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EVALUATION OF THE MOLLUSCICIDAL PROPERTIES OF *EUPHORBIA SPLENDENS* VAR. *HISLOPII* (N. E. B.) (EUPHORBIACEAE) – 1. EXPERIMENTAL TEST IN A LENTIC HABITAT

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The latex of Euphorbia splendens *var.* hislopii, *at concentrations between 5 to 12 mg/l, kills 100% of the population of* Biomphalaria glabrata *in a lentic habitat, after 24 h.*

The lyophilized latex, stocked for 18 months, killed only 34.2% of the snails, at the concentration of 5 mg/l, and 96.0% at 12 mg/l.

No letal effect was observed among Pomacea haustrum *exposed to the same concentrations of the molluscicide.*

Key words: plant molluscicide – schistosomiasis – *Biomphalaria glabrata* – *Euphorbia splendens*

Several species of plants belonging to the family Euphorbiaceae have had their molluscicidal potential tested. Among the species which showed activity on *Biomphalaria glabrata* are *Euphorbia cotinifolia* L. (roxinha) (Pereira et al., 1978), *E. tirucalli* L. (avelós) (Jurberg et al., 1985) and *E. splendens* L. (coroa de cristo) (Vasconcellos & Schall, 1986). Besides these studies on vector snails of schistosomiasis, Singh & Agarwal (1984, 1987) investigated the action of the latex of *E. royleana* and *E. antisyphlitica* on *Lymnaea acuminata.*

The field evaluation of the action of plant-derived compounds demands preliminary investigations, as pointed out by Koeman (1987), such as studies of acute toxicity, as well as of mutagenicity and carcinogenicity tests.

The results of laboratory tests with *E. splendens* latex have so far shown a great specificity of its lethal effect on vector snails. Also, some encouraging results have been detected through other toxicological tests, such as acute toxicity (Matos et al., 1989), cutaneous irritability (Freitas et al., 1990), mutagenicity and cytotoxicity (Schall et al., 1991), carcinogenie assays (Marston & Hecker, 1983, 1984). Other assays have shown a seasonal and geographic stability of the latex (Schall et al., 1992). A phytochemical study of the plant is under way (Zani et al., 1989), which has already revealed an active fraction at 1 μg/l.

In the present study the molluscicidal activity of *E. splendens* latex has been studied, both in an aqueous suspension of the natural extract, and in lyophilized form at 5 and 12 mg/l applied in lentic habitats of *B. glabrata.*

MATERIALS AND METHODS

The natural extract of *E. splendens* latex used in this study was collected in Rio de Janeiro in March 1991, whereas the lyophilized latex was processed in October 1989, and kept in a test tube at 10 °C in refrigerator.

For the preparation of stock solutions at 2500 mg/l, 2.5 ml of crude latex were diluted in 1 l dechlorinated water. Also, 2.5 g of lyophilized latex were suspended in 1 l dechlorinated water, which had also been sonicated for 10 min. The solutions at 5 and 12 mg/l were obtained through aqueous dilution of the stock solutions.

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The solutions of crude and lyophilized latex were tested in artificial puddles, which were inhabited by *B. glabrata* populations, in a vegetable garden of the Centro de Reeducação de Neves, Ribeirão das Neves, Minas Gerais state, 40 km away from Belo Horizonte.

The water volume of each puddle was calculated through the formula $V = \pi \cdot r^2 \cdot h$ (V = volume; r = radius; h = depth). The diameter and depth values were obtained from an average of the three measurements.

Two puddles were treated with crude latex and two were treated with lyophilized latex at 5 and 12 mg/l, respectively. A fifth puddle was held as a control. Water temperature and pH of the puddles were recorded at the beginning and at the end of the experiment. Twenty-four hours after the treatment, the snails of each puddle were collected and taken to the laboratory in separate containers. The number of planorbids collected in the puddles was recorded, their shells were measured and dead animals were discarded. Live molluscs were placed in flasks containing dechlorinated water and lettuce as food, and were kept in observation for 48 h. The same procedure was used for *Pomacea haustrum* (Reeve, 1856) found in the habitat. A cage containing approximately 40 *B. glabrata* snails collected in the study habitats was suspended in every puddle, not being allowed to touch its bottom.

RESULTS

The results obtained in the field are shown in Table. *B. glabrata* snails measured 4 to 19 mm. Water pH in the puddles ranged from 6.5 to 7.0, and the recorded water temperature was 24 °C.

The results showed 100% mortality among *B. glabrata* snails exposed to the 5 and 12 mg/l solutions derived from the natural extract of the latex. On the other hand, the lyophilized latex, which had been kept for 18 months in the laboratory, was more active at 12 mg/l, killing 96.0% of the snails. The lyophilized latex at 5 mg/l caused low mortality among animals kept in the case (5.8%) as compared to the animals in the puddle (61.1%). In the control puddle mortality was minimal (1.5%) (Table).

As regards *P. haustrum*, the other mollusc species studied, there was no considerable lethal effect of the product on it. The 5 mg/l solution of the natural extract of the latex was the only one which caused some mortality (3.5%) on snails of this species (Table).

DISCUSSION

The present field study confirms the molluscicidal properties of *E. splendens*, as previously established by Vasconcellos & Schall (1986) in laboratory tests on *B. glabrata*. The effectiveness of the aqueous solutions at 5 and 12 mg/l suggests that the extraction and application procedures may be directly transferred to the field.

The low molluscicidal activity of the lyophilized latex at 5 mg/l, as shown by the present results, contrasts with the efficacy of the product recorded in the laboratory by Schall & Vasconcellos (1991), which may be attributed to the long time in stock (18 months) until its use in the present study. The loss of activity of the product has been observed after 9 months (Schall et al., 1992). Furthermore, we associate the low mortality rate (5.8%)

TABLE

Molluscicidal activity of crude and lyophilized *Euphorbia splendens* latex at 5 and 12 mg/l on *Biomphalaria glabrata* and on *Pomacea haustrum* in a lentic habitat

Latex	V (l)	C (mg/l)	Number of *B. glabrata* cage dead/total	%	out of cage dead/total	%	total	(%)	Number of *P. haustrum* cage dead/total	%	out of cage dead/total	%	total	(%)
Crude	215	5	44/44	100.0	11/11	100.0	55/55	100.0	2/21	9.5	4/150	2.6	6/171	3.5
Crude	137	12	42/42	100.0	12/12	100.0	54/54	100.0	0/ 2	0.0	0/ 65	0.0	0/ 67	0.0
Lyophilized	126	5	2/34	5.8	22/36	61.1	24/70	34.3	0/ 3	0.0	0/ 3	0.0	0/ 6	0.0
Lyophilized	153	12	41/41	100.0	8/10	80.0	49/51	96.0	0/ 1	0.0	0/ 7	0.0	0/ 8	0.0
Control	135	–	0/44	0.0	1/26	3.8	1/70	1.5	0/ 7	0.0	0/ 7	0.0	0/ 14	0.0

V: water volume in the pudlle; C: concentration of the latex solution.

among snails kept in the cages and exposed to the lyophilized latex at 5 mg/l with the lower solubility of the product. Therefore, these snails, which were not allowed to graze on the substrate, were not exposed to the active principle of the plant compound, which had probably deposited on the bottom of the puddles.

Preliminary tests in the laboratory have indicated that the action of the aqueous solution of the latex at the LD 90 on laboratory-bred snails is not lethal to several organisms which have at least a life stage in freshwater habitats, such as zooplankton (Lustoza, A., personal communication), fish (*Geophagus brasiliensis*), anophelid larvae and *Schistosoma mansoni* cercariae and miracidia (Vasconcellos et al., unpublished). The active fraction extracted from the latex, and isolated by Zani et al. (1989), proved to be active on *B. glabrata* at 1 μg/l, whereas a 7-fold increase in concentration was necessary in order to reach the LD 90 for *Lebistes reticulatus.*

In the conditions of the present study both the crude and lyophilized latex did not present any lethal effect on *P. haustrum,* as well as on Odonata larvae and adult Coleoptera present in the habitat.

As regards the lethality mechanism associated to the latex of Euphorbiaceae, Singh & Agarwal (1984) suggested a potent active substance with an anti-acetylcholinesterase action both *in vivo* and *in vitro* at an unknown concentration. These authors suggest that death of the molluscs may be caused by a sudden interruption of nervous impulses. However, other studies are still necessary in order to further investigate the activity mechanism of *E. splendens* latex on *B. glabrata.*

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