The fascioliasis caused by *Fasciola hepatica* Linnaeus, 1758, a parasite trematode, is of considerable medical-veterinarian importance, because it assaults breeding of cattle, goats, bubalines, horses, ovines and swines (Ribeiro 1951, França 1967, Siqueira et al. 1970, Ueno et al. 1982, Luengo et al. 1984, Alcaino et al. 1990), resulting in serious losses for the cattle raising economy in many countries.

This disease is widely distributed among the five continents and its consequences are great losses to the herds due to the slower growth of the young infected animals (Oakley et al. 1979), low fertility, abortions and progressive loss in milk production, as well as partial or total dooming of the liver, at the time of slaughtering (Bundy et al. 1984, Hurtado et al. 1992). It causes anorexia and anemia with great losses in the nourishing conversion (Ferrer al. 1984, Hurtado et al. 1992). Consequently, the vegetal-originated products have been widely investigated for possible effective use as a specific molluscicide.

In Brazil, the snail *Lymnaea columella* (Say, 1817) is the most relevant intermediate host of *F. hepatica*, as a result of its wide distribution and because its occurrence is almost always associated to the disease (Rezende et al. 1973, Gonzales et al. 1974, Paraense 1982, 1983, Gomes et al. 1985, Amato et al. 1986). Control measures of this disease may be achieved by decrease in the population of intermediate host snails. However, these snails present high reproduction potential (Krull 1933), omnivorous diet (Rey 1991), and means to survive in different habitats (Malek 1985), which has made its control difficult.

It is in the combat to the intermediate host snail of *F. hepatica* that substances with molluscicidal properties become important tools in the control of the propagation of fascioliasis. However, the synthetic molluscicides used currently present some disadvantages, because they alter the structure of the environment, acting as biocides, destroying the flora and fauna of the place (Andrews et al. 1983). Consequently, the vegetal-originated products have been widely investigated for possible effective use as a specific molluscicide.

The objectives of this study were to research the property of the latex of the *Euphorbia splendens* var. *hislopii* (Christ’s Crown) plant, as a molluscicidal over the snail *L. columella*, determining the effective concentration for...
Molluscicidal against Lymanea columella • MC Vasconcellos, A Amorim

its control (lethal concentration - \( LC_{90} \)), observing the effect of the product collected during the four seasons of the year and evaluating the time of duration of the product effect against the mollusks in the laboratory.

**MATERIALS AND METHODS**

Plant used - Euphorbia splendens var. hislopii N.E.B. [(Sin. Euphorbia mili) Des Moul. var. splendens (Hook.) Ursch & Leandri] (Carter 1994) belonging to the family of the euphorbiaceas and originating from the Region of Inselberge, located in the Central Plateau of Madagascar, Africa. It is an exotic, decorative plant and its distribution is cosmopolitan (Rauh 1983).

In Brazil, it is known as “Well Married” and “Our Lady’s Crown”, in Minas Gerais; “Two Friends” and “Two Brothers”, in Bahia; “Torments” and “Friar’s Crown”, in the other regions, and “Christ’s Crown”, in Rio de Janeiro.

It is commonly used in gardens as live fence; its cultivation is simple and its multiplication is done by means of a vegetative process (asexual reproduction), which does not require frequent watering or application of pesticides or fertilizers, because it is not very demanding as to the enrichment of the soil (Baptista et al. 1997).

The hislopii variety was the one used in the current study because it grows larger and can produce more latex.

**Latex collection** - The samples of the latex of *E. splendens* var. *hislopii* were collected always from species cultivated in beds located next to the Biology Department, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, so that possible variations of factors in the metabolism of the plant, which could alter the concentration of the active substance, could be avoided (Lugt 1987).

The latex collection was performed on the same day the tests were done, through transversal sectioning, about 10 cm below the apical meristem of each branch; the raw latex that sprouted was collected in a closed glass essay tube supplied with a screwable lid, so as to avoid coagulation, and transported to the laboratory. This collection method has the advantage of keeping the integrity of the plant, allowing it to remain continually productive.

**Aqueous solution preparation** - The concentrations used in the bioassays were prepared from raw latex, in successive dilutions with distilled water. As of 1 ml of the in natura latex and filling it up to 1000 ml with distilled water, in a volumetric balloon, we obtained the concentration of 1000 mg/l (= 1000 ppm) (mother solution).

A sample of the solution was analyzed later regarding its physical-chemical parameters.

After the preparation of the mother solution of 1000 mg/l, successive dilutions of the aqueous extract were prepared to obtain the final concentrations of 10; 5; 2.5; 2; 1.5; 1; 0.8; 0.6; 0.4; 0.2 and 0.1 mg/l, in a final volume of 1000 ml in each solution, which were divided into equal volumes of 500 ml per flask. For each test, we used a control with pure distilled water, without latex, with the same volume of the solution.

After the latex dilutions, the temperature of the solutions and the environment, as well as the relative humidity of the air was recorded.

**Snail collection** - Mollusks of the species *L. columella* were used in the experiments, which were identi-
as dead. The animals leaving the solution were separated in glasses containing 50 ml of distilled water. After this procedure, all the other animals, which remained in the solutions, were moved to the containers with the solvent (distilled water), in the same volume as the initial one, for another period of 24 h (recovery period) and fed with small bits of fresh lettuce.

After these 24 h period, that is, 48 h after the beginning of the test, the dead and the living animals were counted again with the help of a stereoscopic microscope.

Characterization of the snails death - The deaths of the animals during the tests were confirmed by the change in the shell color and absence of muscle contractions. In general, the cephalopodal mass was distended in a distinguished fashion.

Statistical analysis of data - The calculations of the lethal concentrations were made through a computer program employing the Probit Analysis (Finney 1971), which includes the Qui-Square test ($X^2$); the mortality rates were analyzed through the Variance Analysis test (ANOVA) of two factors (Sokal & Rohlf 1981).

Bioassay to evaluate the time of duration of the latex effect - The evaluation of the persistence of the latex action of E. splendens var. hislopii over different groups of L. columella was observed during 30 days.

For this essay, on the first day of the test, 30 l of latex solution were prepared at a concentration of 5 mg/l from the mother solution, whose initial concentration was 1000 mg/l. This solution was stored in clear glass bottles, kept at room temperature during all the time the test was being performed. Thirty liters of pure distilled water, which served as control, were maintained under the same conditions.

To perform the tests, 20 samples of L. columella, measuring between 7 and 9 mm in shell length, were divided into two groups of 10 samples each every day. One of the groups was placed in a beaker of 1000 ml, containing 500 ml of the solution and the other 10, in another beaker of the same size, containing 500 ml of distilled water (control group). This procedure was repeated during 30 days.

The essays were performed in an area located outside the building of the Biology Department, Fiocruz, protected from direct sun light and rain.

Mortality observations were carried out 24 and 48 h after each exposure. During the first 24 h, the observations were made with no handling of the animals. After 48 h, the dead animals were counted, removed from the flasks containing the solution and discarded, whereas the living animals remained under observation. The same procedure was done with the animals in the control flasks. Daily readings of room temperature and the solution were performed, as well as the relative humidity of the air.

Physical-chemical analysis of the latex solutions - According to the method employed by Feema (1979), some physical-chemical parameters were analyzed, such as conductivity ($\mu nho/cm$), alkalinity (mg/l CaCO$_3$), chloride (mg/l Cl$^-$), calcium hardness (mg/l) and pH of the latex mother solutions (1000 mg/l) of E. splendens var. hislopii, used in the tests for lethal concentration establishment (experiment 1 = E1) and test for the verification of the durability of the latex effect (experiment 2 = E2).

**RESULTS**

Bioassays to determine lethal concentration - Table I shows the molluscidal activity of the aqueous extracts from the in natura latex of E. splendens var. hislopii, collected on the days of the tests, over the mollusks that came from the field of species L. columella, during the four seasons of the year.

![Fig. 1: mortality (probit) of Lymnaea columella in the concentrations of the latex of Euphorbia splendens var. hislopii, during the seasons of the year (N = 20)](image-url)

<table>
<thead>
<tr>
<th>Lethal concentration (mg/l)</th>
<th>Spring</th>
<th>Summer</th>
<th>Fall</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC$_{90}$</td>
<td>1.51</td>
<td>0.55</td>
<td>0.74</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Significant difference between the seasons of the year ($F = 11.01$, G.L. = 3/33, $p < 0.05$)

The values of the lethal concentrations were: 1.51 mg/l, in the spring; 0.55 mg/l, in the summer; 0.74 mg/l, in the fall and 0.93 mg/l, in winter, which presented significant differences in the molluscidal effect during the four seasons of the year (ANOVA test, $F = 11.01$, G.L. = 3/33, $p < 0.05$).

Fig. 1 shows mortalities (probit) of L. columella in relation to the concentrations (logarithmic) of the latex of E. splendens var. hislopii. The values presented a significant difference among the concentrations (ANOVA test, $F = 27.38$, G.L. = 11/33, $p < 0.05$).

Fig. 2 shows the mortality percentages of L. columella when subjected to latex concentrations. It was observed that in the summer, mortality reached 100% as of a concentration at 0.6 mg/l, similarly during the fall and in the
winter, as of a concentration at 1 mg/l, while, in spring, it only reached 100% of mortality as of a concentration at 2 mg/l.

Mortality in the controls was low, reaching 5% in the summer and in winter and 10% in the fall; in spring, none of the samples died.

Bioassay to evaluate the time of duration of the latex effect - Fig. 3, shows the duration of the molluscicide effect of the latex aqueous solution of *E. splendens* var. *hislopii*, at a concentration of 5 mg/l over samples of *L. columella*, during 30 days.

Mortality of the mollusks, calculated after 24 h exposure, was of 100% up to the 3rd day after the preparation of the solution, the deaths gradually decreasing up to the 22nd day. After the 23rd day until the 30th day no more deaths were recorded.

In the control group, low mortality was observed as of the 6th day from the beginning of the test up to 26th day; in all groups tested, when there was mortality, it did not surpass 10%.

In Fig. 4, it is observed that the duration of the latex molluscicide effect over *L. columella* after 48 h of exposure was extended, resulting in 100% mortality of the animals during the first 15 days after preparation of the solution. As of the 16th day, there was an oscillation in mortality until the 20th day, when a steep drop from 80% to 10% in two days was recorded. From the 23rd day until the end of the experiment, mortality of the mollusks was no longer verified.

In the control group, mortality was observed as of the 2nd day from the beginning of the test, not surpassing 30%; on the 19th day this reached 50%. From the 28th day until the end of the test no more deaths were recorded.

The average of the environmental temperatures, of the latex solution and the percentage of relative humidity of the air, during the 30 days of the test, is illustrated in Fig. 5. We observe that there was a similarity in the curves at an average environmental temperature of 29.15 ± 1.74°C and of the solution of 24.98 ± 1.24°C, during all the experiment. The relative humidity of the air varied between 73% and 100% during the 30 days of the test.

Physical-chemical analysis of the latex solutions - The results of the physical-chemical analyses are presented in Table II. We observe that the data of the parameters analyzed were kept below the maximum values obtained in waters of the habitat of *L. columella*, where the collections were made. As to the calcium value (Ca++), in E2, it was 0 mg/l since the beginning (day 0) until the end of the test (30 days after the solution preparation).

**DISCUSSION**

The molluscicidal activity of the in natura latex of *E. splendens* var. *hislopii* (Christ’s Crown) against snails of the species *L. columella*, intermediate hosts of *F. hepatica*, was demonstrated for the first time in the present work.
While studying this plant’s latex action against the intermediate host species of Schistosoma mansoni, Vasconcellos and Schall (1986) verified that the LC90 remained between 1.07 and 4.04 mg/l, for snails derived from the field and, more recently, for intermediate host snails of Schistosoma haematobium, Bilharz, 1852, Schall et al. (1998) obtained a LC90 between 0.15 and 4 mg/l.

In the present study, the values of the lethal concentrations of the latex aqueous solutions found for L. columella, according to the season of the year (1.51 mg/l in spring; 0.55 mg/l in the summer; 0.74 mg/l in the fall and 0.93 mg/l in winter), showed that there are variations among the seasons of the year as far as the molluscicidal activity goes. These data differ little from the ones de-

![Mortality (%) of Lymnaea columella after 48 h of exposure to a solution of 5 mg/l of the latex of Euphorbia splendens var. hislopii, for 30 days (groups of 10 samples)](image)

**Fig. 4:**

![Solution average temperature (°C), of the environment (°C) and relative humidity of the air (%), during the duration time test of the effect of the latex of Euphorbia splendens var. hislopii, against Lymnaea columella](image)

**Fig. 5:**

**TABLE II**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>E1</th>
<th>E2</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity (µho/cm)</td>
<td>25</td>
<td>50</td>
<td>350</td>
</tr>
<tr>
<td>Alkalinity (mg/l CaCO₃)</td>
<td>2</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>Chloride (mg/l Cl⁻)</td>
<td>3.7</td>
<td>1.4</td>
<td>18.1</td>
</tr>
<tr>
<td>Calcium (mg/l)</td>
<td>0.4</td>
<td>0</td>
<td>11.2</td>
</tr>
<tr>
<td>pH</td>
<td>6.6</td>
<td>6.7</td>
<td>6.7</td>
</tr>
</tbody>
</table>

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scribed by Schall et al. (1992) that showed seasonal stability when they tested the latex with Biomphalaria tenagophila, in the four seasons of the year and with plants of different geographical regions of Brazil. In spite of these observed variations, the high LC$_{90}$ found (1.51 mg/l is spring) is lower than the value established by the World Health Organization (WHO 1965), to a plant which presents an active aqueous extract recommending that it has to kill 90% of the snails exposed for 24 h at a concentration less than 20 mg/l (Mott 1987).

As to the active principles of the latex of E. splendens var. hislopii, milliamines are present (A-I), which are stereopeptides of ingenios (Zani et al. 1993). The L milliamine has already been tested in B. glabrata, at a LC$_{90}$ of 1 µg/l (Zani et al. 1989), showing to be 100 times stronger than Niclosamide.

Regarding the stability, the activity of a molluscicide product is an important characteristic of its use, because this product must keep its strength constant, from its preparation and storing until its effective use; otherwise, its use will be quite limited. This problem happens with derivatives of the anacardic acid, a compound existing in the shell of Anacardium occidentale (cashew-nut) (Kloos & McCullough 1982), which is unstable and can even become explosive when stored in sealed containers.

The stability of a sample of the in natura latex of E. splendens var. hislopii, collected and kept at room temperature storage conditions, was observed by Schall et al. (1992). This sample maintained its molluscicidal activity after 124 days (4 months), being the LC$_{90}$ 0.84 mg/l, while the latex, after going through a lyophilization process and kept in the refrigerator at a temperature between 10-12°C, was able to maintain its molluscicidal activity after 736 days (over 2 years).

When we evaluated the duration time of the molluscicidal effect of an aqueous solution of the latex of E. splendens var. hislopii, at a concentration of 5 mg/l, against L. columella, we noticed that the effect was kept for 15 days, with 100% of mortality, and that it started to lose its strength the 23rd day. These data confirm the observations made by Schall et al. (1992), regarding the same latex solution, which was kept active for 13 days over intermediate hosts of S. mansoni, losing its action completely in 30 days. Such a decrease could suggest that the reduction in the molluscicidal activity might have been caused by extended exposure of the solution to light, which can accelerate the degrading process of the active principle (Oliveira-Filho & Paumgartten 1997) and other environmental factors such as temperature, water hardness and concentration of organic materials (Oliveira-Filho & Paumgartten 1999). This was demonstrated by Gillet and Bruaux (1961) for the Niclosamide, which looses about 80% of its molluscicidal activity against B. glabrata when it is exposed to ultraviolet light for 24 h.

As to the stability of the action of a molluscicide, it is important to point out that the activity of these products must last enough time so that we can get the desired effect, but it is also important not to persist far beyond this period in the places where it is employed. This is one of the main advantages for the use of vegetals in the control of plagues, since its potential degradation assures that an accumulation of residue and/or persistence of the activity for long periods of time will not be produced in the environment. Such fact must be taken into consideration because repetitions of the applications may be called for. Field studies with aqueous solutions latex were performed by Mendes et al. (1992), Baptista et al. (1992) and Schall et al. (2001) that demonstrated 100% of mortality for Biomphalaria species.

Such results indicate that the latex of E. splendens var. hislopii is an effective molluscide that can be used as a natural alternative in the control of L. columella.

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