

The Molluscicidal Activity of Niclosamide (Bayluscide WP70®) on *Melanoides tuberculata* (Thiaridae), a Snail Associated with Habitats of *Biomphalaria glabrata* (Planorbidae)

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The aim of this study was to determine the toxicity of niclosamide (Bayluscide®) on Melanoides tuberculata and Biomphalaria glabrata under laboratory conditions. The latter species is the intermediate host of Schistosoma mansoni (Sambon 1917). M. tuberculata was successfully used as competitor of B. glabrata in biological control programs in French West Indies. Both molluscicide and biological control using M. tuberculata have proved to be successful in reducing the population density of B. glabrata. The associated use of molluscicide in this area would be an effective measure if M. tuberculata were less susceptible to the molluscicide than B. glabrata. Three hundreds individuals each of B. glabrata and of M. tuberculata, collected in Sumidouro, State of Rio de Janeiro, were used in the experiment. The molluscs were exposed to 14 different concentrations of niclosamide as recommended by the World Health Organization. Probit analysis was used to determine the LC 50 and LC 90. The LC 50 and LC 90 values for B. glabrata were 0.077 mg/l and 0.175 mg/l, respectively and the LC 50 and LC 90 values for M. tuberculata were 0.082 mg/l and 0.221 mg/l respectively. As the lethal concentrations of niclosamide were approximately the same to both species, this could be a disadvantage when controlling B. glabrata with niclosamide in an area of M. tuberculata occurrence. It might therefore be preferable to utilize the latex extracted from the Euphorbia splendens, which presented a much higher efficiency for B. glabrata than to M. tuberculata.

Key words: molluscicide - niclosamide - *Melanoides tuberculata* - *Biomphalaria glabrata* - schistosomiasis

The use of molluscicides is one of the procedures recognized by the World Health Organization (WHO 1998) against schistosomiasis. In Sumidouro, State of Rio de Janeiro, Brazil, this method was planned as a control measure against *Biomphalaria glabrata* infected by *Schistosoma mansoni*. However, a reduction in population density of *B. glabrata* occurred following the appearance of the thiarid snail, *Melanoides tuberculata* (Müller 1774), before the molluscicide application. This observation suggests competition between these species. Similar results were obtained in French West Indies by Pointier and McCullough (1989), Pointier et al. (1989), Pointier and Guyard (1992), and Schlegel et al. (1997). Therefore, the two measures could be used concurrently to increase the efficiency of *B. glabrata* control. However, this assumption could only be confirmed if *M. tuberculata* were more resistant to the molluscicide than the target snail *B. glabrata*.

Either niclosamide (Bayluscide®) and latex from *Euphorbia splendens* var. *hislopii* [Sin. *Euphorbia milii* Des Moul. Var. *splendens* (Hook) Ursh & Leandri (Carter 1994)] could be used as molluscicides. The former is already commonly used in various continents (WHO 1984). The latter, a molluscicide of plant origin has been shown to be

effective against species of *Biomphalaria* (Vasconcellos & Schall 1986, Baptista et al. 1992, Mendes et al. 1992, 1997, Schall et al. 1998, 2001). The aim of the present study was to measure the susceptibility of *M. tuberculata* to niclosamide in order to evaluate the possibility of using both chemical and biological control to fight *B. glabrata* population.

MATERIALS AND METHODS

The niclosamide (Bayluscide WP70®, September 1993-stock) was provided by the Schistosomiasis Laboratory of Centro de Pesquisa René Rachou-Fiocruz, Belo Horizonte, State of Minas Gerais. The toxicity experiment was performed simultaneously with 8 to 20 mm diameters *B. glabrata* and 15 to 21 mm long *M. tuberculata*, both collected at Sumidouro. The test was made with *B. glabrata* for comparison with results obtained for *M. tuberculata*, in the same laboratory conditions. The treatments used the following 14 concentrations of niclosamide in 750 ml medium in 1,000 ml beakers: 0.02; 0.04; 0.06; 0.08; 0.10; 0.15; 0.20; 0.25; 0.30; 0.35; 0.40; 0.45; 0.50; 1 mg/l (WHO 1983). The medium used was dechlorinated water. Each treatment and the control (medium only) were duplicated. Ten molluscs were added to each beaker. Thus, 300 individuals of each species were used in the experiments.

The period of exposition to the molluscicide dilutions and control was 24 h. Thereafter, the snails in each replicate were placed in 750 ml dechlorinated water for another 24 h (recovery period). At the end of the first 24 h the number of molluscs withdrawn into their shells was recorded. If the snails remained inactive at the end of the

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recovery period (48 h), they were considered dead. Snails were deprived of food during the experiments.

The LC 50 and LC 90 values were determined by the Probit analysis (Finney 1971) using the Chi-square test to verify the fitting of the obtained mortality pattern with the estimated one.

RESULTS

Mortality was expressed on Probit probabilities and plotted against the log-transformed values of niclosamide concentrations. The regression line obtained from this data was used for LC50 and LC90 determination (Fig. 1).

The majority of *B. glabrata* snails, withdrawn into their shell on the first 24 h, were found to be dead at the end of the experiment. No *B. glabrata* survived at concentrations higher than 0.30 mg/l. The LC 50 and LC 90 values when *B. glabrata* were exposed to niclosamide were 0.077 and 0.175 mg/l, respectively. There was not significant differences between the observed and expected values ($\chi^2 = 8.35$; DF = 13; $p > 0.05$). The water leaving behavior were only observed in three individuals of *B. glabrata*, showing a low capacity of this species to escape to niclosamide action.

In relation to *M. tuberculata*, it was observed that some molluscs that did not retract in the first 24 h, died at the end of the experiment. This fact occurred at concentrations higher than 0.15 mg/l. The LC 50 obtained for *M.*

tuberculata was 0.082 mg/l while the LC 90 was 0.221 mg/l. All *M. tuberculata* individuals died when exposed to niclosamide concentrations higher than 0.25 mg/l, with the exception of one mollusc, which survived to the 0.4 mg/l concentration (Fig. 2). There was not significant difference between the observed and the expected values ($\chi^2 = 18.69$; DF = 13; $p > 0.05$).

DISCUSSION

These tests aimed to verify the fitting of niclosamide application on a specific situation: the natural substitution of *B. glabrata* by *M. tuberculata* in an area of schistosomiasis transmission. But it would be important that the applied molluscicide had low toxicity to *M. tuberculata*, because in the contrary, the population reduction of this species could diminish the competition pressure on *B. glabrata*. The occurrence of this phenomenon was already suggested by Mkoji et al. (1992) in relation to *M. tuberculata* and *B. pfeifferi* in Africa.

In this study, concentrations of niclosamide higher than 0.30 mg/l caused the death of almost all *M. tuberculata* and *B. glabrata* individuals on the first 24 h. The rapid mortality, probably due to an acute toxic effect, is desirable, as it reduces the possibility of escaping behavior by the molluscs (Sarquis et al. 1998). In addition, the LC 90 was reached at small concentrations of the molluscicide for both species. These results confirmed the

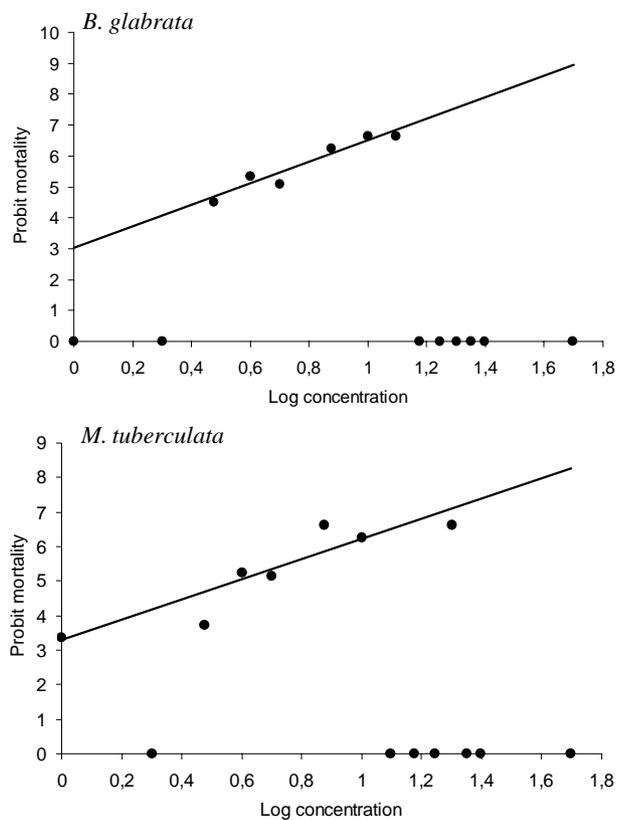


Fig. 1: probit analysis of mortality of *Biomphalaria glabrata* and *Melanoides tuberculata* plotted against the logarithms of concentrations (observed values). The regression line is used for LC 50 and LC 90 determination.

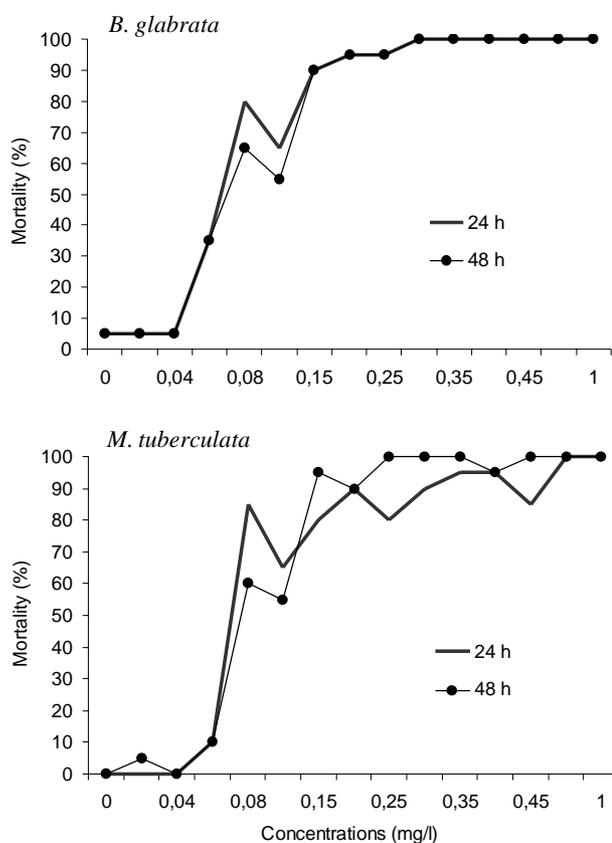


Fig. 2: mortality (%) of *Biomphalaria glabrata* and *Melanoides tuberculata* after being exposed to niclosamide (Bayluscide®) during 24 h, followed by 48 h recovery period in distilled water.

niclosamide efficacy against intermediate host molluscs of *S. mansoni* (Mc Cullough 1992, Souza 1995) and showed that this product affected *M. tuberculata* similarly to *B. glabrata*.

However the LC 50 and LC 90 values obtained on this experiment for *M. tuberculata* and *B. glabrata* were very close to each other, this contraindicates the use of niclosamide when both species were found in the same habitat. In this case, the application of the latex from *E. splendens* var. *hislopii* (crown of Christ), a molluscicide of plant origin, would be more indicated. In laboratory tests performed with *E. splendens*, it was observed that the required LC 90 for *M. tuberculata* was 13.8 times higher than the obtained for *B. glabrata* (Giovannelli et al. 2001). In this way, the competitive pressure of *M. tuberculata*, together with the molluscicide could present a synergistic effect on the reduction of *B. glabrata* population.

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