Molluscicidal Action of the Latex of *Euphorbia splendens* var. *hislopii* N.E.B. ("Christ's Crown") (Euphorbiaceae) against *Lymnaea columella* (Say, 1817) (Pulmonata: Lymnaeidae), Intermediate Host of *Fasciola hepatica* Linnaeus, 1758 (Trematode: Fasciolidae). 1- Test in Laboratory

Mauricio Carvalho de Vasconcellos/+, Alziro de Amorim*

Núcleo de Biologia e Controle de Endo e Ectoparasitas de Interesse Médico e Veterinário, Departamento de Biologia, Instituto Oswaldo Cruz-Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil *Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brasil

The latex action of Euphorbia splendens *var.* hislopii *(Christ's Crown) against snails* Lymnaea columella*, intermediate host of* Fasciola hepatica*, derived from irrigation ditches of the Station of Pisciculture at Universidade Federal Rural do Rio de Janeiro, was studied in the laboratory. Lab bioassays, using aqueous solutions of the latex, varying between 0.1 and 10 mg/l, have proven molluscicidal activity of the product collected on the same day the tests were performed, during the four seasons of the year, finding the following lethal concentrations (LC_{90}): 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, after 24 h exposure of the snails, showing significant differences among the seasons of the year (ANOVA test, $F = 11.01$, G.L.= 3/33, $p < 0.05$), as well as among the concentrations (ANOVA test, $F = 27.38$, G.L.= 11/33, $p < 0.05$). In the summer, mortality reached 100% from concentration at 0.6 mg/l, the same during fall and in winter as of 1 mg/l, while in spring it only reached 100% mortality as of 2 mg/l. Mortality in the controls was low, reaching 5% in the summer and winter and 10% in the fall and spring. None of the samples died.*

During the assay, with an aqueous solution of the latex at a concentration of 5 mg/l, in order to check the time of duration of the product effect, in the laboratory, it was observed that the molluscicidal activity remained stable up to the 15th day after the beginning of the test with 100% mortality of L. columella*, gradually losing its effect until the 23rd day, when we no longer observed animal mortality. In the control group, there was a random daily variation in mortality rate ranging 0-50% after 48 h of observation for 30 days.*

Key words: vegetal molluscicide - *Euphorbia splendens* var. *hislopii* - *Lymnaea columella* - control

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 et al. 1985, Urquhart et al. 1990), coming to the point of causing the death of the infected animals (Reid et al. 1972).

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

+Corresponding author. Fax: +55-21-2560.6474. E-mail: mau@ioc.fiocruz.br.
Received 16 October 2002
Accepted 26 February 2003

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 milii* 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 gets 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 identified according to Paraense (1983) and Ueta (1977). The animals were systematically collected in a cemented irrigation ditch at the Station of Pisciculture at the Universidade Federal Rural do Rio de Janeiro, used to fill tanks meant for the creation of fish for research purposes. The mollusks were collected with the help of forceps, placed in plastic bowls with dampened gauze and transported to the laboratory of the Biology Department, Instituto Oswaldo Cruz-Fiocruz.

Creation and maintenance of L. columella in laboratory - Samples of the mollusks collected from the field were examined so as to proceed to the research on the larvae of *F. hepatica*, placing the animals in glass flasks with a small amount of water under artificial light for 1 h. After such a period of time, they were examined by means of a stereoscopic microscope. A small sample was smashed between the slides in order to check for the existence of redias (Frandsen & Christensen 1984). In the case of a negative result, the mollusks were placed, for creation purposes, in amianthus boxes of 50 liters-capacity, containing only chlorine-free water, outside the building. These boxes had air pumps that worked 24 h a day and polystyrene foam plates of different sizes so that the mollusks could lay eggs. The animals were fed daily in amounts sufficient for their nourishment during such a period; the diet consisted of fresh lettuce leaves (*Lactuva sativa)*, previously left under chlorine-free water for rinsing of potential agrotoxic substances. Cleaning of the tanks was done once a week, by means of a plastic siphon to remove sediments of leftovers, excrements and dead mollusks.

Tests with the mollusks - The bioassays were performed according to the method recommended by the World Health Organization (WHO 1965, 1983) and that Mott (1987) considered essential in research with molluscicide substances.

Bioassays to determine the lethal concentration - The bioassays for the establishment of the LC_{90} were performed during a period of one year, covering the four climatic seasons, so as to investigate possible seasonal variations of the molluscicide effect of the latex, collected from the same place. The animals from the control group were exposed only to distilled water.

The mollusks used in the tests were selected before the beginning of each batch of tests and were between 5 and 9 mm in shell length.

Period of exposure and recovery - Twenty samples of *L. columella* were exposed through concentration in two bequers of 1000 ml (experiment and reproduction), with 10 animals in each flask, containing 500 ml of the solution, during 24 h. The flasks were covered with a plastic screen to allow the air in and keep the snails from escaping; the space between the solution and the screen allowed the animals to leave the solution without leaving the container. During this period, the flasks containing the concentrations were kept at room temperature and the animals were not fed.

After the exposure period, both the animals leaving the solution and the ones remaining in the solution, were removed from the flasks, rinsed with distilled water for removal of residues from the shell solution and counted

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 bequer of 1000 ml, containing 500 ml of the solution and the other 10, in another bequer 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 (µnho/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 molluscicidal 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.

TABLE I

Lethal concentrations (LC_{90}) of latex aqueous solutions of *Euphorbia splendens* var. *hislopii*, against *Lymnaea columella*, during seasons of the year (N = 20)

Lethal concentration (mg/l)	Seasons of the year			
	Spring	Summer	Fall	Winter
LC_{90}	1.51	0.55	0.74	0.93

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 molluscicidal 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

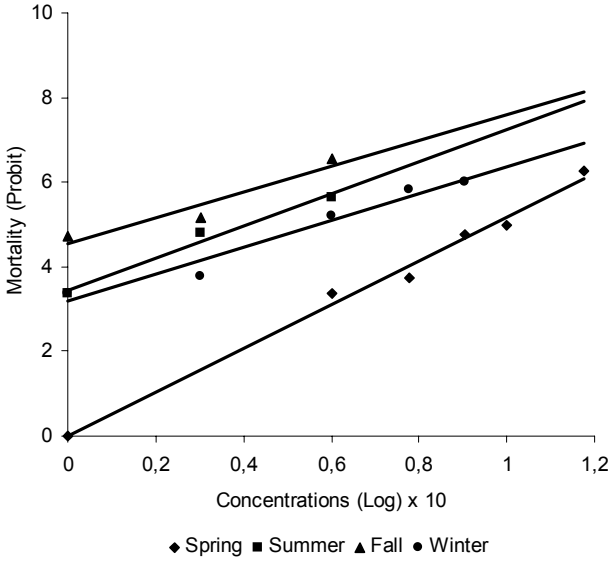

Fig. 1: mortality (probit) of *Lymnaea columella* in the concentrations of the latex of *Euphorbia splendens* var. *hislopii*, during the seasons of the year (ANOVA, F = 27.38, g.l. = 11/33, $p < 0.05$)

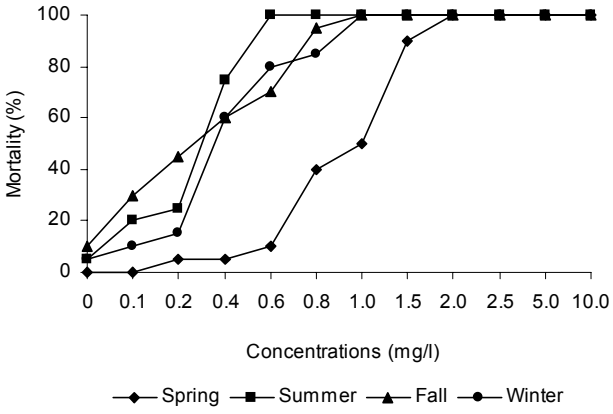

Fig. 2: mortality (%) of *Lymnaea columella* in the concentrations of the latex of *Euphorbia splendens* var. *hislopii*, during the seasons of the year

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. *hisllopii* (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.

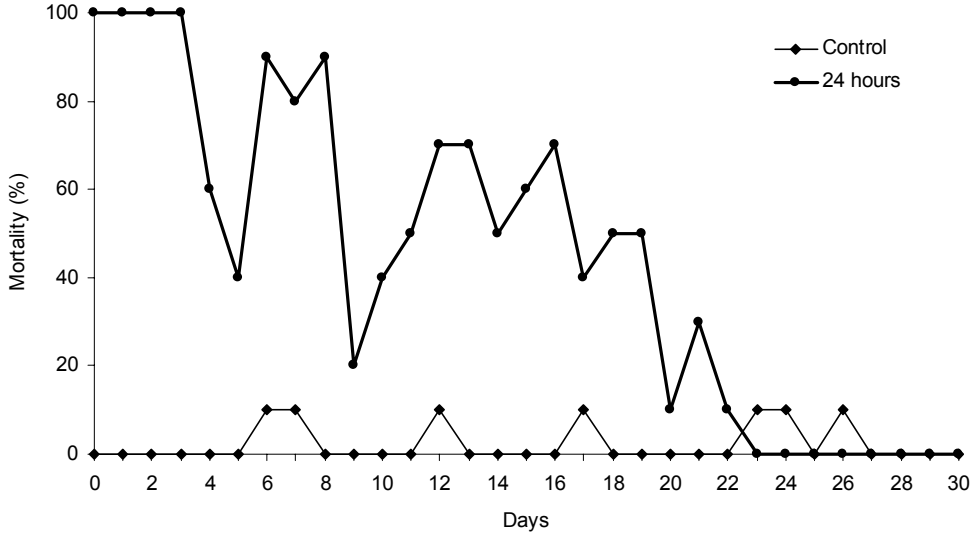

Fig. 3: mortality (%) of *Lymnaea columella* after 24 h of exposure to a solution of 5 mg/l of the latex of *Euphorbia splendens* var. *hislopii*, for 30 days (groups of 10 samples)

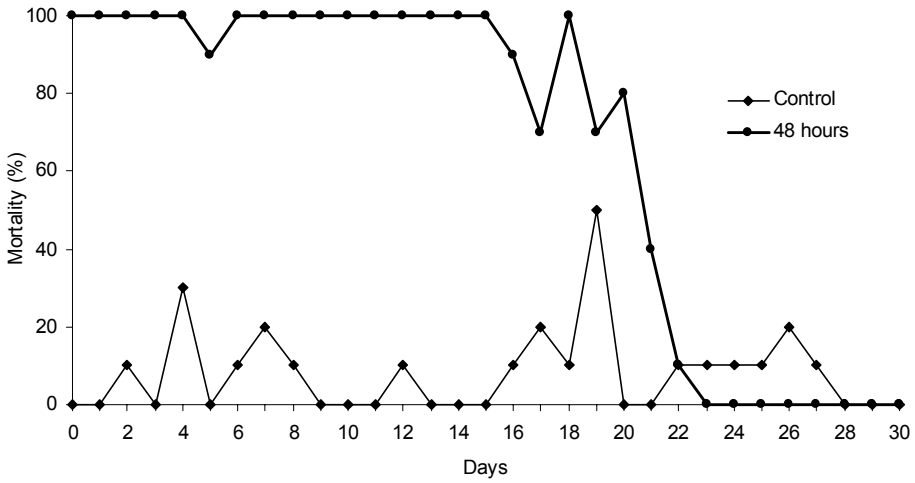

Fig. 4: mortality (%) of *Lymnaea columella* after 48 h of exposure to a solution of 5 mg/l of the latex of *Euphorbia splendens* var. *hislopii*, for 30 days (groups of 10 samples)

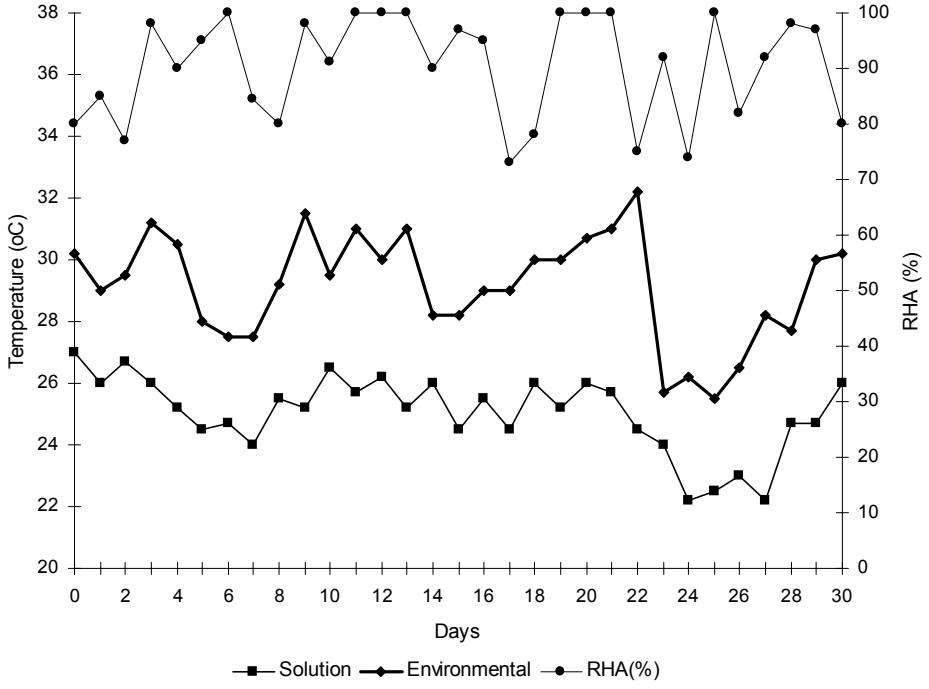

Fig. 5: solution average temperature (ºC), of the environment (ºC) and relative humidity of the air (%), during the duration time test of the effect of the latex of *Euphorbia splendens* var. *hislopii*, against *Lymnaea columella*

TABLE II

Values found in some of the physical-chemical parameters of the mother-solutions used in the lethal concentration tests (E1) and duration time test relative to the effect (E2), with the latex of *Euphorbia splendens* var. *hislopii*, against *Lymnaea columella,* in a concentration of 1000 mg/l

	Maximum values		
	E1	E2	Habitat
Conductivity (µnho/cm)	25	50	350
Alkalinity (mg/l $CaCO_3$)	2	1	28
Chloride (mg/l Cl^-)	3.7	1.4	18.1
Calcium (mg/l)	0.4	0	11.2
pH	6.6	6.7	6.7

While studying this plant's latex action against the intermediate host species of *Schistosoma mansoni*, Vasconcellos and Schall (1986) verified that the LC_{90} 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 LC_{90} 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-

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 ingenois (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 Niclosamida.

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 debasing 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 demonstred 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 molluscicide that can be used as a natural alternative in the control of *L. columella*.

REFERENCES

Alcaino H, Gorman T, Gonzalez V, Vega F, Apt Baruch W 1990. Fascioliasis en caballos, cerdos y conejos silvestres en la provincia de Talca, VII Region, Chile. *Parasitol dia 14*: 9-13.

Amato SB, Rezende HEB, Gomes DC, Serra Freire NM 1986. Epidemiology of *Fasciola hepatica* infection in the Paraíba River Valley, São Paulo, Brasil. *Vet Parasitol 22*: 275-284.

Andrews P, Thyssen J, Lorke D 1983. The biology and toxicology of molluscicides, Bayluscide®. *Pharmacol Ther 19*: 245-295.

Baptista DF, Soares LHJ, Vasconcellos MC, Lopes FH, Paz IS, Schall VT 1997. Vegetative development and seed germination of *Euphorbia splendens* var. *hislopii* - A biomolluscicidal species. *Arq Biol Tecnol 40*: 435-441.

Baptista DF, Vasconcellos MC, Lopes FEF, Silva IP, Schall VT 1992. Evaluation of the molluscicidal property of *Euphorbia splendens* var. *hislopii* (N.E.B.) (Euphorbiaceae) - 2 Investigation in lotic habitat. *Mem Inst Oswaldo Cruz 87*: 549-553.

Bundy DAP, Arambulo Ill PV, Grey CL 1984. La fascioliasis en Jamaica: aspectos epidemiologicos y economicos de una zoonosis parasitaria transmitida por caracoles. *Bol Sanit Panam 96*: 1-19.

Carter S 1994. A preliminary classification of *Euphorbia* subgenus *Euphorbia*. *Ann Missouri Bot Gard 81*: 368-379.

Feema-Fundação Estadual de Engenharia e Meio Ambiente 1979. *Métodos de Análises Físico-Químicas da Água,* Vol. III, Série Didática, 14/79, DICOMT, Rio de Janeiro.

Ferrer JR, Perera G, Yong M 1985. Estudio de los moluscos fluviátiles de una localidad afectada por un brote de fascioliasis. *Rev Cub Med Trop 37*: 155-160.

Finney DJ 1971. *Probit Analisys*, 3rd ed., Cambridge University Press, New Delhi, 333 pp.

França I 1967. Fasciolose hepática em bovinos no Vale do Paraíba, Estado de São Paulo - Brasil. *Arch Esc Vet Minas Gerais 19*: 157-159.

Frandsen F, Christensen NO 1984. An introductory guide to the identification from African freshwater snail with special reference to cercariae of trematoda species of medical and veterinary importance. *Acta Trop 41*: 181-202.

Gillet J, Bruaux P 1961. Essais en laboratoire des nouveaux molluscicides Bayer 73 et ICI 24223. *Bull WHO 25*: 509-517.

Gomes DC, Serra Freire NM, Amato SB, Rezende HEB 1985. Análise do envolvimento de *Lymnaea columella* e *Stenophysa marmorata* na epidemiologia da fasciolose hepática no Vale do Paraíba. IX Cong Soc Bras Parasitol, Resumos, p. 99.

Gonzales JC, Tomé JW, Gonçalves PC, Oliveira CBM 1974. *Lymnaea columella* hospedeiro intermediário de *Fasciola hepatica* (Lin. 1758) no Rio Grande do Sul, Brasil. *Arq Fac Med Vet Rio Grande do Sul 2*: 37-40.

Hurtado AAC, Sánchez IE, Romero HQ 1992. Frecuencia de fasciolasis hepática en bovinos sacrificados en las plantas Tipo Inspección Federal en México de los años 1979-1987. *Vet Méx 23*: 339-342.

Kloos H, Mccullough FS 1982. Plant Molluscicides. *Planta Medica 46*: 195-209.

Krull WH 1933. The snail *Pseudosuccinea columella* (Say) as a potentially important intermediate host in extending on range of *Fasciola hepatica* Linn. *J Was Acad Sci 23*: 389-391.

Luengo J, Arata N, Luengo M, Araya N 1984. Estudio anatomopatológico de hígado de equinos con fasciolasis. *Bol Chile Parasit 39*: 43-46.

Lugt CB 1987. Feasibility of growth and productions of molluscicidal plants. In Mott KE, *Plant Molluscicides,* UNDP/ World Bank/WHO, John Wiley & Sons, New York, p. 231-244.

Malek E 1985. *Snail Hosts of Schistosomiasis and Other Snail-transmitted Diseases in Tropical America: A Manual*, Pan-American Health Org Scientific Publication n. 478.

Mendes NM, Baptista DF, Vasconcellos MC, Schall VT 1992. Evaluation of the molluscicidal properties of *Euphorbia splendens* var. *hislopii* (N.E.B.) (Euphorbiaceae) - 1 Experimental test in a lentic habitat. *Mem Inst Oswaldo Cruz 87*: 21-23.

Mott KE 1987. *Plant Molluscicides,* UNDP/World Bank/WHO, John Wiley & Sons, New York, 326 pp.

Oakley GA, Owen B, Knapp NH 1979. Production effects of subclinical liver fluke infection in growing dairy heifers. *Vet Rec 104*: 503-507.

Oliveira-Filho EC, Paumgartten FJR 1997. Photodegradation of the molluscicidal látex of "Crown-of-Thorns" (*Euphorbia splendens* var. *hislopii*). *Mem Inst Oswaldo Cruz 92*: 657-659.

Oliveira-Filho EC, Paumgartten FJR 1999. The influence of environmental factors on the molluscicidal activity of *Euphorbia milii* latex. *J Environ Sci Health B34*: 289-303.

Paraense WL 1982. *Lymnaea viatrix* and *Lymnaea columella* in the Neotropical Region: a distributional outline. *Mem Inst Oswaldo Cruz 77*: 181-188.

Paraense WL 1983. *Lymnaea columella* in Northern Brazil. *Mem Inst Oswaldo Cruz 78*: 477-482.

Rauh W 1983. Madagascarian euphorbias: life & growth forms. *Euphorbia J 3*: 19-45.

Reid J, Doyle F, Armour JJ, Jennings J 1972. *Fasciola hepatica* infection in cattle. *Vet Rec 90*: 486.

Rey L 1991. *Fasciola hepatica* e fasciolose. In *Parasitologia*, 2ª ed., Guanabara Koogan, Rio de Janeiro, p. 411-416.

Rezende HEB, Araújo JLB, Gomes PAC, Nuernberg S, Pimentel Neto M, Oliveira GP, Mello RP 1973. Notas sobre duas espécies de *Lymnaea* Lamark, 1799, hospedeiros intermediários de *Fasciola hepatica* L, no Estado do Rio de Janeiro (Mollusca, Gastropoda, Basommatophora, Lymnaeidae). *Arq Univ Fed Rur do Rio de Janeiro 3*: 21-23.

Ribeiro PA 1951. Causa de rejeições de suínos abatidos no Brasil Central nos anos de 1936/1949. *Rev Fac Med Vet Univ S Paulo 4*: 421-468.

Schall VT, Vasconcellos MC, Villaça-Coelho AL, Ferreira-Lopes FE, Silva IP 1992. Evaluation of temporal, seasonal and geographic stability of the molluscicidal property of *Euphorbia splendens* latex. *Rev Inst Med Trop São Paulo 34*: 183-191.

Schall VT, Vasconcellos MC, Rocha RS, Souza CP, Mendes NM 2001. The control of the schistosome-transmitting snail *Biomphalaria glabrata* by the plant molluscicide *Euphorbia splendens* var. *hislopii* (syn *milli* Des Moul): a longitudinal field study in an endemic area in Brazil. *Acta Trop 79*: 165-170.

Schall VT, Vasconcellos MC, Souza CP, Baptista DF 1998. The molluscicidal activity of crown of Christ (*Euphorbia splendens* var. *hislopii*) latex on snails acting as intermediate hosts of *Schistosoma mansoni* and *Schistosoma haematobium*. *Am J Trop Med Hyg 58*: 7-10.

Siqueira PA, Serra OP, Rocha UF, Serra RG 1970. Fasciolose hepática em búfalos (*Bubalus bubalis* var. *bubalis* Linnaeus, 1758) no Estado de São Paulo, Brasil. *Bol Indust Anim 27*: 27-28.

Sokal RR, Rohlf FJ 1981. *Biometry*, Freeman HW, San Francisco, 830 pp.

Ueno H, Gutierres VC, Mattos MJT, Müller G 1982. Fascioliasis problems in ruminants in Rio Grande do Sul, Brazil. *Vet Parasiltol 11*: 185-191.

Ueta MT 1977. Variações anatômicas em *Lymnaea columella*. *Rev Soc Brasil Med Trop 11*: 217-237.

Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FN 1990. *Parasitologia Veterinária*, Guanabara Koogan, Rio de Janeiro, 306 pp.

Vasconcellos MC, Schall VT 1986. Latex of "Coroa de Cristo" (*Euphorbia splendens*): an effective molluscicide. *Mem Inst Oswaldo Cruz 81*: 475-476.

WHO-World Health Organization 1965. Molluscicide screening and evaluation. *Bull WHO 33*: 567-581.

WHO-World Health Organization 1983. *Report of Scientific Working Group on Plant Molluscicide and Guidelines for Evaluation of Plant Molluscicide*, (TDR/SCH - SWE(4)/ 83.3), Geneve.

Zani CL, Marston A, Hamburger M, Hostettmann K 1993. Molluscicidal milliamines from *Euphorbia milii* var. *hislopii*. *Phytochemistry 34*: 89-95.

Zani CL, Passos LKL, Souza CP, Oliveira AB 1989. Bioassay guided phytochemical study of the latex from *Euphorbia splendens* (Euphorbiaceae). *Mem Inst Oswaldo Cruz 84* (Suppl. I): 254.