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## A Studies on Chemical Control of *S. mansoni* Intermediate Host

A.G. Arijo, T.A. Qureshi and Z.A. Pathan  
Sindh Agriculture University, Tandojam, Pakistan

**Abstract:** This study was conducted to screen different snail species to determine their susceptibility for different parasitic infections and also to determine  $LC_{50}$  and  $LC_{90}$  values. The screening of snail species infected with Schistosome parasite and mean number of cercariae shed by individual infected snail was  $184 \pm 12$ . The experiment on relationship of snail size and dose of Niclosamide showed that juvenile snails were more susceptible to chemical when compared with adult snails. A series of seven experiments indicated that concentration of 0.067 and 0.109 mg  $L^{-1}$  were required to kill 50 and 90% snails, respectively in an exposure period of 24 h and no death occurred in recovery period.

**Key words:** *Schistosoma mansoni*, *Biomphalaria glabrata*, Niclosamide, intermediate host

### INTRODUCTION

Control of snails through micro-parasites, trematodes, predators and competitors snails although have contributed towards the control of snail host through biological means. However, the disadvantages of biological control cannot be ignored. For example, Carlos (1991) reported that *Marisa cornuarietis* (a micro-parasite) is harmful to young seedlings and *Thiara granifera* (a competitor snail species) has been incriminated as a potential intermediate host of *Paragonimus westermani* (McCullough and Malik, 1984). Also *Biomphalaria stramina* (a competitor snail species) was reported as an important intermediate host of *S. mansoni* in some areas of Brazil and *B. tenagophilla* was found naturally infected with *S. mansoni* (Madsen, 1990).

Moreover, because of the difficulties encountered in the chemotherapy and sanitation approaches, plus the fact that a satisfactory vaccine is not yet available as a prophylactic against many parasitic diseases, chemical control of snail-borne diseases by molluscicide is still a useful proportion (Malek and Cheng, 1974).

Chemical control of the snail host is based on the assumption that the snail is the weakest link in the parasite life cycle and provides the quickest and most efficient means of interrupting transmission (Ritchie, 1973). The chemical control of snail host is done with the use of compounds commonly known as molluscicide.

Niclosamide has been in use as a molluscicide since 1960's and is still the molluscicide of choice, (Anonymous, 1993). Approved by WHO Niclosamide is highly toxic to all stages of the snail life-cycle (Webbe, 1987) killing 100% of *Biomphalaria glabrata*

adults at concentrations as low as 1.5 mg  $L^{-1}$  after a 2 h exposure and *B. glabrata* egg masses at concentrations below 1 mg  $L^{-1}$  after 24 h. It is not toxic to man and has limited bio-cidal effects (Jordan and Webbe, 1982).

In Pakistan, there are no published reports on the chemical control of snail intermediate host. Present research was conducted to determine the  $LD_{50}$  and  $LD_{90}$  of Niclosamide against snail population in Laboratory.

### MATERIALS AND METHODS

**Collection and maintenance of snails:** The protocol for the culture of snail in laboratory was that of Webbe and Sturrock (1964). The snails were collected from fresh water pounds and water courses of Tandojam, Sindh-Pakistan and its surrounding areas. The snail samples (three available species) were collected in jars containing water and were maintained in glass tanks (12 L capacity) in laboratory. The tanks were linked with controlled aeration system in order to oxygenate. Each tank was however, filled with 10 L of water only in order to stop snails crawling out.

Randomly collected snails were kept in water filled glass tank at room temperature and snails were fed *ad libitum* every day with commercial fish flakes. Tanks were cleaned intermittently when deemed to be necessary on visual inspection and any dead snails (if any), was removed from the tanks to prevent the degradation of water quality because dead snails decompose and foul the water rapidly at normal temperature (Webbe and Sturrock, 1964).

**Screening for cercarial out-put:** The protocol used here was same as established by Webbe and Sturrock (1964). Approximately 5 mL of water was filled in small glass

tubes. In each tube, only one snail was gently placed and tubes were kept under lamplight at least for 60 min. The light stimulated the eruption of cercariae from the body of the snail. The tubers were hold in front of light, where cercariae may be seen easily. The presence of cercariae was also conformed under dissecting microscope. Adding a drop of iodine in water stopped the movement of cercariae. The iodine also stains the cercariae and they may be seen easily.

**Test beakers:** Five plastic beakers capacitating 1 L were filled with water unto edge of beakers. The beakers were covered with lid in order to avoid the crawling of the snails out of the beakers. Four beakers were used for different concentrations of Niclosamide and one beaker was used for control.

**Test chemical:** Niclosamide 70% powder containing 70% Niclosamide as the active ingredient was used for this work. A stock solution was prepared by adding 0.40 mg to 100 mL water. From this stock solution further solutions of different concentrations were prepared. Fresh chemical was prepared for each exposure period. The final concentrations to which the snails were exposed were as follows 0.12, 0.09, 0.06 and 0.03 mg L<sup>-1</sup>.

**Exposure to chemical:** Shell diameter of snails was measured using graph paper with 1 mm divisions and the snails were then randomly distributed with an equal number being exposed to the different concentrations of Niclosamide.

Snails were exposed to water containing chemical for 24 h which is called exposure period. After 24 h exposure period, snails were removed, rinsed with chemical-free water three times and were again placed into respective beakers with fresh water and food for a further 24 h period which is called recovery period. During the first exposure to chemical no food was provided.

Mortality was recorded at 8, 24 and 48 h after exposure to the chemical. Control snails were treated the same way, except that they were not exposed to chemical.

## RESULTS AND DISCUSSION

Table 1 show that 7% snails were infected and the mean number of cercariae shed by infected snails was 1850±136. Table 2 reveals that, juvenile snails were more susceptible to the effects of Niclosamide when compared with large size snails, as a higher percentage of snails was found susceptible to the effects of Niclosamide at all concentrations. Table 3 is pool of seven experiments and shows that 0.03 mg L<sup>-1</sup> concentration gave 20%

Table 1: Percentage of infected snails and mean number of cercariae shed per infected snail

Collection	No. of screened	No. infected (%)	Cercariae/snail
1	50	5(10)	1850±136
2	80	3(2.4)	1833±144
Mean±SD	65±21.2	6.2±4.2	1841±120

Table 2: Mortality rate of two different size of snails

Concentrations (mg L <sup>-1</sup> )	Snails	Size of shell diameter (%)	
		5 mm	10 mm
0.03	8	3 (37.5)	2 (25)
0.06	8	7 (87.5)	5 (62.5)
0.09	8	8 (100)	7 (87.5)
0.12	8	8 (100)	8 (100)

mortality rate and this percentage increased to 42.8, 74.2 and 100% at 0.06, 0.09 and 0.12 mg L<sup>-1</sup> concentrations, respectively.

The main purpose of this study was to screen snail host species to understand the susceptibility potential of snails, which act as intermediate host for different species of trematodes. During this study, only three snail species were found from waters of Tandojam and its adjoining areas. The screening of three snail species showed that only *Biomphalaria* spp. snails were susceptible to parasitic infection and shed fasciola and schistosome cercariae. The 6.2% snails were found shedding schistosome cercariae, whereas the mean number of cercariae shed by individual infected snail was 1841±12 (Table 1). The shedding of schistosome cercariae by snail host during present study are confirmation of the reports of Abdulsalam and Sarwar (1952) and Anwer and Gill (1990). They reported schistosome infections from various parts of Punjab province of Pakistan. Based on these findings, it can be concluded that the potential host for schistosomiasis is present in Sindh (Pakistan) and there may be opportunities for the spread of this snail-borne disease.

The second object of this study was to test and determine LC<sub>50</sub> and LC<sub>90</sub>, a dose of Niclosamide that kills 50 and 90% of host population. The uniform sample size is thought to be prerequisite to obtain the consistent results. During this study, only same size of snails was used for which a basic experiment was conducted to see the relationship of snail size with the concentrations of Niclosamide. This study showed that juvenile snails are more affected when compared with adult snails (Table 2). These findings are in agreement with those of Lemma (1970), Ritchie *et al.* (1963), Webbe and Sturrock (1964), Hopf and Muller (1962), Prequito and Fraga de Azezedo (1968), El-Gindy *et al.* (1980) and Malek (1971). They studied the relationship between concentration of

Table 3: Percentage mortality at different concentrations and time periods

Concentration (mg L <sup>-1</sup> )	No. of snail	No. (%) dead			Total dead	Dead (%)	Corrected mortality
		After exposure period		After recovery period			
		8 h	24 h	48 h			
0.03	70	5 (7.1)	14 (20)	14 (20)	13	20.0	20.0
0.06	70	24 (32.8)	30 (42.8)	30 (42.8)	30	42.8	42.8
0.09	70	37 (52.8)	52 (74.2)	52 (74.2)	52	74.2	74.2
0.12	70	51 (72.8)	70 (100)	70 (100)	70	100.0	100.0
Control	70	00	00	00	00	00.0	00.0

% Dead - Control % dead, LC<sub>50</sub> = 0.067 mg/100, 100 - Control % dead, LC<sub>90</sub> = 0.109 mg L<sup>-1</sup>

Niclosamide and the size of the snail host and found that the susceptibility of snails of different sizes for *Biomphalaria* spp. snail to Niclosamide declined with increasing size of snails.

In order to determine the dose of Niclosamide, a series of seven experiments was conducted. The data from all these experiments were pooled (Table 3), which indicated that mortality rate increased with increase in the concentrations of the chemical. Analysis of mortality data revealed that different mortality rates were apparent between 0.03, 0.06, 0.09 and 0.12 mg L<sup>-1</sup> concentrations. A significant difference (p<0.05) in the mortality rate at different concentrations was calculated by Chi-square test. The data were also subjected to Probit Analysis by using a graph paper. The LC<sub>50</sub> and LC<sub>90</sub> values were calculated as 0.067 and 0.109 mg L<sup>-1</sup>, respectively. Tchounwou *et al.* (1991) calculated 0.13 mg L<sup>-1</sup> as a concentration required to kill 90% population, which although is close but not in complete agreement with the present study. This difference may be attributed to the different environmental habitats where from the snails were collected and also due to difference under which the snails were cultured in laboratory.

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