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EVALUATION OF THE MOLLUSCICIDAL PROPERTY OF *EUPHORBIA SPLENDENS* VAR. *HISLOPII* (N. E. B.) (EUPHORBIACEAE) – 2. INVESTIGATION IN LOTIC HABITAT

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The latex of Euphorbia splendens var. hislopii in 12 ppm concentration (12mg/l) caused 100% mortality for a Biomphalaria tenagophila *population in a lotic habitat after 9 hr of drip-feed application. For sentinel snails, in cages placed at the water surface and buried under 0.10 m, the mortality rates varied with the distance from the application point and were: 100% (0 m); 92.6% (50 m) and 94.7% (100).*

*No lethal effects were observed for the other living species in this habitat (*Pomacea haustrum *and* Poecilia reticulata*).*

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

There is a great interest in the use of molluscicidal plants by local communities in a self-supporting system of a plural schistosomiasis control program (Taylor, 1986) since the high costs of synthetic molluscicides as well as some difficulties with their transportation and application are prohibitive to that purpose.

Euphorbia splendens known in Brazil as "coroa de cristo" is among 354 plants already tested for molluscicidal activity in the country (Jurberg et al., 1989).

In the latex of "coroa de cristo" it was identified a ingenols series presented as miliamines (Zani et al., 1991). Until now biological assays did not show any carcinogenic property of these substances (Marston & Hecker, 1983, 1984). Other toxicological tests have already been done like acute toxicity (Mattos et al., 1989), cutaneous and ocular irritability (Freitas et al., 1991), mutagenicity and citotoxicity (Schall et al., 1991), with some encouraging results.

The phytochemical fractionation of the latex had just showed an active fraction at 0.01 ppm (Zani et al., 1989). That is one hundred more potent than niclosamide, the compound that is used in large-scale nowadays. At the present time long-term toxicological studies are being conducted in mammals with some observations on mutagenicity and carcinogenicity.

Laboratory assays have showed seasonal and geographical stability of the latex in Brazil (Schall et al., 1992) in spite of the possible variations in its molluscicidal activity according to different geographical origins.

Considering the control of schistosomiasis vector snails in restricted lentic habitat promising field investigations were done with the latex in Minas Gerais state (Brazil). The mortality of *Biomphalaria glabrata* snails was 100% in the concentrations of 5 and 12 ppm (Mendes et al., 1992).

This work presents the results of a field study using the latex of *E. splendens* as molluscicide for the control of *B. tenagophila* snails in natural lotic habitat in the municipality of Paracambi, state of Rio de Janeiro.

MATERIALS AND METHODS

Study area – The study was carried out in the municipality or Paracambi, a low prevalence area of schistosomiasis.

General observations – The field investigation aimed to test the molluscicidal activity

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of the latex of *E. splendens* in an aqueous solution in a natural lotic habitat. Some known foci of *B. tenagophila* were used for a short-term mortality test. The density of *B. tenagophila* was quantified in November, with maximum population density just before the beginning of the rainfall period. The molluscicide was applied after the snail population quantification. In that period the stream flow was low because of the dry season. This habitat was not connected to any other river so there was no molluscicide dispersion. Although in laboratory investigations LD90 varied from 1 to 4 ppm, the highest concentration (12 ppm) was used in order to compensate some inaccurancy in the measured water volume as well as the effects of silt deposits in the habitat.

Two streams were chosen: one was taken as experimental site and the other one as control. Both of them had low turbidity, slow and continous water flow, pH varying from 5 to 6, water temperature of 27 °C, mud substrate and heavy vegetation along the water edge.

The small experimental stream was approximately 500 m long, 0.70 m wide and 0.10 m deep, with water flow of 3 l/s. The water was collected by a drainage channel of the county sewerage which prevented any human contact for domestic and leisure purposes.

The control stream which was similar to a natural one was a small channel built by man. It was approximately 400 m long, 0.50 m wide, 0.05 m deep, with water flow of 3 l/s. The water is used by the neighboring population for domestic purposes.

Molluscicide plant collection and application – The employed chemical was the latex diluted in water. The latex was collected in the standard locality (Ilha do Governador, RJ) two weeks before the field application. It was extracted by the stem incision method at 10 cm from the top.

The concentration was 12 ppm calculated from the total volume of habitat water to be treated for 9 hr at a water flow of 3 l/s. For this purpose, 933 ml of latex were used. The drip-feed mollusciciding method was chosen: the latex was stored in a stainless steel container placed over the experimental stream.

Snail estimation by direct sampling – The snails were sampled by two persons for 30 min. In both experimental and control sites the

TABLE I

Molluscicidal activity of the latex of *Euphorbia splendens* at 12 pmm on *Biomphalaria tenagophila* in lotic habitat

	Number of *B. tenagophila*				
	Before application		After application		Mortality (%)
	Alive	Dead	Alive	Dead	
Exp.	1107	0	0	1095	100
Cont.	259	0	268	7	2.6

Exp. = experimental; Cont. = control.

TABLE II

Exposure of sentinel snails (*Biomphalaria tenagophila*) to the latex of *Euphorbia splendens* at 12 ppm. The cages were places at the water surface

Distance from application point (in m)	Number of *B. tenagophila*				
	Before application		After application		Mortality (%)
	Alive	Dead	Alive	Dead	
0	40	0	0	40	100
50	40	0	0	40	100
100	39	0	0	39	100

TABLE III

Exposure of sentinel snails (*Biomphalaria tenagophila*) to the latex of *Euphorbia splendens* at 12 ppm. The cages were placed beneath 0.10 m in the substrate of the lotic habitat

Distance from application point (in m)	Number of *B. tenagophila*				Mortality (%)
	Before application		After application		
	Alive	Dead	Alive	Dead	
0	42	0	0	42	100
50	41	0	3	38	92.6
100	38	0	2	36	94.7

molluscs found on the substrate and at the water surface were collected with a forceps.

The snails sampling in the experimental site was done along a 100 m area adjacent to the site where the water was collected by the county sewerage. After the quantification the sampled snails were returned to the habitat. In order to verify some chemical dispersion effects, the snails quantification after the application was done along the stream at sites distant 0.50 and 100 m from the application point.

Sentinel molluscs – Before application of the latex of *E. splendens*, six cages with 40 *B. tenagophila* specimens were placed at 0.50 and 100 m from the application point. Three cages were placed at the water suface and the other three were placed 0.10 m beneath the substrate. This procedure was also done in the experimental stream.

RESULTS

B. tenagophila snails measured 3 to 14 mm. Water pH in the lotic habitat ranged from 5 to 6, and the recorded water temperature was 27 °C.

Data on snail mortality – The results of this first investigation in running water showed that at a 12 ppm concentration (applied by the drip-feed method for 9 hr) there was 100% mortality among snails along the natural tested stream (Table I).

The results for sentinel snails placed beneath 0.10 m showed a slight reduction in the mortality rates as the distance from the application point increased: 0 (100%), 50 (92.6%) and 100 m (94.7%) (Table III). The mortality was 100% for the snails kept in cages on the water surface (Table II).

For the control stream snails, the mortality rate was 2.6%.

The other observed species in the habitat (*Pomacea haustrum* and *Poecilia reticulata*) were not affected by the molluscicide.

DISCUSSION

The present work confirms the efficiency of the molluscicide action of the latex of *E. splendens* under field conditions, as previously indicated by Mendes et al. (1992) in a lentic habitat.

Since mud could be one of the most important factors in the efficient reduction of the molluscicides, we tested its efficiency on sentinel snails placed beneath 0.10 m in the muddy substrate distant 0.50 and 100 m from the applicaton point. Until now the results have indicated some loss in the chemical efficiency as the distance from the appication point increases, with mortality rates of 100%, 92.6% and 94.7%, respectively. Such data may be related to: (a) a great dispersion of the latex in running waters with enough time to penetrate in the substrate and (b) a possible molecular instability of the active fraction after reacting with the substrate. More revealing answers shoud be obtained in experiments to be made regarding the absorption degrees of the active fraction identified by Zani et al. (1991) with samples of deposits exposed to the latex in simulated watercress cultures.

Although the results indicate 100% mortality among *B. tenagophila* exposed to 12 ppm concentrations of the latex for 9 hr, new tests on the time optimization and on the chemical application mode are desirable since the use of low concentrations for longer exposure peri-

TABLE IV

Plant (species)	Common name	Target snails	Mortality (%) concentration (ppm)	Time of observed mortality	Active principle author	Potential toxicologic problems
Ambrosia maritima	damsissa	*Biomphalaria alexandrina*	100% 70 ppm	after 24 hr	sesquiterpenes lactones Shoeb & El Eman (1978)	Allergic contact dermatitis in humans. Possible cytotoxicity (Mitchell & Dupuis, 1971; Kupchan et al., 1971; Lee et al., 1971)
Anacardium occidentale	cashew-nuts	*Bulinus globosus*	86.5 – 97.8% 1 ppm	after 24 hr	anacardic acid alkenul phenols Sullivan et al. (1982)	Unstable compound. Allergic reaction or visicant effects in persons exposed Toxic effects of poison-ivy, poison-ivy and poison-sumac dermatitis (Baer, 1979)
Euphorbia splendens	crown christ's	*Biomphalaria glabrata Biomphalaria tenagophila*	100% 5/12 ppm	after 24 hr	ingenols diterpenes Milliamines Zani et al. (1991)	Possible existence of phorbol esters that promote tumors, although its do not occur in all species of Euphorbiaceae family (Farnsworth et al., 1987)
Phytolacca dodecandra	endod	*Bulinus globosus Lymnaea natalensis*	100% 20 ppm	after 24 hr	saponins Domon & Hostettmann (1984)	Possible active mitogenic lectins (highly found in *P. americana*) (McPherson, 1979; Stobo, 1980)
Swartzia madagascariensis	kipogoro	*Bulinus globosus Biomphalaria pfeifferi*	100% 200 ppm	after 24 hr	oleanolic acid saponin Suter et al. (1986)	Possible rotenoids – piscicidal and adverse effects on the offspring (Haley, 1978; Khera et al., 1982)

ods as well as studies with massive doses can facilitate its use in the field. It is also necessary to follow the possible habitat repopulation along the year in order to verify the optimum interval between the annual applications, to control the snail population and to disrupt schistosomiasis transmission.

Since the latex is the active plant part to be used as molluscicide, there are some advantages regarding its applicability in the field, as follows: extration and application at any season, easy manipulation (latex dilution in water), the chemical storage for long periods and no toxicity to nontarget organisms in the concentrations used.

Concerning the great potential of *E. splendens* as a plant molluscicide when used in running waters we point out some of its applications in the field. *E. splendens* is of great potential considering that it is easy to grow because of its resistance to desiccation, its vegetative reproduction through cuttings, dispersion by stakes, its plague resistance and its use in small and medium-sized irrigation channels where water can be diked. These are remarkable aspects in sites where schistosomiasis is connected to irrigated cultures like sugarcane, rice, watercress and others.

However, its effective action on an African vector specie (*B. pfeifferi*) as note by Vasconcellos et al. (unpublished) and its Madagascar origin makes it suitable for application in the African continent where disease attacks around 120 millions people and the transmission through irrigation channels is quite frequent.

Among other plants were evaluated in the field, some of them presented good results as: *Phytolacca dodecandra,* with 100% mortality to *Bulinus globosus* after 24 hr exposure at a 20 ppm concentration (Nadamba et al., 1989); *Anacardium occidentale,* that kills 97.8% of the snails (*B. globosus*) in irrigated channels at 1 ppm concentration (Rey et al., 1987); *Ambrosia maritima,* with 100% mortality to *B. alexandrina* at a 70 ppm concentration (El Sawy et al., 1983); *Swartzia madagascariensis,* that kills 100% of the snails (*B. globosus* and *B. pfeifferi*) after 24 hr at a 200 ppm concentration (Suter et al., 1986). Effective results were also obtained with *E. splendens* var. *hislopii,* evaluated in a lentic habitat, when the mortality of *glabrata* snails was 100% at a 5 and 12 ppm concentration (Table IV).

Although all of these are potentially effective to use in field as molluscicides, some toxicologic and operational aspects have to been investigated in detail before their application in the control of schistosomiasis. As pointed out in Table IV, there are possible toxicologic effects that have to be very well known in order to guarantee total security to the human population, the environment and the other organisms.

As the results confirmed a total reduction in the snail population in the stream treated for 9 hr, the chemical at a 12 ppm concentration should be tested to verify its effect in the reduction of schistosomiasis prevalence in human populations. Only through this procedure and after the conclusion of the toxicologic studies, the viability of this plant in schistosomiasis control will be achieved.

REFERENCES

BAER, H., 1979. The poisonous Anacardiaceae, p. 161-170. In A. D. Kinghorn, *Toxic Plants*, New York: Columbia University Press.

DOMON, B. & HOSTETTMANN, K., 1984. New saponins from *Phytolacca dodecandra* l'Herit. *Helv. Chim. Acta., 67:* 1310-1315.

EL SAWY, M. F.; DUNCAN, J.; MARSHALL, T. F. C.; BASSIOUNY, H. K. & SHEHATA, M. A. R., 1983. The molluscicidal properties of *Ambrosia maritima* L. (Compositae). 1. Design for a molluscicide field trial. *Tropenmed. Parasit., 34:* 11-14.

FARNSWORTH, N. R.; HENDERSON, T. O. & SOEJARTO, D. D., 1987. Plants with potential molluscicidal acitivity, p. 131-204. In K. E. Mott, *Plant Molluscicides.* New York, John Wiley & Sons Ltd.

FREITAS, J. C. B.; PRESGRAVE, O. A. F.; FINGOLA, F. F.; MENEZES, M. A. C.; VASCONCELLOS, M. C.; SCHALL, V. T. & PAUNGARTTEN, F. J. R., 1991. Toxicological study of the molluscicidal latex of *Euphorbia splendens:* irritant on skin and eye. *Mem. Inst. Oswaldo Cruz, 86:* (Suppl. II): 87-88.

HALEY, T. J., 1978. A review of the literature of rotenone. *J. Environ. Pathol. Toxicol., 1:* 315-337.

JURBERG, P.; VASCONCELLOS, M. C. & MENDES, N. M., 1989. Plantas empregadas como moluscicidas: uma visão crítica. *Mem. Inst. Oswaldo Cruz, 84* (Suppl. I): 76-83.

KHERA, K. S.; WHALEN, C. & ANGERS, G., 1982. Teratogenicity study on pyrethrum and rotenone (natural origin) and Ronnel in pregnant rats. *J. Toxicol. Environ. Health, 10:* 111-119.

KUPCHAN, S. M.; EAKIN, M. A. & THOMAS, A. M., 1971. Tumor inhibitors: 69. Structure-cytotoxicity relationships among the sesquiterpene lactones. *J. Med. Chem., 14:* 2105-2126.

LEE, K. H.; HUANG, E. S.; PIANTADOSI, C.; PAGANO, J. S. & GEISSMAN, T. A., 1971. Citotoxicity of sesquiterpene lactones. *Cancer Res., 31:* 1649-1654.

MARSTON, A. & HECKER, E., 1983. On the active principle of the Euphorbiaceae VI. *Planta Med., 47:* 141-147.

MARSTON, A. & HECKER, E., 1984. Active principle of the Euphorbiaceae VII. *Planta Med., 4:* 285-364.

MATTOS, R. C.; VASCONCELLOS, M. C.; LOPES, M. C.; SOUZA, C. A. M.; ALVES, E. N.; FARIAS, M.; SCHALL, V. T. & PAUNGASTTEN, F. J. R., 1989. Estudo toxicológico do látex moluscicida da "coroa de cristo" (*Euphorbia splendens* var. *hislopii*). I. Ensaios preliminares. In Reunião Anual da Federação de Sociedades de Biologia Experimental, 4, Caxambú, MG. p. 320.

MENDES, N. M.; BAPTISTA, D. F.; VASCONCELLOS, M. C. & SCHALL, V. T., 1992. Evaluation of the molluscicidal properties of *Euphorbia splendens* var. *hislopii* (N. E. B.) (Euphobiaceae). 1. Experimental test in lentic habitat. *Mem. Inst. Oswaldo Cruz, 87:* 21-23.

McPHERSON, A., 1979. Pokeweed and other lymphocytic mitogens, p. 83-102. In A. D. Kinghorn, *Toxic Plants.* New York: Columbia University Press.

MITCHELL, J. J. & DUPUIS, G., 1971. Allergic contact dermatitis from sesquiterpenoids of the Compositae family of plants. *Br. J. Dermatol., 84:* 139-150.

NDAMBA, J.; CHANDIWANA, S. K. & MAKASA, N., 1989. The use of *Phytolacca dodecandra* berries in the control of trematode-transmitting snails in Zimbabwe. *Acta Tropica, 46:* 303-309.

REY, K.; LOURENÇO, M. I. & GARCIA, C. M., 1987. Esquistossomoses: Metodologia de controlo em aldeias comunais de Moçambique. I. Controlo de moluscos, terapeutica e participação comunitária. *Rev. Med. Moçambique, 3:* 1-7.

SCHALL, V. T.; VASCONCELLOS, M. C.; VALENT, G. U.; SATO, M. I. Z.; FURLAN, E. V. & SANCHEZ, P. S., 1991. Evaluation of genotoxic activity and acute toxicity of *Euphorbia splendens* latex, a molluscicide for the control of schistosomiasis. *Brazilian J. Med. Biol. Res., 24:* 573-582.

SCHALL, V. T.; VASCONCELLOS, M. C.; VILLAÇA-COELHO, A. L.; LOPES, F. E. F. & SILVA, I. P., 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.

SHOEB, H. A. & EL-EMAN, M. A., 1978. The molluscicidal properties of substances gained from *Ambrosia maritima,* p. 487-494. In Proceedings of the International Conference on Schistosomiasis, S. O. P. Press. Cairo.

STOBO, J. D., 1980. Mitogens. *Clin. Immunobiol., 4:* 55-77.

SULLIVAN, J. T.; RICHARDS, C. S.; LLOYD, H. A. & KRISHNA, G., 1982. Anacardic acid: Molluscicide in cashew nut shell liquid. *Planta Med., 44:* 175-177.

SUTER, R.; TANNER, M.; BOREL, C.; HOSTETTMANN, K. & FREYVOGEL, T. A., 1986. Laboratory and field trials at Ifkara (Kilombero District, Tanzania) on the plant molluscicide *Swartzia madagascariensis. Acta Tropica, 43:* 69-83.

TAYLOR, P., 1986. Water supply and sanitation programmes to support Schistosomiasis control in Zimbabwe. *Trop. Med. Parasit., 37:* 188.

ZANI, C. L.; PASSOS, L. K. J.; SOUZA, C. P. & OLIVEIRA, A. B., 1989. Bioassay-guided phytochemical study of the latex from *Euphorbia splendens* (Euphorbiaceae). *Mem. Inst. Oswaldo Cruz, 84* (Suppl. I): Abs. 254.

ZANI, C. L.; MARSTON, A.; HAMBURGER, M. & HOSTETTMANN, K., 1991. Isolamento e identificação dos componentes moluscicidas do látex de *Euphorbia splendens.* In Jornada Científica da Fundação Oswaldo Cruz, RJ. Abs. E-103.