Impact of the freshwater operculate snail *Melanoides tuberculata* on Survival and Egg Production of the planorbid snail *Bulinus truncates* and on its transmission of *Schistosoma haematobium* Infection

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ABSTRACT

The biological control of vector snails is an essential component in the fight against schistosomiasis. *Melanoides tuberculata* (Thiaridae: Prosobranchia) was evaluated experimentally for its impact on the survival, egg production, of *Bulinus truncatus*, and its infection rate and cercarial production of *Schistosoma haematobium. M. tuberculata* has been proved to have a considerably negative effect on the survival and egg production of *B. truncatus*. The results show also that this suppressive effect becomes much higher as the relative density of *Melanoides* to *Bulinus* snails was increased. Besides, this was associated with considerable decline of *Bulinus* egg production.

The rate of infection of *Bulinus* snails with *S. haematobium* was found to be significantly reduced by the presence of *Melanoides* during miracidial exposure of snails. The same was also observed if *Melanoides* was maintained with miracidially exposed *Bulinus* throughout its life span. The rate of infection of *Bulinus* with *S. haematobium* and the cercarial production was much reduced in comparison with the control group and therefore the total periodic cercarial production was reduced by 78.2%. The effects of *Melanoides* are additive and thus could lead to considerable depression in schistosomiasis transmission. This supports the utilization of *Melanoides* in the biocontrol of schistosomiasis in Egypt.

Keywords: *Melanoides tuberculata, Bulinus truncatus,* infection, *Schistosoma haematobium,*

INTRODUCTION

The control of vector snails is an essential component in the fight against schistosomiasis. One of the methods for this control is by utilizing biological agents which can negatively affect the survival and infection of the vector snails. In this respect, many organisms were reported to be useful such as bacteria (El Emam *et al*., 1996; El Bardicy *et al*., 2005), predators such as the leech *Helobdella punctatolineata* (Yousif *et al*., 2006 a & b), larvae of the

sciomyzid fly *Sepedon scapularis* (Maharaj *et al*., 1992), and the cay fish *Procambrus clarkii* (Ibrahim *et al*., 1995 and Sleem & El-Hommosany 2008). Several attempts to control schistosomes included also the antagonistic effect of rediae of certain echinostomes such as *Echinostoma liei* on sporocysts of schistosomes (Yousif *et al*., 1991).

The present snail *Melanoides tuberculata* Muller (Thiaridae: Prosobranchia) was claimed to be a competitive snail of the schistosome vector snail *Biomphalaria* (Pointier *et al*., 1991; Pointier & Guyard, 1992; Pointier, 1993; Pointier & Giboda, 1999). In Brazil, studies carried out in the Caribbean region showed reduction and even disappearance of *Biomphalaria glabrata* and *B. straminea* populations in breeding places, where *M. tuberculata* was introduced (Guimaraes *et al*., 2001). *M. tuberculata* was reported also to reduce considerably the infection rate of *B. alexandrina* with *S. mansoni* under laboratory conditions (Yousif *et al*., 1998 a & b) and similar effect was also obtained under stimulated natural conditions (Yousif *et al*., 1999 a&b). This information initiated the present authors to evaluate the potential effect of *M. tuberculata* as a biocontrol agent against *Bulinus truncatus*, the snail vector of *Schistosoma haematobium* in Egypt. This was carried out by studying, under laboratory conditions, the impact of this snail may have on the survival and egg production of *Bulinus* snail as well as on its infection with *Schistosoma haematobium* transmission in Egypt.

MATERIAL AND METHODS

Melanoides tuberculata snails were collected from the River Nile and its branches near Cairo while *Bulinus truncatus* snails were obtained from the colonies of the Schistosome Biological Supply Centre (SBSC) at Theodor Bilharz Research Institute. The snails were maintained under laboratory conditions in dechlorinated water tap (26°C± 1°C) and provided with blue green algae and boiled lettuce as food.

The potential competitive effect of *M. tuberculata* on *B. truncatus*:

The potential competitive effect of *M. tuberculata* on *B. truncatus* was studied under standardized laboratory conditions, by maintaining both snail spp. together at different ratios for 12 weeks in suitable aquaria. Throughout the experiment, dead snails were replaced by living ones of the same size to maintain the starting relative densities constant. This experiment was carried out using 3 relative densities of *M. tuberculata* and *B. truncatus* namely 1:1, 2:1 and 3:1respectively. Dead snails of both spp. were counted daily and the survival rate was calculated weekly. The eggs of *B. truncatus* were collected weekly and counted.

Effect of *Melanoides tuberculata* on infection of *Bulinus truncatus* with *Schistosoma haematobium*

The potential effect of *Melanoides* on infection of *Bulinus* snails was studied using two methods: firstly by exposing *Bulinus* snails to *S. haematobium*

miracidia in presence of *Melanoides* and secondly by exposing *Bulinus* snails to *S. haematobium* miracidia and then after maintaining the miracidially exposed snails with *Melanoides* throughout their life. Starting from day 39 post miracidial exposure, surviving *Bulinus* snails were examined individually for cercarial shedding twice weekly by exposing them for two hrs to a 100 watt-filament light at height of 40cm. The obtained cercarial suspension was poured in graduated Petri dish, a few drops of Bouin's fluid were added and all cercariae were counted using a dissecting microscope.

Maintaining *Melanoides* with *Bulinus* snails during miracidial exposure only:

Sixty *Bulinus* snails (3-5 mm shell height) were divided into two groups, 30 snails each. One group was distributed in 3 Petri dishes (10 snails each). Each Petri dish was provided with 50 ml dechlorinated water and 10 adult *Melanoides* snails (10-15 mm shell height). The other group of *Bulinus* was similarly distributed in Petri dishes but without *Melanoides* snails and used as control. All *Bulinus* snails were exposed to *S. haematobium* miracidia in mass (150- 200 miracidia/ dish) and were maintained overnight (24 hrs). *Melanoides* snails were then removed from the Petri dishes. *Bulinus* snails of each group were placed in separate aquaria and maintained in the laboratory under standard conditions.

Exposing *Bulinus* snails to *Schistosoma* miracidia and then after maintaining the exposed snails with *Melanoides* throughout life.

Sixty *Bulinus* snails were exposed to *S. haematobium* in mass as mentioned above. The snails were divided into two groups, 30 snails each. One group was maintained with *Melanoides* snails (10-15mm shell height) starting from the next day of miracidial exposure. The snails were distributed into 3 aquaria, 10 snails/ aquarium and 10 *Melanoides* snails were placed in each aquarium. The aquaria used for maintaining the snails were (8x16x22cm) each containing 2 liters of dechlorinated aerated tap water which was changed weekly. The ratio of *Melanoides* to *Bulinus* was maintained constant throughout the duration of the experiment, either by replacing dead *Melanoides* by living ones or removing a number of *Melanoides* snails as some *Bulinus* snails died. The other miracidially exposed group of *Bulinus* snails was maintained similarly but without *Melanoides* snails for comparison. All aquaria were maintained under laboratory conditions.

RESULTS

The potential competitive effect of *Melanoides* on *Bulinus*

The results (Fig. 1) show that *Melanoides* caused considerable increase in the mortality of *Bulinus* snails, and this effect depends on the relative density of snails. Thus, as the relative density of *Melanoides* to *Bulinus* increased from 1:1 to 2:1 and 3:1 respectively, the mortality of *Bulinus* was highly increased. Meanwhile, during the whole experimental period (12 weeks) no considerable

change in mortality of *Melanoides* and a few deaths of *Bulinus* in the control group were observed.

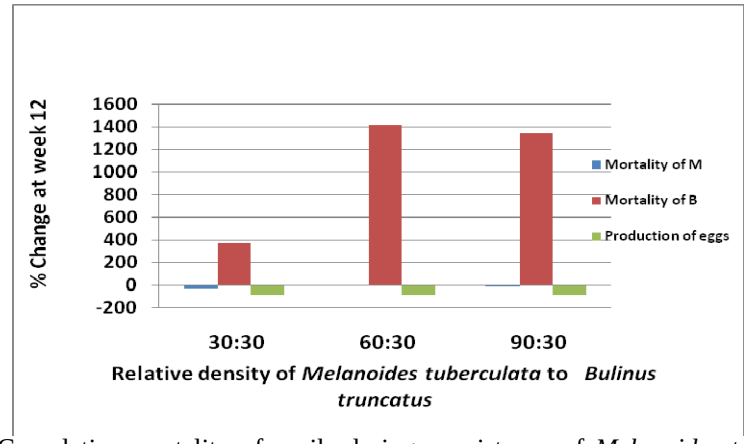

Fig (1): Cumulative mortality of snails during coexistence of *Melanoides tuberculata* (M) with *Bulinus truncatus* (B) at various relative densities.

Survival, infection rate and cercarial incubation period of *Bulinus* snails

The results (Tables 1&2 and Figs 2 &3) show that there is insignificant difference in the survival of exposed snails at the time of the first cercarial shedding, between groups exposed to *Schistosoma* miracidia in presence and absence of *M.tuberculata.* Meanwhile, the rate of infection of *Bulinus* snails was significantly reduced by the presence of *M. tuberculata* during miracidial exposure in comparison with the control group.

Table (1): Effect of *Melanoides tuberculata* on survival at first cercarial shedding, infection rate and cercarial incubation period of *Bulinus truncatus*.

Treatment	Survived *Bulinus* at First cercarial shedding			Infection of surviving snails				Cercarial incubation period (day)
	*Total Number	Mean No. of snails± SD	% Survival	Total Number of survived snails	Mean No. of snails± SD	% change	Infection %	Mean ± SD
Bulinus snails miracidially exposed in absence of *Melanoides* (control)	28	9.3 ± 0.6	93.3	25	9.3 ± 0.6		89.3	43. ± 3.4
Bulinus snails miracidially exposed in presence of *Melanoides*	25	8.3 ± 1.2	83.3	18	6± 1	-19.4	72	42.6 ± 2.1
Bulinus miracidially exposed and thenafter maintained with *Melanoides* throughout their life	22	7.3 ± 1.5	73.3	8	2.7 ± 0.6	36.9	36.4	47.1 ± 4.4

*Number of snails exposed (3 replicates x 10) = 30

Table (2): Total periodic cercarial production / snail during the whole life of infected snails

		Number of shedding snails	Mean total periodic number of cercariae/ snail ± SD	Total periodic number of cercariae
Bulinus snail miracidially exposed in presence of *Melanoides*	Control	25	770± 442.2	21560
	Experimental	18	452± 266.5	8136
	% change	-28	-41.3	-62
Bulinus snail miracidially exposed and thenafter maintained with *Melanoides* throughout their life	Control	15	1446.5± 843	21697.5
	Experimental	8	588.9± 529.9	4711.2
	% change	-46.7	-59.3	-78.2

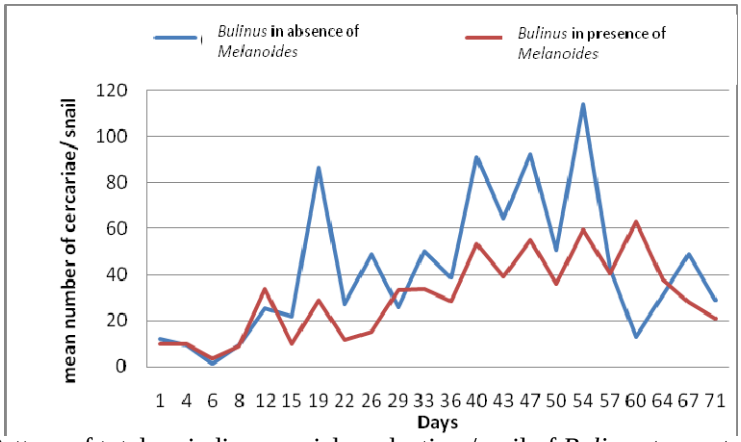

Fig (2): Pattern of total periodic cercarial production /snail of *Bulinus truncatus* exposed to *S. haematobium* in presence or absence of *Melanoides tuberculata.*

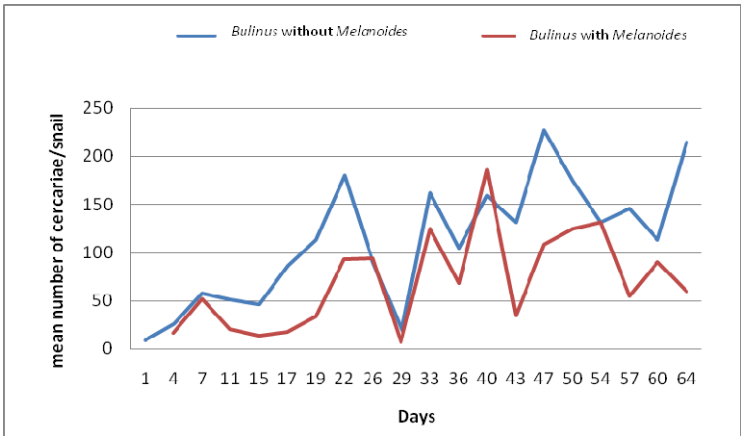

Fig (3): Pattern of total periodic cercarial production /snail of *Bulinus truncatus* infected with *S. haematobium* and maintained with or without *Melanoides* snails.

In the case of miracidially exposed *Bulinus* snails which were maintained continuously with *Melanoides* throughout snails life, the infection rate was also greatly reduced (59.2%). The mean cercarial incubation period showed no significant difference in both of experimental and control groups.

DISCUSSION

The present results indicate that *M. tuberculata* has a negative impact on the survival and egg production of *B. truncatus* and on its infection with *S. haematobium* under laboratory conditions. These findings are in agreement with what was claimed by Gomez *et al*. (1990), in laboratory experiments, that thiarid snails act as competitor of *Biomphalaria* spp.and exert a negative effect on them. Similarly, El Sayed (1996) reported that the mean life span of *B. alexandrina* was reduced by the presence of *Melanoides* and its fecundity was highly decreased. Under natural conditions, Pointier & Mc Cullough (1989), Pointier *et al*. (1991), Pointier & Guyard (1992), Pointier (1993), Pointier & Giboda (1999) and Pointier & Jaurdane (2000) reported that thiarid snails have proven their competitive efficiency in limited populations of *Biomphalaria* snails in Guadeloupe. Pointier (2001) and Guimaraes *et al*. (2001) showed reduction of schistosome intermediate hosts and even disappearance of their populations in breeding spaces. Rocha- Miranda and Martins- Silvia (2006) concluded that *M. tuberculata* may prey on Planobid snails of Parana River Basin in Brazil.

The present results show clear impact of *M. tuberculata* on infection of *B. truncatus* with *S. haematobium.* Thus the rate of infection of *Bulinus* with *S. haematobium* was significantly reduced both when: *Bulinus* snails were miracidially exposed in presence of *Melanoides* and when the former snails were miracidially exposed and then maintained with *Melanoides* throughout infection. Meanwhile, the mean total number of cercariae / shedding snail during the entire

life- span in the above two cases was also much reduced. These findings agree with the fact that various non target snails reduce the capacity of schistosome miracidia to infect the target snails (Chernin, 1968; Combes and Mone, 1987 and Yousif *et al*., 1991 & 1998 a & b). *M. tuberculata* was reported also to cause the highest reduction in miracidial infection by Thomas and Tait (1984), Pointier (1993) and Yousif *et al*. (1998 a&b). The effect *Melanoides* separately and combined with other non target snails on the capacity of *S. mansoni* miracidia to locate and infect vector snails was proved to show highly reducing effect on infection rate of *Biomphalaria* with *S. mansoni* miracidia (Yousif *et al*. 1999 a &b).

The addition of the negative impact of *Melanoides* on the survival and egg production of *B. truncatus* snails and on its infection with *S. haematobium* could lead to a considerable reduction of cercarial production which is the main critical factor of the epidemiology of schistosomiasis in endemic areas. Therefore, the present study gives a strong evidence that *M. tuberculata* could be used as a biocontrol agent against schistosomiasis *haematobium* because of its capability to exert suppressive effect on the transmission of this medically important parasite.

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