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www.ambi-agua.net
E-mail: ambi-agua@agro.unitau.br
Tel.: (12) 3625-4212

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Determination of the lethal doses LD_{50} and LD_{90} of *Euphorbia splendens* var. *hislopii* latex on *Physa cubensis* Pfeiffer

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José Augusto Albuquerque dos Santos; Valdinei Valin; Aloysio da Silva Ferrão Filho.

Laboratory for Evaluation and Promotion of Environmental Health, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Av. Brasil 4365, CEP 21045-900, Manguinhos, Rio de Janeiro, RJ, Brazil.
e-mail: santosja@ioc.fiocruz.br, nei@ioc.fiocruz.br, aloysio@ioc.fiocruz.br

ABSTRACT

The genus *Physa* are among the most common and widespread fresh-water snails and are frequently associated with the genus *Lymnaea and Biomphalaria*, typically occupying the same habitat and being hosts of trematodes that can cause disease in humans. Several studies have evaluated the control of snails through the use of plant-derived molluscicides; however, few such studies exist regarding the genus *Physa*. Determining the correct lethal doses of [latex of *Euphorbia splendens*] is important for the control of the *Physa cubensis* snail. In one experimental model, 150 snails from Jacarepaguá, Rio de Janeiro, RJ were used. After acclimatization for 72 hours, the snails were divided into groups of 10 and placed into beakers containing different doses, each at a temperature of 26±1ºC and a relative humidity of 80±10%. The results demonstrated LD_{50} of 1.08 µL/L and LD_{90} of 3.41 µL/L, without significant changes in the physical or chemical parameters of the solutions. We conclude that *E. splendens* latex had a significant effect on *P. cubensis* in the laboratory setting.

Keywords: Molluscicidal activity, *Physa cubensis* Pfeiffer, *Euphorbia splendens*.

Determinação das doses letais DL_{50} and DL_{90} do latex da *Euphorbia splendens* var. *hislopii* sobre *Physa cubensis* Pfeiffer.

RESUMO

O gênero *Physa* está entre os mais comuns e difundidos animais de água doce, estando frequentemente associado com os gêneros *Lymnaea* e *Biomphalaria* e geralmente ocupando o mesmo habitat, sendo hospedeiros de trematódeos que podem causar doenças ao homem. O controle de moluscos por meio do uso de moluscicidas de origem vegetal foi avaliado por diversos estudos, no entanto, existem poucos estudos para o gênero *Physa*. As doses letais do látex da *Euphorbia splendens* são importantes para o controle dos moluscos *Physa cubensis*. No modelo experimental foram utilizados 150 moluscos, procedentes de Jacarepaguá, Rio de Janeiro, RJ. Após o processo de aclimatação dos moluscos (72 horas), eles foram divididos em grupos de 10 e colocados em béqueres contendo doses diferentes, na temperatura de 26±1ºC e umidade relativa de 80±10%. Os resultados demonstraram DL_{50} de 1,08 µL/L e DL_{90} de 3,41 µL/L, sem alterações significativas nos parâmetros físico-químicos das soluções. Concluímos que o látex da *E. splendens* apresentou atividade sobre *P. cubensis* em laboratório.

Palavras-chave: Atividade moluscicida, *Physa cubensis* Pfeiffer, *Euphorbia splendens*.

1. INTRODUCTION

The search for an effective, inexpensive, and environmentally friendly molluscicide has motivated the study of plants as a possible source of such substances. (Mott, 1987; Vasconcellos et al., 2003; Mello-Silva et al., 2007, 2010, 2011). According to Baptista et al. (1994), and as noted by Kloos and McCullough (1987), Kuo (1987) and Jurberg et al. (1989), it is estimated that 1426 plant species with molluscicidal properties have been analyzed to date. One of the most promising is the species *Euphorbia splendens* var. *hislopii* (Vasconcellos and Schall, 1986), which has been evaluated for its toxic effects in both field and laboratory studies (Schall et al., 1991) that confirmed its molluscicidal properties. (Schall et al., 1992). In Brazil, this plant is known as Christ's Crown ("Coroa de cristo") and "Bride Mattress" (Joly, 1979).

A study regarding the molluscicidal properties of the latex of *E. splendens* var. *hislopii* on *Melanoides tuberculata*, a snail associated with the habitats of *Biomphalaria glabrata*, demonstrated that in the presence of thiarid snail the molluscicide demonstrated a synergistic effect on the reduction of *Biomphalaria* populations (Giovanelli et al., 2001).

Latex of *E. splendens* was proven to be an effective molluscicide against *Lymnaea columella*, an intermediate host of *Fasciola hepatica*. (Vasconcellos et al., 2003), and altered not only the reproductive activity of *B. glabrata* (Mello-Silva et al., 2007), but also the glucose content *B. glabrata*'s haemolymph and carbohydrate deposits (Mello-Silva et al., 2010), as well as concentrations of proteins and nitrogen products in snails infected with *Schistosoma mansoni* (Mello-Silva et al., 2011).

Previous studies on phytochemical compounds of the species *E. splendens* have demonstrated the presence of triterpenes, flavonoids, ingenol, phorbolsters, lasiodiplodin and eight types of milliamines that have molluscicidal properties (Leet et al., 1982; Zani et al., 1993).

Plant-derived molluscicides are important for the control of snail-borne parasitic infections and intestinal helminthiases. However, the use of niclosamida, a synthetic product recommended by the World Health Organization for snail-control in the schistosomiasis control program (WHO, 1998), is a controversial because of its toxic effects on non-targeted organisms, environmental contamination and the tendency of the mollusks to develop resistance.(Andrews et al., 1982).

Experimental infection of *Physa cubensis* Pfeiffer and *L. columella* with *F. hepatica* miracidiae has demonstratedthe presence of esporocysts in the ephalopodal mass and mantle in the first days of the infection. A malacological survey of waterways to detect foci of transmission of schistosomiasis and other parasitic diseases found mollusks infected by trematodes, *Biomphalaria* sp. infected by *Schistosoma mansoni*, Echinostomatidae, Strigeidae, Derogenidae, and Clinostomatidae cercariae; and *Physa* sp. infected by Echinostomatidae (Souza et al., 1998). There are records of the genus *Physa* possibly hosting larval forms of trematodes that may interfere in the mollusks' community structure (Vigueras and Moreno, 1938; Richards, 1964; Anderson and Fried, 1987; Guimarães et al., 1987; Kanev, et al., 1998; McCarthy, 1999; Degueurce et al., 1999; Snyder and Esch, 1993; Kosanke et al., 1988;).

It is believed necessary to determine lethal doses of chemical compounds or vegetal extracts in order to assess the possible environmental impact when used the control of mollusks coexisting other non-targeted aquatic organisms, since the indiscriminate use of molluscicides may lead to changes in local wildlife. For this reason, the test for evaluating molluscicidal properties, used herein, represents an important alternative in the control of mollusk disease vectors.

Thus, this work aims to investigate the molluscicidal activity and to determine the lethal doses of latex of *Euphorbia splendens* on *Physa cubensis* in laboratory conditions.

2. MATERIAL AND METHODS

2.1. Plant

According to Pio Correa (1931), *Euphorbia splendens* var. *hislopii* is an ornamental plant native to Madagascar, introduced and cultivated in gardens for use as hedges. It is commonly known in Rio de Janeiro as “”Christ’s Crown”’ and is characterized as being a low, contorted shrub, with sprigs and numerous spines, red flowers, and leaves gathered at the apex of its branches.

2.2 Snails

Specimes of *Physa cubensis* Pfeiffer, Jacarepaguá, Rio de Janeiro, RJ, were collected and identified according to Paraense (1983). The specimens were then separated into groups of 10 and placed in 6-liter plastic boxes filled with chorine–free water in order to for acclimatize. They were fed lettuce during this period. (*Lactuca sativa* L. 1758).

2.3. Collection of latex

Latex was collected with covered test tubes by cutting through the stem of *Euphorbia splenden* plants that were cultivated on the Oswaldo Cruz Foundation campus, Rio de Janeiro, RJ and allowing the latex to drip into the tubes.. The material was then taken to the laboratory for preparation of the dilutions.

2.4. Preparation of the doses

From a stock solution with a concentration of 100 µL/L of latex of *Euphorbia splendens*, aqueous solutions were prepared in the following doses: 0.5 µL/L, 1.0 µL/L, 1.2 µL/L, 1.4 µL/L, 1.5 µL/L, 1.6 µL/L, 1.8 µL/L, 2.0 µL/L, 2.5 µL/L, 3.0 µL/L, 3.5 µL/L, 4.0 µL/L, 4.5 µL/L and 5.0 µL/L.

2.5. Bioassays

Groups of 10 snails with a shell size between 5–10 mm were used for each exposure dose of *E. splendens*’ latex plus control, totaling 150 snails, for a period of 24 h. The recently collected latex was used in the bioassays. The groups were kept in 1000 mL beakers containing a 500 mL dose of the solution, while a control group was exposed only to the dilutant (distilled water) at a temperature of 26±1^{o}C and a relative humidity of 80±10%. No food was provided to the animals during that period.

After the exposure period, the animals were removed from the experimental and control solutions, rinsed with distilled water to remove the molluscicide residue and placed in beakers with distilled water, and kept for an additional 24 h.

After this phase, a stereoscopic microscope was used to count the surviving and deceased animals, as determined by the appearance and layout of shells, and the odors of the soft parts of animals. The lethal doses of LD_{50} and LD_{90} were determined in accordance with Vasconcellos and Amorim (2003), as recommended by the World Health Organization (1983). The bioassays were carried out in triplicate.

2.6. Physical and chemical parameters of solutions

The parameters of alkalinity were determined by the titration method with indicators; total hardness and calcium hardness, titration method by EDTA; parameters of chlorides by the Mohr titration method; conductivity by the electrometric method using a conductivity

meter; and pH by the potentiometric method, using a pH meter (FEEMA, 1982; FUNASA, 2006).

2.7. Statistical analysis

Lethal doses (LD_{50} and LD_{90}), were determined probit analysis (Finney, 1971). The plot probit of kill against log of doses (µL/L) provides a simple graphic representation of the dose-to- response ratio. The results of physical and chemical analyses were obtained by analysis of variance (ANOVA) to the significance level of 5% and indexes of correlation and regression were determined (Instat version 1.0).

3. RESULTS AND DISCUSSION

We believe that the use of vegetal molluscicides against *Physa cubensis* should be postponed pending further research in view of the existence of other trematodiosis of medical and veterinary importance.

This study showed the effect of different doses of latex on the significant behavioral changes on lethality of snail in the test. The experimental groups confirmed the molluscicidal effect. Increase in mortality rate due to exposure periods and doses of the latex as well as several factors may be acting the molluscicidal effect of the latex.

There was a relatively dose-dependent response in mortality rates of *P. cubensis* from 0.5 to 1.6 µL/L and the percentage of mortality reached 100% at latex doses of 1.6 µL/L, 3.0 µL/L, 3.5 µL/L, 4.5 µL/L and 5.0 µL/L, as shown in Table 1.

Table 1. Mortality rates of *Physa cubensis*, submitted to various doses of *Euphorbia splendens* var. *hislopii*'s látex.

Dose (µl/L)	Dead (Mean ± SD)	Mortality (%)
CG	0 ± 0	0
0.5	3 ± 1	30
1.0	3 ± 1	30
1.2	5 ± 1	50
1.4	8 ± 1	80
1.5	4 ± 1	40
1.6	10 ± 0	100
1.8	9 ± 1	90
2.0	9 ± 1	90
2.5	9 ± 0	90
3.0	10 ± 0	100
3.5	10 ± 0	100
4.0	8 ± 1	80
4.5	10 ± 0	100
5.0	10 ± 0	100

CG (control grupo); means of three replicats, **SD** = standart deviation, **N** = 10.

Table 2, shows the result of probit analysis snail experiments, with doses of LD_{50} of 1.08 µL/L and a LD_{90} of 3.41 µL/L.

Table 2. LD_{50} and LD_{90} of *Euphorbia splendens* on *Physa cubensis Pfeiffer*.

Snail	**LD_{50} (µl/L)** **X ± SD**	**LD_{90} (µl/L)** **X ± SD**
Physa cubensis	1.08 ± 0.04	3.41 ± 0.12

X = mean of three replicates, **SD** = standard deviation. Analysis with t test unpaired.

Linear regression analysis of the percentage of dead snails in relation to various doses of plant latex found a correlation coefficient of (r) = 0.7688; F value = 18.793 and p-value = 0.0008, which is considered very significant.

The Euphorbiaceae plants are known to have widespread molluscicidal properties (MacBae et al., 1988), though activity varies greatly with respect to species, plant part and even the method of extraction.

In the present study, the latex of *E. splendens* was active at a dose of 1.6 µL/L. The latex of *E. splendens* extracted by driping showed a LD_{50} of 1.08 µL/L and LD_{90} of 3.41 µL/L against *P. cubensis* in laboratory bioassays (Table 2), which is considerably higher than the minimum level recommended for plants to be considered for use as molluscicidal (WHO, 1983).

The varying environmental factors with changes in pH, hardness of water, conductivity, chlorides, alkalinity and different pollutants including pesticides and molluscicides, suggest that physical and chemical parameters of the aquatic medium must be consistent and controlled.

In the present study, we evaluated the physical and chemical parameters used in the latex test solutions of *E. splendens* on *P. cubensis* in laboratory conditions (Table 3). Correlation and regression analysis showed no relationship relationship between the latex doses used and the parameters of total hardness, calcium hardness, mg/L Ca, pH, total alkalinity, conductivity, bicarbonate alkalinity and mg/L HCO^-, and no significant differences were found (Table 4).

In the experimental model using *E. splendens*'s latex *in natura* on *P. cubensis* we found a CL_{90} of 3.41 µL/L. According to Mott (1987), to be considered as a molluscicide, the plant's activity must fall in concentrations up to 100 mg/L, and must be able to kill 90% of the mollusks in the concentration range tested after a 24 hour contact Thus, our results demonstrated that *E. splendens* is an effective molluscicide against these snails, corroborating once again the high potential indicated by Vasconcellos and Schall (1986).

Although this study was performed to evaluate molluscicidal properties in laboratory conditions, previous results demonstrated the activity of *E. splendens* latex under field conditions using both *in natura* and freeze-dried material (Vasconcellos and Schall, 1986). Toxicity studies were also conducted with latex from plants of the genus (Oliveira-Filho and Paumgartten, 2000), and established their levels of safety and acceptability as molluscicides.

Toxicological studies using *E. splendens* as molluscicides showed that the latex has no carcinogenic or mutagenic effect, low embryotoxic, teratogenic and ecotoxicological effects (Delgado et al., 2003).

Table 3. Values of the chemical and physical parameters and linear regression analysis of aqueous solutions with latex of *Euphorbia splendens* var hislopii, with *Physa cubensis* Pfeiffer (N = 10) in laboratory.

Parameters	**GC**	**0.5 µL/L**	**1.0 µL/L**	**1.2 µL/L**	**1.4 µL/L**	**1.5 µL/L**	**1.6 µL/L**	**1.8 µL/L**	**2.0 µL/L**	**2.5 µL/L**	**3.0 µL/L**	**3.5 µL/L**	**4.0 µL/L**	**4.5 µL/L**	**5.0 µL/L**
Total hardness (mg/L $CaCO_3$)	16	18	12	14	24	14	22	18	34	20	22	18	20	22	18
Calcium hardness (mg/L $CaCO_3$)	6	6	8	4	4	10	10	10	12	10	8	6	14	8	12
Magnesium hardness (mg/L $CaCO_3$)	10	12	4	10	20	4	12	8	22	10	14	12	6	14	6
mg/L Ca	2.4	2.4	3.2	1.6	1.6	4	4	4	4.8	4	3.2	2.4	5.6	3.2	4.8
mg/L Mg	2.4	2.9	1.0	2.4	4.8	1	2.9	1.9	5.3	2.4	3.4	2.9	1.4	3.4	1.4
Chlorides (mg/L Cl^-)	3.4	4.4	3.4	2.4	3.4	3.4	2.4	3.4	5.4	5.4	3.4	3.4	3.4	3.4	3.4
pH	6.3	6.2	6.7	6.4	6.2	6.5	6.3	6.2	6.3	6.3	6.4	6.3	6.3	6.6	6.5
Conductivity	12.5	12.5	12.5	15	15	15	16.2	16.2	17.5	15	15	15	16.2	13.7	15
Total alkal. (mg/L $CaCO_3$)	14.4	14	14	18	18	18	21.6	18	19.2	16.2	19.2	24	16.2	14.4	14.4
Hydroxide alkal. (mg/L $CaCO_3$)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mg/L OH^-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carbona alkal. (mg/L $CaCO_3$)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
mg/L $CO^=$	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bicarb. alkal. (mg/L $CaCO_3$)	14.4	14	14	18	18	18	21.6	18	19.2	16.2	19.2	24	16.2	14.4	14.4
mg/L HCO^-	17.6	17.1	17.1	22	22	22	26.3	22	24.1	19.8	24.1	29.3	19.8	17.6	17.6

GC (control group); Total alkal. (total alkalinity); Hydroxide alkal. (Hydroxide alkalinity); Carbona alkalinity (Carbonate alkalinity); Bicarb. alkal. (Bicarbonate alkalinity). Each value is mean of three replicates.

Table 4. The linear regression analysis of physical-chemical parameters in the doses of the latex of *Euphorbia splendens* var hislopii.

Parameters	r	R^2	F	P	significance
Total hardness	0.2190	0.04796	0.6549	0.4329	ns
Calcium hardness	0.4959	0.2460	4.241	0.0601	ns
Magnesium hardness	0.0615	0.003789	0.04944	0.8275	ns
Chlorides	0	0	0	1	ns
pH	0.0143178	0.000205	0.002671	0.9596	ns
Conductivity	0.37855	0.1433	2.175	0.1640	ns
Total alkalinity	**0.06685**	**0.004469**	**0.05836**	**0.8129**	**ns**

r = correlation coefficient**,** $\mathbf{R^2}$ = regression coefficient, **p** = probability, **ns** = not significant.

The stability of the molluscicidal property of the in natura latex of *E. splendens*, collected and stored at room temperaturewas observed in laboratory by Schall et al. (1992). Our results showed that the in natura latex of *E. splendens*, collected was considered active on *Physa cubensis*.

Therefore, due the molluscicidal activity of latex on planorbids, such as *Physa cubensis*, further research should be developed in view of the existence of other trematodiosis of medical and veterinary importance.

4. CONCLUSION

Our results showed molluscicidal activity and lethal doses of the latex of *Euphorbia splendens* on *Physa cubensis* in laboratory conditions and may be an effective alternative in the reduction of the mollusks disease vectors for humans. However, further studies are needed to better investigate the mechanism of molluscicidal activity of latex of *Euphorbia splendens* on *Physa cubensis.*

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