

Molluscicidal Activity of Crown of Christ (*Euphorbia splendens* var. *hislopii*) (Euphorbiaceae) Latex Submitted to pH Variation

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ABSTRACT

Laboratory and field bioassays have confirmed the specificity of the molluscicidal activity of the *Euphorbia splendens* var. *hislopii* latex (crown of Christ) (Euphorbiaceae) over snails of the species *Biomphalaria glabrata*, *B. tenagophila*, *B. straminea*, *B. pfeifferi* and *Bulinus* sp. in the control of *Schistosoma mansoni*. In the present study, the effect of the pH variation on lethal concentration (LC₉₀) over *B. tenagophila* was evaluated. Bioassays with the aqueous solutions of the latex ranging from 0.4 to 12 µl/l were adjusted for pH of 5.0; 6.0; 7.0 and 8.0, and tested in accordance with methods standardized by World Health Organization. The results obtained indicated that the minor concentration of the latex occurred at pH 6.0 (LC₉₀ = 3.2 µl/l) and the maximum at pH 8.0 (LC₉₀ = 10.3 µl/l). Lethal concentrations adjusted for pH 5.0 and 7.0 were 3.4 µl/l and 4.7 µl/l, respectively. From the results it could be concluded that the molluscicidal toxicity was not altered when the concentrations were adjusted for pH 5.0 and 6.0, as we observed that mortality rate was 100% starting at a concentration of 2.0 µl/l, not the same for the concentrations with adjustment for pH 7.0 and 8.0.

Key-words: *Euphorbia splendens* var. *hislopii*; molluscicidal activity, pH variation, *Biomphalaria* sp., *Bulinus* sp.

INTRODUCTION

Studies on vegetable materials with molluscicidal activity have been carried out since 1930s aiming to control *Schistosoma mansoni* intermediate hosts. Such studies have improved in the last years due to the high costs of acquisition of synthetic molluscicidal as well as operational difficulties of transportation and application, which makes the products prohibitive. The molluscicidal activities of *Euphorbia splendens* var. *hislopii* were firstly studied by Vasconcellos and Schall (1986), who

showed the lethal action for *Biomphalaria glabrata* and *B. tenagophila* under laboratory conditions in concentrations ranging from 0.5 to 4.0 mg/l. Originally from Madagascar island (Pio Corrêa, 1931), this plant is known in Brazil as crown of Christ. In literature, few references on molluscicidal activity, when submitted to pH variation, are found. Some of them only observed data such as those of Gönnert (1961), demonstrating no difference in Niclosamida biological activity (Baylucid®) over adult snails with pH ranging from 5.0 to 9.0. Fox et al. (1963)

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observed that the maximum toxicity range of the Bayluscid occurred when pH ranged from 7.0 to 8.0, and a higher concentration of the molluscicide was required when pH was between 5.0 and 9.0. Souza and Paulini (1967) reported that both Pentaclorophenol (PCF) and Bayluscid presented a higher toxicity in water with pH 6.0 and their action decreased when pH varied from 7.0 to 8.0. Ducan and Pavlik (1970) studied the molluscicidal property of 51 aromatic compounds, pH ranging from 5.5 to 7.7, and only five significant presented molluscicidal activity, with no difference in toxicity at different pH values. In the various works on molluscicidal or records about ecological aspects of snail habitats, pH tended to be acidic. Rumi and Harmann (1992) analyzed the structural age of the *Biomphalaria occidentalis* population in a stagnant pond of the Riachuelo river (Argentina), recording its pH, which varied from acidic to neutral. They observed a linear correlation between pH and snail abundance. Lopez et al. (1993) studied, under laboratory conditions, the molluscicidal activity of *Agave legrelliana* over *Biomphalaria havanensis* and the pH obtained from such solutions was 5.47. The current study aimed at assessing the pH variation over molluscicidal activity of the *E. splendens* var. *hislopii* latex on *B. tenagophila*, pointing out the influence of such parameter over lethal action of different concentrations of vegetal latex.

MATERIAL AND METHODS

Snail Collection and Latex Extraction: For the biological assay, *Biomphalaria tenagophila* snails were used. They were randomly collected from watercress beds in Água Santa district – Rio de Janeiro (RJ - Brazil), through the scoop method. After collection, snails were transported to the laboratory and kept in an aquarium with distilled water and fed with fresh lettuce for at least 48 hours for acclimatization. During this period, they were checked for elimination of cercariae (Frandsen and Christensen, 1984). Animals used in this test measured between 12 to 14 mm of shell diameter. *In natura* latex was obtained from *E. splendens* var. *hislopii* plantation, from horticulture of the Oswaldo Cruz Foundation – Rio de Janeiro, by transversal section at approximately 10 cm below apical meristema of the main stem and its branches. After clipping, latex was recovered by dropping in test tubes with thread lids and taken to the laboratory for desirable concentration acquisition.

Buffer Preparation: The method employed for the Sørensen buffer solution preparation was based on Duncan and Pavlik (1970). Na_2HPO_4 and KH_2PO_4 were used at 5mM. Salts were weighted for specific pH, aimed to obtain a final volume of 10 liters in both kinds of salt solution in distilled water (Table 1).

Table 1 - Mass of the salts (mg) to Sørensen buffer preparation.

	pH			
	5.0	6.0	7.0	8.0
Solutions 0.005M				
Na_2HPO_4	0.1775	1.4198	4.9693	6.744
KH_2PO_4	6.6349	5.444	2.0415	0.3403
distilled water q.s.p.	101	101	101	101

Experimental Procedures: Biological assays were carried out during the fall months and performed in accordance with methods standardized by WHO (1965). From the latex collected, it was obtained 0.1 ml to dilute Sørensen buffer to obtain stock solution at 10 $\mu\text{l/l}$. From this solution, dilutions were performed at each concentration: 0.4; 0.6; 0.8; 1.0; 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5 5.0 and 12.0 $\mu\text{l/l}$. In the control group, animals were submitted only to the diluent, i.e., distilled water. Solutions were placed in 14 glass

beakers, and 10 snails, per concentration, were exposed. A replication of each concentration was done. In first stage, the snails were exposed to the concentrations for 24 hours at room temperature and were not fed. After such period, animals were taken out from the solutions, rinsed with distilled water to remove excess of solution, and then replaced into beakers that had been also washed with distilled water. At this stage all animals were exposed to distilled water, only, for more 24 hours, being fed with fresh lettuce pieces. After this

period, quantitative analyses of surviving and dead snails were counted. The pH of the concentrations was recorded before exposing the snails to molluscicidal and 24 hours after exposure.

Statistical analysis: Lethal dose (LC₉₀) was calculated from statistic software using Probit Analysis (Finney, 1971). Comparison between mortality rates was performed through analysis of variance (ANOVA).

RESULTS

Table 2 shows mortality rates of *B. tenagophila* specimens, submitted to different concentrations of *E. splendens* var. *hislopii* latex, as well as the values of the lethal dose (LC₉₀) obtained from corrected concentrations for pH 5.0, 6.0, 7.0 and 8.0, which were 3.4; 3.2; 4.7 and 10.3 µl/l,

respectively. Such concentrations presented significant difference. The results showed that there was no alteration in molluscicidal activity among the latex concentrations by being fitted at pH 5.0 and 6.0. At these pH values, the mortality rate was 100% starting at a concentration of 2.0 µl/l. No significant difference between pH values was observed (F= 0.62; p>0.05). The adjustment performed in the concentrations with pH 7.0 and 8.0 showed a gradual loss of the molluscicidal activity reaching a maximum of 90% of mortality among the animals submitted to the concentrations with pH 8.0, showing a significant difference among such concentrations (F=2.74; p<0.05). By comparing the concentrations at pH 5.0 and 8.0, it could be also observed that there was no significant difference among the values of the concentrations (F=2.67; p<0.05).

Table 2 - Mortality (%) of *Biomphalaria tenagophila* (n=10) submitted to pH variations of the concentrations of *Euphorbia splendens* var. *hislopii* latex.

Concentrations (µl/l)	5.0	6.0	pH	7.0	8.0
0.0	0(0)	1(10)		0(0)	0(0)
0.4	0(0)	4(40)		0(0)	0(0)
0.6	1(10)	1(10)		0(0)	0(0)
0.8	3(30)	5(50)		0(0)	0(0)
1.0	7(70)	6(60)		0(0)	0(0)
1.5	7(70)	8(80)		0(0)	1(10)
2.0	9(90)	10(100)		0(0)	2(20)
2.5	10(100)	10(100)		1(10)	4(40)
3.0	8(80)	10(100)		6(60)	4(40)
3.5	9(90)	10(100)		6(60)	5(50)
4.0	9(90)	10(100)		7(70)	4(40)
4.5	10(100)	10(100)		10(100)	6(60)
5.0	10(100)	10(100)		9(90)	8(80)
12.0	10(100)	10(100)		10(100)	9(90)
LC ₉₀	3.4	3.2		4.7*	10.3*

* ANOVA (p<0.05)

DISCUSSION

Lethal dose (LC₉₀) obtained from laboratory for *B. tenagophila* in previous studies were 1.07 mg/l for animals from Água Santa locality – RJ and 4.04 mg/l for the animals from Pendotiba – RJ (Vasconcellos and Schall, 1986). In the present

work, lethal dose (LC₉₀) of the concentrations submitted to pH variation were 3.4 µl/l, 3.2 µl/l, 4.7 µl/l and 10.3 µl/l (pH 5.0, 6.0, 7.0 and 8.0, respectively). For all pH adjustments, lethal doses were obtained with values varying between 3 to 10 fold above the CL₉₀ found by Vasconcellos and Schall (1986) for the animals from the same

locality. These data differed from those obtained by Schall et al. (1992), who observed stability of the product related to seasonal variation of the lethal substance contained in the latex, with the species *B. tenagophila*, finding: LC₉₀ of 1.14 mg/l in the spring; 1.07 mg/l in the summer; 1.02 mg/l in the autumn and 1.09 in the winter, with plants from different localities in Brazil. However, these values were in accordance with WHO standardization, in which an ideal natural molluscicidal had been a plant with active aqueous extract when the concentration was 20 mg/l or less to kill 90% of the snails exposed for 24 hours (Mott, 1987). The current study showed that although the pH values of 5.0 and 6.0 did not alter the molluscicidal activity, a gradual loss of this action could be observed in solutions with pH 7.0 and 8.0. These data corroborated those by Souza and Paulini (1967), who analyzed the behavior of PCF and Bayluscid, and related their chemical constitution. They also associated PCF and Bayluscid solubility to the presence of a phenol group providing an acidic feature to both products, consequently, minor solubility which may increase the molluscicidal concentration together with membrane of the cephalopod mass of the snail, providing higher absorption of the molluscicidal and so higher mortality to the animals exposed. By comparison of such data with concentrations of *E. splendens* var. *hislopii* latex, we could suggest a similar acidic feature, although the chemical constitution of this product was not known. Concentrations of latex diluted only in distilled water, presented pH 6.95 at the minor concentration (0.4 µl/l) and pH 5.0 at higher concentration (12 µl/l), totaling 13 concentrations ($\chi = 6.36 \pm 0.32$), then emphasizing the acidic character of this product. The gradual loss of lethal action of alkaline pH values also suggested occurrence of degradability of some chemical substances present in the latex, when submitted to physic/chemical variations of the environment. Such fact have been supposed by Gillet and Bruaux (1961), emphasizing a 80%-loss of Bayluscid molluscicidal activity over *B. glabrata*, when exposed to ultraviolet light for 24 hours, later confirmed by Oliveira-Filho and Paumgarten (1997) who obtained a reduction of 10% in *B. glabrata* mortality rate. Another factor that could alter the latex action was the high concentration of dissolved organic matter in it, as well as the temperature of the solution influencing *B. glabrata*

mortality (Oliveira-Filho et al., 1999). Such results allowed us to conclude that the lethal action of the *E. splendens* var. *hislopii* latex over *B. tenagophila* was possible enabled in acidic environment, which could be remarked by the significant differences in mortality rates between acidic and alkaline pH. In neutral pH, there was a slight variation in mortality rate, maximum of 100%, starting at a concentration of 4.5 µl/l. For alkaline pH, a higher concentration of the latex was required (12 µl/l) in order to obtain the same mortality rate of 100%, obtained in the adjusted concentrations for pH 5.0 and 6.0.

RESUMO

Bioensaios de laboratório e de campo têm comprovado a especificidade da ação moluscicida do látex da *Euphorbia splendens* var. *hislopii* (coroa-de-Cristo) (Euphorbiaceae) para os moluscos das espécies *Biomphalaria glabrata*, *B. tenagophila*, *B. straminea*, *B. pfeifferi* e *Bulinus* sp. No presente estudo foi investigado o efeito da variação do pH na concentração letal (CL₉₀) testada para *B. tenagophila*. Bioensaios com soluções aquosas do látex variando de 0,4 a 12,0 µl/l foram ajustadas aos valores de pH 5,0; 6,0; 7,0 e 8,0, e testadas de acordo com os métodos padronizados pela Organização Mundial da Saúde. Os resultados obtidos indicam que a menor concentração letal do látex ocorreu em pH 6,0 (CL₉₀ = 3,2 µl/l) e a maior em pH 8,0 (LC₉₀ = 10,3 µl/l). As concentrações letais (LC₉₀) ajustadas em pH 5,0 e 7,0 foram 3,4 µl/l e 4,7 µl/l, respectivamente. A partir destes dados conclui-se que a toxicidade do moluscicida não se alterou quando as concentrações foram ajustadas em pH 5,0 e 6,0, pois observou-se que a mortalidade foi de 100% a partir da concentração de 2,0 µl/l, o que não ocorreu nas concentrações com ajuste de pH 7,0 e 8,0.

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