Evaluation of the Molluscicidal Properties of *Euphorbia splendens* var. *hislopii* (N.E.B.) Latex: Experimental Test in an Endemic Area in the State of Minas Gerais, Brazil

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Following the positive results obtained regarding the molluscicidal properties of the latex of Euphorbia splendens *that were corroborated in laboratory and field tests under restricted conditions, a field study was conducted in experimental streams located in an endemic area. After recording the average annual fluctuations of vectors in three streams, a solution of* E. splendens *latex at 12 ppm was applied in stream A, a solution of niclosamide at 3 ppm that was applied in stream B and a third stream (C) remained untreated for negative control. Applications of* E. splendens *and niclosamide resulted in a mortality of 100% among the snails collected in the streams A and B. No dead snails were found in the negative control stream. A monthly follow-up survey conducted during three consecutive months confirmed the return of vectors to both experimental streams treated with latex and niclosamide. This fact has called for a need to repeat application in order to reach the snails that remained buried in the mud substrate or escaped to the water edge, as well as, newly hatched snails that did not respond to the concentration of these molluscicides. Adults snails collected a month following treatment led us to believe that they had migrate from untreated areas of the streams to those previously treated.*

Key words: Euphorbiaceae - *Euphorbia splendens* - molluscicide plant - *Biomphalaria glabrata* - schistosomiasis

Since the 1930s, researches have investigated the molluscicidal properties of various plants attempting to develop natural substances that could be used by communities (Mozley 1939). The idea was to create a self-sustaining way of producing and using natural molluscicides within an integrated program of control, that is, substances that could supplant unhandy and costly synthetic products (Taylor 1986).

The molluscicidal properties of the different species of Euphorbiaceae have already been studied, using different parts of the plant and extraction processes (Table I). All species, *Euphorbia cotinifolia* (Pereira et al. 1978), *E. pulcherrima* (Mendes et al. 1984) and *E. tirucalli* (Jurberg et al. 1985) have a potential effect on snails of the genus *Biomphalaria*. However, the latex extracted from *E. splendens* was found to be effective in a much lower concentration than other parts of the plant or other plants of the same genus previously studied (Table I).

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E. splendens (Syn *E. milii* - Zani et al. 1993) is an ornamental plant from Madagascar. It was introduced in Brazil with the purpose of fencing in gardens. It is popularly known as "Coroa de Cristo" (Crown of Thornes), "Coroa de Nossa Senhora" (Our Lady's crown), "Duas amigas" (Two friends), "Colchão de noiva" (Bride's mattress) and "Martírios" (Martyrs) (Pio Corrêa 1984) being one more plant among 360 to be tested for molluscicidal properties in Brazil (Jurberg et al. 1989).

From the phytochemical fractionation of the latex of *E. splendens*, eight different miliamines were isolated and identified through biological assays performed on adult snails of the genus *B. glabrata*. Three of these miliamines had already been reported in publications, whereas the other five were reported for the first time. The miliamine L. (a dianthraniloyl peptide ester of ingenol) was found to be effective in a dilution of 0,001 ppm (Zani et al. 1993). It is worth recalling that this dilution is roughly 100 times more potent than niclosamide at 0.3 ppm (Martin & Worthing 1977), a synthetic compound currently used worldwide against the vector of schistosomiasis.

So far toxicological assays made with some miliamines have not shown any carcinogenic properties (Marston & Hecker 1983). Other tests con-

TABLE I

Euphorbia species from Brazil presenting molluscicidal activity on *Biomphalaria glabrata*

Species	Popular name	Tested part	Type extract	Activity ppm	Reference
E. cotinifolia L.	Roxinha	Leaves	Hexanic	< 50	Pereira et al. 1978
		Fruits	Pure	< 50	
		Stem	Pure	<50	
		Seed's coating	Pure	<50	
		Latex	-	<50	
E. pulcherrima Wild	Bico de papagaio	Leaves	Hexanic	Inactive	Mendes et al. 1984
			Ethanolic	Inactive	
		Flowers	Hexanic	Inactive	
			Ethanolic	Inactive	
E. splendens Bojer	Coroa de Cristo	Leaves	Hexanic	Inactive	
			Ethanolic	100	
		Stem	Hexanic	100	
			Ethanolic	100	
		Stem's coating	Hexanic	100	
			Ethanolic	100	
		Latex	-	<0,5	Vasconcellos & Schall 1986
E. tirucalli L.	Avelós	Latex	-	100	Jurberg et al. 1985

ducted with the latex *in natura* and/or lyophilized have shown encouraging results such as acute toxicity (Mattos et al. 1989), cutaneous and ocular irritability (Freitas et al. 1991) as well as mutagenicity, cytotoxicity (Schall et al. 1991, Zamith et al. 1996), embroyofeto-toxicity (Souza et al. 1997) and ecotoxicity (Oliveira-Filho & Paumgartten 1997). Cruz et al. (1996) described a tumor promoter-like activity *in vitro*. Studies have been carried *in vivo* to establish the possibility of using latex in field conditions. It was also demonstrated a photodegradation of the molluscicidal latex, which prevents cumulative effects.

Further laboratory tests have confirmed that the latex of *E. splendens* is seasonably and geographically stable and thus adequate for use in Brazil (Schall et al. 1992).

The field tests conducted in lotic and lentic habitats under restricted conditions have shown a mortality of 100% for *B. glabrata* (lentic) and *B. tenagophila* (lotic) using concentrations of 5 ppm and 12 ppm, respectively (Mendes et al. 1992, Baptista et al. 1992).

The purpose of this study is to assess the potential effect of *E. splendens* in the field and its impact on the natural fluctuation of vector snails through a survey that was conducted during 13 months prior and 3 months after treatment, comparing its effect with that of niclosamide.

MATERIALS AND METHODS

A field study was conducted in the rural area of Comercinho, a municipality located in Vale do Jequitinhonha, in the northeast region of the State of Minas Gerais, 713 km far from the city of Belo Horizonte. It is an hyperendemic area for schistosomiasis, whose prevalence in urban area was of the order of 70%, in 1981, prior to initiating large scale specific treatments. A late survey has shown that the prevalence of the disease had dropped to 13% (Rocha 1995). Comercinho has 10,000 inhabitants, but only 2,000 of them live in the urban area. The remaining population is distributed in 66 rural communities where sewage systems are inexistent. In the urban area, 87.6% of the dwellings are connected to waterworks.

The field study was carried out in three experimental streams. They were selected for not posing any risk to the population through contact with the water. Latex was applied in the stream Córrego dos Macacos (A) - Fazenda Alegre and niclosamide in the stream Córrego Água Belas (B) - Fazenda Novo Mundo (Tables II and III). No drug was applied in the stream Córrego da Lage (C) - Fazenda Novo Mundo, selected as a negative control of the streams A and B (Table IV).

Malacological survey was made in the three experimental streams, employing the method of ten scoopfuls at previously select stations and the snails collected were sent to the laboratory to be counted, measured and then, disposed of. From November 1993 to December 1994, prior to any treatment, these streams were surveyed and from January 1995 to March 1995 a follow-up survey was made.

In December 1994, 48 hr after applying the drugs, snails were collected by scoopfuls and

TABLE II

Monthly fluctuation of *Biomphalaria glabrata* in the stream Córrego dos Macacos - Fazenda Alegre, where latex of *Euphorbia splendens* was applied in December 1994

Variables	1993 (months)		1994 (months)										Alive	Dead		1995 (months)		
	11	12	1	2	3	4	5	6	7	8	9	10	11	12	12[a]	1	2	3
Diameter of snails (mm)																		
0-6	13	6	0	0	0	1	0	0	1	7	18	6	0	0	0	0	33	0
≥ 7	117	37	0	1	0	4	0	9	19	24	51	130	43	411	674	23	135	33
Total	130	43	0	1	0	5	0	9	20	21	69	136	43	411	674	23	168	33
Ambient temperature (°C)	23	23	27	27	26	27	26	23	26	25	32	25	26	26	-	26	27	26
Water temperature (°C)	22	25	26	23	25	26	27	21	25	24	32	25	24	25	-	26	27	24
Fluvial index (mm)	0	35	211	0	313	82	0	0	26	20	0	0	81	97	-	109	81	0

a: number of snails caught 48 hr after treatment.

TABLE III

Monthly fluctuation of *Biomphalaria glabrata* in the stream Córrego Águas Belas - Fazenda Mundo Novo, where niclosamide was applied in December 1994

Variables	1993 (months)		1994 (months)										Alive	Dead		1995 (months)		
	11	12	1	2	3	4	5	6	7	8	9	10	11	12	12[a]	1	2	3
Diameter of snails (mm)																		
0-6	0	7	8	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
≥ 7	38	22	68	35	0	23	4	28	28	37	133	60	99	268	316	26	131	80
Total	38	29	76	35	0	23	7	28	28	37	133	60	99	268	316	26	131	80
Ambient temperature (°C)	24	27	28	29	26	27	34	24	22	23	23	23	23	28	-	26	26	27
Water temperature (°C)	26	26	26	28	25	27	26	22	22	23	23	23	23	28	-	25	25	26
Fluvial index (mm)	5	59	212	0	224	80	10	0	8	18	0	0	100	97	-	90	104	0

a: number of snails caught 48 hr after treatment.

TABLE IV

Monthly fluctuation of *Biomphalaria glabrata* in the stream Córrego da Laje - Fazenda Mundo Novo, selected as negative control of the streams where latex and niclosamide were applied

Variables	1993 (months)		1994 (months)										Alive	Alive		1995 (months)		
	11	12	1	2	3	4	5	6	7	8	9	10	11	12	12[a]	1	2	3
Diameter of snails (mm)																		
0-6	2	4	0	0	0	0	0	1	0	2	5	14	7	0	0	6	34	24
≥ 7	89	15	0	4	0	2	0	6	0	4	76	78	49	56	83	71	155	163
Total	91	19	0	4	0	2	0	7	0	6	81	92	56	56	83	77	189	187
Ambient Temperature (°C)	26	28	29	28	26	27	35	23	23	24	25	25	24	28	-	26	26	27
Water Temperature (°C)	24	24	26	29	25	26	26	22	23	23	24	24	23	28	-	25	25	25
Fluvial index (mm)	0	59	212	0	224	80	9	0	7	18	0	0	100	97	-	90	104	0

a: number of snails caught 48 h after treatment.

caught from sieves placed at different sites of the streams and sent to the laboratory where they were under observation and examined three days after treatment to evaluate their mortality.

The solutions of latex and niclosamide (diluted at 12 ppm and 3 ppm, respectively) were applied with a watering can. Safety instructions provided by McCullough (1992), were followed in all stages of the applications in order to prevent any direct contact with the skin and the eyes of the technician. The latex was extracted by stem incision from *E. splendens* grown in the gardens surrounding the Mineirão Stadium in Belo Horizonte.

RESULTS

In Tables II to IV, the results of the malacological study carried out from 1993 to 1995 can be observed. A higher density of snails was detected from September to December and a natural decrease in the population occurred from January to June in the stream Córrego dos Macacos (A), from March to May in the stream Córrego Águas Belas (B) and from January to August in the stream Córrego da Laje (C). Ambient temperatures varied from 22°C to 35°C over the year, averaging 26°C, s=0.52 in the stream Córrego dos Macacos, 25.8°C, s=0.76 in the stream Córrego Águas Belas and 26.4°C, s=0.72 in the stream Córrego da Laje. Water temperature was lower in relation to the ambient temperature. The density of vectors population did not seem to be affected by temperature and rainfalls, which oscillated throughout the months.

As can be seen in Table V, 100% of mortality were observed after latex and niclosamide application. Although the success of this treatment snails returned to the area a month later. In the stream treated with latex there were an increase in the number of snails in the second month, decreasing in the third month (Table II). In the stream where niclosamide was applied a slight increase in the number of snails during the second month was detected with no modification in the third month (Table III). No snail mortality was reported in the negative control stream (Table IV). Fish mortality was observed only in the stream where niclosamide was applied.

DISCUSSION

The results confirm the lethal action of latex in lentic environment, whose effect can be compared to that of niclosamide. However, we may infer that both substances are incapable of contributing to erradicate snails. Their defensive behavior such as the burial in the mud substrate and the escape to the water edge contribute to them be unaffected by molluscicidal substances returning soon to the former infestation. In the case of the latex of *E. splendens*, the lethal concentration required to exterminate egg masses is approximately 1,000 times higher than that required for adult snails (Vasconcellos et al. 1986). Newly hatched snails may infest an habitat over a brief lapse of time and one single snail may generate thousands of offsprings, in a period of three months, since they are hermaphrodites and can lay hundreds of eggs.

In this study, besides all facts mentioned above, it is worth noting that the snails collected during the period of three months following application were wider in diameter than the average of two/three months old specimens. This has led us to believe that the snails migrated or were washed away by heavy rainfalls to the places where latex or niclosamide were applied. These facts call for a need to conduct further tests, which will consist in repeating applications in order to reach newly hatched snails. However, considering that snails may still survive in the mud substrate or at the water edge, environmental measures must be taken into consideration. Since migration is likely to recur including migration against currents, the streams infested will have to undergo extensive treatment, encompassing larger regions than those where people usually has water contact.

TABLE V

Results of the moluscicidal activity of the latex of *Euphorbia splendens* and niclosamide on *Biomphalaria glabrata* applied in two streams

Streams	Length (m)	Product	Dilution (ppm)	Snails			
				Before treatment	After[a] treatment	% mortality	Mean diameter
Macacos	200	Latex	12	411	674	100	8 - 33
Águas Belas	200	Niclosamide	3	268	316	100	12 - 35
Laje	500	Control	-	56	83	0	11 - 30

a: snails caught 48 hr after treatment and examined in the laboratory 3 days after treatment.

Despite the fact that niclosamide can exterminate 100% of snails, late follow-up surveys have confirmed that vectors will invariably re-infest areas approximately a month following treatment (Pieri et al. 1995). Basing on the areas studied, Pieri has admitted that to maintain a low prevalence of schistosomiasis moluscicide application must be repeated monthly along with treatment of the infected people. According to Pieri, the use of niclosamide as a control strategy would be too costly as well as unfeasible at such intervals. Thus, the use of the latex of *E. splendens* as an alternative drug has to be assessed and may in turn be implemented in endemic areas for being more convenient to handle and apply. Cost estimates reported by Baptista et al. (1994) indicate that the cultivation, extraction and application of *E. splendens* by communities of endemic areas are feasible. However, it is necessary to conduct experimental studies to assess its use through a variety of approaches in order to confirm such possibilities.

On the other hand, studies will be conducted in areas where water is used by the population only after the conclusion by toxicological tests that the latex of *E. splendens* is free of negative effects.

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