Effect of Niclosamide (Bayluscide WP 70®), *Anacardium occidentale* Hexane Extract and *Euphorbia splendens* Latex on Behavior of *Biomphalaria glabrata* (Say, 1818), under Laboratory Conditions

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The repellent effect of the molluscicides Niclosamide (Bayluscide WP 70®), Anacardium occidentale *and the latex of* Euphorbia splendens *on* Biomphalaria glabrata *was observed through the investigation of the occurrence of escape behavior among molluscs that were exposed to dosages lower than the LD 50.*

The total number of individuals out of water among the surviving snails in the control group provided a "Natural Escape Index". The comparison between this total and the total number of surviving snails in each group exposed to the different dosages of the molluscicides after 24 hr provided the "Molluscicide Escape Index" and the detection of a "Repellency Range" to these snails.

The escape indexes for Niclosamide, A. occidentale *and* E. splendens *were 10%, 6.22% and 6.44% respectively. Repellency occurred at the following concentration ranges: 0.01, 0.02 and 0.03 ppm Bayluscide, 0.1, 0.2 and 0.3 ppm* A. occidentale *and 0.05, 0.10, 0.15 and 0.20 ppm* E. splendens. *The Natural Escape Index obtained in the control group was zero.*

Key words: *Biomphalaria glabrata* - molluscicide - schistosomiasis - Bayluscide - *Euphorbia splendens* - *Anacardium occidentale*

The control of schistosomiasis transmission might be accomplished by the reduction of the populations of vector molluscs through the use of molluscicides. Despite being widely employed, molluscicides have their efficacy reduced by physicochemical conditions of the environment or by the molluscs' activities which reduce partially or completely the contact with these substances, thus favoring their survival. These activities were classified as protective behavior (Pieri & Jurberg 1981a, Jurberg 1987) and among them are the retraction of the mollusc into the shell and the movement away from the sites treated with molluscicides.

Since *Biomphalaria glabrata* is capable of responding to sub-lethal dosages of molluscicides, moving away when exposed to these substances (Nolan et al. 1953, Etges 1963, Etges & Gilbertson 1966, Souza & Paulini 1967, Pieri & Jurberg 1981b, Jurberg et al. 1985, 1988) and since one does not know this species' behavior when in contact with molluscicides of plant origin that might be highly promising, such as *Anacardium occidentale*, studied by Rey et al. (1987) and *Euphorbia splendens*, studied by Vasconcellos and Schall (1986) and Mendes et al. (1991), the present study aims (1) to study the repellency of these molluscicides as compared to Niclosamide, a widely employed product and the sole commercially available molluscicide (McCullough 1992); (2) to establish a technique in order to determine the Molluscicide Escape Index, the Natural Escape Index of the population and the Repellency Range of the molluscicides, based on the occurrence of the emigration behavior of *B. glabrata*.

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MATERIALS AND METHODS

One thousand four hundred and seventy melanic *B. glabrata* specimens were utilized, measuring 12 to 17 mm, originated from Touros, Rio Grande do Norte, Brazil, and maintained for several generations at the Departamento de Biologia, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.

The molluscicides were employed in decreasing concentrations from the LD 50, calculated in pre-experiments through a probit analysis procedure (Finney 1971). The minimum concentrations corresponded to those concentrations beyond which no significant differences were detected. The employed concentrations were (a) Niclosamide (Bayluscide WP 70®) - 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07 and 0.08 ppm (the last one corresponding to the LD 50); (b) *A. occidentale* - 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ppm (the last one corresponding to the LD 50); (c) *E. splendens* var. *hislopii* - 0.025, 0.030, 0.035, 0.040, 0.045, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 ppm (the last one corresponding to the LD 50).

Three groups of 10 animals (n=30) were exposed to each of the concentrations for 24 hr in 1000 ml glass vials (exposition phase). To every tested substance corresponded a control group of 60 molluscs kept in distilled water, which provided the Natural Escape Index of the population studied. At the end of this phase the molluscs found out of the aqueous solutions were counted, washed under water and maintained for another 24 hr in vials containing distilled water and food (recovery phase), after which the surviving and the dead snails were counted. The same procedure was employed on snails that did not exhibit the emigration behavior.

The percentage of molluscs observed out of the water after exposure to concentrations lower than the LD 50 of each substance and which survived the recovery phase was considered the "Molluscicide Escape Index". The percentage of snails in the control group that were observed out of the water after the treatment corresponding to the recovery phase of the experimental groups and considered alive constituted the Natural Escape Index.

The repellency range of the molluscicides was characterized by the identification of the concentrations at which the Molluscicide Escape Index was higher than the Natural Escape Index.

The significance of the differences between the escape of snails from the water at each molluscicide concentration and at the control group or among the three groups of 10 specimens at each concentration were established by the Fisher test (Siegel 1975). The chi-square test was employed for pairwise comparisons involving the different molluscicides.

During the experiment the room temperature was 25 ± 2 °C, the lighting period consisted of 12 hr light, 12 hr dark and the water pH was 6.5.

RESULTS

No specimen among the 180 snails in the control group exhibited the behavior of exiting the water.

The results of the tests comparing the occurrence of the behavior of exiting the water by *B. glabrata* in the presence of the three molluscicides at the various concentrations are listed in Tables I, II, III.

TABLE I

Results of the comparisons done through the Fisher test between the percentage of *Biomphalaria glabrata* snails exiting the water in the presence of Niclosamide (Bayluscide WP 70®) (n 30 in each concentration) and the percentage of snails exiting the water in the control group. The escape behavior was not observed in the control group (ns: not significant)

Concentration (ppm)	Escape (%)	Fisher test
0.005	6.7	ns
0.006	10	ns
0.007	10	ns
0.008	10	ns
0.009	10	ns
0.01	26.7	p<0.01
0.02	16.7	p<0.05
0.03	16.7	p<0.05
0.04	10	ns
0.05	3.3	ns
0.06	3.3	ns
0.07	3.3	ns
0.08	3.3	ns

Snails exited the water at all Niclosamide concentrations tested. However, the occurrence of this behavior was statistically significant only at three of these concentrations (0.01 ppm: p=0.0023; 0.02 ppm: p=0.026; 0.03 ppm: p=0.026) **p < 0.05**. The Molluscicide Escape Index was 10%.

Snails did not exit the water at all *E. splendens* concentrations. However, at four of them the occurrence of this behavior was statistically significant (0.05 ppm: p=0.012; 0.010 ppm: p=0.026; 0.15 ppm: p=0.026 and 0.20 ppm: p=0.026) **p < 0.05**. The Molluscicide Escape Index was 6.44%.

Snail exits from the water were observed at practically every concentration of *A. occidentale*. However, the difference between the number of animals which exited the solution and those which did not was statistically significant at the concentrations of 0.1 ppm: p=0.26; 0.2 ppm: p=0.026; 0.3 ppm: p=0.12 **p < 0.05**. The Molluscicide Escape Index was 6.22%.

A significant difference was detected between the total number of escape instances under Niclosamide and under *A. occidentale* (χ^2=4.07; p<0.05). No significant difference in the total number of escape instances was detected under the treatments with Niclosamide and *E. splendens* (χ^2=3.52; p>0.05).

TABLE II

Results of the comparisons done through the Fisher test between the percentage of *Biomphalaria glabrata* snails exiting the water in the presence of *Anacardium occidentale* (n=30 in each concentration) and the percentage of snails exiting the water in the control group. The escape behavior was not observed in the control group (ns: not significant)

Concentration (ppm)	Escape (%)	Fisher test
0.05	0	ns
0.06	3.3	ns
0.07	3.3	ns
0.08	3.3	ns
0.09	6.7	ns
0.1	16.7	p< 0.05
0.2	16.7	p<0.05
0.3	20	p<0.05
0.4	10	ns
0.5	6.7	ns
0.6	3.3	ns
0.7	3.3	ns
0.8	0	ns
0.9	0	ns
1.0	0	ns

TABLE III

Results of the comparisons done through the Fisher test between the percentage of *Biomphalaria glabrata* snails exiting the water in the presence of *Euphorbia splendens* (n:30 in each concentration) and the percentage of snails exiting the water in the control group. The escape behavior was not observed in the control group (ns: not significant)

Concentration (ppm)	Escape (%)	Fisher test
0.025	0	ns
0.030	3.3	ns
0.035	0	ns
0.040	3.3	ns
0.045	3.3	ns
0.05	20	p<0.05
0.10	16.7	p<0.05
0.15	16.7	p<0.05
0.20	16.7	p<0.05
0.25	10	ns
0.30	6.7	ns
0.35	3.3	ns
0.40	0	ns
0.45	0	ns
0.50	0	ns

DISCUSSION AND CONCLUSION

The present results provide an estimate of the number of snails that might escape the action of molluscicides, which may be used in planning snail control with molluscicides. Since these molluscs are prolific, hermaphroditic and able to carry out self-fertilization, and considering the present data and further knowledge on population ecology of planorbids, it is possible to estimate the time taken for them to repopulate their breeding sites, and consequently, the necessary periodicity of the application of the product.

The results also provide data about (1) the concentrations lower than the LD 50 at which the exiting behavior is exhibited, which favors survival (Molluscicide Escape Index); (2) the concentration at which the molluscicides are repellent (Repellency Range); (3) the number of snails that naturally exit the water, thus being able to avoid the action of molluscicides applied to the water (Natural Escape Index).

The behavior of exiting the water is a common phenomenon among planorbids and it may occur due to the following factors: (1) unfavorable conditions of the environment (overpopulation, lack of food or dissolved oxygen, high temperature or eutrophication of the water) (Paraense 1957). These factors may happen isolated or in association (Green et al. 1992); (2) in lamellate populations, due to genetic factors (Paraense 1957, Richards 1968) or induced by low temperatures on juvenile snails (Pieri & Thomas 1986, Dannemann & Pieri 1991); (3) through the repellent action of the molluscicides. Nolan et al. (1953) observed this kind of response in relation to the action of phenolic compounds in the laboratory, and Rey (1973) observed the same kind of response with sodium pentachlorophenate under field conditions.

Based on the repellency of molluscicides Jurberg et al. (1985) proposed the Exit Index, which consisted of the comparison between the percentage of snails that exited the water in the control group, without molluscicide, and the percentage of snails that exited the water at the various molluscicide concentrations after 24 hr. Subsequently, Jurberg et al. (1988) verified, through cinematographic recordings, that the snails that exited the water and remained longer away from the *Phytolacca dodecandra* concentrations had their survival favored.

The Molluscicide Escape Index adopted in the present study has some advantages over the Exit Index. Firstly, only molluscicide concentrations below the LD 50 are considered, as above it lethality increases and snails no longer exit the water. Secondly, it is easier to detect it because only those snails are counted, which remain alive out of the molluscicide solutions after 24 hr of test.

The three molluscicides present concentration ranges at which they are repellent, although only in the group exposed to Bayluscide did snails exit the water at all concentrations, indicating repellency to some specimens even at low concentrations.

The results obtained with distilled water showed that the population tested did not exhibit a Natural Escape Index under laboratory conditions.

A relevant fact is the high frequency of natural exits from the water, recorded in some populations (Etges & Gilbertson 1966, Pieri & Jurberg 1981b), which may mask the repellent effect of a molluscicide. In the present study, as new molluscicide substances are involved, it would be profitable to test them on laboratory-bred *B. glabrata* populations that exhibit this behavior at a low frequency. Nevertheless, it must be stressed that if the objective of a study is to determine the action mode of a known molluscicide on a population in the field, then a sample of this population should be utilized. The control group will reveal, through the Natural Escape Index, if this population is characterized by a high frequency of exits from the water. This will be an important factor in planning a control campaign, as it will include an estimate of that part of the population which will not be exposed to the molluscicide due to the above mentioned behavior.

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