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EVALUATION OF POTENTIAL PLANT MOLLUSCICIDES FROM THE AZORES. LIST OF THE PLANT SPECIES TESTED ON *LYMNAEA TRUNCATULA*

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ABSTRACT: A list of twenty-one plants from the Azores, six endemic and fifteen non-endemic, assayed for their molluscicidal activity on *Lymnaea truncatula*, is presented. Aqueous and organic extracts (obtained by Soxhlet with dichlorometane, ethanol and toluene) were used. From all species tested, the endemic ones, *Euphorbia stygiana* and *Monostroma* sp., showed higher molluscicidal properties (LC 100 at 25 ppm in 8 hours and LC 100 at 20 ppm in 2 hours, respectively) than the others. Among the non-endemic species assayed, *Rumex obtusifolius* presented more efficacy (LC 100 at 1300 ppm in 8 hours for the boiled extract of seeds). Some considerations are made about the use of extracts from some plants at concentrations higher than those which are normally recommended. Such plants considered as real vegetal plagues in the Azores do not run the risk of extinction. Also, these plants showed more efficacy in aqueous extracts and so can contribute to a less expensive process of snail control.

KEY WORDS: Plant molluscicides, *Lymnaea truncatula*, Azores.

INTRODUCTION

After the first epidemiological surveys on fasciolosis in the Azores recognized the snail *Lymnaea truncatula* as the intermediate host of *Fasciola hepatica* responsible for fasciolosis in the Island of S. Miguel (MENDONÇA, 1987), the need to study the means of the snail control was acknowledged.

The impossibility of applying chemical synthetic molluscicides in certain types of habitats, owing to their effects on the other ecosystem elements, led us to try to find molluscicidal activity in some plants.

Added to that, economic reasons increase the need for such studies (MC CULLOUGH *et al.*, 1980). So, many plant species have been tested to evaluate their molluscicidal properties, some of them showing great efficacy. About 1100 species were assayed, some 600 only in the Chinese region. Unfortunately, a small number of them have been used regularly in the field of snail control (KLOOS & MC CULLOUGH, 1987).

In the present study we give a list of some plant species from the Azorean flora tested for molluscicidal activity on *Lymnaea truncatula*.

MATERIAL AND METHODS

Snails: The specimens of *Lymnaea truncatula* were collected from pastures, marshs and sloughs in S. Miguel and kept in the refrigerator at 4° C till the laboratory experiments.

Azorean plant species tested: Since 1988 twenty-one different species of plants were analyzed, six of them considered as endemic and the other ones (15) non-endemic. The endemic species (*Chaerophyllum azoricum, Euphorbia stygiana, Ilex perado, Lactuca watsoniana, Monostroma* sp., *Myriophyllum* sp.) were exclusively assayed in organic extracts obtained from dichlorometane, ethanol and toluene; from the non-endemic species, three (*Colocasia esculenta, Pittosporum undulatum, Tropaeolum majus*) were tested by organic extracts, six (*Azalea* sp., *Carpobrotus edulis, Hydrangea macrophylla, Ranunculus repens, Scrophularia scorodonia, Viscum album*) by aqueous extracts and six (*Cryptomeria japonica, Gunnera tinctoria, Hedychium gardnerianum, Mentha* sp., *Pteridium aquilinum, Rumex obtusifolius*) by both type of extracts.

Aqueous extracts: The aqueous extracts were obtained from maceration or boiling of root, stem, fresh or dried leaves and flowers, fruits or seeds. The material was ground by an electric mixer and prepared in a concentrated aqueous extract, by macerating directly in water or boiling during fifteen minutes. From each kind of these standard solutions, successive dilutions were later obtained (MENDONÇA, 1989).

Organic extracts: The whole plants were dried out, pulverized and extracted by Soxhelet during 24 hours by increasing polarity solvents (toluene, dichlorometane and ethanol). The solvents were then removed under reduced pressure (MEDEIROS, 1986).

Bioassays: The bioassays were realized at different concentrations of each type of extracts. Five ml of each dilution were poured in crystallization dishes (Ø 5 cm) where ten snails had previously been placed. The test group was made up of ten snails placed in pure water. The assays were carried out at 20° C and repeated twice. Observations were made hourly from two till ten hours of exposure, and at 24 hours. The snails that seemed to be dead were placed in pure water for two hours. Having verified death by pricking the foot and the mantle, the snails were measured and dissected to observe the effect of the product on larval forms of *Fasciola hepatica* in snails.

RESULTS

Regarding Table 1, concerning endemic plants, the ethanolic extract from *Euphorbia stygiana* can be con-

sidered one of the most promising. The other extracts need very high concentrations to produce almost the same effect. Nevertheless, and because *E. stygiana* is an endemic plant in danger of extinction, it would not be very advisable to use high concentrations which would threaten the survival of the species. The same reasons, or more serious ones, apply to *Monostroma sp.*

Among the non-endemic species reported in Table 2, the ethanolic extract of *Pittosporum undulatum* presented more efficacy (100% mortality after two hours of snail exposure at 3125 ppm).

From the six non-endemic plants analyzed by aqueous extracts (Table 3) two of them showed molluscicidal effect: *Carpobrotus edulis* and *Viscum album*. The first one, by using its pure latex, gave a high mortality (90%) in a lapse of time of two hours (100% at three hours); the second one produced, in the same time, 50% mortality and the lethal dose L100 was reached after four hours (MENDONÇA, 1993 b). In this case a maceration of leaves submitted to boiling was used (the concentration was 2000 ppm).

Table 4 shows the results of tests made in both extracts (organic and aqueous) on six non-endemic plants. Altogether, the organic extracts presented less efficacy than the other ones, mainly because the same effect has been produced by higher concentrations (MENDONÇA, 1993 a). Among all species, the seeds of *Rumex obtusifolius* in the boiled extract killed 100 % of snails at 1300 ppm in eight hours while the similar extract obtained from dried leaves of *Gunnera tinctoria* did so in half this time, but at a concentrations of 4000 ppm. However, the latter extract can eliminate 40 % of snails in two hours after exposure. These results had already been studied by MENDONÇA (1989).

DISCUSSION

In so far as we know, most of the species listed in this paper have been evaluated here for the first time for their molluscicidal properties. Only three of them, *Hedychium gardnerianum, Tropaeolum majus* and *Viscum album* had already been reported by other authors. Thus, *H. gardnerianum*, studied by SAI EH, SHABANA & TORKI (1982) as a potential molluscicide, was refered to by FARNSWORTH, HENDERSON & SOEJARTO (1987) as active at 100 ppm or less, in the list of Monocots tested for molluscicidal effect. This result may be due to the presence of a triterpene belonging to the monodesmosidic saponins group. This might be the reason for the difference bet-

PLANT SPECIES	EXTRACT	CONC (ppm)	% MORT (2h)	OTHER % MORT %	OTHER % MORT T(h)
Chaerophyllum azoricum	D	22400	30	100	6
	E	22400	0	100	10
	To	22400	0	30	24
Euphorbia stygiana	D	3200	0	100	7
	E	25	40	100	8
	To	1600	20	100	5
Ilex perado	E	40000	70	100	3
Lactuca watsoniana	D	20000	0	10	3
	E	20000	0	10	5
	To	20000	0	20	8
Monostroma sp.	To	20	100		
Myriophyllum sp.	E	10000	0	100	24

Table 1.— Evaluation of molluscicidal activity from Azorean endemic plants in organic extracts. D= dichlorometane; E= ethanol; To toluene; T(h)= time (hours).

PLANT SPECIES	EXTRACT	CONC (ppm)	% MORT (2h)	OTHER % MORT %	OTHER % MORT T(h)
Colocasia esculenta	E	50000	10	40	24
Pittosporum undulatum	D	50000	40	100	24
	E	3125	100		
Tropaeolum majus	E	25000	20	100	24

Table 2.— Evaluation of molluscicidal activity from Azorean non-endemic plants in organic extracts. D= dichlorometane; E= ethanol; T(h)= time (hours).

PLANT SPECIES	PARTS TESTED	CONC (ppm)	% MORT (2h)	OTHER % MORT	
				%	T(h)
Azalea sp.	Fl:ME	100000	0	10	24
	Fl:BE	100000	0	100	24
Carpobrotus edulis	L:lat		90	100	3
Hydrangen macrophylla	S,L,Fl:BE	70000	0	40	24
Ranunculus repens	L,Fl:ME	20000	0	0	24
	L,Fl:BE	20000	0	0	24
Scrophularia scorodonia	S,L,Fl:ME	100000	0	0	24
	S,L,Fl:BE	100000	0	0	24
Viscum album	L:ME	2000	0	80	24
	L:BE	2000	50	100	4
	L*:ME	2000	0	50	24
	L*:BE	2000	0	100	8

Table 3.— Evaluation of molluscicidal activity from Azorean non-endemic plants in aqueous extracts. Fl = flower; L = leaf; S = stem; lat = latex; * = fresh plant material; ME = macerated extract; BE = boiled extract; T(h) = time (hours).

PLANT SPECIES	EXTRACTS	PARTS TESTED	CONC (ppm)	% MORT (2h)	OTHER % MORT	
					%	T(h)
Cryptomeria japonica	W	L:ME	25000	0	0	24
	W	L:BE	15000	0	10	24
	W	F:ME	25000	0	0	24
	W	F:BE	15000	0	0	24
	E	F,L	100000	0	0	24
Gunnera tinctoria	W	L*:ME	30000	0	100	7
	W	L*:BE	30000	0	100	4
	W	L:ME	4000	0	100	6
	W	L:BE	4000	40	100	4
	D	wp	30000	40	100	5
	E	wp	30000	100		
Hedychium gardnerianum	W	L:ME	4800	0	20	24
	W	L:BE	4800	0	100	24
	D	wp	30000	0	30	24
	E	wp	30000	70	100	24
Mentha sp.	W	R,S,L:ME	10000	0	0	24
	W	R,S,L:BE	10000	0	30	24
	E	wp	25000	10	30	24
Pteridium aquilinum	W	L:ME	10000	0	30	24
	W	L:BE	10000	0	30	24
	E	wp	25000	30	70	24
Rumex obtusifolius	W	Se:ME	1300	0	30	24
	W	Se:BE	1300	0	100	8
	W	Fl:ME	3600	0	60	24
	W	Fl:BE	3600	0	100	4
	W	L*:ME	25000	0	100	8
	W	L*:BE	25000	0	100	10
	D	wp	10000	20	70	24
	E	wp	10000	20	70	24
	To	wp	10000	10	40	24

Table 4.— Evaluation of molluscicidal activity from Azorean non-endemic plants in organic and aqueous extracts. F = fruit; Fl = flower; L = leaf; R = root; S = stem; Se = seeds; wp = whole plant; * = fresh plant material; D = dichlorometane; E = ethanol; To = toluene; W = water; ME = macerated extract; BE = boiled extract; T(h) = time (hours).

ween the aqueous and organic extracts verified in *H. gardnerianum* (MENDONÇA, 1993 a).

In fact, DOMON & HOSTETTMANN (1984) observed, for instance, a difference in the saponins obtained by water and by methanolic extraction from *Phytolacca dodecandra*: in the first extract, more monodesmosidic saponins were obtained, whereas in the second the saponins were bidesmosidic. After the studies carried out by GAFNER, MSONTHI & HOSTETTMANN (1985), it seems that the monodesmosidic saponins are seen as strong molluscicides in relation to the bidesmosidic ones.

Concerning *Tropaeolum majus*, both KUO (1987) and FARNSWORTH, HENDERSON & SOEJARTO (1987) described it as inactive. Similarly, *Viscum album* is described by KUO (1987) as a plant with no molluscicidal effect on *Oncomelania* snails. The author considers all the plants which showed no activity at concentrations higher than 10000 ppm to be non-toxic. Not knowing what kind of extracts were used for KUO's conclusions concerning the inefficacy of *Viscum album*, we cannot compare our own results with his (MENDONÇA, 1993 b). KUO (1987) lists plants with high molluscicidal activity at 10000 ppm regarding only the mortality produced. Nevertheless, we have obtained LC 100 in much less time and at much lower concentrations than he described.

The toxic effect shown by endemic plants (*E. stygiana and Monostroma* sp.) led us to consider them to be possible candidates for molluscicides (MENDONÇA *et al.*, 1992). However, their future application as such in S. Miguel is problematic owing to their scarcity. Indeed, the endemic plants on this island, as on the others of the Archipelago of the Azores, have suffered the consequences of the introduction of other plant species.

Among the non-endemic species reported here, *G. tinctoria, H. gardnerianum, P. undulatum* and *R. obtusifolius* can be promising plant molluscicides. Although the lethal concentrations are not so low as desirable, these plants are widely spread over certain areas of the island, even considered as real plagues. For this reason the question of specimen devastation does not apply in this case. Added to that, *G. tinctoria, H. gardnerianum* and *R. obtusifolius* showed more activity in aqueous extracts, which will contribute to a less expensive control of the intermediate host of *Fasciola hepatica*, the amphibious snail *Lymnaea truncatula*.

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REFERENCES

DOMON (B.) & HOSTETTMANN (K.), 1984.— New saponins from *Phytolacca dodecandra* l'Herit. *Helvetica Chimica Acta*, 67: 1310-1315.

FARNSWORTH (N.R.), HENDERSON (T.O.) & SOEJARTO (D.D.), 1987.— Plant with potential molluscicidal activity. In: *Plant Molluscicides* (K.E. Mott edit.), UNDP/WORLD BANK/WHO, Special Programme for Research and Training in Tropical Diseases: 131-204.

GAFNER (F.), MSONTHI (J.D.) & HOSTETTMANN (K.), 1985.— Phytochemistry of African medicine plants. Part 3. Molluscicidal saponins from *Talinum tenuissimum* Dinter. *Helvetica Chimica Acta*, 68: 555-558.

KLOOS (H.) & MC CULLOUGH (F.S.), 1987.— Plants with recognized molluscicidal activity. In: *Plant Molluscicides* (K.E. Mott edit.), UNDP/WORLD BANK/WHO, Special Programme for Research and Training in Tropical Diseases: 45-108.

KUO (Y.H.), 1987.— Plant molluscicide studies in the People's Republic of China. In: *Plant Molluscicides* (K.E. Mott edit.), UNDP/WORLD BANK/WHO, Special Programme for Research and Training in Tropical Diseases: 289-297.

MC CULLOUGH (F.S.), GAYRAL (P.), DUNCAN (J.) & CHRISTIE (J.D.), 1980.— Molluscicides in schistosomiasis control. *Bulletin of the WHO*, 58: 681-689.

MEDEIROS (J.), 1986.— *A search for agrochemicals from Peruvian plants*. Ph. D. Thesis, Mississipi State University, 208 pp.

MENDONÇA (M.M. DE), 1987.— *A fasciolose por Fasciola hepatica L., 1758 em S. Miguel (Açores). Contribuição para o estudo das condições ecológicas da sua transmissibilidade*. Tese para candidatura a Investigador Auxiliar, IICT, Lisboa, 365 pp.

MENDONÇA (M.M. DE), 1989.— Actividade moluscicida de três espécies florísticas de S. Miguel. Ensaios preliminares. *Garcia de Orta, sér. Zoologia*, 16 (1-2): 91-95.

MENDONÇA (M.M. DE), 1993 a.— Comparison of the molluscicidal activity on *Lymnaea truncatula* of aqueous and organic extracts obtained from *Gunnera tinctoria, Hedychium gardnerianum* and *Rumex obtusifolius*. *Research and Reviews in Parasitology*, 53: 39-42.

MENDONÇA (M.M. DE), 1993 b.— Molluscicidal effect of *Viscum album* extracts on *Lymnaea truncatula* from the Azores. *Research and Reviews in Parasitology*, 53: 43-45.

MENDONÇA (M.M. DE), MEDEIROS (J.), BARATA (M.C.), LIMA (F.) & RAUTER (A.P.), 1992.— Actividade moluscicida de algumas plantas endémicas dos Açores. *Açoreana*, 7: 371-378.

SALEH (M.M.), SHABANA (M.) & TORKI (M.A.), 1982.— Phytochemical and molluscicidal studies on *Hedychium gardnerianum* and possible use in control of bilharzia. *Planta Medica*, 45: 138-139.