Reproductive activity alterations on the *Biomphalaria glabrata* exposed to *Euphorbia splendens* var. *hislopii* latex

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The reproductive activity of Biomphalaria glabrata *exposed to* Euphorbia splendens *var*. hislopii *latex was evaluated. Parameters related to fecundity and fertility were observed. The snails were exposed to the* LD_{50} *(1 mg/l) of crude latex. At the first week post exposure (p.e.), the egg laying was reduced. After the fourth week p.e., an increase of the number of eggs/snail occurred. The results showed a marked reduction in the hatching of the snails, revealing an interference of latex exposure with the reproductive process of* B. glabrata of *E. splendens* var. *hislopii*. *The* LD_{50} *of the latex may be used as an alternative method to control the size of the populations of* B. glabrata *in field.*

Key words: *Biomphalaria glabrata* - plant moluscicide - reproductive activity alteration - snail population control

The reproductive potential of *Biomphalaria glabrata*, the main intermediate host of *Schistosoma mansoni* in Brazil, is straightly related to environmental conditions (Magalhães & Lucca 1971, Kawazoe 1977).

The physiological stress factors, as temperature variations (Pimentel-Souza et al. 1990), lightness (Barbosa et al. 1987), starvation (Livingstone & Zwaan 1983), and parasitism by larval trematodes (Looker & Etges 1979, Sullivan et al. 1985, Cooper et al. 1994, Cousin et al. 1995) have been pointed as able to influence the reproductive biology of the snails.

There are few studies on the interference of plants that exhibit moluscicide action with the reproductive activity of the snails, intermediate host of helminthes. The reproductive capacity of *B. alexandrina* is reduced when it was exposed to *Allium sativum*, *A. cepa*, and *Atriplex halimus* (Mantawy 2001, Tanatawy 2002).

Many molluscicide have been used to schistosomiasis control, and one of the most promising and widely studied in Brazil, is the crude latex of *Euphorbia splendens* var. *hislopii*. The latex obtained from this plant, under laboratory and field conditions, showed that it attends to queries needed to be used as a natural molluscicide (Schall et al. 2001, Vasconcelllos & Amorim 2003 a,b).

The lethal effects on the embryos in the egg masses were obtained using elevated concentrations of the latex (870 a 1500 mg/l) (Schall et al. 1998), but, until the moment, there were no studies focusing the latex effects on the reproductive biology of *B. glabrata*.

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The objective of this study was evaluating the influence of sub lethal dose (LD_{50}) of latex of *E. splendens* var. *hislopii* on reproductive parameters of *B. glabrata*.

MATERIALS AND METHODS

Collection of E. splendens var. *hislopii latex* - Samples of *E. splendens* var. *hislopii* latex were collected in the autumn from plants cultivated in plots near Departamento de Biologia, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brazil. The latex was collected as described by Vasconcellos and Amorim (2003a), on the same day of tests conduction.

Rearing of B. glabrata in the laboratory - Specimens of *B. glabrata* (Belo Horizonte, BH lineage) were reared at the Laboratório de Esquistossomose Experimental, Instituto Oswaldo Cruz-Fiocruz, RJ, Brazil. The snails were placed in 30 l polyethylene aquaria, with dechlorinated water. The average water temperature was 28 ± 1ºC and relative humidity varied from 70 to 78% throughout the experiment.

Three times a week the aquaria were cleaned and the snails were fed ad libitum with lettuce leaves (*Lactuca sativa* L.). All the specimens of *B. glabrata* used in the experiments had shell diameters between 8-10 mm and exhibit ovipository activity.

Determination of sublethal concentrations of latex of E. splendens var. *hislopii* - The determination of LD_{50} were made according to Vasconcellos and Amorim (2003a), in accordance to WHO recommendations (WHO 1983) and Mott (1987), being obtained LD_{50} value equal to 1 mg/l (Mello-Silva 2005, Mello-Silva et al. 2006).

Reproductive biology of B. glabrata exposed to LD_{50} *of E. splendens* var. *hislopii latex* - Sixty specimens of *B. glabrata* were exposed to LD_{50} of *E. splendens* var. *hislopii* latex by 24 h and, after this time, they were

washed in dechlorinated tap water and 30 of these animals were maintained in three aquaria, with 10 snails each. Thirty snails were used as control group (unexposed).

The snails were weekly observed until the fifth week p.e. the parameters related to the reproductive biology analyzed were: number of live snails, number of egg masses laid by snail, number of eggs/egg mass, number of eggs laid/snail, and number of hatched snails/week.

Statistical analysis - The results were expressed as mean ± standard deviations and subjected to a variance analysis test (ANOVA), to the Tukey-Kramer test, and to Student's t-test for unpaired data for comparing the means ($\alpha = 5\%$). Linear regression tests was used to verify the significance of the relation between the parameters observed and the time post exposed (p.e), except to the data on survival to which the Chi-Square test was used (Graph Pad Prism, v.3.00, and GraphPad InStat, v. 3.06, GraphPad Software Inc.).

RESULTS

The number of survivorship of unexposed snails was significantly higher than those exposed to *E. splendens* latex (Table I), with a percentual survival of 60%, 10 snails died in the initial 24 h (p.e), while to unexposed snails the survival was equal to 93.3% (Chi-Square = 7.54; $P < 0.01$). This parameter was negatively related to the time p.e. in both groups (exposed and unexposed), but the survival of the exposed snails was always lower than the observed to the unexposed snails.

The latex exposition caused a reduction of 25.01% in the total number of the egg masses of *B. glabrata* (Table I). When the number of eggs masses/snail was compared between the both groups (exposed and unexposed), a reduction of 25.14% was observed to exposed snails in relation the unexposed animals (Table II). The linear regression showed a positive relation between the number of egg masses/snail and the time post exposure, in weeks (control: y = 1.67 + 0.75x; exposed: y = 0.30 + 0.92x), which was marked significant to the exposed snails (Table II).

The mean number of eggs laid/snail was reduced in 17.3% when *B. glabrata* was exposed to *E. splendens* var. *hislopii* latex (Table II) in relation to control group. But, when these data were analyzed weekly, it was possible verify that this reduction occurred until the third week p.e. Onwards, the number of eggs laid/snail to this group was higher than that observed to unexposed *B. glabrata*. Similar response was observed when the number of eggs/egg mass laid by *B. glabrata*, being observed an increase of 13.04% in relation to control group at the end of the period analyzed, but this difference was not significant. A reduction was also observed to the number of eggs/egg mass until the third week p.e. and an increase occurred from the fourth week onwards. As the number of eggs/egg mass (control: y = 317 + 2.2x; exposed: y = 3.16 + 2.67x), as the number of eggs laid/snail (control: y = 24.6 + 6.4x; exposed: y = -0.20 + 13.08x) presented a positive relation with the time after exposition to latex.

The mean number of hatched snails came from eggs laid by *B. glabrata* unexposed to *E. splendens* var. *hislopii* latex was significantly different and higher than that observed to the exposed snails (Table II). The linear regression analysis revealed a positive relation between these values and the period of observation to both groups (y = 7.86 + 9.5x), but this relation was strongest to the snails unexposed to *E. splendens* var. *hislopii* latex (y = 57.49 + 18.25x). The statistic analysis for parameters should be observed by different letters (Table II).

DISCUSSION

The use of sub lethal dose (LD_{50}) of the crude *E. splendens* var. *hislopii* latex, in the present study, caused a marked mortality of 60% of *B. glabrata* at the first 24 h post exposure. This mortality percentual was observed after exposure to *E. splendens* latex in *Lymnaea columella* (intermediate host of *Fasciola hepatica*) by Vasconcellos and Amorim (2003b).

Studies about duration of the molluscide effects of the aqueous solution of *E. splendens* var. *hislopii* latex on freshwater snails showed that the moluscicide effect of latex was lost due sunlight exposition (Schall et al. 1992). Vasconcellos and Amorim (2003a) showed that the latex is photosensible and biodegradable.

In this study, an important statement was that even after *E. splendens* var. *hislopii* latex looses it mollusci-

TABLE I

Total values of the reproductive biology of *Biomphalaria glabrata* exposed (E) and unexposed (C) to *Euphorbia splendens* var. *hislopii* latex, after 24 h of exposition and observed through five weeks

	Total snails surviving		Egg masses		Eggs		Snails hatched	
Time	C	E	C	E	C	E	C	E
Day 0	30	30	-	-	-	-	-	-
1 st day	30	20	5	-	31	-	20	-
1 st week	29	19	117	16	974	194	517	100
2 nd week	28	18	115	65	1216	541	459	133
3 rd week	28	18	128	66	1470	695	118	77
4 th week	28	18	141	77	1603	1116	195	166
5 th week	28	18	115	74	1457	1040	435	155
Total	-	-	621	298	6751	3586	1744	631

TABLE II

Reproductive biology of *Biomphalaria glabrata* control (C) and exposed (E) to sublethal dose (LD_{50}) *Euphorbia splendens* var. *hislopii* latex by 24 h and observed through five weeks

	Snails surviving		Egg mass/snail		Eggs/snail		Eggs/egg mass		Snails hatched	
Time	C	E	C	E	C	E	C	E	C	E
1 st day	10.0 ± 0.0	6.6 ± 1.5	0.2 ± 0.3^{a}	0^{a}	1.0 ± 1.8^{a}	0^{a}	2.0 ± 3.6^{a}	0^{a}	$6.66 \pm 11.5^{a,b}$	0^{a}
1 st week	9.6 ± 0.5	6.3 ± 1.2	4.0 ± 0.3^{b}	$0.8 \pm 0.5^{a,b}$	33.5 ± 7.9^{b}	$10.2 \pm 3.3^{a,b}$	8.3 ± 1.6^{b}	14.1 ± 4.0^{b}	172.3 ± 86.1^{b}	33.3 ± 16.4^{a}
2 rd week	9.3 ± 1.2	6.0 ± 1.7	4.0 ± 0.3^{b}	3.5 ± 0.9^{b}	43.5 ± 3.0^{b}	$29.3 \pm 9.7^{a,b,c}$	10.6 ± 0.9^{b}	$8.1 \pm 0.6^{a,b}$	153.0 ± 38.5^{b}	44.3 ± 39.5^{a}
2 rd week	9.3 ± 1.2	6.0 ± 1.7	4.5 ± 0.7^{b}	3.7 ± 0.6^{b}	52.8 ± 7.6^{b}	38.8 ± 7.2^{b}	11.5 ± 0.6^{b}	$10.6 \pm 2.2^{a,b}$	$136.6 \pm 69.6^{a,b}$	17.0 ± 22.7^{a}
4 th week	9.3 ± 1.2	6.0 ± 1.7	5.0 ± 0.6^{b}	3.6 ± 1.9^{b}	57.0 ± 7.0^{b}	$60.1 \pm 12.2^{c,d}$	11.3 ± 1.2^{b}	$16.0 \pm 9.2^{a,b}$	65.0 ± 5.0^{b}	55.3 ± 34.1^{a}
5 th week	9.3 ± 1.2	6.0 ± 1.7	4.1 ± 0.9^{b}	4.3 ± 1.3^{b}	52.7 ± 17.3^{b}	58.2 ± 13.5^{c}	12.5 ± 1.4^{b}	$13.7 \pm 2.2^{a,b}$	145.0 ± 34.7^{b}	51.6 ± 41.6^{a}
Regression (r^2)	-	-	0.66	0.95	0.77	0.99	0.87	0.91	0.74	0.88

a,b,c,d: means followed by different letters are significantly different ($\alpha = 5\%$).

cide activity, at the concentration used (LD_{50}), caused interference on the reproductive function of the *B. glabrata*. This interference had direct relation with the alterations in glucose level of the hemolymph, with previous observations in snails exposed to latex (Mello-Silva 2005, Mello-Silva et al. 2006). The regulation of glicemia in snails was observed by Thompson and Lee (1986) and in the present study, probably occurred a conversion of galactogen in glycogen, which was redirected from the reproduction to nutrition, to maintain the glucose levels to survival during the time of experiment.

In the present study, the decreasing of the number of egg masses associated to the increase in the number of eggs laid/snail and the number of eggs/egg mass, clearly reflect an attempt to compensate the reproductive losses occurred early, before the fourth week, this fact is commonly observed in physiologically stressed snails. The snails presented a minor energetic waste in their process of reproduction, laying a higher number of eggs in each egg mass, resulting in an increased efficiency of this process and a lower spent of energy, similar phenomenon was observed in *S. mansoni* infected snails (Minchella 1985), with a major number of eggs produced in the beginning of the infection as a strategy to compensate the reduction that will occur at the following phases of the larval intramolluscan development of the parasite. The reduction on fecundity associated to the intermediate and final phases of the *S. mansoni* larval development, coinciding with the depletion of the energy stores, as the glycogen deposits of the digestive gland, verifying that the eggs production decreases after 20 days post infection (Looker & Etges 1979, Crews & Yoshino 1989, 1991).

The *E. splendens* latex, as a moluscicide substance, has been shown satisfactory results to the control of the hatched snails population. So, when this substance is tested against egg masses, Schall et al. (1998) observed that a higher concentration was needed, 1500 ppm, to kill the embryos. In the present study, the sublethal dose, 1 mg/l, altered significantly the fertility of the adult snails, acting indirectly on the population control.

Many natural products have been used in high concentrations, interfering with different reproductive parameters (Schall et al. 1992, 1998, Souza et al. 1992, Mantawy 2001, Bacchetta et al. 2002, Tantawy 2002). But, by the first time it was showed that the concentration of *E. splendens* var. *hislopii* latex (1mg/l) used in this study, must be efficient to the control of *B. glabrata* populations even after the residual time p.e.

The control of the population size of *B. glabrata* in field has been used by the competitor snails, with relevance for *Melanoides tuberculatus* (Giovanelli et al. 2005). In the present study, the control did not eliminate the snail of the aquatic environments, but may be used as an alternative method to control the size of the populations of *B. glabrata* in field by interfere with its reproductive process and will be utilized to transmission control for schistosomiasis according stated by King et al. (2006).

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