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Short Communication

Molluscicidal Activity of Different Organic Solvent Latex Extracts of Some Common Euphorbiales against Freshwater Harmful Snails

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Abstract

Molluscicidal activity of different organic solvent extracts of latex of *Euphorbia pulcherima* and *Euphorbia hirta* (Family-Euphorbiaceae) against two freshwater harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus* has been reported. Both the snails are the intermediate hosts of *Fasciola hepatica* and *Fasciola gigantica*, which cause endemic fascioliasis in cattle and livestock of Eastern part of India. Toxicity of all the extracts of both the plants was time as well as dose dependent against both the snails. There was significant negative correlation between LC values and exposure periods. Thus, the LC values of latex of *Euphorbia pulcherima* of chloroform and carbon tetrachloride extracts were decreased from 0.51 mg/L (24 h) to 0.24 mg/L (96 h) and 0.51 mg/L (24 h) to 0.21 mg/L (96 h) respectively, against *Lymnaea acuminata* and from 0.46 mg/L (24 h) to 0.11 mg/L (96 h) and 0.49 mg/L (24 h) to 0.17 mg/L (96 h) against *Indoplanorbis exustus*. Similar trend was also observed in case of *Euphorbia hirta* of all the solvent extracts. The doses that can be used for killing the 90% population of snail *Lymnaea acuminata* are safe for non-target fish *Channa punctatus*, which shares the habitat with these snails. We thus believe that further purification of active compounds present in *Euphorbia pulcherima* and *Euphorbia hirta* latex may eventually be of great value for the control of harmful snails and other aquatic pests.

Keywords: *Euphorbia pulcherima*; *Euphorbia hirta*; Molluscicide; *Indoplanorbis exustus*; *Channa punctatus*

Introduction

A large population of snails, however, inhabits freshwaters, where the larvae of parasitic trematodes also pass part of their life. Many aquatic snails act as vectors for the larvae of trematodes and thereby cause a number of diseases. Two diseases carried by aquatic snails, fascioliasis and schistosomiasis caused immense

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harm to man and his domestic animals [1-5].

The method of controlling the schistosomiasis and fascioliasis is to kill the intermediate host snail with molluscicides [6]. Widespread and heavy use of synthetic pesticides, have been found to affect water bodies due to their high toxicity, bioaccumulation and long term persistence [7,8]. The hazardous nature of synthetic pesticides has prompted the scientists to find out the least disruptive newer options in the field of pest control technologies. Molluscicides of plant origin are widely used, because the selective toxicity of these products is high, easily biodegradable in nature, safe for users and low cast [9].

The aim of the present study is to investigate molluscicidal properties of different organic solvent extracts of latex of *Euphorbia pulcherima* and *Euphorbia hirta* (Family-Euphorbiaceae), two common medicinal plants of India against two freshwater harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus*. Both the snails are the intermediate hosts of *Fasciola hepatica* and *Fasciola gigantica*, which cause endemic fascioliasis in cattle and livestock in Northern part of India.

Materials and Methods

The freshwater harmful snails *Lymnaea acuminata* (2.6±0.3 cm in shell height) and *Indoplanorbis exustus* (0.85±0.03 cm in shell height) and non-target fish *Channa punctatus* (14.8±1.3 cm total body length) were collected from the local freshwater bodies of Gorakhpur district, in India. The collected animals were stored in glass aquaria containing de-chlorinated tap water for acclimatization to laboratory conditions. Experimental conditions of water were determined in the beginning of the experiments by the method of APHA/WPCF [10]. Dead animals were removed from the aquaria to avoid any contamination. Average sized adult animals were used for the experiments.

Plants *Euphorbia pulcherima* and *Euphorbia hirta* (family-Euphorbiaceae) were collected from Botanical Garden of D.D.U. Gorakhpur University, Gorakhpur, Uttar Pradesh, India and identified by Prof. S.K. Singh, Plant taxonomist, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur, India, where a voucher specimen is deposited. The latex of both plants was collected in test tube by cutting stem apices and lyophilised at −40°C and the lyophilised powder was used for further study. Wet weight of 1 mL latex of *Euphorbia pulcherima* was 1.44 mg and the weight of lyophilised powder was 480 mg and the wet weight of 1 mL latex of *Euphorbia hirta* was 1.34 mg and the weight of lyophilised powder was 460 mg.

Extraction of Active Moiety

The lyophilised latex powder was extracted with 50 mL of each organic solvent. Centrifugation for 20 min at 2000 g was carried out in a refrigerated centrifuge, at −4°C, after extraction. The solvent fraction was decanted and the solvent allowed to evaporate. The dried soluble fraction was re-dissolved in water for further experiments.

Toxicity experiments were performed by the method of Singh and Agarwal [4]. Ten experimental animals either *Lymnaea acuminata* or *Indoplanorbis exustus* were kept in glass aquaria containing 3 L de-chlorinated tap water for 24 h, 48 h, 72 h and 96 h. These were exposed to four different concentrations of latex of *Euphorbia pulcherima* (0.20, 0.30, 0.40, 0.50 mg/L and 0.10, 0.20, 0.30, 0.40 mg/L) for *Lymnaea acuminata* and *Indoplanorbis exustus*, respectively and four different concentrations of latex of *Euphorbia hirta* (0.40, 0.50, 0.60, 0.70 mg/L and 0.30, 0.40, 0.50, 0.60 mg/L) for the *Lymnaea acuminata* and *Indoplanorbis exustus*, respectively. 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 24 h during the observation period of 96 h. The values were calculated by the probit log method of Russel *et al.* [11].

For environmental toxicity, if any caused by the extracts of both the plants, the toxic effect of different organic solvent latex extracts of *Euphorbia pulcherima* and *Euphorbia hirta* was also studied in mixed populations of fish and snails. In these experiments, group of 10 snail *Lymnaea acuminata* and 10 fish *Channa punctatus* were put together in 6 L de-chlorinated tap water. These mixed populations were exposed to previously determined LC_{90} (24 h) of snail *Lymnaea acuminata* for 24 h. Mortality was recorded after 24 h exposure periods.

Results and Discussion

Experimental conditions of water were determined by the method of APHA/WPCF [10]. Atmospheric and water temperatures were 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.

Exposure to the different organic solvent latex extracts of *Euphorbia pulcherima* and *Euphorbia hirta* caused significant behavioural changes in the freshwater harmful snails *Lymnaea acuminata* and *Indoplanorbis*

exustus. Behavioural changes appeared with 5 to 10 min of exposure. The initial 30-40 min was a period of hyperactivity during which, sluggish snails moved rapidly in the aquarium water. After some time they started crawling on each other. As the poison enters in the snails body a muscular twitching happens and the snails become spirally twisted, which resulted in ataxia, convulsion, paralysis and finally death of snails. Prior to death, there was complete withdrawal of the body inside the shell that indicates nerve poisoning.

In the present study different organic solvent latex extracts of *Euphorbia pulcherima* and *Euphorbia hirta* was tested against both the freshwater harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus*. Snail mortality was used as a bioassay for monitoring the molluscicidal activity. The LC_{50} values of the different organic solvent latex extracts of *Euphorbia pulcherima* and *Euphorbia hirta* for periods ranging from 24 h to 96 h of both the snails *Lymnaea acuminata* and *Indoplanorbis exustus* are shown in Tables 1 and 2. The toxicity was time as well as dose dependent, as there was a significant negative correlation between LC_{50} values and exposure times. Thus, the LC_{50} of chloroform latex extracts for *Lymnaea acuminata* decreased from 0.51 mg/L (24 h) to 0.24 mg/L (96 h) and for *Indoplanorbis exustus* it decreased from 0.46 mg/L (24 h) to 0.11 mg/L (96 h) (Table 1). Similar trend of toxicity was observed in case of other different solvent latex extracts of *Euphorbia pulcherima* against both the snails *Lymnaea acuminata* and *Indoplanorbis exustus* (Table 1).

Table 1. Toxicity (LC_{50}) of different organic solvent extracts of latex of *Euphorbia pulcherima* against freshwater harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus* at different time intervals

Solvents	Exposure periods	Effective dose (LC_{50}, mg/L)	Limits (mg/L)		Slope value	'g' factor	't' ratio	Hetero-geneity
			LCL	UCL				
			Lymnaea acuminata					
Chloroform	24 h	0.51	0.44	0.66	3.45±0.68	0.15	5.10	0.19
	96 h	0.24	0.19	0.28	3.09±0.59	0.14	5.25	0.19
Ethyl alcohol	24 h	0.52	0.44	0.73	2.88±0.64	0.19	4.53	0.09
	96 h	0.25	0.14	0.27	2.64±0.59	0.20	4.44	0.23
Acetone	24 h	0.53	0.47	0.67	4.04±0.76	0.13	5.36	0.27
	96 h	0.26	0.15	0.29	2.09±0.56	0.28	3.69	0.12
Diethyl ether	24 h	0.54	0.44	0.93	2.30±0.61	0.27	3.80	0.11
	96 h	0.26	0.20	0.28	3.61±0.61	0.11	5.90	0.36
Carbon tetrachloride	24 h	0.57	0.47	0.90	2.75±0.21	0.21	4.26	0.09
	96 h	0.27	0.14	0.25	2.56±0.59	0.20	4.35	0.12
			Indoplanorbis exustus					
Chloroform	24 h	0.46	0.35	0.77	1.99±0.43	0.18	4.57	0.09
	96 h	0.11	0.06	0.14	1.74±0.39	0.19	4.52	0.16
Ethyl alcohol	24 h	0.47	0.35	0.45	1.59±0.41	0.26	3.88	0.11
	96 h	0.12	0.06	0.16	1.45±0.38	0.26	3.88	0.28
Acetone	24 h	0.48	0.38	0.75	2.48±0.50	0.16	4.97	0.23
	96 h	0.15	0.10	0.19	1.58±0.37	0.21	4.24	0.18
Diethyl ether	24 h	0.48	0.35	0.91	1.69±0.41	0.23	4.10	0.14
	96 h	0.12	0.06	0.13	1.88±0.40	0.17	4.72	0.45
Carbon tetrachloride	24 h	0.49	0.37	0.93	1.86±0.43	0.21	4.30	0.09
	96 h	0.17	0.07	0.19	1.80±0.39	0.18	4.68	0.18

Effective dose are the final concentrations (w/v) in aquarium water. Each set of experiment was replicated six times. Mortality was recorded every 24 h. Regression coefficient showed that there was significant ($P<0.05$) negative correlation between exposure time and different LC value. LCL & UCL = lower and upper confidence limit.

Table 2. Toxicity (LC_{50}) of different organic solvent extracts of latex of *Euphorbia hirta* against freshwater harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus* at different time intervals

Solvents	Exposure periods	Effective dose (LC_{50}, mg/L)	Limits (mg/L)		Slope value	'g' factor	't' ratio	Hetero-geneity
			LCL	UCL				
			Lymnaea acuminata					
Diethyl ether	24 h	0.69	0.62	0.84	4.41±0.99	0.20	4.43	0.07
	96 h	0.41	0.36	0.45	5.73±1.04	0.13	5.52	0.17
Ethyl alcohol	24 h	0.70	0.64	0.85	4.89±1.04	0.17	4.73	0.09
	96 h	0.43	0.34	0.46	4.36±0.97	0.19	4.49	0.13
Chloroform	24 h	0.73	0.65	0.96	4.14±1.01	0.23	4.13	0.14
	96 h	0.43	0.37	0.47	4.65±0.97	0.17	4.81	0.09
Acetone	24 h	0.74	0.68	0.88	6.08±1.18	0.14	5.14	0.22
	96 h	0.47	0.42	0.51	4.77±0.95	0.15	5.01	0.15
Carbon tetrachloride	24 h	0.75	0.66	1.01	4.26±1.03	0.23	4.13	0.13
	96 h	0.43	0.37	0.47	5.01±0.98	0.15	5.11	0.15
			Indoplanorbis exustus					
Diethyl ether	24 h	0.56	0.53	0.76	3.70±0.81	0.19	4.55	0.08
	96 h	0.30	0.23	0.34	3.68±0.80	0.18	4.60	0.40
Ethyl alcohol	24 h	0.58	0.52	0.73	3.79±0.81	0.18	4.66	0.12
	96 h	0.32	0.26	0.36	3.93±0.80	0.16	4.93	0.29
Chloroform	24 h	0.62	0.55	0.81	3.83±0.83	0.18	4.60	0.12
	96 h	0.32	0.21	0.37	2.75±0.76	0.29	3.63	0.19
Acetone	24 h	0.64	0.57	0.78	5.01±0.95	0.14	5.25	0.26
	96 h	0.36	0.29	0.40	3.42±0.76	0.19	4.49	0.23
Carbon tetrachloride	24 h	0.67	0.54	0.76	4.04±0.84	0.17	4.79	0.08
	96 h	0.38	0.27	0.36	4.22±0.81	0.14	5.21	0.30

Details are as given in Table 1.

Same trend of toxicity was also observed in case of all the different solvent latex extracts of *Euphorbia hirta* against both the snails *Lymnaea acuminata* and *Indoplanorbis exustus* (Table 2).

It is clear from the present study that the different organic solvent latex extracts of *Euphorbia pulcherima* and *Euphorbia hirta* have higher molluscicidal activity than any of the prevalent synthetic molluscicides like carbamate, organophosphate and synthetic pyrethroids. Thus, the 24h LC_{50} of mexacarbate (3.5 mg/L), aldicarbe (30.00 mg/L), farmothion (27 mg/L), cypermethrin (2.5 mg/L), permethrin (0.82 mg/L) and fenvalerate (2.5 mg/L) against the freshwater harmful snail *Lymnaea acuminata* [12,13] are higher than those of the *Euphorbia pulcherima* (0.51 mg/L) and *Euphorbia hirta* (0.69 mg/L) which are about 23.00 and 19.00 times stronger than the standard molluscicide niclosamide LC_{50} (11.8 mg/L) [14].

Statistical analysis of the data on toxicity brings out several important points. The χ^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 slope values. The steepness of the slope line indicates that there is a large increase in the mortality of snails with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the target animal to the pesticides

used. A steep slope is also indicative of rapid absorption and onset of effects. Even though the slope alone is not a very reliable indicator of toxicological mechanism, yet it is a useful parameter, for such a study. Since the LC_{50} of the latices of different euphorbiales lay within the 95% confidence limits, it is obvious that in replicate test of random samples, the concentration response lines would fall in the same range [15]. The LC_{90} doses, of snail have no apparent mortality against freshwater non-target fishes. In conclusion we can say that finding of present communication have great potential as molluscicides. Low toxicity to non-target animals, make it more suitable for snail control programme and development of indigenous bio-insecticides.

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