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A Comparative Study of Toxic Effect of a Euphorbia's Plant *Euphorbia tirucalli* Against Two Freshwater Harmful Snails in Laboratory as Well as in Pond and its Effect on Their Reproductive Physiology

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Abstract: In the present study molluscicidal activity of methanol extract of stem bark of plant *Euphorbia tirucalli* (Family: Euphorbiaceae) against two freshwater harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus* were evaluated in the laboratory as well as in the pond. Both snails are intermediate hosts of liver fluke, *Fasciola hepatica* and *Fasciola gigantica*, which causes endemic fascioliasis in cattle and livestock. Toxicity experiment was performed by the method of Singh and Agarwal (1988). The toxicity of methanol extract was time as well as dose dependent and there was a significant negative correlation between LC values and exposure periods, thus the LC_{50} value of methanol extract was decreased from 8.18 mg/L (24h) to 3.43 mg/L (96h) in the case of *Lymnaea acuminata* and from 10.24 mg/L (24h) to 5.06 mg/L (96h) in the case of snail *Indoplanorbis exustus* in laboratory. Similar trends of toxicity were also observed in the case of pond but the toxicity of methanol extract was lowered about 3.0-4.7 times in pond than the laboratory. The LC_{90} (24h) doses of methanol extract against snail have no apparent killing against non target organism i.e. fish *Channa puctatus* in the treatment of mixed population of snail and fish which shares the habitat of these snails. The effects of different sub lethal concentration of this extract on the reproductive physiology of both the snails i.e. *Lymnaea acuminata* and *Indoplanorbis exustus* were performed up to 28 days in laboratory. Concentration-dependent effects of this extract on snail's embryogenesis were noted. It was found that fecundity, hatchability and survivability was significantly reduced and no survival was observed at higher dose after 28 days in the snail *L. acuminata.* Hence it can be concluded that methanol extract of plant *E. tirucalli* was toxic against all the stages of both the snails and acts as potential source of safer molluscicides.

Keywords: *E. tirucalli* · *Lymnaea acuminata* · *Indoplanorbis exustus* · Methanol Extract · Fecundity

INTRODUCTION

India possesses one of the largest livestock populations in the world. Livestock are an integral component of agriculture in India and make multifaceted contributions to the growth and development of the agricultural sector and are also an important source of income for the rural poor [1]. Livestock diseases are widely distributed and one of the major causes of livestock mortality [2]. Fasciolosis is an important disease caused by the liver flukes *Fasciola hepatica* and *F. gigantica*, infecting mammalian species, including cattle and sheep. According to a World Health Organization report in 2007 the fascioliasis infection was limited in the past to specific and typical geographical areas but is now widespread throughout the world [3]. A large population of snails inhibit freshwater where the parasitic treamatode's larvae pass their life. Many aquatic snails act as vectors of these larvae and cause a number of diseases. Two freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus* are known for their role as intermediate hosts in the life cycle of *F. gigantica* or *F. hepatica* [4].

The control of vector snail populations is done either by killing snails or disruption in their reproductive physiology. Because reproduction is the single most important function in the life cycle of an organism and disruption in the reproduction will affect the abundance and distribution of the species. The inability of an organism to complete any one of the reproductive process

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severely reduces its lifetime reproductive success [5]. Synthetic and natural molluscicides have played a significant role in restricting the population of the snail [6]. Even though chemical pesticides are target specific and effective, but have bad impact on the environment. Plant based pesticides contain active principles with low half-period and their effects on the environment are not too detrimental [7]. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems [8].

In the present investigation molluscicidal activity of methanol extract of medicinally important plant *Euphorbia tirucalli* (Family: Euphorbiaceae) was evaluated against two freshwater harmful snails *L. acuminata* and *I. exustus* were tested in laboratory as well as in pond and their sub-lethal effects were also observed on fecundity, hatchability and survivability of hatchlings of these snails. *E. tirucalli* is commonly known as "pencil tree". It has been used as an application for asthma, cough, earache, neuralgia, rheumatism, toothache and warts.

MATERIAL AND METHODS

Plant: The stem bark of the plant *E. tirucalli* was collected locally from Gorakhpur, India. First of all, stem bark was washed with tap water and then dried in shade. Then dried stem bark was powdered with the help of mechanical device.

Extraction of compounds: About 50 gram powder of stem bark was subjected to extraction through Soxhlet apparatus in methanol solvent for about 72 hours, after 72 hours concentrated solution was obtained. This concentrated solution was filtered and the solvent was evaporated at low temperature by using vacuum pump till active compound was dried. The extracted compound was stored in air-tight desiccators and further used for experiments.

Experimental Animals: The two fresh water harmful snails *Lymnaea acuminata* (3.65 ± 1.00 cm total shell length) and *Indoplanorbis exustus* (0.85 ± 0.037 cm in shell length) were collected from the fresh water bodies of Gorakhpur district (India). Prior to experiment snails were allowed to acclimate to laboratory conditions for 72h.

Toxicity Experiment

In Laboratory: Toxicity experiment was performed by the method of Singh and Agarwal [9]. Thirty animals were kept in glass aquaria containing 3L de-chlorinated tap water. Snails were exposed for 24h, 48h, 72h or 96h at four different concentrations of methanol extract. Each set of experiments, were replicated six times. Mortality was recorded after every 24h during the observation period of 96h.

In Pond: The experiment was conducted in freshwater ponds 29.28 m^3 in area and 9.19 m^3 in water volume. Each pond was stocked with 100 snails with a size difference not greater than 1.5 times [10]. The experimental animals were exposed continuously for 96h to four different concentrations.

Contraction of the snail body within the shell and no response to a needle probe were taken as evidence of death of snails. Dead animals were removed to prevent the decomposition of body in experimental aquarium. Control animals were kept in similar condition without any treatment.

The effective doses (LC values), upper and lower confidence limits, slope values, 't' ratio and heterogeneity were calculated by the probit log method of Robertson *et al.* [11]. Student's 't' test was applied to determine the significant ($p<0.05$) differences between treated and control animals. Product moment co-relation coefficient was applied in between exposure time and lethal concentrations [12].

Toxicity Against Non-Target Organism: Mixed population of snails (*L. acuminata/I. exustus*) and fishes (*Channa punctatus*) were treated in dechlorinated water with the LC_{90} (24h) of methanol extracts of stem bark of *E. tirucalli* caused no mortality amongst non target fish *Channa punctatus* which shares the habitat with these snails.

Fecundity, Hatchability and Survivability Experiment: These experiments were performed according to the method of Presing [13]. In this experiment, fresh water adult snails *L. acuminata* and *I. exustus* were exposed to different sub-lethal [20% & 40% of LC_{50} (24h)] doses of methanol extract. For fecundity experiments, aquariums were filled with 5L de- chlorinated tap water and required amount of extracts were mixed in each aquarium. Ten adult snails were placed in each aquarium. Six replicates were

used for each set of experiment, water temperature were kept at 25±1°C during the entire time of experiments. No food was given to the snails during the experimental period. Control groups of snail were kept in similar conditions without any treatment, for smooth spawning fresh lotus leaf was let floated in each aquarium.

The eggs of *L. acuminata* were attached in ribbon like egg masses (spawns) and eggs of *I. exustus* were attached in round shape egg masses, both egg spawns containing variable number of eggs to the back surface of lotus leaf and inner wall of the aquarium. The egg masses produced by the snails in the experiment were removed after every 24 hours up to 96 hours and the number of eggs counted under compound microscope. All the spawns of each group were transferred into separate petri-dishes containing one litter de- chlorinated tap water for hatching under the same exposure condition as above and kept at 25±1°C for development of embryo in B.O.D. incubator. The number of hatched young snails was counted and their survival rate was recorded continuously after 7 days, 14 days, 21 days and 28 days after hatching. Disintegration of embryos or absence of movement of the embryo was considered for calculating the percent mortality of eggs.

RESULTS

Toxicity: LC_{50} values of methanol extract of *Euphorbia tirucalli* stem bark against *L. acuminata* and *I. exustus* in the laboratory and in pond for periods 24h to 96h are shown in (Table 1 and 2) respectively. Toxicity of methanol extracts of *E. tirucalli* stem bark was time as well as dose dependent and there was a significant negative correlation between LC values and exposure periods. The LC_{50} values of methanol extract of stem bark of *Euphorbia tirucalli* decreased from 08.18 mg/L (24) to 03.43 mg/L (96h) against *Lymnaea acuminata* and from 10.24 mg/L (24) to 5.06 mg/L (96) against *Indoplanorbis exustus* (Table 1) in laboratory and LC_{50} values decreased from 38.30 mg/L (24) to 11.97 mg/L (96) against *Lymnaea acuminata* and from 29.98 mg/L (24) to 16.59 mg/L (96) against *Indoplanorbis exustus* in pond respectively (Table 2).

Effect on the Fecundity, Hatchability and Survivability: After treatment of snail *L. acuminata* with sub lethal doses (20% and 40% of LC_{50} of 24h) of methanol extract of *E. tirucalli* stem bark, fecundity was reduced to 72% and 71% than control group after 96h exposure period and the number of hatched eggs was reduced to 70% and 63% than control group respectively (Table 3). Survival of hatched young snails was reduced to 64%, 41%, 25% and 9% than control group after 7, 14, 21 and 28 days of hatching in 20% treatment, respectively and it was further reduced to 45%, 30% 14% after 7, 14, 21days and no survival was observed after 28 days of hatching than control group, respectively (Table 3).

Significant reduction in the fecundity of snails *I. exustus* was observed after exposure to sub lethal doses (20% and 40% of LC_{50} of 24h) of methanol extract of *E. tirucalli* stem bark for 96h exposure period to 77% and 70% and the number of hatched eggs was reduced to 71% and 68% of the control respectively. Survival of hatched young snails was reduced to 60%, 54%, 44% and 32 % of control after 7, 14, 21 and 28 days of hatching in 20%

Table 1: Toxicity (LC_{50} value) of different concentrations of methanol extracts of *Euphorbia tirucalli* stem bark against two freshwater harmful snails *L. acuminata* and *I. exustus* at different time intervals in laboratory.

Exposure periods	LC_{50} Value (mg/L)	Limits (mg/L) LCL-UCL	Slope value	't' ratio	Hetero-geneity
		L. acuminata			
24h	8.18	6.32-11.86	2.13 ± 0.55	3.83	0.29
48h	5.11	03.50-6.52	2.24 ± 0.54	4.12	0.43
72h	4.09	02.74-5.14	2.64 ± 0.57	4.59	0.73
96h	3.43	02.08-4.42	2.66 ± 0.59	4.45	0.69
		I. exustus			
24h	10.24	08.30-13.98	2.63 ± 0.73	5.64	0.01
48h	8.71	06.70-11.09	2.55 ± 0.72	6.15	0.02
72h	6.19	04.32-7.48	3.12 ± 0.75	5.68	0.12
96h	5.06	03.34-6.17	3.68 ± 0.85	7.36	0.22

•Batches of thirty snails were exposed to four different concentrations of the extract.
•Concentrations given are the final concentration (w/v) in the aquarium water containing de-chlorinated tap water.
•Each set of experiment was replicated six times. Mortality was recorded after every 24h.
•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.
•There was no mortality in the control group.

Table 2: Toxicity (LC_{50} value) of different concentrations of methanol extracts of *Euphorbia tirucalli* stem bark against two freshwater harmful snails *L. acuminata* and *I. exustus* at different time intervals in pond.

Exposure periods	LC_{50} Value (mg/L)	Limits (mg/L) LCL-UCL	Slope value	't' ratio	Hetero-geneity
		L. acuminata			
24h	38.3	30.34-59.14	1.57±0.30	5.46	0.8
48h	27.63	23.17-35.52	1.70±0.30	6.25	0.03
72h	16.74	13.22-20.16	1.55±0.28	7.25	0.28
96h	11.97	09.69-13.91	2.34±0.30	7.98	0.42
		I. exustus			
24h	29.98	26.10-38.42	2.49±0.48	6.04	0.35
48h	26.45	23.21-32.67	2.30±0.46	7.35	0.12
72h	22.19	19.91-25.04	2.72±0.46	5.74	0.1
96h	16.59	14.52-18.28	3.25±0.47	6.83	0.33

Hundred snails were exposed to four different concentrations of the extract. Other details are given in Table 1.

Table 3: Number of laid eggs, duration of hatching, hatched eggs and survivability of hatched young snails (hatchlings) after treatment of 20% and 40% of LC_{50} (24h) of methanol extract of *E. tirucalli* stem bark against freshwater snails, *L. acuminata*.

	Control	20% of LC_{50} (24h)	40% of LC_{50} (24h)
No. of eggs laid			
(after 96 hrs. treatment)	208.50±0.83 (100)	150.50±0.83 (72)	147.83±1.03 (71)
Duration of hatching (in days)	11-13	14-17	14-18
No. of hatched eggs	204.33±0.96 (100)	143.83±0.65* (70.38%)	129.50±0.83* (63.38%)
		Survivability of hatchlings	
After 7 days	202.1±0.65 (99)	91.50±0.83** (64)	58.50±0.63** (45)
After 14 days	199.50±0.83 (98)	59.50±0.83** (41)	38.50±0.83** (30)
After 21days	197.50±0.83 (97)	35.50±0.83** (25)	18.50±0.83** (14)
After 28 days	196.50±0.83 (96)	12.50±0.83** (9)	-

•All experiments were replicated six times.
•Values are means ± SE of six replicates.
•Values in parentheses are percentages of the corresponding value with control taken as 100%.
•*, Significant (P<0.05), when Student's 't' test was applied between control and treated groups.
•**, Significant (P<0.05), when Student's 't' test applied between number of hatched eggs and survivability of hatchlings in corresponding treated groups.
•-, shows no survival.

Table 4: Number of laid eggs, duration of hatching, hatched eggs and survivability of hatched young snails (hatchlings) after treatment of 20% and 40% of LC_{50} (24h) of methanol extract of *E. tirucalli* stem bark against freshwater snails, *I. exustus*.

	Control	20% of LC_{50} (24h)	40% of LC_{50} (24h)
No. of eggs laid			
(after 96 hrs. treatment)	235.6±0.96 (100)	180.5±0.83 (77)	152.3±0.73 (70)
Duration of hatching (in days)	10 -13	12-17	14-19
No. of hatched eggs	235.5±0.83 (100)	167.8±0.66* (71)	120.5±0.833*(68)
Survivability of hatchlings			
After 7 days	231.5±0.83 (98)	100.8±0.66** (60)	65.5±0.83** (54)
After 14 days	229.6±0.96 (99)	91. 3±0.83** (54)	45.50±0.83** (38)
After 21 days	229.5±0.83 (97)	74.30±0.83** (44)	25.30±0.96** (21)
After 28 days	228.5±0.83 (97)	54.80±0.66** (32)	6.5±0.83** (5)

Details are given in Table 3.

treatment and it was further reduced to 54%, 38%, 21% and only 5% after 7, 14, 21 and 28 of days hatching than control group in 40% treatment respectively (Table 4).

DISCUSSION

From result section it is apparent that there was a positive correlation between exposure period and mortality. The increases in mortality with increase in exposure period could be due to several factors, which may be acting separately or conjointly. The uptake of the active moiety of methanol extract of plant *E. tirucalli* could be time dependent leading to a progressive increase in the titer of the active ingredients and it effects in both the snails [14, 9]. The toxic effect of same extract was practiced in pond for its effectiveness because under natural circumstance many factors such as temperature, sunlight, adsorption by soil particles etc. influences on

toxicity (i.e. 3-4.7 times lower) and toxicant degradation. Dawson *et al*. [15] found that rotenone disappeared two to three times faster in earthen ponds than in concrete ponds. Perchbacher and Sarkar [16] reported that the detoxification of Phostoxin (Phosphine) occurred in 4 days in laboratory tank at 23°C and in 1 day in earthen ponds at 37°C. Similar result of toxicity of various plants viz. *Euphorbia pulcherima*, *Lantana indica*, *Azadirachta indica* and *Annona squamosa* was also reported by many researchers against snail *Lymnaea acuminata* [17-20].

Statistical analysis of the data on toxicity brings out several important points. The x^2 test for goodness of fit 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 graph exhibits steep slope values. The steepness of slope line indicates that there is a large increase in the mortality of vectors population with relatively small increase in the toxicant. The slope is thus an index of the susceptibility of the target animal i.e. snails to the methanol extract of *E. tirucalli* used. A steep slope is also indicative of rapid absorption and onset of effects. Since the LC_{50} of the plant extracts 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 [21].

From the developmental studies it was clear that the sub-lethal doses of this plants extracts were also affect the reproductive cycle of the snail *L. acuminata* and *I. exustus* and affect the fecundity, hatchability and survivability in dose dependent way. Similar trends of result were also observed by several workers [19, 22].

The fecundity of both snails was found to be significantly reduced, given that facts that effect of methanol extract on reproduction. The effect included significant dose-dependent reduction in fecundity means that at higher doses more reduction of fecundity was observed. The delay and reduction in hatching was also observed than control group it may be due to the fact that methanol extract interfere the physiological activities of these eggs hence delay and reduction in hatching in young snails [23-25].

The survival of hatchlings of both the snails was also reduced and it reached to zero percent at higher doses after 28 days of hatching in *L. acuminata*. The rapid action of the plant extract in killing in test organisms is perhaps due to its toxic effect on the respiratory function of the snails by acting as uncouple of oxidative phosphorylation at the mitochondrial level [26, 27].

CONCLUSION

It can be concluded from the result section that the methanol extracts of *E. tirucalli* plant was toxic against all the stages of both the snails. It is also safe for non target organism (especially fishes which shares the habitat with these snails), hence it can be used a potential source of safer molluscicides.

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