten experimental fish were kept in laboratory condition containing 10 L de-chlorinated tap water for 24 to 48h. These were exposed to four different concentrations of aqueous latex extracts of E. hirta plant (05, 06, 07, 08 mg/L in laboratory condition and 25, 26, 27, 28 mg/L in cemented ponds). The experiments with acetone solvent latex extracts of E. hirta plant (01, 02, 03, 04 mg/L in laboratory condition and 05, 06, 07, 08 mg/L in cemented pond conditions). 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 a 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.,(2007).

2.5 Experimental conditions of experimental water

Experimental conditions of water were determined in the beginning of the experiments (APHA, 2005). Atmospheric and water temperature was ranging from 30.7-31.6°C and 28.0-29.0°C, respectively, pH of water was 7.3-7.5, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were ranging from 6.9-7.7 mg/L, 4.5-6.6 mg/L and 105.0-109.0 mg/L, respectively, during the experiments in laboratory conditions.

In the cemented ponds the atmospheric and water temperature from 32.6-33.8°C and 29.0-30.0°C, respectively, the pH of water was 7.6-7.7, and the dissolved oxygen, free carbon dioxide and bicarbonate alkalinity from 7.5-8.7 mg/L, 5.6-7.8 mg/L and 108.0-113.0 mg/L, respectively.

3. Results

The LC values of the aqueous and acetone latex extracts of E. hirta for periods ranging from 24 to 48h of fingerlings of L. rohita is shown in Table 1 and 2; figure 1. The toxicity was time as well as dose dependent, as there was a significant negative correlation ($P<0.05$) between LC50 values and exposure periods. Thus, the LC50 of aqueous latex extracts of E. hirta plant decreased from 6.37 mg/L (24h) >5.59 mg/L (48h) in laboratory conditions, respectively (Table 1; Figure 1) and 26.29 mg/L (24h) >25.52 mg/L (48h) in cemented pond conditions (Table 2; Figure 1). The LC50 of acetone latex extracts of E. hirta plant for fingerlings of L. rohita decreased from 2.11 mg/L (24h) > 1.47 mg/L (48h) in laboratory conditions, respectively (Table 1; Figure 1) and 6.38 mg/L (24h) >5.75 mg/L (48h) in cemented pond conditions, respectively (Table 2; Figure 1).

The aqueous latex extracts of this plant was least effective in comparison to acetone latex extracts in both the experiments. 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 L. rohita in both conditions (Tables 1 and 2; Figure 1).

Statistical analysis of the data on toxicity brings out several important points. The X2 test for goodness of fit (Heterogeneity) demonstrated that the mortality counts were not significantly heterogeneous and other variables, e.g. resistance etc. did not significantly affect the LC50 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 L. 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.

The LC50 values given in the (Table 1 and 2) were steep and heterogeneity factor was less than 1.0 indicates that the result found to be 95% confidence limits of LC50 values. The regression test (‘t’ ratio) was greater than 1.96 at all the probability levels.

Table 1
Toxicity (LC10,50,90) of aqueous and acetone latex extracts of E. hirta plant against freshwater fingerlings of L. rohita in laboratory condition at different time intervals.

Solvents	Exposure periods	Effective dose (mg/L)	Limits (mg/L) LCL	UCL	Slope value	‘t’ ratio	Hetero-geneity
Aqueous Extract	24h	LC10=3.86 LC50=6.37 LC90=10.54	4.93	7.31	5.86±1.53	3.28	0.05
	48h	LC10=3.57 LC50=5.59 LC90=8.75	3.98	6.39	6.59±1.67	3.31	0.21
Acetone Extract	24h	LC10=0.55 LC50=2.11 LC90=8.09	1.03	3.03	2.19±0.36	3.21	0.20
	48h	LC10=0.42 LC50=1.47 LC90=5.09	0.55	2.13	2.37±0.35	3.28	0.35

- Batches of 10 fishes were exposed to four different concentrations of E. hirta.
- 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 (LC10,50,90) of aqueous and acetone latex extracts of E. hirta plant against freshwater fingerlings of L. rohita in pond condition at different time intervals.

Solvents	Exposure periods	Effective dose (mg/L)	Limits (mg/L) LCL	UCL	Slope value	‘t’ ratio	Hetero-geneity
Aqueous Extract	24h	LC10=23.98 LC50=26.29 LC90=28.83	25.21	27.02	32.08±11.63	3.94	0.18
	48h	LC10=23.56 LC50=25.52 LC90=27.65	24.10	26.21	36.81±14.97	3.49	0.12
Acetone Extract	24h	LC10=4.33 LC50=6.38 LC90=9.41	5.39	7.12	7.60±1.65	3.87	0.55
	48h	LC10=3.98 LC50=5.75 LC90=8.29	4.61	6.43	8.06±1.80	3.69	0.52

- Batches of 50 fishes were exposed to four different concentrations of E. hirta.
- 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.

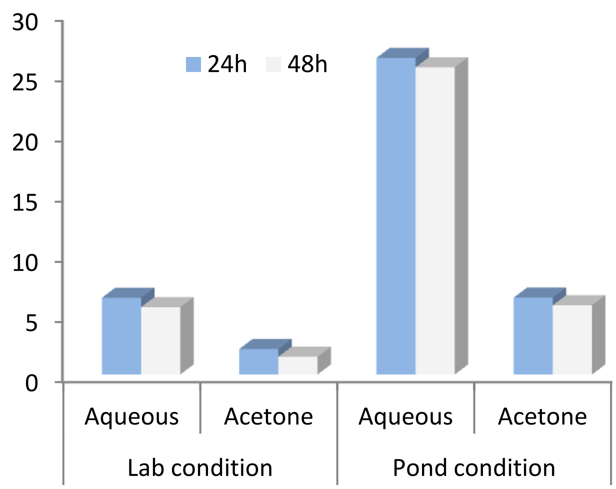

Fig. 1. Toxicity (LC50) of aqueous and acetone latex extracts of E. hirta plant against freshwater fingerlings of L. rohita in laboratory and pond condition at different time intervals.

4. Discussion

The present study indicates that the aqueous and acetone latex extracts of E. hirta plant have a high piscicidal activity in laboratory and cemented pond conditions. The toxicity against the fingerlings of L. rohita was both time as well as dose dependent. There was a significant negative correlation between LC50 values and exposure periods, thus, increasing the exposure time, the LC50 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 L. rohita resulting in a time dependent effect. The results of this study were similar to those of several workers (Kulakkattolickal, 1989; Van Andel, 2000) 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; Maesa ramentacea and Sapindus emarginatus are the most effective plants against the Moina sp. Oreochromis niloticus and Anabas testudineus (Chiayuareesajja, 1997). The same result was also found in case of E. royleana against Channa punctatus (Singh and Singh, 2002; 2005) and E. royleana plant is most effective against Channa punctatus (Tiwari et al., 2008).

Different species of plants employed as piscicides have different effects, depending on the species of fish targeted (Fafioye et al., 2004). 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 (Sambasivan et al., 2003). The E. hirta extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Chika et al., 2007; Patil et al., 2009).

In laboratory conditions, the LC50 values of the tested plant against fingerlings of L. rohita was 2.11 mg/L (24h) > 1.47 mg/L (48h) in acetone latex extracts of E. hirta plant. In a cemented pond conditions, the toxicity of E. hirta plant acetone extracts was 6.38 mg/L (24h) >5.75 mg/L (48h) against the fingerlings of L. rohita. In pond condition LC50 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 (Dawson et al., 1991) or acceleration of the toxicant degradation process by temperature. A similar trend was reported by Perchbacher and 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. Ophiocephalus striatus and Anabas testudineus that is generally more tolerant of toxicants than other (Perchbacher and Sarkar, 1992). Yadav and Singh, (2007) also reported that the E. pulcherima plant is toxic to snail L. acuminata in pond condition. Similar trend of toxicity was also reported that E. pulcherima and Thevetia peruviana plant is toxic to fingerlings of L. rohita in laboratory and pond conditions by (Singh and Singh, 2009; 2010). In recently the T. peruviana plant is toxic to fish C. punctatus in laboratory condition by (Singh et al., 2013).

Statistical analysis of the data on toxicity brings out several important points. The X2 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 LC50 values, as these were found to lie within the 95% confidence limits (Rand and 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 fish to the plant origin pesticides used.

5. Conclusion

There are a very large number of plants, which contain compounds lethal to target as well as non-target organism at doses, which are much below those for synthetic pesticides. Use of such products has the additional advantage that these contain biodegradable compounds, which are less likely to cause environmental contamination. After all such compounds are not only confined to the plants in which they are founds but also possibly gets distributed in the environment. We strongly feel that if these herbaceous products are used as molluscicides, piscicides and larvicides they would not only control the vector snail would also have the advantage of easy availability, low cost biodegradability and greater acceptance amongst the users. Further, more we feel that with further progress in biotechnology, such products could be raised form, sources other than that plant in which they are currently found. Production of plant pesticides could, in long run also become an important industry using biotechnological methods.

Further studies going on the plant to elaborate the more activity in plant constituents, therefore there are many plants uses are mentioned in ayurveda on that base go for further studies.

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