J. Egypt. Soc. Parasitol., 36 (3): 979 - 992

EFFECT OF NON TARGET SNAILS ON SOME BIOLOGICAL OF *LYMNAEA NATALENSIS* SNAILS AND THEIR INFECTION TO *FASCIOLA GIGANTICA*

By

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Abstract

The influence of non-target freshwater snails (*Melanoides tuberculata* and *Planorbis planorbis*) on the capacity of *Fasciola* egg production *F. gigantica* miracidia to infect *Lymnaea natalensis* and their effect on mortality and growth rates showed that the snails exhibited a competitive ability against *L. natalensis*. The mortality rate existed in mixed cultures with snails was greatly increased, and increased with increase of snails number. The egg production and growth rate were negatively affected by the presence of *M. tuberculata* and *P. planorbis* which was more pronounced when snails were at higher ratio 1L: 10D. Also, the snails showed significant degree of reduction in infection rate of *L. natalensis* with *F. gigantica* miracidia.

Introduction

Fascioliasis caused by infection with either *Fasciola hepatica* or *F. gigantica* has a worldwide distribution including Egypt (Haseeb *et al.*, 2002). It is an increasing zoonotic disease (El-Shazly *et al.*, 1991). The parasite is transmitted to its final host through the snail intermediate host *Lymnaea natalensis* (El-Shabrawi *et al.*, 1997) and *Lymnaea* sp. Several studies were carried out on the prevalence of fascioliasis among humans, livestock and the intermediate hosts and their role in the transmission of infection (El-Shabrawi *et al.*, 1997; Mas-Coma *et al.*, 1999). The parasites obtain their nutrients and many of their structural elements from the host for growth and energy generation (Thompson *et al.*, 1993; Tielens, 1994). In general, the most effective method for schistosomiasis and fascioliasis control is by the use of chemical molluscicides (Shiff, 1961). The chemical control of snails is still costive and dangerous. This emphasizes strongly the need for other approaches of vector control such as biological method which may be safer, more specific, cheaper, with relatively permanent results and less harm to the environment (Jobin and Berrios, 1970). One method for biological control of snails hosts of schistosomiasis is the competitive displacements through the introduction of non-vector snails that have similar ecological requirements but with higher potentially and adaptability (Frandsen and Madsen, 1979). Mandahi-Barth (1970) claimed that *Helisoma duryi* might be useful as a potential agent against Schistosome vector snails. No impo-rtant trematodes have been recorded from the genus *Helisoma* and all attempts to infect *H. duryi* with schistosome were unsuccessful (Frandsen, 1976; Haroun *et al.*, 1985). The introduction of competitor snails has been also successfully utilized such as *Marisa conuarietis*, *Pomacea haustru*, *H. duryi.* Mkoji (1998) concluded that *Pila ovata* may prove useful in the biological control of medically important pulmonate snails. *Melanoides tuberculata*, a potential competitor which is considered one of the most promising candidates to control *Biomphalaria glabrata*, the intermediate host of *Schistosoma mansoni* (Pointier and Augustin, 1999; Giovanelli *et al.*, 2002; Pointier and Jordan, 2000).

The present study was initiated with two main objectives; firstly, the competitive effect of *Melanoides tuberculata* and *Planorbis planorbis* on *Lymnaea natalensis* under laboratory conditions. Secondly, these snails on the infection rate of *L. natalensis* with *Fasciola gigantica* miracidia

Material and Methods

Lymnaea natalensis (4-6 mm in length) and *Fasciola gigantica* ova used were obtained from the Schistosome Biological

Supply Program (SBSP), Theodor Bilharz Research Ins-titute (TBRI). Laboratory-bred snails were exposed individually to *F. gigantica* miracidia (3-5 miracidia/snail). The snails were left over-night to ensure maximum infection at room temperature (25-27°C) for 24 hrs. Non target snails such as *Melanoides tuberculata* (Muller, 1774) (Thiaridae: Mesogastropoda) and *Planorbis planorbis* (Linnaeus, 1758) (Planorbidae: Basommatophora) were collected from the Nile and irrigation schemes in Giza and Qalyoubia Governorates. In the laboratory, snails were washed thoroughly with dechiorinated tap water and examined for natural trematod-infection. Healthy snails were put into aquaria (26.5×20.5× 13.5cm), provided with glass plates and maintained in an air-conditioned room at 25±1°C in fluorescent light.

To test the competitive effect that. *M. tuberculata* (5-8 mm in length) and *P. planorbis* (5-8mm in length) my exert on *L. natalensis.* The decoy snails were used in two ratios in pro-portion to the target *L. natalensis.* The ratios were 5 decoy :1 target and 10 decoy :1 target. The 3 replicates, each of 50 target snails with 10 *L. natalensis* or 100 target snails to 10 *L. natalensis* and another 3 replicates for controls, each of 10 *L. natalensis* alone were used. Boils or oven dried lettuce leaves and blue-green algae *Nostoc muscorum* were used for snails feeding. Each aquarium was provided with polyethylene sheets for oviposition. The egg masses and eggs laid were counted using a binocular stereo-microscope. Dead snails were removed daily and counted. The mortality rate was calculated weekly for each species. The shell diameter (mm) of *L. natalensis* was measured weekly using a caliper (El-Emam and Madsen, 1982). The egg production/snail and growth rate of *L. natalensis* were calculated in the presence and absence of decoy snails.

P. planorbis and *M. tuberculata*, usually associated with *L. natalensis* in nature were used here as decoy for *F. gigantica* miracidia. The decoy snails were used in two ratios in proportion to *L. natalensis*. The ratios were 5 decoy: 1 target snails and 10 decoy :1 target. *L. natalensis* were exposed to *F. gigantica* miracidia as10/snail for 24 hrs in the presence and absence of decoy snails. So, 3 replicates, each of 50 target snails with 10 *L. natalensis* or 100 target snails to 10 *L. natalensis* and another 3 replicates for controls, each of 10 *L. natalensis* alone. After exposure, snails were maintained in separate aquarium of dechlorinated water at 25°C±1°C. Starting from day 25 post miracidial exposure, surviving *L. natalensis* and target snails were examined individually twice weekly for cercarial shedding by exposure to fluorescent light in 2 ml of water for 3 hr at 25°C.

Statistical analysis: Data was analyzed by student's *t*-test to compare the means of experimental and control groups (Spiegel, 1981).

Results

The results are in tables (1, 2, 3, 4, 5& 6) and figures (1 &2).

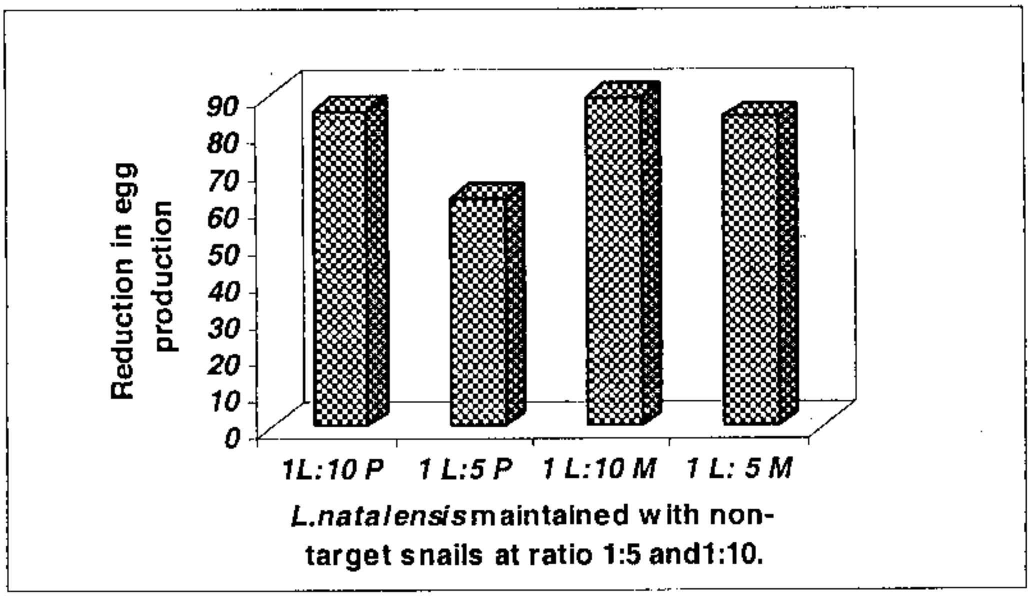

Fig. 1: Percent reduction of egg production of *L. natalensis* exposed to *F. gigantica* miracidia in presence *P. planorbis and M. tuberculata* at 1L:5D & 1L:10D).

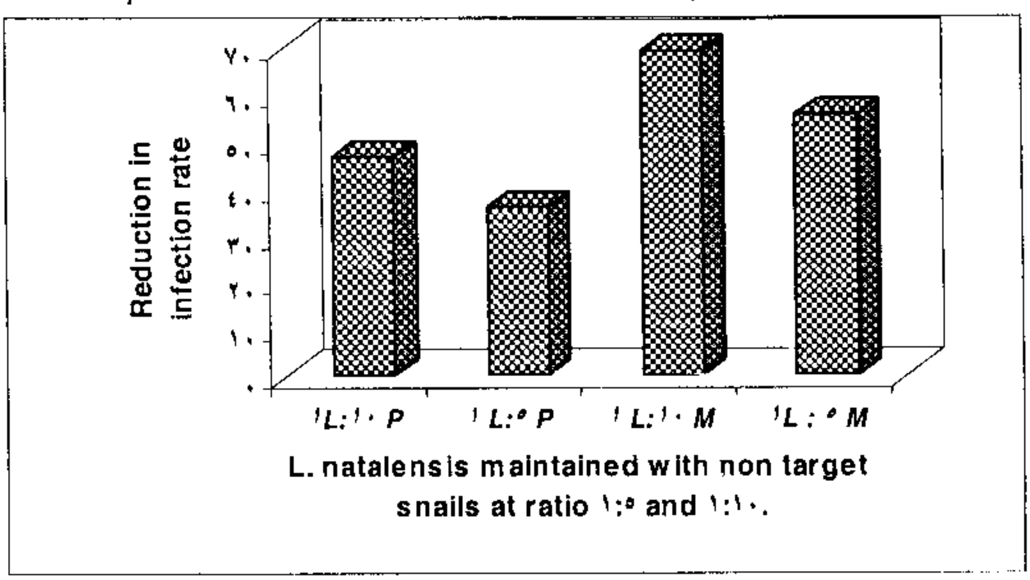

Fig. 2: Percent reduction of infection rate of *Lymnaea natalensis* exposed to *F. gigantica* miracidia in presence of *P. planorbis and M. tuberculata* at 1L:5D & 1L:10D

Table 1: Interaction between *L. natalensis* and *M. tuberculata* together as1L:5D

Time (Weeks)	Mortality of control				Mortality of Mixed culture at proportion 1: 5			
	L. natalensis		*M. tuberculata*		*L. natalensis*		*M. tuberculata*	
	M± SD	Cumulative	M± SD	Cumulative	M± SD	Cumulative	M± SD	Cumulative
1	0	0	1.1±1.5	1.1	4.3±0.8	4.3	1.2±0.6	1.2
2	4± .94	4	3.3±1.2	4.4	7.2±1.6	11.5	2.3±0.5	3.5
3	4.2±1.52	8.2	4.2±1.1	9.2	9.4±1.6	21.1	2.2±0.82	5.7
4	2.5±1.4	10.7	2.4±1.6	11.6	12.2±0.6	33.3	2.5±0.84	8.2
5	4.6±1.1	15.3	2.8±0.82	14.4	8.6±2.1	41.9	3.1±1.5.6	11.3
6	9± 0.82	24.3	2±1.15	16.4	8.4±1,2	40.3	4.2±1.2	15.5
7	6.2±1.4	30.5	3.8±0.2	20.2	0	50.3	2.1±0.83	17.6
8	8.2±0.82	38.7	4.2±0.7	24.4	0	50.3	3.8±1.6	21.7
9	9.3±0.58	48	2.6±1.7	27	0	50.3	1.4±0.62	23.1
10	2±0.32	50	2.4±0.42	29.4	0	50.3	2.3±1.6	25.4
11	0	50	1.4±0.4	30.8	0	50.3	2.4±0.72	27.8
12	0	50	1.6±1.1	32.4	0	50.3	1.6±1.5	29.4

Table 2: Interaction between *L. natalensis* and *M. tuberculata* together as 1L:10D.

Time (Weeks)	Mortality of control				Mortality of Mixed culture at proportion 1: 10			
	L. natalensis		*M. tuberculata*		*L. natalensis*		*M. tuberculata*	
	M± SD	Cumulative	M± SD	Cumulative	M±SD	Cumulative	M± SD	Cumulative
1	0	0	1.1±1.5	1.1	10.6±0.34	10.6	1.4±0.72	1.4
2	4± .94	4	3.3±1.2	4.4	13.5±2.1	24.1	1.8±0.42	3.2
3	4.2±1.52	8.2	4.2±1.1	9.2	16.2±2.4	40.3	2.4±0.4	5.6
4	2.5±1.4	10.7	2.4±1.6	11.6	9.8±3.1	50.1	1.8±1.5	7.4
5	4.6±1.1	15.3	2.8±0.82	14.4	0	50.1	4.2±0.6	11.6
6	9± 0.82	24.3	2±1.15	16.4	0	50.1	5.8±2.1	17.4
7	6.2±1.4	30.5	3.8±0.2	20.2	0	50.1	2.6±1.1	20

8	8.2±0.82	38.7	4.2±0.7	24.4	0	50.1	3.1±0.98	23.1
9	9.3±0.58	48	2.6±1.7	27	0	50.1	2.8±1.4	25.9
10	2±0.32	50	2.4±0.42	29.4	0	50.1	3.6±1.1	29.5
11	0	50	1.4±0.4	30.8	0	50.1	2.8±1.6	32.3
12	0	50	1.6±1.1	32.4	0	50.1	2.4±0.65	34.7

(3) Interaction between *L. natalensis* and *P. planorbis* snails maintained together as 1L:5D

Time (Weeks)	Mortality of control				Mortality of Mixed culture at proportion 1: 5			
	L. natalensis		*P. planorbis*		*L. natalensis*		*P. planorbis*	
	M± SD	Cumulative	M± SD	Cumulative	M± SD	Cumulative	M± SD	Cumulative
1	0	0	0	0	1.6±0.4	1.6	1.4±0.42	1.4
2	4± .94	4	1.3±0.6	1.3	7.4±2.1	9	2.8±0.18	4.2
3	4.2±1.52	8.2	2.4±0.6	3.7	8.4±1.6	17.4	2.9±0.42	7.1
4	2.5±1.4	10.7	1.8±0.42	5.5	12.2±0.5	29.	1.2±0.12	8.3
5	4.6±1.1	15.3	2.5±1.2	8	9.3±0.57	38.3	2.5±0.24	10.8
6	9± 0.82	24.3	3.7±0.64	11.7	5.6±1.32	43.9	2.8±0.25	13.6
7	6.2±1.4	30.5	5.3±0.84	17	6.3±0.43	50.2	3.4±1.2	17
8	8.2±0.82	38.7	4.2±1.6	21.2	0	50.2	4.1±1.4	21.1
9	9.3±0.58	48	1.6±0.52	22.8	0	50.2	1.3±0.31	22.4
10	2±0.32	50	2.8±1.7	25.6	0	50.2	3.2±0.5	25.6
11	0	50	1.2±0.21	26.8	0	50.2	1.2±0.21	26.8
12	0	50	2.2±0.82	29	0	50.2	0.5±0.1	27.3

(4) Interaction between *L. natalensis* and *P. planorbis* maintained together as 1L:10D.

Observation period (Weeks)	Mortality of control				Mortality of Mixed culture at proportion 1: 10			
	L. natalensis		*P. planorbis*		*L. natalensis*		*P. planorbis*	
	Mean± SD	Cumulative	Mean± SD	Cumulative	Mean± SD	Cumulative	Mean± SD	Cumulative
1	0	0	0	0	5.3±1.1	5.2	1.6±0.75	1.6
2	4± .94	4	1.3±0.6	1.3	9.7±1.41	14.9	3.2±1.1	4.8
3	4.2±1.52	8.2	2.4±0.6	3.7	11.8±1.7	26.7	2.3±1.43	7.1
4	2.5±1.4	10.7	1.8±0.42	5.5	14.2±2.3	40.9	1.8±0.84	8.9
5	4.6±1.1	15.3	2.5±1.2	8	9.3±1.6	50.3	2.1±0.65	11
6	9± 0.82	24.3	3.7±0.64	11.7	0	50.3	3.1±1.32	14.1
7	6.2±1.4	30.5	5.3±0.84	17	0	50.3	4.5±2.3	18.6
8	3.2±0.82	38.7	4.2±1.6	21.2	0	50.3	1.6±0.65	20.2
9	9.3±0.58	48	1.6±0.52	22.8	0	50.3	1.2±0.42	21.4
10	2±0.32	50	2.8±1.7	25.6	0	50.3	1.8±0.65	23.2
11	0	50	1.2±0.21	26.8	0	50.3	0.75±0.2	23.95
12	0	50	2.2±0.82	29	0	50.3	1.4±0.42	25.35

Table 3: Effect of *M. tuberculata* and *P. planorbis* on eggs production of *L. natalensis* maintained together as 1L:5D & 1L:10D (Mean No. of eggs/snail).

Period (Weeks)	*L.natalensis* (control)	*L.natalensis* & *M. tuberculata* at 1:5	*L.natalensis* & *M. tuberculata* at 1:10	*L.natalensis* & *P. planorbis* at 1:5	*L.natalensis* & *P. planorbis* at 1:10
1	1.2	0	0	0.85	0
2	3.6	0.74	0.58	1.7	0.76
3	6.1	2.3	1.82	2.3	1.1
4	7.3	1.8	1.6	3.4	2.1
5	5.5	1.2	0	2.8	1.4
6	4.6	0	0	2.1	0
7	3.6	0	0	1.3	0
8	2.8	0	0	0	0
9	2.2	0	0	0	0
10	1.45	0	0	0	00
11	0	0	0	0	0
Eggs/snail	38.35	6.04	4	14.45	5.36
Reduction		84.3%	89.6%	62.3%	86%

Table 4: Mean shell diameter (mm) of *L. natalensis* maintained with *M. uberculata* and *P. planorbis* together as1L:5D & 1L:10D.

Period (Weeks)	*L. natalensis* (control)	*L natalensis* & *M. tuberculata* at 1:5	*L. natalensis* & *M. tuberculata* at 1:10	*L.natalensis* & *P.planorbis* at 1:5	*L.natalensis* & *P. planorbis* at 1:10
1	3.2± 0.12	3.2±1.1	3.2± 0.75	3.2± 1.5	3.2± .5
2	4.2 ± 0.8	3.6±0.54	3.4± 1.61	3.9± 0.82	3.3±1.2
3	5.6 ± 1.5	4.8.±0.42	4.2± 1.8	5.2± 0.67	4.6 ± 0.62
4	6.1 ± 1.2	5.1±0.56	4.4± 1.2	5.6± 0.54	4.8±1.8
5	7.2 ± 2.1	5.4±0.41	0	6± 1.7	5±0.76
6	8.2 ± 1.6	0	0	6.1± 2.1	00
7	8.9 ± 1.1	0	0	0	00
8	8.61 ± 2.3	0	0	0	0
9	9.2 ± 1.72	0	0	0	0
10	9.6 ± 0.84	0	0	0	0

Table 5: Growth rate % of *L. natalensis* through snail life span maintained with *M. tuberculata* and *P. planorbis* together as 1L:5D &1L:10D.

Growth phase	*L.natalensis* (control)		*L.natalensis* & *M.tuberculata* at 1:5		*L.natalensis* & *M.tuberculata* at 1:10		*L.natalensis* & *M.tuberculata* at 1:5		*L.natalensis* & *P.planorbis* at 1:10	
	Period	Grow.	Period	Grow.	Period	Grow.	Period	Grow.	Period	Grow.
First	6	125	3	50	3	31.25	3	62.5	3	43.8
Second	5	33.3	2	12.5	1	4.76	3	17.3	2	8.7

Period in week, Grow. = Growth rate.

Table 6: Effect of *M. tuberculata* and *P. planorbis* on infection of *L. natalensis* with miracidia of *F. gigantica* at two ratios (1L:5D&1L:10D).

Snail	*L. natalensis* (control)	*L.natalensis* & *M. tuberculata* at 1:5	*L.natalensis* & *M.tuberculata* at 1:10	*L.natalensis* & *P.planorbis* at 1:5	*L.natalensis* & *P.planorbis* at 1:10
Exposed	50	50	50	50	50
Survived	32	18	13	25	20
Infected	24	6	3	12	8
% Infection	75%	33.3%	23.1%	48%	40%
% Reduction		55.6%	69.2%	36%	46.7%

Discussion

The results indicated that the mortality rate of *L. natalensis* was greatly increased by the presence of *M. tuberculata* and *P. planorbis* and this effect increased with the increase of the number of non target snails to *L. natalensis* at ratio 1L:10 D. *M. tuberculata* exhibited a strong competitive ability against *L. natalensis* than *P. planorbis*. Similar results of different species of competitive snails on survival of *S. mansoni* vectors were reported by Abou El-Hassan (1974). He found that survival of *Biomphalaria* was reduced by *Physa* than *Helisoma*. The effect of *Melanoides* on the survivorship of *B. pfeifferi* and *Biomphalaria* peregrine was re-ported (Pointier, 1983; Thomas and Tait, 1984; Perera *et al.*, 1992). The present results agreed with El-Sayed and Sharaf El-Din (2000) who showed considerable increase in mortality of *B. truncatus* by *Cleopatra* sp. Also, the present results showed that egg production of *L. natalensis* in ratios (1L:5D &1L:10D) was greatly reduced than that of the controls. The reduction was increased with the increase of the number of non-target snails relative to *L. natalensis*. Similar reducing effect of egg production was reported by *H. duryi* (Madsen and Frandsen, 1979; Madsen, 1979,1980, 1985; Mostafa and Sharaf El-Din, 2000), El-Sayed and Sharaf El-Din (2000) added that *C. bulimoides* exerted a harmful effect on *B. truncatus* egg production.

The present results showed that the growth of *L. natalensis* was negatively affected by *M. tuberculata* and *P. planorbis*. This effect was more pronounced when *M. tuberculata* and *P.*

planorbis were at ratio 1L: 10D. This finding agreed with many authors working on different species of *Helisoma* and vector snails. Joubert and De Kock (1989) found that the growth rate of *Biomphalaria africanus* was reduced in the presence of *H. duryi*. The present data showed that decoy snails at a ratio of 1L:5D and 1L:10D of *L. natalensis* and decoy high significantly reduced the infection rate of *L. natalensis* with *F. gigantica*. This reduction is assumed to be due to the interfering effect of the non-target snails with the host finding capacity of *F. gigantica* miracidia to find and locate *L.. natalensis*. The non-target snails protect most target ones from infection and the miracidia were irreversibly damaged by abortive attempts to penetrate them (Chernin, 1968). A similar reducing effect on the infection rate of *B. truncatus* with *S. haematobium* by *C. bulimoides* was reported (El-Sayed and Sharaf El-Din, 2000) and by *H. duryi* (Mostafa and Sharaf El-Din, 2000) under laboratory conditions. Rasmussen (1974) and Jobin and Faracuent (1979) reported that infection rate of *B. pfeifferi* and *B. glabrata* with *S. mansoni* was reduced by using *H. duryi* and *M. cornuarietis* as decoy snails during their exposure to *S. mansoni* miracidia under natural conditions. Yousif *et al.* (1990) who found that infection of *B. alexandrina* with *S. mansoni* was highly affected in all experimental snails by the presence of *H. duryi* and the reduction in infection ranged from 53%-79%. Yousif *et al.* (1998) found that the infection rate of *B. alexandrina* with *S. mansoni* miracidia was significantly reduced by *P. planorbis* and *M. tuberculata*. Bakry and Abd El-Monem (2005) found that *M. tuberculata, P. planorbis* and *P. acuta* gave significant reduction in infection rate of *B. truncates* with *S. haematobium* miracidia.

The present results showed that *M. tuberculata* was more effective in reducing *L. natalensis* infection with miracidia than *P. planorbis*. This may be due to trapping of miracidia by the snails or the miracidia were exhausted by unsuccessfully penetration trials to the non-vector snails. Similar reducing effect of some non-target snails on the capacity of *S. mansoni* miracidia to locate and infect target snails, under laboratory conditions was reported by Combes and Mone (1987) using *M. tuberculata*, and Yousif *et al.* (1990) using *H. duryi*. None of *L. natalensis* was infected *F. gigantica* in the presence of *M. tuberculata* at ratio 1L: 10D.

It was concluded that *M. tuberculata* and *P. planorbis* exhibited a competitive ability against *L. natalensis*. The egg production and survival and growth rates of *L. natalensis* in cultures with non-target snails was greatly reduced, and reduction increased with increase of non-target snails, which had high significant reduction of *L. natalensis* infection to *Fasciola gigantica.*

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