

Toxic Effect of two Common Euphorbiales against freshwater target snail *Lymnaea acuminata* and *Indoplanorbis exustus* in ponds

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Abstract: Leaf extracts of the medicinal euphorbious plants *Codiaeum variegatum* and *Croton tiglium* were tested as molluscicides in natural ponds against the freshwater snail *Lymnaea acuminata* and *Indoplanorbis exustus*. The molluscicidal activity of leaf extracts of both the plants was time and dose dependent at all the exposure periods. A significant negative correlation between LC values of leaf extracts and exposure periods existed, thus LC₅₀ values decreased from 434.79 mg/L (24h) to 212.04 mg/L (96h) and 497.65 mg/L (24h) to 224.15 mg/L (96h), respectively against *Lymnaea acuminata* and 338.74 mg/L (24h) to 130.30 mg/L (96h) and 410.86 mg/L (24h) to 207.72 mg/L (96h) respectively against *Indoplanorbis exustus*. Both the snails are intermediate host of trematode *Fasciola hepatica*, which causes endemic fascioliasis in cattle and live-stock in northern part of India. The leaf of both the euphorbious plants may probably be used as potent ecofriendly molluscicides in natural ponds for managing the harmful snail population. Non-target freshwater fish *Channa punctatus* (which share the habitat with these snails) the extract of both plants are at higher doses were also lethal, but the doses LC₉₀ (24h) of snails are safe for fish.

[Yadav RP, Singh A. **Toxic Effect of two Common Euphorbiales against freshwater target snail *Lymnaea acuminata* and *Indoplanorbis exustus* in ponds.** *NY Sci J* 2013;(6):18-22]. (ISSN: 1554-0200).

<http://www.sciencepub.net/newyork>. 3

Keywords: Molluscicides, Fascioliasis, *Croton tiglium*, *Lymnaea acuminata*

1. Introduction

Many aquatic snails act as vectors for the larvae of trematodes and thereby cause a number of diseases. Fascioliasis is caused by *Fasciola hepatica*, the large liver fluke, common in sheep, cattle, goat and other herbivorous animals throughout the World (Froyed, 1975) reported that about 21% cattle and 7% sheep were infected with liver fluke in Great Britain. In India, the freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus* are the intermediate hosts of *Fasciola hepatica* and *Fasciola gigantica* (Hyman, 1970), which cause immense harm to domestic animals of this country (Singh and Agarwal, 1981; Singh et al., 1996; Yadav and Singh, 2007; Yadav and Singh, 2011).

In recent times the use of plant products has gained unprecedented impetus all over the World. The people of the northeastern region of India, in particular the rural and tribal people living in some remote areas, primarily depend upon folk and traditional medicine, and indigenous knowledge of how these plant are used for different purposes in different areas. A large number of plant families have furnished many classes of product, which may vary in the degree of pesticidal activity. Several countries have promoted the use of plant products due to their wide range of ideal properties, such as high target toxicity, low mammalian toxicity, low cost, solubility in water, easy biodegradability, abundant growth in endemic areas and operator safety (Kinghorn and

Evans, 1975; Marston and Hostettman, 1985; Singh et al., 1996; Singh et al., 2000).

In the present study the molluscicidal effect of leaf extracts of two common euphorbious plants i.e. *Codiaeum variegatum* and *Croton tiglium* against the harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus*. Toxicity experiments have also carried out on freshwater fish *Channa punctatus* (Which shares the habitat with snails) for environmental toxicity, if any.

2. Materials and Methods

Plant: Plants *Croton tiglium* and *Codiaeum variegatum* (family-Euphorbiaceae) was collected from Botanical Garden of DDU Gorakhpur University, Gorakhpur Uttar Pradesh, India and identified by taxonomist, Department of Botany, DDU Gorakhpur University, Gorakhpur (U.P), where a voucher specimen is deposited.

Animal: The freshwater vector snails *Lymnaea acuminata* (2.6±0.3 cm in total shell height) and *Indoplanorbis exustus* (0.87±0.035 cm in total shell height) were collected from the local freshwater bodies of Gorakhpur district (U.P), in India. The collected animals were stored in glass aquaria containing de-chlorinated tap water for acclimatization to laboratory conditions. Dead animals were removed from the aquaria to avoid any contamination. Average sized animals were used for the experiments.

Extraction of Active moiety: The leaf of the plants *C. tiglium* and *C. variegatum* was minced in 5.0 mL distilled water, homogenized for 5 min. The supernatant was used as water extracts for the molluscicidal activity.

Toxicity experiments were done using the method of (Singh and Agarwal, 1988), using two freshwater ponds, 29.28 m² in area and 9.19 m³ in water volume. Each pond was stocked with 100 snails and these experimental ponds were exposed continuously for 96h to four concentrations of leaf extracts. Control group ponds were kept in similar conditions without treatment. Experimental condition of water determined by the method of (APHA, 1998) Water analysis for temperature, pH, dissolved O₂, total ammonia, free CO₂ and total alkalinity. Atmospheric and water temperature was ranging from 30.5-31.5°C and 27.0-28.0°C, respectively. The pH of water was 7.3-7.5, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were ranging from 6.8-7.6, 4.4-6.5 and 105-109.0 mg/L.

Toxic effect of aqueous extracts of leaf of *C.tiglium* and *C.variegatum* was also studied in mixed populations of fishes and snails. In these experiments a group of 10 snails (*Lymnaea acuminata*) and 10 fishes (*Channa punctatus*) were put together in 50L dechlorinated tap water in pond. These mixed populations were exposed to previously determined LC₉₀ doses of snails for (24h).

The mortality was recorded at 24h intervals up to 96h. Lethal concentration (LC₅₀) values, upper and lower confidence limits (UCL, LCL) and slope values were calculated by the probit log method using POLO computer programme of (Robertson et al., 2007). The regression coefficient was determined between exposure time and different values of LC₅₀ (Sokal and Rohlf, 1973).

Exposure to the aqueous extracts of leaf of *Croton tiglium* and *Codiaeum variegatum* caused significant behavioural changes in the freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus*. Behavioral changes appear with 5-10 min of exposure. The initial 30-45 min was a period of hyperactivity during which sluggish snails moved rapidly in the ponds. After some time, the snails started crawling on each other. As the poison entered the snails's a muscular twitching occurred and the snail became spirally twisted, causing ataxia, convulsion, paralysis and finally death of the snail.

Prior to death, there was complete withdrawal of the body inside the shell, indicating nerve poisoning.

The LC₅₀ values of the aqueous leaf extracts of *Croton tiglium* and *Codiaeum variegatum* for periods ranging from 24h or 96h of snails *Lymnaea acuminata* and *Indoplanorbis exustus* are shown in (Table 1, 2). Thus increase in exposure time the LC₅₀ of *Croton tiglium* leaf decreased from 434.79 mg/L (24h); > 335.74 mg/L (48h); > 249.21 mg/L (72h); > 212.04 mg/L (96h) in case of *Lymnaea acuminata* and 338.74 mg/L (24h); > 272.33 mg/L (48h); > 202.51 mg/L (72h); > 130.30 mg/L (96h) *Indoplanorbis exustus*, respectively (Table 1). Same trend was also observed in case of leaf extracts of *Codiaeum variegatum* (Table 2).

At the higher dose, active moiety of plants, which were effective against the snails, would also cause death amongst the fish. Consequently, a mixed population of 10 snails (*Lymnaea acuminata*) and 10 fish (*Channa punctatus*) were treated with the 24h, LC₉₀ doses for snail *Lymnaea acuminata* there was no mortality amongst fish (Table 3).

The slope values given in the (Table 1-2) were steep and heterogeneity factor was less than 1.0 indicates the result found to be within the 95% confidence limits to LC₅₀ values. The regression test ('t' ratio) was greater than 1.96 and the potency estimation test ('g' values) was less than 0.5 at all probability levels (Table 1-2).

4. Discussion

It is evident from the result presented here that both the plant extracts are highly toxic to the freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus*. The most obvious sign of distress in the treated snails were muscular twitching and spiral twisting of the body, followed by crawling on each other.

The nature and rapid onset of the behavioural response indicates that, the leaf perhaps contains some neurotoxins, which amongst other think might be active at the neuromuscular system of exposed animals. Similar behavioural responses were also observed (Singh and Agarwal, 1990), in their study on acute toxicity of *Euphorbia royleana*, *Euphorbia antisiphilitica* and *Jatropha gossypifolia* on snail *Lymnaea acuminata*. No such behavioural symptoms and death occurred in control groups indicating that no factor other than plant moieties was responsible for altered behaviour and mortality.

Table 1. Toxicity (LC₅₀) of aqueous leaf extracts of *Croton tiglium* (Family- Euphorbiaceae) against *Lymnaea acuminata* and *Indoplanorbis exustus* at different time intervals.

Exposure period	Effective dose (mg/L)	Limits		Slope value	't' Ratio	'g' Factor	Heterogeneity
		LCL	UCL				
<i>Lymnaea acuminata</i>							
24h	434.79	374.23	638.39	5.14±1.12	4.14	0.22	0.24
48h	335.74	301.06	417.89	4.73±1.02	4.63	0.17	0.19
72h	249.21	231.47	266.53	6.06±0.97	6.21	0.99	0.60
96h	212.04	194.36	224.86	6.85±1.03	6.61	0.88	0.66
<i>Indoplanorbis exustus</i>							
24h	338.74	307.22	407.48	4.57±0.99	4.60	0.18	0.35
48h	272.33	247.58	301.10	4.25±0.93	4.55	0.18	0.42
72h	202.51	179.16	244.59	3.92±0.94	4.14	0.22	0.77
96h	130.30	39.72	172.35	3.58±1.16	3.04	0.40	0.34

- Batches of hundred snails were exposed to four different concentrations of *Croton tiglium*
- Concentrations given are the final concentrations (w/v) in natural ponds.
- Regression coefficient showed that there was significant (P<0.05) negative correlation between exposure time and different LC values.

Table 2. Toxicity (LC₅₀) of aqueous leaf extracts of *Codiaeum variegatum* (Family-Euphorbiaceae) against *Lymnaea acuminata* and *Indoplanorbis exustus* at different time intervals.

Exposure period	Effective dose (mg/L)	Limits		Slope value	't' Ratio	'g' Factor	Heterogeneity
		LCL	UCL				
<i>Lymnaea acuminata</i>							
24h	497.65	394.97	717.58	3.77±1.15	3.25	0.36	0.17
48h	337.92	308.84	397.90	4.93±1.00	4.96	0.10	0.36
72h	257.35	233.06	279.80	4.60±0.93	4.90	0.16	0.44
96h	224.15	201.92	240.38	6.09±1.81	6.00	0.10	0.47
<i>Indoplanorbis exustus</i>							
24h	410.86	368.39	503.49	4.23±0.80	5.26	0.13	0.10
48h	328.89	304.26	365.63	4.67±0.75	6.16	0.10	0.24
72h	246.47	230.63	262.05	6.52±0.79	8.20	0.05	0.88
96h	207.72	188.02	234.11	4.74±0.78	7.32	0.07	0.59

- Details as given in table 1

Table 3. Per cent mortality (mean ± SE) of *Lymnaea acuminata* and *Channa punctatus* caused by aqueous extracts of leaf (i.e. 24h LC₉₀ of snail) of *Codiaeum variegatum* and *Croton tiglium* after 24h exposure periods. Each aquarium contained ten fish *Channa punctatus* and ten snails *Lymnaea acuminata* in 6L dechlorinated tap water. There was no mortality in case of control group.

Plants	Concentration (mg/L)	Experimental animals	% Mortality
<i>Codiaeum variegatum</i>	808.46 (LC ₉₀)	<i>C. punctatus</i>	-
		<i>L. acuminata</i>	92.5±1.57
<i>Croton tiglium</i>	771.67 (LC ₉₀)	<i>C. punctatus</i>	-
		<i>L. acuminata</i>	90.0±2.00

The leaf extracts of *Croton tiglium* and *Codiaeum variegatum* showed a significant negative correlation between LC values and exposure periods e.g LC₅₀ of leaf extracts of *Croton tiglium* were decreased from 434.79 mg/L (24h); > 335.74 mg/L (48h); > 249.21 mg/L (72h); > 212.04 mg/L (96h) and 338.74 mg/L (24h); > 272.33 mg/L (48h); > 202.51 mg/L (72h); to 130.30 mg/L (96h)

in the case of *Lymnaea acuminata* and *Indoplanorbis exustus* respectively (table 1).

The correlation between dose and mortality in all cases was noted become increase concentration of pesticides in aquarium water resulted in more intake or entry of pesticides in the body of animals. This trend is also in dependent upon several factors such rate of penetration, nature of slope, variability

and maximal effects of active moieties. The increased in mortality with increased in exposure periods could be affected by several factors, which may be acting separately or conjointly. For example, uptake of active moiety is time dependent, which leads progressive increase the entrance of the drug and its effects, in the snail body (Singh and Agarwal, 1990).

The present study demonstrates that the leaf of *Croton tiglium* and *Codiaeum variegatum* have higher molluscicidal activity than any of the prevalent synthetic pyrethroids. Thus, the 24h LC₅₀ of mexacarbamate (3.4 ppm), aldicarb (30.0 ppm), farmothion (27.0 ppm), cypermethrin (2.5 ppm), permethrin (0.82 ppm) and fenavalerate (2.5 ppm) against the *Lymnaea acuminata* (Singh and Agarwal, 1981; Singh and Agarwal, 1986; Singh and Agarwal, 1987; Singh and Agarwal, 1988; Singh and Agarwal, 1988; Singh and Agarwal, 1991; Sahay et al., 1991) is higher than that of the *Croton tiglium* (0.06 ppm) which is about 196 times stronger the standard molluscicides niclosamide (LC₅₀ 11.8 ppm) (Singh and Agarwal, 1984).

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 LC₅₀ 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 LC₅₀ 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 (Rand and Petrocelli, 1988).

The plant products are less expensive, easily available, easily solubles in water and fewer hazards to the non-target animals than the synthetic molluscicides. In the light of these results, the extract of leaf of *Codiaeum variegatum* and *Croton tiglium* appears to be a promising molluscicidal agent. So it may be concludes that the extracts of above plants may be used as a potent source of molluscicides.

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5/2/2013