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VEGETATIVE DEVELOPMENT AND SEED GERMINATION OF Euphorbia splendens VAR. hislopii, A BIOMOLLUSCICIDAL SPECIES

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ABSTRACT

A total of 1426 species have been tested for molluscicidal activity against the snail vectors of Schistosoma mansoni. Euphorbia splendens is one of the plants with greater molluscicidal potential. In the present paper we describe some of the propagating characteristics of E. splendens in terms of seed germinative viability under different conditions of temperature an luminosity and under field vegetative conditions. Two peaks of E. splendens fructification were recorded in August and December. The cardinal temperatures for germination of the December seed lot were: optimum temperature of 25°C in the light, with 80% germination; minimum temperature of 15°C in the dark, with 20% germination; maximum temperature of 35°C in the light, with 5% germination. The plant reaches a mean height of 75 cm after two years of cultivation in organically fertilized sandy soil and can be used for extraction of the active principles starting on the 13th month. These results suggest that the plant presents a high potential for cultivation in Brazil for use as a molluscicide of plant origin and can be grown throughout the area of distribution of the vector snails of schistosomiasis in the country.

KEY WORDS: Euphorbia splendens, seed germination, biomolluscicidal potential.

INTRODUCTION

The World Health Organization (WHO) recommends the simultaneous use of different methods for the control of schistosomiasis such as chemical treatment of patients, basic sanitation, health education and the use of molluscicides against the vector snails of Schistosoma mansoni (1).

Today there is a growing interest in the use of molluscicides of plant origin so that affected communities may participate in a self-sustaining system of control of vector snails using substances extracted from plants within a multiple program of schistosomiasis control.

The mollucicidal activity of the "coroa de cristo" (Christ's crown) (Euphorbia splendens var. hislopii) latex has been demonstrated (2), proving that this plant contains a natural substance with great potential for large-scale use in the control of the vector snails of schistosomiasis (3).

E. splendens var. hislopii, a plant of African origin (Madagascar) is the species with the highest mollucicidal activity among the 354 plants species tested in Brazil (4) and also among the approximately 1100 plants tested all over the world (5).

A series of ingenol compounds present as milliamines has been identifies in the latex of "coroa de cristo" (6,7). Thus far, biological assays have shown that these substances have no carcinogenic property (6,8). Other toxicologic tests have also been concluded, such as acute toxicity (9), skin and eye irritability (10), mutagenicity and cytotoxicity (11), with no detection of adverse effects.

This plant could be potentially used technologically in two different ways: first by synthesizing one of its 8 compounds identified thus far, preferably the fraction known as milliamine L witch is 100 times more active than Niclosamide, currently the only molluscicide commercially sold worldwide. However, the process of chemical synthesis of this group of substances (ingenols in the form of milliamines) has not yet been dominated by man. Thus, because if the barrier imposed by this difficulty, the other perspective for the short-term use of this plant is the application of its latex in natura.

E. splendens var. hislopii can be propagated by vegetative or sexual reproduction. Within this context, the objective of the present study was to provide information about the forms of propagation of this plant in Brazil since, in addition to the fact that plant is exogenous, the vector snails are distributed throughout a variety of habitats with different types of climatic conditions where the reproductive limitations of the plant are unknown. Thus, we determined the germinative viability of E. splendens var. hislopii seeds under different conditions of luminosity and temperature, as well as the cultivation conditions by vegetative propagation in the field.

MATERIAL AND METHODS

Vegetative reproduction in the field

To test the growth performance of Euphorbia splendens var. hislopii in the field, plant cuttings from Ilha do Governador (Rio de Janeiro) were transplanted to an experimental area on the campus of the Oswaldo Cruz Foundation.

The trial was carried out on three different soil types: sandy soil with a particle size of 2-0.5 mm, clay soil with a particle size of 0.5-0.02 mm, and sandy soil enriched with an organic compound of plant origin. Small stem stakes (20 cm) were left to dry in the open air for a period of 3 to 7 days and then planted to a depth of 10 cm in well drained soil, with 20 cm spacing between cuttings. Plant growth was monitored for 24 months from May 1990 to May 1992. Monthly mean growth rates an flowering and fructification periods were recorded.

Germination in the laboratory

The seeds of Euphorbia splendens var. hislopii used in the present study were collected on the campus of the Nuno Lisboa Faculty (Recreio dos Bandeirantes) in the city of Rio de Janeiro in the months of August and December 1993, stored in sealed glass containers protected from light and kept in the laboratory at 25°C.

For the germination tests, 20 seeds were placed on 10 Petri dishes measuring 50 mm in diameter and containing 2 layers of filter paper soaked in 6 ml distilled water. Five of the ten dishes were exposed to white light (15 W fluorescent bulbs, Philips, Brazil) and the remaining 5 were wrapped with 2 layers is aluminum foil and kept in the dark throughout the experiment (7 days). This procedure was followed for all isothermal incubations from 10 to 40°C, at 5°C intervals. The experiments were carried out in B.O.D. incubators (FANEM, Brazil).

All experiments were repeated at least twice and the figures illustrate one of the experiments, since similar patterns were obtained.

RESULTS

Vegetative establishment in the field

Observation of the process of vegetative growth of E. splendens var. hislopii indicated that the vertical growth of the plant only occurred after the appearance of the first leaves at the end of the first month.

The plant grew on average 30 cm per year, with a mean monthly growth rate of 2 cm. The highest growth rates were recorded during the months of December and January, reaching 4.5 cm/month.

With respect to the different types of soil, the lowest growth rates occurred in clay soil, with a mean growth of 65 cm after two years, and the highest occurred in the other two types of soil, i.e., organically fertilized and sandy soil, with a mean growth of 75 cm after two years (Figure 1).

E. splendens var. hislopii cultivated under the environmental conditions described above presented two flowering periods, on in the months of June, July and August (with a fructification peak in August) and the other in November, December an January (with a fructification peak in December).

Germination in the laboratory

Figure 2 shows that seeds of December lot presented the following characteristics of germinative ability in terms of the determination of cardinal temperatures: optimum temperature of 25°C in the light, with 80% germination; minimum temperature of 15°C in the dark, with 20% germination; maximum temperature of 35°C in the light, with 5% germination. Figura 2 also shows that there was no variation in germinative power for the light and dark conditions within each temperature. On this basis, the seeds of E. splendens var. hislopii may behave as photoblastic positive or indifferent to light depending on the incubation temperature.

In contrast to the December lot, the seed lot collected in August showed low germination rates with a maximum of 4% at 25°C in the dark, behaving as photoblastic negative seeds. Only the experiment performed as 30°C did we recorded a small germination rate (less than 2%) under conditions of luminosity (Figure 3).

Figure 1 - Mean total growth of *Euphorbia splendens* var. *hislopii* in 3 beds cultivated between May 1990 and May 1992.

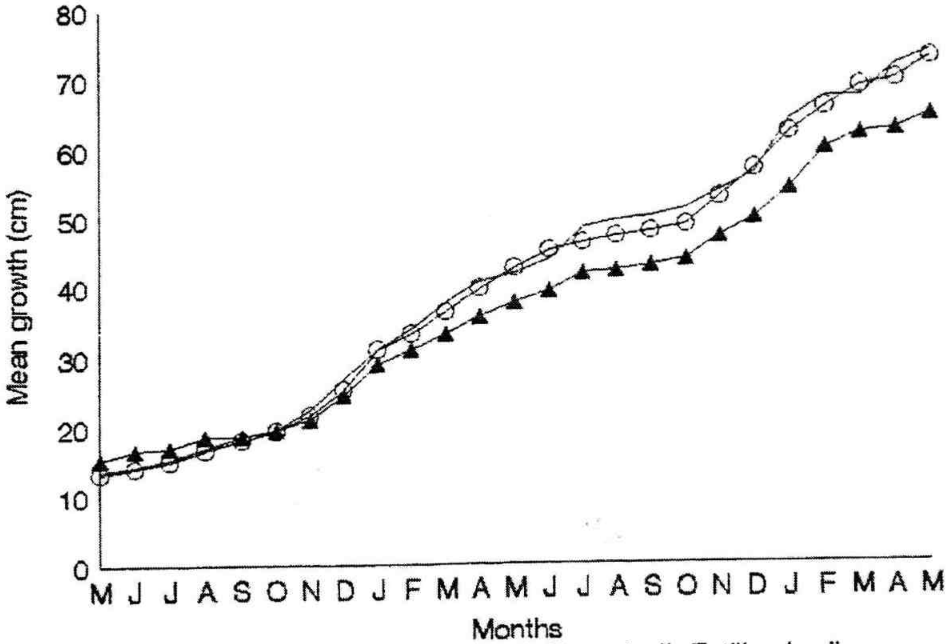


Figure 2- Germination of *Euphorbia splendens var. hislopii* at different temperatures (December, 1993 lot).

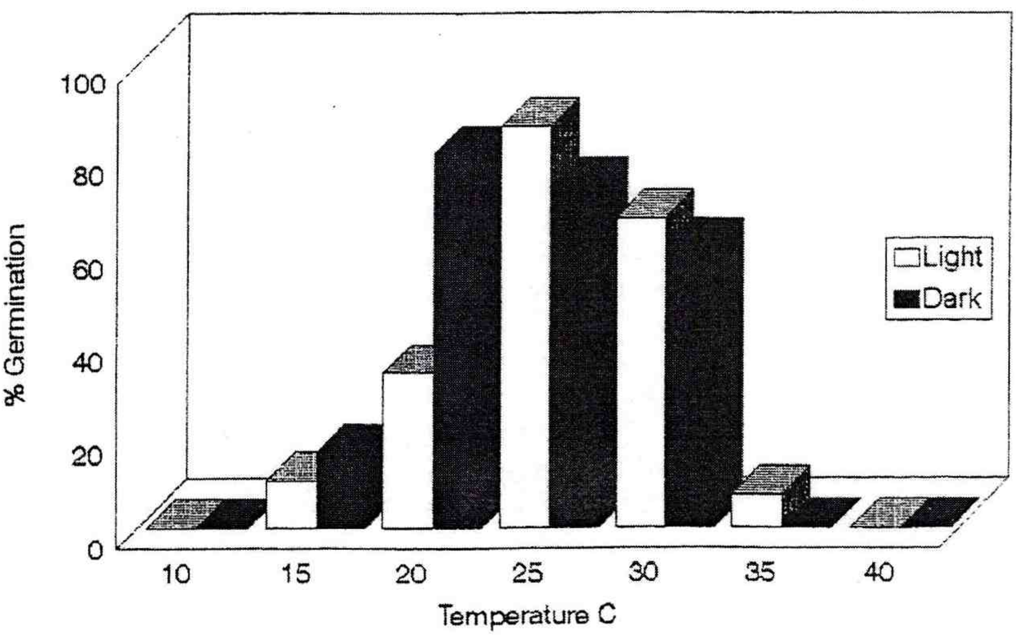
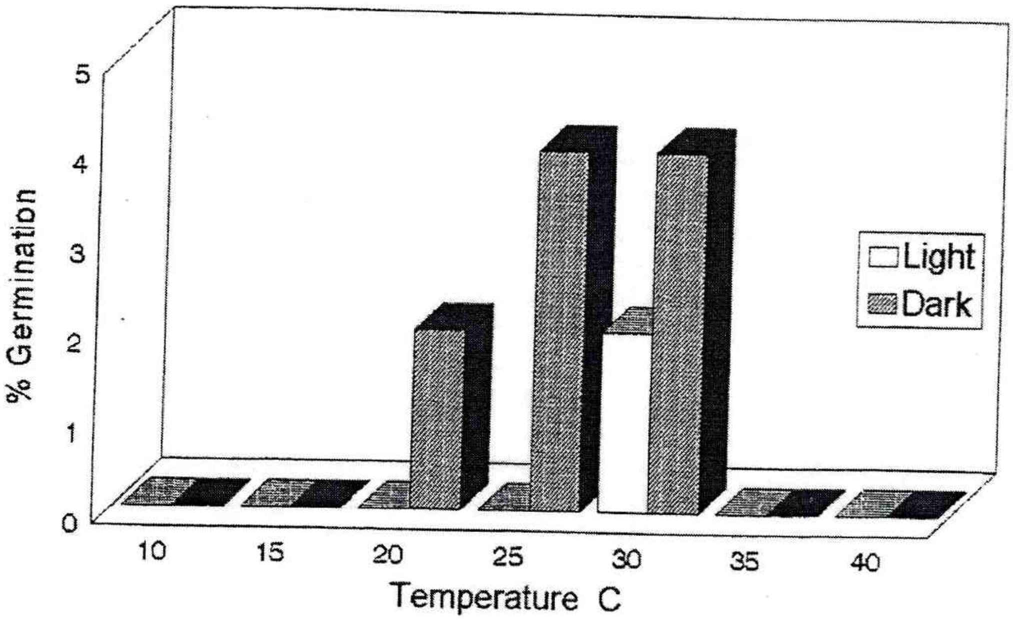


Figure 3 -Germination of *Euphorbia splendens var. hislopii at* different temperatures (August, 1993 lot).



DISCUSSION

According to Rauh (12), Euphorbiacea of the E. splendens var. hislopii group originate from the Inselberge region, a site characterized as a dry "island" in the middle of the central plateau of Madagascar, which is considered to be a relatively humid region with two annual rainfall periods. These climatic conditions at the site of origin must have shaped the genetic trait of twice yearly fructification observed here in Brazil, with fructification peaks in August and December.

Propagation of E. splendens var. hislopii by the vegetative process proved to be highly favorable for plant development, requiring no frequent watering or applications of fertilizers or pesticides, since the plant has few needs in terms of soil enrichment and its latex protects it against predators.

These characteristics would permit its use as a molluscicidal plant in water-deficient areas such as the areas where schistosomiasis is endemic in Northeastern Brazil, facilitating its cultivation by the communities affected by schistosomiasis.

On the basis of the present results, we may conclude that the seeds of E. splendens var. hislopii are highly adaptable to the different climates of Brazil since they presented good germinative ability at temperatures from 20 to 30°C, values corresponding to the mean ambient temperature for most of the places where schistosomiasis mansoni is endemic. Observations of the plant at its site of origin

(Inselberge) have indicated that, although the plant produces many seeds, few of them become viable (12) due to predation by coleopters. This behavior is not observed in Brazil, a fact that would facilitate plant cultivation from seeds. Later studies will be conducted to determine the germinative performance of seeds under field conditions.

The nature of the molluscicidal material extracted from the plant in the form of latex has a series of advantages for the immediate use of the plant, such as the permanent availability of the active principles (milliamines) in the latex throughout the year, the small volume of plant material to be manipulated during the propagation process, easy extraction of the product and storage for as long as 18 months at 10°C (13).

After the plant is definitively established in the field, the product can be applied in three different ways: i) as crude latex, ii) as plant extract, and iii) in natura, i.e., by punching the plant (field studies are currently in the final phase).

On the basis of our personal experience with latex collection for laboratory bioassays, we observed that a 40-cm plant is of the appropriate size for continued latex extraction. Thus, on the basis of our results, we may state that plant can become productive within approximately 13 months. Later studies will be conducted to determine the mean period of plant production when the plant is submitted to continuous latex extraction, since plant yield may be influenced by resistance to pathological agents. Indeed, as mentioned earlier, according to Rauh (12), the latex confers protection against predators but does not protect the plant against fungi or bacteria (14). On this basis, during the act of latex extraction by apical cutting, part of the plant is continuously exposed to invasion by pathological agents that might interfere with the mean productive life of the plant.

The creation of operational models that will respect the cultural, religious and economic characteristics of the population will determine how and who will apply the product derived from E. splendens var. hislopii.

As an example of an operational model with the participation of the community we may mention the use of the structure of agricultural schools for plant cultivation and extraction of the crude product. These schools could also serve as cutting for transplantation by the community itself to the margins of rivers and ponds that serve as focal point of transmission of the disease. Another model may involve the municipal administration through urbanization projects in which the plant could be grown in parks and gardens since it is normally used in different countries as an ornamental plant.

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