

## REVIEW

### PERSPECTIVES OF USING *EUPHORBIA SPLENDENS* AS A MOLLUSCICIDE IN SCHISTOSOMIASIS CONTROL PROGRAMS

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**Abstract.** A total of 1,426 species of plants had been tested in order to find out a vegetal-derived molluscicide against the vectors of *Schistosoma mansoni*. *Euphorbia splendens* is one of the most promising molluscicides. In this article we briefly present the main aspects that have to be considered to use this plant as a molluscicide, which include some agricultural aspects and operational methods for application. This can be done by the extraction of the active principles (milliaminas) present in the latex or in the raw juice obtained through total maceration of plants.

The culture of *E. splendens*, which spreads out by vegetative sprouts, showed an annual average growth of 30 cm. After 1.5 years the average productivity of crude latex with a DL90 of 12 ppm concentration, was 1 liter by 8m<sup>2</sup> by cultivated area. This amount is enough to treat a volume of water of 97,200 liters.

A comparative analysis between the cost of snail control through synthetic molluscicides was carried out based upon the data presented by Jobim (1979) for six controlling methods in several countries, and some cost aspects for *E. splendens*. This analysis was done considering a geographic parameter expressed by the index cubic meters of treated habitat per square kilometer, from which we can obtain an estimate dimension of the geographic area to be cultivated with *E. splendens* necessary to treat the same volume of water treated by synthetic molluscicides.

#### INTRODUCTION

Many plant species have been tested as molluscicides all over the world, as indicated by Kloos and McCullough (1987); Kuo (1987) and Jurberg *et al* (1989), the total figure being 1,426 studied species.

There have been few initiatives involving the use of plant molluscicides in western countries, which attained the experimental stage in the field. There have been no records so far of national control programs employing plants with molluscicidal properties (WHO, 1993).

*Euphorbia splendens* is native to Madagascar, but it is abundant in the Americas and in some countries in Africa as well. The utilization of this species in popular medicine in India was reported by Rao and Sussela (1984). Its cosmopolitan distribution is mainly related to its wide utilization as an ornamental plant.

In Brazil this plant is known by the common names of "coroa de cristo" (Christ's crown) and "colchão de noiva" (bride's mattress) (Joly, 1979).

The molluscicidal properties of *Euphorbia splendens* were initially studied through an objective per-

spective by Vasconcello and Schall (1986), who demonstrated the lethal action of the plant's latex on *Biomphalaria tenagophila*, *B. glabrata* and *B. straminea* under laboratory conditions.

The phytochemical fractioning of the latex revealed the existence of an active fraction, out of which a series of eight substances were isolated, known to be ingenols. One of these substances, milliamine L is active at 0.01 ppm (Zani *et al*, 1993), being 100 times as active as niclosamide, a chemical compound largely used in the control of schistosomiasis vector snails at present.

Out of the eight substances already identified, three were tested by Marston and Hecker (1983, 1984) and did not present any carcinogenic activity. Other toxicological tests, such as acute toxicity (Mattos *et al*, 1989), cutaneous and ocular irritability (Freitas *et al*, 1991), mutagenicity and toxicity (Schall *et al*, 1991) produced satisfactory results.

The lethal doses (LD<sub>50</sub>) for the following species of schistosomiasis mollusc hosts have been established: *Biomphalaria glabrata* (0.99 ppm); *Biomphalaria tenagophila* (1.0 ppm); *Biomphalaria straminea* (4.0 ppm); *Biomphalaria pfeifferi* (4.0 ppm) and *Bulinus* sp

(0.15 ppm) (Vasconcellos *et al*, 1993).

Laboratory tests have demonstrated the seasonal and geographic stability of the latex in Brazil (Schall *et al*, 1992). The control of vector snails in lotic and lentic natural habitats corresponded to 100% death rates of *B. glabrata* and *B. tenagophila* exposed to 12 ppm of crude latex (Mendes *et al*, 1992; Baptista *et al*, 1992).

The adequacy of the utilization of *Euphorbia splendens* in integrated schistosomiasis control programs will depend on studies of the operational models of cultivation and application in the communities involved.

#### CULTIVATION ASPECTS OF *EUPHORBIA SPLENDENS*

The viability of utilization of a plant with molluscicidal properties depends on several factors, such as the potency of the extract; the nature of the plant material to be applied in the snail habitats; the ease of cultivation and handling; the price; costs and benefits; the availability to be used when necessary; stocking possibilities. Although these factors are connected, we will approach them separately in order to understand the whole process of cultivation of the plant as suggested by Lugt (1987).

#### NATURE OF THE MATERIAL

The permanent availability of the active principles (milliamines) in the latex of *Euphorbia splendens* makes it a suitable product to be used as a molluscicide. This implies that the applications of the product can be carried out at any time of the year, depending only on planning the cultivation according to the seasonal transmission pattern of the disease.

#### PERCENTAGE OF THE COMPOUND

Schall *et al* (1992) showed that the LD 90 of *Euphorbia splendens* on snails is seasonally stable (1.07 ppm in summer; 1.02 ppm in autumn; 1.09 ppm in winter and 1.14 ppm in spring) and that only minor variations in the yield of crude latex prevail along the seasons, probably influenced by the hydric balance of

the plant. After lyophilization of the latex the action of the LD remains at the expected concentration, giving support to that supposition.

#### HANDLING - STOCKING - EXTRACTION

One of the greatest advantages of *Euphorbia splendens* is the small volume of plant material that is necessary to handle during plant multiplication and extraction stages as well as the small volume of the extracted product needed to stock. Handling the plant is simple but it requires some care with the numerous thorns along its stems, and with possible squirting of the crude latex into the eyes. Adoption of security measures, such as wearing appropriate gloves and goggles during handling, are advised.

The crude latex can be kept in stock for at least 18 months when refrigerated at 10°C (Schall *et al*, 1992). The product should not be stocked inside plastic containers, the ones made of glass being preferable. The final molluscicide may be obtained when the active plant substances are extracted either from the crude latex or the whole plant extract.

The crude latex is obtained from the incision along the stems of the plant 10 cm below the main apical meristemata. The latex is collected in drops in small glass containers after the plant is cut. The advantage of this process is the maintenance of the integrity of the plant, which is kept continuously productive.

The whole plant extract is the most profitable extraction product in terms of volume of extract active principle per cultivated area. To obtain the whole plant extract one must only cut the plant 10 cm from the soil (a reasonable size for regeneration of the plant). The stem is mashed in an electrical blender with some water being added. This extract is then filtered either through a plastic sieve or through a piece of cloth. When the whole plant extract is passed through a sieve its crude production per square meter is approximately 3.61 yielding an LD 90 at 250 ppm for 1.5 year-old plants. The whole plant extract may be either dripped or sprinkled on the snail habitats. The results of the field tests using the whole plant extract showed a great drop in the acitivity caused by the time of storage.

The plant may also be utilized *in natura*, ie, the whole plant may be introduced in schistosomiasis transmission sites, acting as a slow-release matrix of the product. The studies which are concerned with this are under way, through a series of field experiments.

## ESTABLISHMENT AND MAINTENANCE IN THE FIELD

### Production of shoots, spacing and cutting

The most common process of multiplication of this plant species is through asexual reproduction. In this process, small sections of the stem measuring 20 cm are left to dry under natural conditions for a period of 7 days at the most, before being planted. The planting should be done in well-drained terrain in 10 cm-deep holes spaced at 20 cm intervals. The plant growth was followed for 24 months in a cultivate area in the Fundação Oswaldo Cruz campus (Rio de Janeiro). The plant nursery was initiated with shoots brought from the locality of Ilha do Governador (Rio de Janeiro). The multiplication of the plants was done by asexual reproduction. An initial period of latency was observed, which included a slow growth of the plants until the first leaves began sprouting after one month. The highest growth rates were observed in December and January. The plant grows on the average 30 cm a year. The monthly growth rates are 2 cm, with the exception of the ones observed in December and January, which were up to 4.5 cm. With regard to the soil type the slowest growth rates occurred in argilaceous soil and the fastest one occurred in fertilized soil, in spite of no significant differences. At the end of 2 years the average plant height was 80 cm (Fig 1). However, as the plant attains a height of 50 cm at 1.5 year it may be employed in the extraction of the product.

Under favorable growing conditions in the field the maintenance of *Euphorbia splendens* is very simple, not requiring either frequent watering or the application

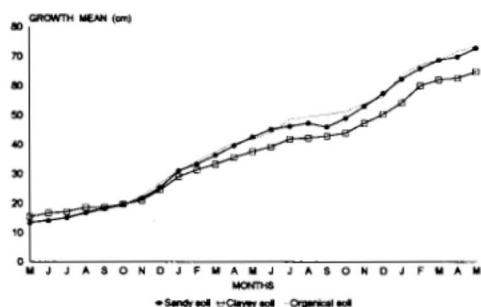


Fig 1—Mean of *Euphorbia splendens* total growth registered from 3 flower-bed cultivated during May/90 to May/92.

of fertilizers or pesticides. These characteristics render the employment of specialized manpower unnecessary.

Although *Euphorbia splendens* is adapted to survive against adverse conditions, complementary studies should concentrate on the viability of growing this plant species in water deficient areas, such as the xerophytic ones in North-east Brazil. The main laboratory and field observed characteristics of this plant are summarized in Table 1.

### APPLICATION OF THE PRODUCT

The control operations involving the use of molluscicide plants include, as in the case of synthetic chemical products, some planning phases. These should be able to deal with the questions of where, who, when and how to employ the plants, as suggested by WHO (1992).

The use of a plant molluscicide should comply with two general strategies: (1) focal and seasonal applications and (2) total area applications. As schistosomiasis transmission follows a spatio-temporal pattern in almost every endemic focus, the application of the molluscicide should be carried out on a focal and seasonal basis and be restricted to the main transmission sites. These sites are generally associated either with domestic and recreational activities or irrigated agriculture and/or fishing activities. The peri-domiciliary foci in semi-urbanized areas are also extremely important (Kloetzel, 1990).

The creation of operational models which comply with the cultural, religious and economical characteristics of the communities involved will determine how and by whom the product derived from *E. splendens* will be applied.

As an example of operational model with the participation of the community we mention the employment of the structure of agricultural schools in order to grow, multiply and handle plants. Another model could involve the municipal administration, through which an urbanization project would be carried out, involving the cultivation of the plant in parks and gardens.

### PRODUCTION - PRICE COSTS AND BENEFITS

As with any plant cultivation, the projected

Table 1

Main characteristics of *Euphorbia splendens*.

Active principles	Ingenols - (8) milliamines
Physical properties	Solubility in water
<b>Toxicity on:</b>	
snail (*) (LD 90)	0.15-4.0 ppm
snail eggs (*) (LD 90)	1.200 ppm
<i>Schistosoma mansoni</i> cercaria (LD 90)	under investigation
fish (LD 90)	4.0 ppm
mice (LD 90) - acute oral	non-toxic up to 2.0 mg/kg
Herbicidal activity	none
Genotoxicity (chromotest)	non-toxic up to 445 mg/l (lyophilized)
Cytotoxicity (CHO)	Ec50 toxic effect at 148 mg/l
Mutagenic activity	non-toxic up to 200 µg/ml non-mutagenic up to 12 ppm
<b>Stability affected by:</b>	
turbidity, sediment	none
Algae, plants, zooplankton	none
stocking	none
Ultraviolet (UV) light	under investigation
pH	under investigation
Cultivation	simple
Multiplication	asexual or seeds
Formulation	latex <i>in natura</i> or whole plant extract
<b>Field dosage:</b>	
crude latex	12 ppm
whole plant extract	250 ppm

(\*) *Biomphalaria glabrata*, *B. tenagophila*, *B. straminea*, *B. pfeifferi*, *Bulinus globosus*

production per cultivated area is necessary in order to determine the viability of the final costs of the product in accordance with the demand. Considering *E. splendens*, the volume basis to do the calculations was one liter of crude latex extracted from 8 m<sup>2</sup> of cultivated area. This quantity of crude latex is sufficient to treat 97,200 liters of water in 12 ppm concentration. These data were compared with the ones presented in an analysis of costs by Jobin (1979). As this analysis takes into account the costs of employment of synthetic molluscicides in control projects of several countries, we

made a projection as to the necessary cultivated area of *E. splendens* to treat the same volume of water as the one treated with synthetic molluscicides in each of those projects. As the projects analyzed by Jobin (1979) spanned a wide variety of endemic areas and habitats, this author analyzed the costs according to several geographic parameters. This was due to the impossibility of establishing a *per capita* basis in the human population since the costs of control of molluscs have no intrinsic relation to the human population. Thus, the analysis of costs and benefits for the control

Table 2

Estimates of *Euphorbia splendens* cultivation area in six control projects.

Project	Volume of habitat treated per area of the endemic zone (m <sup>3</sup> /km <sup>2</sup> )	Dimension of the geographic area cultivated with <i>Euphorbia splendens</i> (m <sup>2</sup> /km <sup>2</sup> )	Control area (km <sup>2</sup> )	Populational density (no. of persons/km <sup>2</sup> )
<b>Puerto Rico</b>				
Viesques	500	41	130	64
Patillas	730	60	122	140
Guayama	514	42	207	227
<b>Santa Lucia</b>				
Cul-de-sac	834	68	218	333
<b>Brazil</b>				
São Lourenço	1,000	82	80	54
Belo Horizonte	195	16	200	100
Taquarendi	6,000	493	2.5	600
<b>Egypt</b>	16,000	1,316	52	330
<b>Iran</b>	2,300	189	220	82
<b>Tanzania</b>	2,000	164	100	43

of molluscs relates the benefits only to prevention of disease transmission and environmental impacts. The costs are related to the distribution of snail habitats in each project, characterized by an index of "cubic meters of habitat per kilometer". Considering this same index we may estimate the dimension of the geographic area to be cultivated with *E. splendens* in order to treat the endemic areas in the six projects mentioned by Jobin (1979).

According to these projections we may prescribe an operational model for the control of vector molluscs through the introduction of the concept of "patchy cultivation". This model does not require the need of cultivation of the plant in a continuous and restricted area. It would rather be cultivated in a decentralized way, close to the transmission sites and in small patches.

Table 2 shows that the projection of results is very promising. As an example we could take the control project carried out in São Lourenço (Brazil), involving 4,320 inhabitants. The projected demand of total cultivated area of *E. splendens* is 6,560 m<sup>2</sup>, in order to produce 820 l of the latex crude. The adoption of the concept of patchy cultivation would demand the maintenance of a cultivated area as small as 68 m<sup>2</sup>/km<sup>2</sup>. This

would represent insignificant costs for a municipality of that size, if the relation between costs and number of persons benefiting from the control measures is taken into account.

The conclusions of studies which are under way (on toxicology and restricted field) could allow for the use of *E. splendens* under natural conditions in the near future. The effective action of the plan as a molluscicide in the control of schistosomiasis could then be evaluated in endemic areas.

The results, analyses and projections of estimates presented here support the evidence that *E. splendens* is a plant species with a great potential for use in the control of schistosomiasis vector snails.

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