Egypt. J. Aquat. Biol. & Fish., Vol. 11, No.1:1 - 21 (2007) ISSN 1110-6131

EFFECT OF SOME ENVIRONMENTAL PARAMETERS ON CROSSING PROCESS OF TARGET SNAILS TO *SCHISTOSOMA MANSONI* INFECTION

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Keywords: *Schistosoma mansoni*, *Biomphalaria*, biological control, environmental parameters.

ABSTRACT

The biological control of the snails intermediate hosts of *Schistosoma mansoni*, is one of the accepted options to fight schistosomiasis. For the control of schistosomiasis, one strategy is based on the premise that resistant snails to parasite infection could be used as biological competition agents to replace existing susceptible snails in endemic areas. Therefore, the current study was designed to investigate the influence of environmental factors on copulation behaviour of *Biomphalaria* snails under various laboratory conditions. These factors are : light, darkness, starvation, pH and association with non- target snail species ~~in order~~ to obtain information on the ecological conditions for reproduction of the resistant species.

The data obtained showed that, copulation behavior was enhanced by different environmental factors and revealed the best modular system for snail reproduction. ~~It was also~~ noticed that, non-target snail species or the resistant snails affect crossing among the target snails. Moreover, resistant snails recorded more tolerance against abnormal environmental conditions.

INTRODUCTION

Outcrossing and self – fertilization are common modes of reproduction in pulmonate gastropods (Jarne & Stadler, 1995). *Biomphalaria* snails are simultaneous hermaphrodite with ability of self – fertilization, however, in the presence of partner (when paired) cross –

fertilization is preferred (Vidigal *et al.*, 1994 & 1998 a & b) . Selfing snails often have a lower fecundity than cross – fertilizing snails (Bayomy & Joose , 1987) .

Biological methods for control of freshwater snails were reviewed by Madsen (1992), who concluded that more emphasis should be put on searching for pathogens or microparasites as agent for control that can affect directly or indirectly on the intermediate snail hosts . These include bacteria , fungi , Protozoa , Amphibia and fish (Slootweg *et al.*, 1993) , other competitor snails (Abdel – Hamid & Ali , 1998) and Muschovy ducks (*Cairina maschata*) (Ndlela & Chimbari , 2000) .

Although physical, chemical, and biological factors have a significant effect on population dynamics, temperature, water chemistry, current speed, light, shade, snail 's feeding and association among species are of particular importance in the natural history of planorbid snails (Brown , 1994) . Water chemistry includes calcium concentration , total dissolved chemical elements and oxygen. The densities of all snail species appear very low in salt water, high in medium water and somewhat lower in hard water (Beadle, 1981). The level of oxygen may have an important influence on distribution within habitats.

Certain environmental factors have pronounced effects on the susceptibility of snails to infection. For example, a decrease in the temperature has a substantial influence on the development of *S. mansoni* infection in *B. glabrata* (Coelho & Bezerra , 2006) . However, Richards (1984) suggested that , the susceptibility of snails to infection is a hereditary character. Unfavourable conditions , however , can be caused by dense floating vegetation, preventing snail from reaching the surface when there is a shortage of dissolved oxygen (Brown , 1994) . Snail hosts for schistosomes were reported for several generations in almost total darkness (Appleton , 1978) . Shaded sites were unfavourable for *Biomphalaria pfeifferi* and shading by trees was suggested as a mean for controlling this snail . Dense shade beneath mats of floating vegetation is generally unfavourable for snails (Nidifon & Ukoli , 1989). Most aquatic habitats contain rich microflora which , together with decaying vegeTable matter , provide the snails diet and are probably important in conditioning the habitat (Malek , 1958) . Food in the habitat seemed to influence the reproduction of the snail hosts and their population dynamics.

MATERIAL AND METHODS

Target and non-target snail species for *Schistosoma* infection (*Biomphalaria alexandrina, Biomphalaria glabrata* , *Lymnaea truncatula* and *Physa acuta*) were obtained from the field (Giza governorate , Egypt) . Five – liter glass aquaria were used in the experiment . To breed the snails , 500 ml beakers and some crystallizing dishes were used . Snail conditioned water (SCW) was used in the aquaria and at weekly intervals , the aquaria were cleaned and their water changed . The snails were fed on fresh lettuce leaves supplemented with tetramin (fish food) . After careful selection on the basis of health , size and age , methods of separating schistosome resistant and susceptible strains from both *B. alexandrina* and *B. glabrata* were performed according to the method described by Zanotti- Magalhaes *et al.* (1997) .

Adult snails were exposed individually to 10 freshly hatched miracidia of *S. mansoni*-Egyptian strain obtained from Schistosome Biological Supply Project (SBSP) ,(Theodur Bilharz Research Institute , Egypt) in vials containing 3 ml water for 3 – 4 hours . Each tested snail was returned and reared singly in a beaker containing 500 ml SCW .

Snails were examined at 10 days post – exposure and checked for infection with a binocular microscope . Snails with developing sporocysts were transferred to another flask and kept for cercarial emergence for 10 weeks post - exposure . As described by Theron & Coustau (2005), snails that became infected are considered susceptible hosts and those which are not infected are traditionally determined as resistant .

Selfing / out crossing :

- Both susceptible and resistant parent stocks of either *B. alexandrina* or *B. glabrata* snails were isolated and reared singly for self – fertilization These self – fertilized snails were used for breeding and for current studies as following :

Different developmental stages ; juvenile (J) , adult (Ad) and senescent (Se) from either susceptible or resistant strains were obtained in successive generations by rearing snails singly each in 500 ml plastic container containing SCW . On the other hand , crosses among adult and senescent snails were performed .

To confirm hybridization phenomenon within both *B. alexandrina* and *B. glabrata* , the following experiment was conducted . Four groups of snails each contains one *B. alexandrina* (either susceptible or resistant) and one *B. glabrata* (susceptible or resistant) were left to live in pairs in clean plastic containers (500 ml). Crossing numbers and duration of each cross were recorded during 6 hours of pairing with 15 min. interval . The experiments were repeated 5 times.

Effect of some environmental conditions on the snails reproduction:

1 - Effect of light and darkness:

a) Both *B. alexandrina* and *B. glabrata* , either susceptible or resistant were maintained individually for 24 hours in a clean plastic containers (each of 250 ml) containing sew in order to acclimatize the snails to environmental conditions before starting laboratory experiments.

b) The snails of each species and strain were divided into two groups: the first group was allowed to live in pairs under light, while the second one was put in darkness for 6 hours. Twenty four observations with intervals of 15 min. during 6 hours were recorded for each group and the number of crossing and the duration of each cross were recorded for each species, strain and stage.

2 - Effect of starvation:

The effect of starvation on reproduction of both susceptible and resistant within *B. alexandrina* and *B. glabrata* snails populations was examined according to the method described by Pinheiro *et al.* (2001) .

The first group was fed on fresh lettuce leaves supplemented with tetramin (fish food) [ingredient statement: fish meat, shrimp meat, aquatic plants, oat flour yeast various vitamins, chlorophyll], while the second one was starved for 24 hours .

3 - Effect of pH :

Adult stages of both *B. alexandrina* and *B. glabrata* snails were left to live in pairs under suiTable environmental conditions except pH levels.

Six pairs of the acclimatized experimental snails were maintained in clean plastic containers, each contains 500 ml sew at different pH that ranged from 4 - 9.

The numbers of crossing and duration of each cross were recorded with intervals of 15 min. for 6 hrs. . The experiment was repeated 3 times with exchanging the place of the containers.

4-Influence of non-target snail species on the ability of both *B. alexandrina* and *B. glabrata* to crossing:

Each pair of snails (either *B. alexandrina* or *B. glabrata*) was left to live in clean plastic container containing 500 sew under the suiTable environmental conditions. *Physa acuta* and *L. truncatula* (as non- target snails to *Schistosoma* parasite) were added to live in the containers together with the target snail of *B. alexandrina* or *B. glabrata* . Numbers of crossing and the duration of each cross were recorded for 6 hrs. with intervals of 15min.

Statistical analysis

Statistical analysis of results was carried out using analysis of variance (ANOVA) according to Campbell (1989) .

RESULTS

Factors affecting the crossing process :

1 – Effect of light and darkness :

The longest total crossing period was recorded under lighted conditions in groups of both *B. alexandrina* and *B. glabrata* as shown in Tables (1 & 2) .The resistant individuals at adult stage of each group has longest total crossing period which recorded 64.5 and 83.3 min. for *B. alexandrina* and *B. glabrata* respectively .However , the susceptible forms at senescent stage had the shortest total crossing period which recorded 10.4 and 14.7 min. of *B. alexandrina* and *B. glabrata* respectively .

It was also observed that , *B. glabrata* was more tolerant to darkness than *B. alexandrina* , since there was no recorded crossing period in most *B. alexandrina* groups in the darkness .

The longest crossing period in darkeness was found in adult groups of *B. glabrata* of resistant individuals , where it recorded 24.1 min. and the shortest was found in senescent susceptible ones which recorded 0.57 min. .

2 – Effect of starvation :

From Tables (3 & 4) , the total crossing period in the feeding groups of both *B. alexandrina* and *B. glabrata* was longer than in the corresponding starved group .

It was also noticed that , adult resistant snails had the longest total crossing period in all tested groups (89.2 & 62.1 min.) . In contrast , the senescent susceptible ones had the shortest total crossing period (11.8 & 22.0 min.) . Data listed showed that , the resistant individuals of both snail species were more tolerant to starvation than susceptible ones. Adult snails were also more tolerant to starvation than senescent stages (19.8 & 0.65min.).

From Tables (1 , 2 , 3 & 4) , it was observed that the total crossing period was affected by snail species , where in case of *B.*

glabrata , the total crossing period was longer than those corresponding in *B. alexandrina* . The snail type can also affect total crossing period , where total crossing periods in all resistant strains within the two snail species were longer than those corresponding in susceptible ones . It was also noticed that, the total crossing periods were affected by snail age , where crossing periods in adult groups were longer than those corresponding in senescent groups.

Furthermore, the results showed that tolerance of *B. glabrata* to abnormal conditions (e.g. darkness – starvation) was higher than those in *B. alexandrina* .

3 – Effect of pH :

In Table 5 , *B. alexandrina* snails showed the longest crossing period within 6 hours at pH 7 , since they recorded 64.8 min. for susceptible progeny and 84.8 min. for resistant ones . Total crossing period recorded an obvious decrease at lower pH as well as at pH 8 then it reverted to slight increase at pH 9.

Table 6 illustrates the effect of different pH on *B. glabrata* crossing rate. The longest total crossing period was recorded around pH 6 for susceptible snails and at pH 7 for resistant ones . Remarkable decrease was also observed in acidic media . In alkaline media, the total crossing period tended to decrease in pH 8 but it reverted to increase slightly at pH 9 for resistant snails and largely for susceptible one.

4 – Target / non-target snail compatibility:

Tables 7 and 8 demonstrate the crossing process of *B. alexandrina* and *B. glabrata* in snail modular system (SMS) containing both *P. acuta* and *L. truncatula* as non-target snails to *Schistosoma* infection .

The results showed a decrease in total crossing period of target snail species, while a remarkable decrease was observed in the presence of non-target species compared with the control.

In the presence of the non-target snails of *P. acuta* in SMS, resistant individuals of both target snail species (*B. alexandrina* & *B. glabrata*) recorded more total crossing period than susceptible ones .

5 – Hybridization between *B. alexandrina* and *B. glabrata* :

The recorded data showed decrease in crossing period in all the tested groups than the corresponding control groups and the crossing ability between the two *Biomphalaria* species was less than those corresponding within individuals of the same species .

The data also showed that, crossing between different *Biomphalaria* species is affected by snail type, where the crossing period within resistant individuals was more than those in susceptible ones (48.18 & 13.78 min) as described in Table (9).

DISCUSSION

The precise distribution of snails is considered of potential importance, while understanding of factors regulating snail distribution could be valuable for control purposes. So, the present study provides further evidence that the distribution of these snails is a result of more complex interaction of different factors such as nutrition, light and darkness , pH and association with other snail species that is reflected on snail mating system .

Vernon (1995) suggested that , absence of social facilitation may reduce the reproductive output of *B. glabrata* . This result agrees with the present data about the effect of changing natural habitat on crossing process , where a significant decrease was reported in copulation behavior by modifying snail habitat .

Furthermore, *Biomphalaria* snails can copulate at high rate in pH 6 – 7 and the crossing rate decreased at higher or lower limits. This agrees with Donnelly *et al.* (1984) who observed prevention of establishment of intermediate hosts for schistosomes in water at high salinity.

In addition, the starvation of snails showed lower crossing rate compared with the fed ones. Similarly, O'keefe (1985) concluded that poor food quality appeared to limit populations of *B. globosus* during most of the year . In contrast , Vianey – Liaud and Dussart (1994) found that after all periods of starvation and desiccation , albino snail parents were still producing significant numbers of pigmented offsprings , suggesting preferential cross – fertilization with *B. glabrata* using stored allosperm .

On the other hand , *Biomphalaria* snails prefer to cross at higher rate than those associated with other non-target freshwater snails . This result describes the occurrence of two or more species in natural habitat with each other and negative association as reported by Thomas and Tait (1984). Furthermore, our experimental study shows high decrease in crossing response between *B. alexandrina* and *B. glabrata* , whereas *P. acuta* and *L . truncatula* in the same water medium with *B. alexandrina* and *B. glabrata* suppressed their rate of egg production .

Therefore , the snail species affects the crossing process , where its duration time was larger in *B. glabrata* than those corresponding in *B.*

alexandrina. Moreover, the snail type affects the crossing process, where total crossing periods in all resistant groups were larger than those corresponding in all susceptible ones. Furthermore, the snail age of the same species and strain affected the crossing process , where all adult groups recorded higher crossing time than the corresponding senescent ones . These observations confirm those of Coulellec – Vreto *et al.* (1997) who reported that the mating system of such species has been proven to be highly variable either between populations or between families within populations (Ritland & Ganders , 1985 ; Willis , 1993) .

It was also found that the snails preferred to cross at moderately lighted habitat. This agrees with Brown (1994) who reported that shaded sites are unfavourable for *B. pfeifferi* and shading by trees was suggested as a means for controlling these snails. So, from our results, we can suggest the best modular system for snail breeding, which enhance the egg production and distribution of the intermediate host snail .

Furthermore, the current study indicated that hybridization could occur between *B. alexandrina* and *B. glabrata* as regarded by Yousif *et al.* (1998) who reported that hybrid snails were found naturally and participated in schistosomiasis transmission in Egypt .In contrast , Lotfy *et al.* (2005) reported that there was no evidence of the presence of hybridization of *B. alexandrina* with *B. glabrata* in the Nile Delta in Egypt .

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Table (1): Effect of light and darkness on crossing process of *B. alexandrina* snails under laboratory conditions within 6 hrs of observation.

Snail phenotype	In Light			In Dark			T. test for total crossing period
	Crossing Number/ 2 snail ± SE	Duration time/ one cross ± SE	Total crossing period (min) ± SE	Crossing number/ 2 snail ± SE	Duration time/ one cross ± SE	Total crossing period(min) ± SE	
G_1	6.67 ± 0.67	9.67±0.88	64.50±1.76	2.30±0.01	5.51±0.33	12.67±1.33	$P < 0.001$
G_2	3.33 ± 0.67	11.00±0.58	36.63±6.11	0.00	0.00	0.00±0.00	$P < 0.05$
G_3	2.67 ± 0.67	13.00±1.00	34.70±2.00	0.00	0.00	0.00±0.00	$P < 0.005$
G_4	5.33 ± 0.67	11.30±0.88	60.30±4.05	0.00	0.00	0.00±0.00	$P < 0.001$
G_5	4.0 ± 0.29	3.80±0.44	15.20±0.78	1.8±0.13	2.2±0.03	4.00±0.57	$P < 0.001$
G_6	1.3 ± 0.44	8.00±0.60	10.40±1.31	0.00	0.00	0.00±0.00	$P < 0.001$
G_7	2.33 ± 0.33	19.66±0.33	45.10±5.67	2.30±0.43	2.17±0.25	5.00±0.57	$P < 0.05$
G_8	2.33 ± 0.33	13.33±0.88	31.0±2.90	0.00	0.00	0.00±0.00	$P < 0.001$
G_9	4.67 ± 0.67	6.20±0.60	28.5±1.15	0.00	0.00	0.00±0.00	$P < 0.001$

G_1: Pairing of two adults of resistant snails.
G_2: Pairing of one adult (resistant) and one senescent (resistant).
G_3: Pairing of two senescent resistant snails.
G_4: Pairing of two adult susceptible snails.
G_5: Pairing of one adult (susceptible) and one senescent (susceptible).
G_6: Pairing of two senescent susceptible snails.
G_7: Pairing of one adult (susceptible) and the other resistant.
G_8: Pairing of one adult (resistant) & one senescent (susceptible).
G_9: Pairing of one adult (susceptible) & one senescent (resistant).

Table (2): Effect of light and darkness on crossing process of *B. glabrata* snails under laboratory conditions within 6 hrs observation.

Snail type	In Light			In Dark			T. test for total crossing period/min
	Crossing Number/2 snail ± SE	Duration time/ one cross ± SE	Total crossing period/min ± SE	Crossing number/ 2 snail ± SE	Duration time/ one cross ± SE	Total crossing period/min ± SE	
G_1	5.67±0.33	14.70±0.27	83.30±6.33	2.30±0.33	10.50±0.50	24.10±2.96	$P < 0.001$
G_2	4.60±0.67	5.60±0.88	62.80±4.21	6.67±0.67	2.70±0.37	18.00±0.93	$P < 0.001$
G_3	6.60±0.67	8.30±0.33	55.00±4.66	5.33±0.67	1.53±0.38	8.15±1.58	$P < 0.001$
G_4	3.33±0.67	23.40±0.95	77.90±2.40	5.000±0.58	3.96±0.33	19.80±0.78	$P < 0.0001$
G_5	4.60±0.67	8.30±0.88	38.20±3.05	4.67±0.67	1.46±0.29	6.80±0.74	$P < 0.001$
G_6	2.80±0.33	5.20±0.58	14.70±0.33	1.33±0.33	0.43±0.03	0.57±0.12	$P < 0.0001$
G_7	6.60±0.67	11.00±1.00	72.60±6.35	3.33±0.33	5.60±0.67	18.87±2.33	$P < 0.001$
G_8	5.08±0.44	8.60±0.33	50.30±2.72	1.33±0.33	5.80±0.60	7.75±1.32	$P < 0.0001$
G_9	4.60±0.67	10.60±0.88	49.70±2.91	5.33±0.67	1.30±0.33	6.93±0.66	$P < 0.0001$

G_1: Pairing of two adults of resistant snails .
G_2: Pairing of one adult (resistant) and one senescent (resistant) .
G_3: Pairing of two senescent resistant snails .
G_4: Pairing of two adult susceptible snails .
G_5: Pairing of one adult (susceptible) and one senescent (susceptible) .
G_6: Pairing of two senescent susceptible snails .
G_7: Pairing of one adult (susceptible) and the other resistant .
G_8: Pairing of one adult (resistant) & one senescent (susceptible) .
G_9: Pairing of one adult (susceptible) & one senescent (resistant) .

Table (3): Effect of starvation on crossing process of *B. alexandrina* under laboratory conditions within 6 hrs of observation.

Snail type	Feeded groups			Starved groups			T. test for total crossing period/ min
	Crossing Number/ 2 snail ± SE	Duration time/ one cross ± SE	Total crossing period/min ± SE	Crossing number/ 2 snail ± SE	Duration time/ one cross ± SE	Total crossing period/min ± SE	
G_1	5.33±0.67	11.67±0.88	62.10±5.89	2.30±0.33	8.60±0.67	19.79±2.30	$P < 0.005$
G_2	5.6±0.33	6.30±0.33	35.70±0.33	1.30±0.33	4.10±0.44	5.33±0.88	$P < 0.001$
G_3	1.70±0.33	18.70±0.88	31.79±5.49	1.30±0.33	3.80±0.60	4.98±0.72	$P < 0.05$
G_4	5.66±0.33	10.66±0.33	60.40±3.17	3.30±0.67	3.30±0.88	10.89±1.15	$P < 0.001$
G_5	2.67±0.67	6.00±0.58	16.00±2.40	1.80±0.33	1.80±0.29	1.99±0.16	$P < 0.05$
G_6	3.30±0.33	2.80±0.31	11.88±0.20	1.30±0.33	0.50±0.00	0.65±0.16	$P < 0.001$
G_7	5.00±0.58	9.30±0.33	46.50±4.09	1.30±0.33	4.60±0.67	5.98±1.15	$P < 0.001$
G_8	1.80±0.17	17.30±1.20	31.2±2.29	1.67±0.33	1.67±0.33	2.79±0.66	$P < 0.001$
G_9	3.60±0.33	5.80±0.73	21.30±1.25	1.33±0.33	1.67±0.17	2.22±0.44	$P < 0.001$

G_1: Pairing of two adults of resistant snails .
G_2: Pairing of one adult (resistant) and one senescent (resistant) .
G_3: Pairing of two senescent resistant snails .
G_4: Pairing of two adult susceptible snails .
G_5: Pairing of one adult (susceptible) and one senescent (susceptible) .
G_6: Pairing of two senescent susceptible snails .
G_7: Pairing of one adult (susceptible) and the other resistant .
G_8: Pairing of one adult (resistant) & one senescent (susceptible) .
G_9: Pairing of one adult (susceptible) & one senescent (resistant) .

Table (4): Effect of starvation on crossing process of *B. glabrata* under laboratory conditions within 6 hrs of observation .

Snail type	Feeded groups			Starved groups			T. test for total crossing period/ min
	Crossing Number/ 2 snail ± SE	Duration time/ one cross ± SE	Total crossing period(min) ± SE	Crossing number/ 2 snail ± SE	Duration time/ one cross ± SE	Total crossing period(min) ± SE	
G_1	4.67±0.67	19.10±0.49	89.20±10.66	3.33±0.33	6.67±0.88	22.20±1.29	P < 0.05
G_2	8.30±0.88	8.60±0.33	71.90±4.91	3.33±0.33	2.33±0.33	7.76±0.89	P < 0.0001
G_3	4.60±0.67	12.00±0.58	56.00±5.45	2.00±0.00	3.00±0.29	6.00±0.57	P < 0.01
G_4	6.67±0.67	12.10±1.12	85.70±2.09	2.67±0.33	7.00±0.58	18.69±1.45	P < 0.0001
G_5	3.67±0.33	8.50±0.29	31.20±2.08	1.33±0.44	1.50±0.17	3.24±0.62	P < 0.0001
G_6	3.00±0.58	7.33±0.33	22.00±3.48	1.00±0.00	1.67±0.17	1.66±0.16	P < 0.005
G_7	8.67±0.67	8.50±0.76	73.70±1.76	9.00±0.58	1.30±0.33	11.70±2.18	P < 0.0001
G_8	5.30±0.67	9.60±0.33	51.50±5.92	1.30±0.33	3.83±0.60	4.98±0.75	P < 0.001
G_9	5.33±0.67	9.33±0.88	49.70±2.90	1.60±0.33	3.00	4.80±0.67	P < 0.0001

G_1: Pairing of two adults of resistant snails .
G_2: Pairing of one adult (resistant) and one senescent (resistant) .
G_3: Pairing of two senescent resistant snails .
G_4: Pairing of two adult susceptible snails .
G_5: Pairing of one adult (susceptible) and one senescent (susceptible) .
G_6: Pairing of two senescent susceptible snails .
G_7: Pairing of one adult (susceptible) and the other resistant .
G_8: Pairing of one adult (resistant) & one senescent (susceptible) .
G_9: Pairing of one adult (susceptible) & one senescent (resistant) .

Table (5) : Ability of *B. alexandrina* to cross at different pH under laboratory conditions within 6 hrs of observation .

Snail type	*B. alexandrina* (Susceptible)				*B. alexandrina* (Resistant)				T. test within 2 snails
	Crossing number/ 2 snails ±SE	Duration time/ one cross ±SE	Total crossing period (min) ±SE	T. test within control	Crossing number/ 2 snails ±SE	Duration time/ one cross ±SE	Total crossing period (min) ±SE	T. test within control	
pH = 4	1.30±0.33	1.8±0.12	2.34±0.33	P < 0.001	5.30±0.67	3.30±0.33	24.49±0.67	P<0.0001	P<0.000 1
pH = 5	1.60±0.00	6.30±0.33	10.08±2.02	P < 0.001	3.30±0.15	10.3±0.98	33.99±2.00	P<0.0001	P<0.001
pH= 6	4.60±0.10	13.3±0.68	60.96±7.92	N.S.	4.60±0.33	16.6±1.25	76.60±6.76	N.S.	N.S.
pH = 7	5.30±0.56	12.30±0.5	64.80±4.67	Control	4.10±0.14	20.5±1.58	84.80±2.92	Control	P<0.05
pH = 8	1.60±0.16	2.00±0.00	3.20±0.33	P<0.0001	1.30±0.15	5.30±0.15	6.89±1.52	P<0.0001	P<0.05
pH = 9	3.30±0.17	3.30±0.15	10.89±1.33	P < 0.001	2.30±0.10	7.50±0.34	17.25±1.77	P < 0.001	P < 0.05

*Control: The snails left to cross under normal condition (pH = 7.00).
N. S.: Non-significant

Table (6): Ability of *B. glabrata* to cross at different pH under laboratory conditions within 6 hrs of observation .

Snail type	*B. glabrata* (Susceptible)				*B. glabrata* (Resistant)				T. test within 2 snails types
	Crossing number / 2 snails ±SE	Duration Time / one cross ±SE	Total crossing period (min) ±SE	T. test within control	Crossing Number / 2 snails ±SE	Duration Time / one cross ±SE	Total crossing period (min) ±SE	T. test within control	
pH = 4	2.30±0.13	8.60±0.33	20.20±0.88	P<0.0001	2.60±0.01	19.5±0.67	52.00±11.33	P<0.05	N.S.
pH = 5	2.60±0.33	16.60±0.19	44.40±6.66	N.S.	2.60±0.31	20.3±0.72	52.86±11.30	P<0.05	N.S.
pH = 6	2.60±0.05	23.60±0.87	63.10±13.08	N.S.	4.60±0.23	17.6±0.58	80.96±0.27	P<0.05	N.S.
pH =7	23.67±0.97	16.50±0.58	60.50±2.78	Control	4.00±0.06	23.0±0.10	92.00±8.00	Control	P<0.005
pH = 8	1.30±0.01	2.30±0.00	3.10±0.57	P<0.005	1.33±0.09	2.33±0.93	3.10±0.57	P<0.05	N.S.
pH =9	4.66±0.21	12.66±0.35	58.20±2.67	N.S.	2.33±0.33	4.33±0.01	10.09±0.33	P<0.05	P<0.005

*Control :The snails left to cross under normal condition (pH = 7.00).
N. S.: Non-significant

Table (7): Influence of non-target snail species on the crossing process of *B.alexandrina* under laboratory conditions within 6 hrs of observation.

Snail type	*B. alexandrina* (susceptible)			T. test within control	*B. alexandrina* (resistant)			T. test within control	T. test within 2snails types
	Crossing Number / 2 snails ± SE	Duration Time / one cross ± SE	Total crossing period (min) ± SE		Crossing Number / 2 snails ± SE	Duration Time / one cross ± SE	Total crossing period (min) ± SE		
Control	5.33±0.67	11.30±0.88	60.30±5.80	control	6.67±0.67	9.67±0.67	9.67±0.88	Control	N.S.
P. acuta	1.00±0.00	0.60±0.10	0.60±0.10	P<0.0001	1.33±0.33	0.60±0.17	1.33±0.28	P<0.0001	P<0.05
L. trunctula	1.67±0.33	3.33±0.33	5.33±0.667	P<0.0001	1.33±0.33	1.33±0.33	1.77±0.33	P<0.0001	P<0.05

*Control : snails were left to cross at normal conditions in absence of non- target species.
N. S.: Non-significant

Table (8): Influence of non-target snails species on the crossing process of *B. glabrata* under laboratory conditions within 6 hrs of observation.

Snail type	Susceptible *B. glabrata*			T. test within control	Resistant *B. glabrata*			T. test within control	T. test within 2 snails types
	Crossing Number / 2 snails ± SE	Duration Time / one cross ± SE	Total crossing period (min) ± SE		Crossing Number/ 2 snails ± SE	Duration Time / one cross ± SE	Total crossing period (min) ± SE		
Control	3.33±0.67	24.4±0.95	77.9±2.43	Control	5.67±0.33	14.7±0.27	83.30±6.33	Control	N.S.
P. acuta	1.33±0.33	0.70±0.07	0.93±0.14	P<0.0001	1.33±0.33	2.33±0.33	3.10±0.58	N.S	P<0.05
L. trunctula	1.33±0.33	10.67±0.67	14.20±1.33	P<0.0001	1.33±0.33	2.50±0.29	3.30±0.44	N.S	P<0.0001

*Control : snails were left to cross at normal conditions in absence of non- target species
N. S. : Non – significant .

Table (9): Hybridization between *B. alexandrina* and *B. glabrata* under laboratory conditions within 6 hrs. of observation.

Snail type	Crossing Number/ 2 snails ±SE	Duration Time/ one cross ±SE	Total crossing period/min ±SE	Control	Crossing Number/ 2 snails ±SE	Duration Time/ one cross ±SE	Total crossing period/min ±SE
G_1	2.60±0.67	5.30±0.88	13.78±1.6	C_1	5.33±0.67	11.37±0.88	60.30±5.81
				C_2	3.33±0.67	23.40±0.95	77.90±2.43
G_2	3.30±0.67	14.60±0.88	48.18±2.67	C_3	6.67±0.07	9.67±0.88	64.50±1.76
				C_4	5.67±0.33	14.70±0.27	83.30±6.33
G_3	2.30±0.33	1.75±0.38	4.02±0.44	C_5	2.33±0.33	19.66±0.33	45.10±5.67
G_4	1.60±0.33	5.30±0.88	8.48±0.88	C_6	6.60±0.67	11.00±0.10	72.60±6.35

G_1: *B. alexandrina* and *B. glabrata* (susceptible snails) .
G_2: *B. alexandrina* and *B. glabrata* (resistant snails) .
G_3: *B. alexandrina* (susceptible) and *B. glabrata* (resistant) .
G_4: *B. alexandrina* (resistant)and *B. glabrata* (susceptible) .
C_1: two *B. alexandrina* (susceptible) .
C_2: two *B. glabrata* (susceptible) .
C_3: two *B. alexandrina* (resistant) .
C_4 two *B. glabrata* (resistant) .
C_5: *B. alexandrina* one resistant and one susceptible .
C_6: *B. glabrata* one resistant and one susceptible .