

**Activity of *Euphorbia splendens* var. *hislopii* N.E.B.
(Euphorbiaceae) Latex against *Lymnaea columella* (Say, 1817)
(Pulmonata: Lymnaeidae), Intermediate Host of *Fasciola hepatica*,
Linnaeus, 1758 (Trematoda: Fasciolidae). 2: Limited Field-testing**

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The molluscicidal evaluation of Euphorbia splendens var. hislopii (Crown of thorns) against Lymnaea columella snails, intermediate host of Fasciola hepatica, in irrigation ditches of the Pisciculture Station at Universidade Federal Rural do Rio de Janeiro, was studied under limited field conditions. An aqueous solution of the latex at 5 mg/l was tested in two irrigation ditches (experimental and control ditches), after initial sampling of the snail population present. Twenty-four hours after application of the product, it was verified that 97.4% of free L. columella snails and 100% of snails of the same species captive in cages and used as sentinels at three points equidistant from the application site in the experimental ditch, died. For Biomphalaria tenagophila and Melanoides tuberculata snails, present in the experimental ditch, the mortality was 100%, for the species Pomacea spp. the mortality was 40%. No mortality was verified in the free mollusks, or in the sentinels in the ditch used as control. E. splendens var. hislopii latex is thus an efficient natural molluscicide, which may be used as an alternative control agent against L. columella.

Key words: latex - *Euphorbia splendens* var. *hislopii* - *Lymnaea columella*-control - *Fasciola hepatica*

Fasciola hepatica Linnaeus, 1758, is a trematode causing fascioliasis, a disease that affects zootechnical breeding (Gordon 1955), resulting in serious damage to many countries' cattle-raising economy.

As regards Public Health, fascioliasis is worthy of somewhat closer attention, since man, as an occasional host, while being little susceptible to it, can catch the disease when eating aquatic or plants carrying or containing metacercariae (Oliveira 1932, Giovannoni & Kubiak 1947, Freire & Di Primio 1948).

In herds, this disease may bring about economic losses both by retarding growth in infected young animals (Oakley et al. 1979), and resulting in low fertility and abortions, as well as progressive loss in the production of meat and milk, and of the liver (Bundy et al. 1984, Ferrer et al. 1985, Urquhart et al. 1990, Hurtado et al. 1992) as well as leading to the death of infected animals (Reid et al. 1972).

In Brazil, fascioliasis was first described by Lutz (1921) and the first human case was reported by Rey (1958), in Mato Grosso do Sul, followed by references made by Santos and Vieira (1965), Correa and Fleury (1971), Amato Neto and Silva (1977), Baranski et al. (1977), Amaral and Bussetti (1979, 1980), Bussetti (1982).

The states of Rio Grande do Sul (Ueno et al. 1982), Paraná and São Paulo (França 1967), Rio de Janeiro and Minas Gerais (De Rezende 1979), and Santa Catarina (Serra Freire & Nuernberg 1992) are the most important endemic areas in our country.

Lymnaea columella (Say, 1817) is the most important intermediate host of *F. hepatica*, because of its wide distribution and because its occurrence is almost always associated with the disease (Rezende et al. 1973, Gonzales et al. 1974, Paraense 1982, 1983, Gomes et al. 1985, Amato et al. 1986).

The main preventive measures are based on control of the transmission of the disease by earth embankments around waterbeds containing host snails or by killing the snails. Some synthetic molluscicides in current use have the disadvantage of being general biocides, which destroy other species in the water. Plant molluscicides have therefore been widely investigated as possible substitutes, among these the latex of *Euphorbia splendens* var. *hislopii*.

The objective of the present study was to investigate the properties of the latex of *E. splendens* var. *hislopii* (Crown of thorns) plant, as a molluscicidal against *L. columella* snails, characterizing its activity range against snails under a limited field situation.

MATERIALS AND METHODS

Plant used - The species tested was *Euphorbia splendens* var. *hislopii* N.E.B. (syn. *Euphorbia milii* Des Moul. var. *splendens* (Hook.) Ursch & Leandri) (Carter, 1994). The *hislopii* variety was the one used in the present research because it grows larger and can produce more latex.

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Latex collection - Samples from the latex of *E. splendens* var. *hislopii* were collected from plants cultivated in beds located near the Biology Department, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ.

The raw latex that exuded on transversal sectioning, around 10 cm below the apical meristem of each branch was collected in a glass test tube closed with a screwcap and transported to the laboratory.

Evaluation of the molluscicidal effect in the field - This bioassay was performed in an irrigation ditch at the Pisciculture Station of the Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ.

The cemented ditch used was divided into two sections each measuring 80 m long by 0.36 m wide with 0.17 m depth of water. The water inflow was located between the sections, in a cemented box from which it entered the control section in a small and continuous flow with low turbulence and outflow leading into large natural tanks used for fish breeding. Both sections had marginal substrate and vegetation.

The molluscicidal latex was applied in one of the sections (the experimental ditch); the other served as control (control ditch).

The volume of water in the experimental ditch was estimated at 4900 l and this was maintained by closing the entries and exits of the ditch. In the control ditch, the flow was kept continuous as an outlet for the flow, which was around 0.68 l/s.

Both ditches presented samples of the target snails *L. columella* and the non-target species of mollusk *Melanoides tuberculata* (Müller, 1774), *Biomphalaria tenagophila* (Orbigny, 1835), and *Pomacea* sp., identified by the Department of Malacology, IOC, as well as the teleostean fish *Lebistes reticulatus* and aquatic insect larvae.

Samples of the water in the experimental and control ditches were collected before and after latex application so as to enable a comparative analysis of physicochemical parameters.

The concentration of the latex solution used in the test was 5 mg/l (3 times larger than that determined in the laboratory (Vasconcellos 1996), prepared from a stock-solution of 1000 mg/l of the latex (collected on the same day of the experiment and from the same original location as used by Vasconcellos 1996). This concentration was calculated from the total volume of water to be treated in the experimental ditch, using 24.5 ml of in natura latex, with water from the experimental ditch.

The latex solution was applied with a watering can, over the entire experimental ditch, so that it would be essentially homogenous. This solution stayed in the experimental ditch during 24 h after application, after this period, the regular circulation of water in and out of the ditch was restored.

Snails sampling - Sampling of the snails was made from both ditches before application and 24 h after application of the latex solution in the experimental ditch.

Samplings were performed by three individuals, at three specific points (0 m; 40 m; 80 m), in 1 m², for a period of 20 min, in both ditches (man/time sampling) (Olivier & Schneiderman 1956).

The snails gathered before application in the substrate and on the water surface were collected by forceps, separated by species, quantified, and returned to their habitat, at the same spot where they had been collected.

In the sampling effected 24 h after latex application, all of the collected animals (live and dead) were packaged and taken to the laboratory to be quantified by species. The ones that were alive were placed in tanks for 48 h to recover.

Sentinel snails - Before application of the latex solution, six perforated plastic containers measuring 5 x 3 cm (protection flasks of photographic rolls), were immersed in the ditches, as cages, each containing 10 samples of *L. columella*, three in each ditch at the three sampling locations.

Physicochemical analysis of the latex solutions - Following the method employed by the State Environmental Laboratory Feema (1979), some physicochemical parameters were analyzed, such as: conductivity ($\mu\text{mho/cm}$), alkalinity ($\mu\text{g/l CaCO}_3$), chlorides (mg/l Cl⁻), calcium hardness (mg/l) and pH of the latex stock-solution (1000 mg/l), used in the field test and prepared using the water from the snail's habitat.

RESULTS

Evaluation of the molluscicidal effect of the latex in the field - Table I shows the molluscicidal activity of *E. splendens* var. *hislopii* latex, at a concentration of 5 mg/l, against *L. columella*, in a lentic habitat.

The results showed 97.4% mortality of *L. columella*, 24 h after application of the aqueous latex solution in the experimental ditch. In the control ditch, without treatment, there was no snail mortality.

Table II shows the mortality results in samplings of *L. columella*, snails immersed in underwater cages in the treated ditch. Mortality was 100% after 24 h contact, at all three locations. There was no mortality of caged snails in the control ditch (untreated) (Table III).

Table IV shows the results of the latex solution action at 5 mg/l against other mollusk species (*B. tenagophila*, *M. tuberculata*, and *Pomacea* sp.), present in the ditches. It is seen that mortality was 100% for the species *B. tenagophila* and *M. tuberculata*; and 40% for *Pomacea* sp. In the untreated ditch, no mortality was observed in these species. No mortality was observed either among fish (*Lebistes reticulatus*) or in larvae of aquatic insects present in the ditches (experimental and control ditches).

TABLE I

Molluscicidal action of the latex of *Euphorbia splendens* var. *hislopii* at a concentration of 5 mg/l, against *Lymnaea columella* in a natural habitat (irrigation ditch)

Groups	Number of <i>L. columella</i>				Mortality (%)
	Before application		After application		
	Alive	Dead	Alive	Dead	
Control	223	0	129	0	0
Experimental	179	0	4	153	97.4

TABLE II

Exposure of *Lymnaea columella* (sentinel snails) at three points of an experimental ditch to the latex of *Euphorbia splendens* var. *hislopii*, at a concentration of 5 mg/l, for 24 h. The cages were placed under the water

Distance between cages (m)	Number of <i>L. columella</i>				Mortality (%)
	Before application		24 h after application		
	Alive	Dead	Alive	Dead	
0	10	0	0	10	100
40	10	0	0	10	100
80	10	0	0	10	100

TABLE III

Exposure of *Lymnaea columella* (sentinel snails) at three points of the untreated control ditch for 24 h. The cages were placed under the water

Distance between cages (m)	Number of <i>L. columella</i>				Mortality (%)
	Before application		24 h after application		
	Alive	Dead	Alive	Dead	
0	10	0	10	0	0
40	10	0	10	0	0
80	10	0	10	0	0

Physicochemical analysis of the latex solutions - Table V shows the results of the physical-chemical analysis. It is observed that the data for the parameters analyzed were always kept below the maximum values obtained in the water of habitat of *L. columella* where collections were carried out.

DISCUSSION

This study was carried out with snails in the field, in irrigation ditches at the Pisciculture Station of the Universidade Federal Rural Campus in Rio de Janeiro. The season of the year when the test was performed was considered to be atypical due to the long period of drought from winter to spring, which considerably altered the habitat, resulting in a reduced mollusk population.

The fact that *E. splendens* var. *hislopii* latex showed high activity in a field situation makes it a promising natural

TABLE V

Values found in some physicalchemical parameters of the mother-solution used in the field test (E), with the latex of *Euphorbia splendens* var. *hislopii*, against *Lymnaea columella*, at a concentration of 1000 mg/l

	Maximum values	
	E	Habitat
Conductivity (µmho/cm)	150	350
Alkalinity (mg/l CaCO ₃)	16	28
Chloride (mg/l Cl ⁻)	10.7	18.1
Calcium (mg/l)	10.4	11.2
pH	6.5	6.7

molluscicide. The result is in accord with Mendes et al. (1992, 1997) and Baptista et al. (1992), who have tested the latex in lentic and lotic environments, respectively, in concentrations between 5 and 12 mg/l against intermediate hosts of *Schistosoma mansoni*, where they also found 100% mortality.

The mortality rate for the free animals (97.4%) suggests that the surviving mollusks (2.6%) may have escaped the product's action because they could have been out of the water or else protected in some way at the time of its application. However, the utilization of sentinel snails in underwater cages, in three locations in the treated ditch, confirmed the efficiency of the product with 100% mortality.

With reference to the difference in the number of animals collected in samplings before and after the ditch treatment, it is assumed that these animals might have moved, while trying to escape the product's action.

As to the remaining species of mollusk present in the habitat of *L. columella*, the mortality rate of 100% for *B. tenagophila* and *M. tuberculata* can be considered satisfactory, since these are intermediate hosts of *S. mansoni* and *Paragonimus westermanii* (Kelbert 1978), respectively. Now, for *Pomacea* sp., the mortality rate was lower (40%), also observed by Mendes et al. (1992) and Baptista et al. (1992), which indicates that this species is more resistant to the molluscicide than *M. tuberculata*, since both of them contain an operculum that can remain closed for as long as adverse conditions to the means persist.

TABLE IV

Mortality of *Biomphalaria tenagophila*, *Melanoides tuberculata* and *Pomacea* sp. present in a habitat of *Lymnaea columella* before and after application of the latex of *Euphorbia splendens* var. *hislopii*, at a concentration of 5 mg/l

	Number of snails									
	Control group					Experimental group				
	Before		After		Mortality (%)	Before		After		Mortality (%)
	Alive	Dead	Alive	Dead		Alive	Dead	Alive	Dead	
<i>B. tenagophila</i>	1	-	20	-	0	37	-	-	76	100
<i>M. tuberculata</i>	215	4	250	-	0	251	21	-	425	100
<i>Pomacea</i> sp.	4	-	6	-	0	-	-	12	3	40

With regard to the toxicology of *E. splendens* var. *hislopi* latex Marston & Hecker (1983, 1984) identified several diterpene esters, considered to be irritants, but did not confirm carcinogenic properties. Pharmacologically active substances, such as citrostadiene (Ia), present in extracts of this plant, have already been stated by Rao and Sussela (1982), as having anti-inflammatory action, in addition to the presence of a macrolide, lasiodiplodin, which has shown antileukemic activity in mice, according to Lee et al. (1982). When performing a test of skin and ocular irritability in rabbits, Freitas et al. (1991) observed that the latex was not irritating in concentrations below 3500 mg/l (0.35%), nonetheless the use of gloves and protective glasses when handling the raw product was recommended.

Schall et al. (1991) observed that the latex does not present mutagenic activity in *Salmonella typhimurium* (Ames Test) or acute toxicity in the presence of *Photobacterium phosphoreum* (Microtox); the acute oral toxicity in mice was low and the lethal dosage (LC₅₀) was above 5 g/kg (Mattos et al. 1989). Now the sub-acute toxicity test (14 days) resulted in diarrhoea and weight loss in rats, although no deaths occurred in dosages lower than 0.25 g/kg (Lopes et al. 1992). However, Souza et al. (1994) showed that at dosages above that level, the latex proved to be embryotoxic, although no fetal or genotoxic malformation (teratogenicity), was observed by Zamith et al. (1994).

In eco-toxicological studies, using fish, algae and bacteria, no toxic activity was observed with these species, which were considered as non-target, in low concentrations (Oliveira-Filho & Paumgarten 1994).

Regarding the cost-benefit in the use of *E. splendens* var. *hislopii* as a molluscicide, perspectives are quite satisfactory, according to Baptista et al. (1994), because, after one year and a half of the planting, the average productivity of the latex was 1 l per 8 m², which would be enough to treat as much as 97,200 liters of water, in a concentration of 12 mg/l, and which could be done by the rural communities themselves.

These results indicate that *E. splendens* var. *hislopii* latex is an effective molluscicide that can be used as an alternative method to control *L. columella*.

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