Biological studies on fresh water snails target to *Schistosoma mansoni* infection

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Abstract

In this study ,upon exposure of laboratory Egyptian *B. alexandrina* and *B. glabrata* snails to its homologus strain of *S. mansoni* , two different phenotype strains " susceptible and resistant " were distinguished . The aim of this study is to investigate the biological differences between the two types of *Biomphalaria* snails either susceptible or resistant at different ages . The investigated parameters included mortality , survival , percentage of susceptibility / resistance to parasitic infection and fecundity . The results of the preliminary exposure experiment revealed that parental snail stock was not completely susceptible and tend to be partially refractory to infection . The results also showed a strong association between the levels of egg production and susceptibility to parasite infection . Where breeding experiment revealed that , total egg number , E/M and EM values produced by resistant phenotype of both snail species were more than those produced by susceptible progeny snails . Also , it was noticed that , egg production is associated inversely proportion with snail age .

Introduction

Schistosomiasis is a serious public health problem and second most important parasitic disease (WHO , 2004) . More than 600 million people in 74 countries are at risk while more than 200 million were infected (Gibodate and Bergquist , 2000) . The schistosome parasites are transmitted by snail intermediate hosts found in water bodies like lakes , ponds , streams , rivers , irrigation canals and dams .Snails prefer lightly shaded areas , though they show considerable adaptability to light conditions . The snails may live up to 18 – 24 months , unless infected with schistosomes or other parasites , where they may die earlier . Young snails reach sexual maturity in about two months and under favorable conditions can lay 10 – 15 eggs daily (Ayad , 1974) .

Certain environmental factors have pronounced effects on the susceptibility of snails to infection. For example , temperature affects the penetration of miracidia to snails and their development to cercaria (Pitchford , 1981 ; Coelho & Bezerra , 2006) . While , Richards (1984) suggested that the susceptibility of snails to infection is hereditary character. Outcrossing and self-fertilization are common modes of reproduction in pulmonate gastropods (Jarne and Stadler , 1995) Some species reproduce predominantly by self-fertilization , others are facultative self-fertilizers (selfing only occurs when no mating partners are available), and others reproduce exclusively by cross-fertilization . Furthermore , *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.*, 1998) . Selfing snails often have a lower fecundity than cross-fertilizing snails (Bayomy and Joosse ,1987) . It is important to verify the influence of self-fertilization performance and copulation behaviour in breeding of both *B. alexandrina* and *B. glabrata* . The purpose of this study was to determine the different biological parameters of the two *Biomphalaria* species under laboratory conditions .

Material And Methods

Maintenance of snails :

Target snail species for *Schistosoma* infection (*Biomphalaria alexandrina* and *Biomphalaria glabrata*) were obtained from the field (Giza governorate , Egypt) . Five-liter glass aquaria were used in the experiment . To bread 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 daily with fresh lettuce leaves supplemented with tetramin (fish food) .

Two hundreds snail from each species were exposed individually to 10 freshly miracidia of Egyptian strain obtained from Theodur Bilharz Research Institute, Cairo , Egypt according to Langand and Morand (1998) technique .

Snails were examined for infection after 2 weeks and continued thereafter weekly up to 10 weeks post-miracidial exposure. As described by Larson *et al*.(1996), any snails in which high infection frequencies were observed are considered susceptible and those uninfected are considered as resistant. Snails in which developing parasites are not evident were re – exposed individually to 10 miracidia / snail .

Selfing / out crossing :

Selection on the basis of health , size and age , methods of separating schisto-some resistant and susceptible strains from both *B. alexandrina* and *B. glabrata* were performed according to the method described by Zanotti-Magalhaes *et al.* (1997) . Both susceptible and resistant parent stocks of either *B. alexandrina* or *B. glabrata* snails were reared singly for self-fertilization . These self-fertilized snails were used for breading 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 .

In self and cross-fertilization , snails readily deposited their eggs on a plastic sheets floating on the surface of water . Eggs of each snail group were transferred into other plastic containers contain SCW . Oviposition was measured by counting the number of egg mass and the number of eggs in each clutch . This was undertaken for a period of one week . Five replicates were performed for each group . Egg masses were incubated in containers containing SCW until hatching completely .

Newly hatched snails were calculated and transformed into petri-dishes (25 snails / dish) containing mud , source of calcium carbonate (chalk) and SCW . The snails were noticed weekly up to 8 weeks to record the growth and maturity rates .

The results were analyzed by the ANOVA test (Zar , 1996) .

Results

Susceptibility / resistance of *Biomp-halaria* snails towards its homologous strain of *S. mansoni.*

The result pointed out that , about 36.7 % & 30.4 % of the examined *B. alexandrina* and *B. glabrata* were resistant respectively , in contrast with 63.3 % & 69.6 % of these snails yielded high infection frequencies as shown in figure (1) . As it was noticed , the curve representing percentages of susceptible snails within both two snail species started at 6^{th} week post miracidial exposure and reached the highest peaks by approximately the 8^{th} week . While the curve of refractory snails gradually decreased toward the 8^{th} week and up to 8^{th} post-exposure no changes in percentages of refractory snails occurred , and no marked differences were noticed at that time up to the 10th week . The ratio of susceptibility / resistance within the same snail species at a time was fixed , although the number of snails could changes depending on the increase of mortality within susceptible ones (Table 1) .

Furthermore , data listed in Table (1) presents a dramatically decrease in cumulative numbers and percentage of refractory snails until reach 36 (36.7 %) for *B. alexandrina*,31 (30.4 %) for *B. glabrata* and it was fixed during 9th & 10th week post miracidial exposure.

Mortality rate and survival of the host :

The mortality and survival rates of the exposed *B. alexandrina* and *B. glabrata* snails were shown in Table (1) from which, it can be noticed that , the increase in mortality rate was associated with potent *Schistosoma* infection , since there was no death among snail species under normal condition (control) . The total cumulative survival rate decrease gradually in both snail species where it recorded 73 % for *B. alexandrina* and 87 % for *B. glabrata* at 4th week and reach 49 % and 51 % for both species at 9th week post-exposure , respectively .

Egg production of cross and self fertilized parental snails :

Table (2) showed that , the production of egg masses of resistant and susceptible strains of both *B. alexandrina* and *B. glabrata* snail ranges from 7.0 – 9.3 , 7.3 – 9.3 , 5.3 – 8.7 and 6.3 – 7.67, respectively . The recorded values of egg masses (M / snail) produced by either cross or self – fertilization of resistant *B. alexandrina* snail group [Rc (9.0) , Rs (9.3)] being greater than the corresponding of susceptible snail groups [Sc (8.6) , Ss (7.0)] .The mean number of eggs per one clutch (E/M) of two resistant strains being slightly higher or lower than those corresponding in each snail group .

The mean number of eggs per each snail group produced in one week by cross or self- fertilized resistant *B. alexandrina* [Rc (111.00) , Rs (108.88)] were greater than those of the two susceptible snail groups [Sc (101.11) , Ss (86.33)] . On the other hand , the number of eggs per adult snail produced by cross-fertilization [Sc (101. 11) & Rc (111.00)] was slightly greater than those of corresponding self-fertilized snail groups [Ss (86.30) & Rs (108.88)] . The results showed also that the total egg number of both susceptible and resistant strains by either self or cross-fertilization decreased gradually by the age of parental snail . It was observed that , the number of eggs in parental adult snail (111.00) was greater than that produced from crossing between adult and senescent (88.89) which was greater than the two parental senescent snails (72.33) .

Moreover , Table (2) showed gradual decrease in number of clutches by increasing snail age . Where adult parental snails produced egg clutches (9.00) was greater than that obtained by crossing between adult and senescent (8.33) which was greater than senescent stage (7.00) . Although , the ratio of E / M was not more affected by snail age . It was also noticed that , the values of egg masses produced by cross or self-fertilized resistant *B. glabrata* snail groups [Rc (9.3) & Rs (8.0)] being more greater than those two susceptible snail groups [Sc (7.0) & Ss (7.3)] .On the other hand , the numbers of eggs per one clutch (E/M) produced by Sc & Ss (18.0 & 17.0) is not significantly different from those produced by Rc & Rs (17.3 & 18.0) .

Furthermore , the mean total numbers of eggs per one week produced , either by cross or self-fertilized susceptible *B. glabrata* snail groups [Sc (129.00) & Ss (126.00)] were lower than those produced by the two refractory snail groups [Rc (161.78) & Rs (144.00) . It was also observed that , within resistant and susceptible adult snails , the egg production per snail produced by cross fertilization [Sc (129.00) & Rc (161.78)] were greater than those corresponding to self-fertilized snail groups [Ss (126.00) & Rs (144.00)]. Also , the total number of eggs produced by susceptible fertilized (Sc & Ss) snail groups was lower than those produced by resistant fertilized (Rc & Rs) snail groups.

In Table (3) and Fig. 2 , the total egg number produced per one week was inversely proportional to snail age in the case of cross-fertilization of both *B. alexandrina* and *B. glabrata* . Where it was recorded the largest values produced by crossing between two adult parental snails (104.78 & 141.33) which was greater than those produced from adult resistant and senescent susceptible cross-fertilization (82.67 & 135.44) . Moreover, total egg number obtained by crossing between adult susceptible and senescent resistant was greater (78.22 & 121.33) than those produced from two senescent parents (66.67 & 106.67) .

Furthermore , in all *B. glabrata* snail groups either susceptible or resistant produced by cross-fertilization , their total egg number and egg number per mass was greater than those corresponding *B alexandrina* snail groups (Table 3 & Fig. 2) .

Hatchability of *Biomphalaria* snails eggs :

In Table (4) , the most observation was the number of hatched snails and its percentage in relation to total egg number produced by self-fertilization (63.8 %) . It was significantly lower than those produced by cross-fertilization (67.5 %) . The recorded values showed that the number and percentage of newly hatched snails in relation to total egg number produced in susceptible strain of either *B. alexandrina* or *B. glabrata* was significantly lower (66 %) than those produced in resistant snail phenotype (68.3 %) . It was also noticed that , hatchability rate and number of snails reaching maturity in *B. glabrata* snails recorded larger values than those corresponding in *B. alexandrina* (Table 5 & Fig. 3) .

Growth rate of neonatal offspring derived by cross and self- fertilization of parental *Biomphalaria* snails :

The shell diameter of each snail progeny was measured weekly and the data were listed in tables (6 ,7 & 8) . The results pointed out that , there was a gradual increase in the mean shell diameter of all tested groups and this increase was statistically non significant within the same snail species .

It was also noticed that , the growth rate of *B. glabrata* snails was faster than those of *B. alexandrina* especially at 4^{th} and up to 8^{th} week of experiment (Tables 7 & 8) .

It was observed that , the phenotype of the parent snails , the type of fertilization and age of parent snails did not affect the growth rate of neonatal snails within the two snail species (*B. alexandrina* and *B. glabrata*) .

Table (1): Susceptibility/Resistance of *B. alexandrina* and *B. glabrata* parental snail population towards *S. mansoni* under laboratory conditions.

Snail phenotype		Time of examination after miracidial exposure													
		28 days		35 days		42 days		49 days		56 days		63 days		70 days	
		B. a	*B.g*	*B. a*	*B.g*	*B. a*	*B.g*	*B. a*	*B.g*	*B. a*	*B.g*	*B. a*	*B.g*	*B. a*	*B.g*
Cumulative Total survival	No %	146 (73)	174 (87)	134 (67)	163 (81.5)	124 (62)	132 (66)	114 (57)	120 (60)	105 (52.5)	109 (54.5)	98 (49)	102 (51)	98 (49)	102 (51)
Cumulative Total susceptible	No %	0 0	0 0	0 0	0 0	48 (38.7)	62 (46.9)	57 (50)	69 (57.5)	62 (59.1)	71 (65.1)	62 (63.3)	71 (69.6)	62 (63.3)	71 (69.6)
Cumulative Total refractory	No %	146 (100)	174 (100)	134 (100)	163 (100)	76 (61.3)	70 (53.1)	57 (50)	51 (42.5)	43 (40.9)	38 (34.9)	36 (36.7)	31 (30.4)	36 (36.7)	31 (30.4)
Mortality Total Rate	No %	54 (27)	26 (13)	66 (33)	37 (18.5)	76 (38)	68 (34)	86 (43)	80 (40)	95 (47.5)	91 (45.5)	102 (51)	98 (49)	102 (51)	98 (49)

Note: Groups of 200 snails (each of the same age and size) were used in each experiment.
B.a.: *B. alexandrina.* *B. g.*: *B. glabrata.*

Table (2): Mean number of eggs and egg masses produced by self and cross-fertilization of both susceptible and resistant *B. alexandrina* and *B. glabrata* at different ages under laboratory conditions.

Snail phenotype	Self-fertilization						Cross-fertilization								
	Adult			Senescent			Ad & Ad			Ad & Se			Se & Se		
	EM	E/M	E	EM	E/M	E	EM	E/M	E	EM	E/M	E	EM	E/M	E
B. alexandrina Resistant	9.33 ± 2.00	11.67 ± 1.15	108.88	8.33 ± 0.58	12.00 ± 1.00	100.00	9.00 ± 1.00	12.33 ± 1.53	111.00	8.33 ± 0.58	10.67 ± 1.15	88.89	7.00 ± 1.00	10.30 ± 0.58	72.33
B. alexandrina Susceptible	7.00 ± 1.00	12.33 ± 1.15	86.33	6.00 ± 1.00	11.00 ± 1.00	66.00	8.67 ± 1.52	11.67 ± 1.53	101.11	6.33 ± 0.58	13.33 ± 0.58	84.44	5.3 ± 0.58	12.00 ± 1.00	64.00
B. glabrata Resistant	8.00 ± 1.53	18.00 ± 1.00	144.00	7.33 ± 0.58	17.00 ± 1.00	124.67	9.30 ± 1.52	17.33 ± 1.50	161.78	8.67 ± 1.53	17.33 ± 0.58	150.22	8.00 ± 1.00	16.33 ± 0.58	130.67
B. glabrata Susceptible	7.33 ± 0.53	17.00 ± 0.50	126.00	6.67 ± 0.58	16.67 ± 0.57	111.10	7.00 ± 1.00	18.00 ± 1.70	129.00	7.67 ± 0.58	17.33 ± 1.58	132.89	6.33 ± 0.58	16.33 ± 0.57	103.44
Anova	N.S	P < 0.001		N.S	P < 0.001		N.S	P < 0.001		N.S	P < 0.001		N.S	P < 0.001	

E = Mean total number of eggs E/M = Number of eggs per egg mass. EM = Number of egg masses
Ad = Adult Se = Senescent N.S.= Non-significant

Table (3): Mean number of eggs and egg masses produced by cross-fertilization between susceptible and resistant strains of both *B. alexandrina* and *B. glabrata* at different ages under laboratory conditions.

Snail phenotype	*B. alexandrina*			*B. glabrata*		
	EM Mean±S.D.	**E/M Mean±S.D**	**E**	**EM Mean±S.D.**	**E/M Mean±S.D**	**E**
Ad_S & Ad_R	7.67±1.52	13.67±1.52	104.78	8.00±1.00	17.67±0.58	141.33
Se_S & Se_R	6.67±1.15	10.00±1.00	66.67	6.67±1.15	16.00±1.00	106.67
Se_R & Ad_S	7.33±1.15	10.67±1.53	78.22	7.00±1.00	17.33±0.58	121.33
Ad_R & Se_S	8.00±1.00	10.33±1.15	82.67	7.67±1.53	17.67±1.15	135.44
Anova	N.S.	P<0.001		N.S.	P<0.001	

E = Mean total number of eggs . EM = Number of egg masses .
N.S.= Non-significant . E/M = Number of eggs per egg mass

Table (4): Total number of eggs, hatching offspring and offspring reaching maturity and their percentage produced by self and cross fertilization of either susceptible or resistant *B. alexandrina* and *B. glabrata* at different ages.

Snail phenotype	Self-fertilization						Cross-fertilization								
	Ad			Se			Ad & Ad			Ad & Se			Se & Se		
	E	H (M±SD)	RM (M±SD)	E	H (M±SD)	RM (M±SD)	E	H (M±SD)	RM (M±SD)	E	H (M±SD)	RM (M±SD)	E	H (M±SD)	RM (M±SD)
B. alexandrina Resistant	108.88	69.30 ± 0.40 (63.8%)	23.30 ± 2.50 (34.6%)	100.0	61.80 ± 7.00 (61.8%)	20.00 ± 3.00 (33.3%)	111.00	7.50 ± 9.20 (67.5%)	20.33 ± 3.50 (27%)	88.89	59.33 ± 4.00 (66.7%)	17.33 ± 2.00 (29.2%)	72.33	44.33 ± 1.50 (61.1%)	14.67 ± 1.50 (33.0%)
B. alexandrina Susceptible	86.30	54.50 ± 5.00 (62.5%)	16.30 ± 2.50 (31.3%)	66.0	40.67 ± 6.00 (61.6%)	11.60 ± 2.00 (28.5%)	101.11	68.33 ± 8.50 (67.2%)	20.3 ± 3.00 (29.7%)	84.44	49.67 ± 4.50 (58.8%)	17.33 ± 1.50 (34.8%)	64.00	38.0 ± 3.20 (59.3%)	13.33 ± 1.50 (35.0%)
B. glabrata Resistant	144.00	98.30 ± 7.60 (68.3%)	23.67 ± 1.50 (24%)	124.7	76.67 ± 7.60 (61.4%)	21.00 ± 2.00 (27.3%)	161.78	117.67 ± 9.30 (72.7%)	27.00 ± 2.00 (22.9%)	150.2	106.67 ± 12.58 (71.0%)	25.0 ± 1.50 (23.4%)	130.70	93.30 ± 6.50 (69.1%)	23.67 ± 1.50 (25.0%)
B. glabrata Susceptible	126.00	85.67 ± 7.00 (66%)	20.30 ± 1.50 (23.6%)	111.1	65.0 ± 5.60 (58.5%)	19.00 ± 1.70 (29.2%)	129.00	87.00 ± 8.50 (69.0%)	25.67 ± 5.20 (29.5%)	132.9	91.67 ± 9.60 (68.9%)	18.70 ± 1.50 (20.4%)	103.44	68.00 ± 4.60 (65.7%)	14.33 ± 1.50 (21.0%)

E = Mean total number of eggs (Mean of Egg masses X No. of eggs / mass) .
H = Number of hatching snails.
RM = Number of new offspring reaching maturity .

Table (5): Total number of eggs, hatching offspring and offspring reaching maturity and their percentage in relation to total egg number produced by cross fertilization between susceptible and resistant strains of eithher *B. alexandrina* or *B. glabrata* at different ages.

Snail phenotype	*B. alexandrina*			*B. glabrata*		
	E	**H (M±SD)**	**R (M±SD)**	**E**	**H (M±SD)**	**R (M±SD)**
Ad_S & Ad_R	104.78	65.67±7.00 (62.6%)	19.33±1.52 (29.4%)	135.44	91.67±3.50 (67.7%)	25.00±2.60 (27.2%)
Se_S & Se_R	66.67	49.00±6.25 (73.4%)	13.67±1.50 (27.9%)	106.67	69.67±10.00 (65.3%)	16.67±1.50 (23.9%)
Se_R & Ad_S	78.22	48.67±4.00 (62.2%)	15.00±1.00 (30.8%)	121.33	86.00±3.60 (70.8%)	17.00±2.00 (19.7%)
Ad_R & Se_S	82.67	50.00±6.00 (60.5%)	16.33±1.50 (32.7%)	141.33	98.67±5.50 (69.8%)	16.33±0.58 (16.5%)

E = Total number of eggs (Mean of egg masses x No. of eggs / mass) .
H = Number of hatchling snails .
R = Number of new offspring reaching maturity .

Table (6): Growth rate of neonatal offspring derived by self and cross fertilization of *B. alexandrina* at different ages.

Snail phenotype		**Time of Examination / week**															
		***B. alexandrina* resistant**								***B. alexandrina* susceptible**							
		1st (M±SD)	**2nd (M±SD)**	**3rd (M±SD**	**4th (M±SD)**	**5th (M±SD)**	**6th (M±SD**	**7th (M±SD**	**8th (M±SD**	**1st (M±SD**	**2nd (M±SD**	**3rd (M±SD**	**4th (M±SD**	**5th (M±SD**	**6th (M±SD**	**7th (M±SD**	**8th (M±SD**
Self-fertilization	Ad	1.26 ± 0.15	2.13 ± 1.53	2.70 ± 0.20	3.47 ± 1.53	4.06 ± 0.20	4.77 ± 0.15	5.93 ± 0.25	6.90 ± 0.40	1.20 ± 0..20	2.06 ± 0.15	2.58 ± .1.2	3.33 ± 1.53	3.96 ± 1.53	4.67 ± 0.15	5.80 ± 0.25	6.90 ± 0.30
	Se.	1.18 ± 0.15	2.0 ± 0.20	2.55 ± 0.16	3.27 ± 0.15	3.83 ± 0.13	4.60 ± 0.30	5.67 ± 0.35	6.77 ± 0.35	1.10 ± 0.20	2.1 ± 0.10	2.27 ± 0.25	3.27 ± 0.15	3.70 ± 0.20	4.60 ± 0.20	5.53 ± 0.47	6.70 ± 0.40
Cross-fertilization	Ad & Ad	1.123 ± 0.15	2.06 ± 0.20	2.73 ± 0.15	3.633 ± 0.15	4.00 ± 0.15	4.77 ± 0.25	5.77 ± 0.35	6.83 ± 0.30	1.18 ± 0.15	2.18 ± 0.16	2.63 ± 0.25	3.36 ± 0.15	3.80 ± 0.25	4.67 ± 0.25	5.77 ± 0.25	6.80 ± 0.40
	Se & Ad	1.230 ± 0.15	2.00 ± 0.20	2.70 ± 0.15	3.60 ± 0.10	4.00 ± 0.20	4.70 ± 0.30	5.77 ± 0.20	6.79 ± 0.25	1.17 ± 0.15	2.30 ± 0.26	2.60 ± 0.20	3.33 ± 0.15	3.70 ± 0.26	4.63 ± 0.20	5.70 ± 0.20	6.77 ± 0.40
	Se & Se	1.16 ± 0.15	2.0 ± 0..20	2.70 ± 0.16	3.53 ± 0.15	3.93 ± 0.13	4.70 ± 0..30	5.73 ± 0.35	6.87 ± 0.35	1.10 ± 0.20	2.10 ± 0.1	2.57 ± 0.25	3.27 ± 0.15	3.70 ± 0.20	4.60 ± 0.20	5.53 ± 0.47	6.70 ± 0.40
Anova	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.: Non-significant .

Table (7): Growth rate of neonatal offspring derived by self and cross-fertilization of *B. glabrata* at different ages.

Snail phenotype		Time of Examination / week															
		***B. glabrata* resistant**								***B. glabrata* susceptible**							
		1^{st} (M±SD)	**2^{nd} (M±SD)**	**3^{rd} (M±SD)**	**4^{th} (M±SD)**	**5^{th} (M±SD)**	**6^{th} (M±SD)**	**7^{th} (M±SD**	**8^{th} (M±SD**	**1^{st} (M±SD**	**2^{nd} (M±SD**	**3^{rd} (M±SD**	**4^{th} (M±SD**	**5^{th} (M±SD**	**6^{th} (M±SD**	**7^{th} (M±SD**	**8^{th} (M±SD**
Self-fertilization	Ad	1.33 ± 0.12	2.13 ± 0.17	2.93 ± 0.15	3.70 ± 0.20	4.50 ± 0.30	5.77 ± 0.30	7.10 ± 0.50	8.16 ± 0.45	1.38 ± 0.14	2.15 ± 0.15	2.91 ± 0.10	4.20 ± 0.20	4.57 ± 0.25	5.68 ± 0.25	7.00 ± 0.40	8.18 ± 0.40
	Se.	1.26 ± 0.20	2.03 ± 0.20	2.87 ± 0.12	4.43 ± 0.35	4.67 ± 0.30	6.00 ± 0.50	7.0 ± 0.50	8.07 ± 0.45	1.28 ± 0.10	2.10 ± 0.10	2.80 ± 0.20	3.67 ± 0.20	4.40 ± 0.10	5.40 ± 0.30	6.93 ± 0.40	8.10 ± 0.10
Cross-fertilization	Ad & Ad	1.23 ± 0.15	2.08 ± 0.20	2.87 ± 0.15	3.93 ± 0.15	4.60 ± 0.15	5.77 ± 0.25	7.00 ± 0.35	8.23 ± 0.30	1.27 ± 0.10	2.23 ± 0.16	2.90 ± 0.25	3.80 ± 0.15	4.60 ± 0.20	5.67 ± 0.25	7.00 ± 0.35	8.10 ± 0.40
	Se & Ad	1.23 ± 0.15	2.70 ± 0.15	2.83 ± 0.15	3.80 ± 0.20	4.53 ± 0.15	5.73 ± 0.30	7.00 ± 0.20	8.20 ± 0.20	1.17 ± 0.15	2.88 ± 0.10	2.93 ± 0.15	3.70 ± 0.25	4.57 ± 0.25	4.70 ± 0.30	7.03 ± 0.25	8.00 ± 0.30
	Se & Se	1.21 ± 0.15	2.03 ± 0.20	2.83 ± 0.15	3.83 ± 0.15	4.53 ± 0.20	5.67 ± 0.35	6.99 ± 0.25	8.20 ± 0.30	1.20 ± 0.10	2.17 ± 0.20	2.90 ± 0.10	3.77 ± 0.25	4.56 ± 0.20	5.60 ± 0.40	6.97 ± 0.35	8.00 ± 0.30
Anova	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

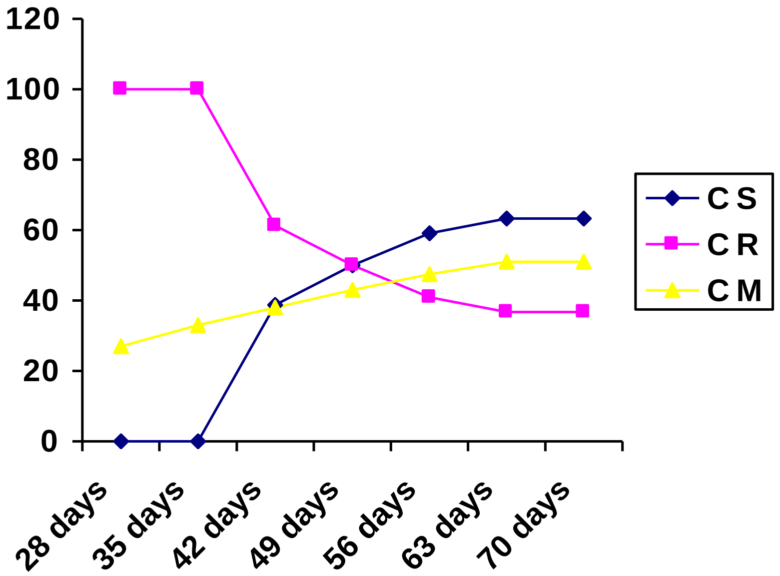
N.S.: Non-significant

Table (8): Growth rate of neonatal offspring derived by cross-fertilization of different strains within the same snail species (either *B. alexandrina* or *B. glabrata)* at different ages.

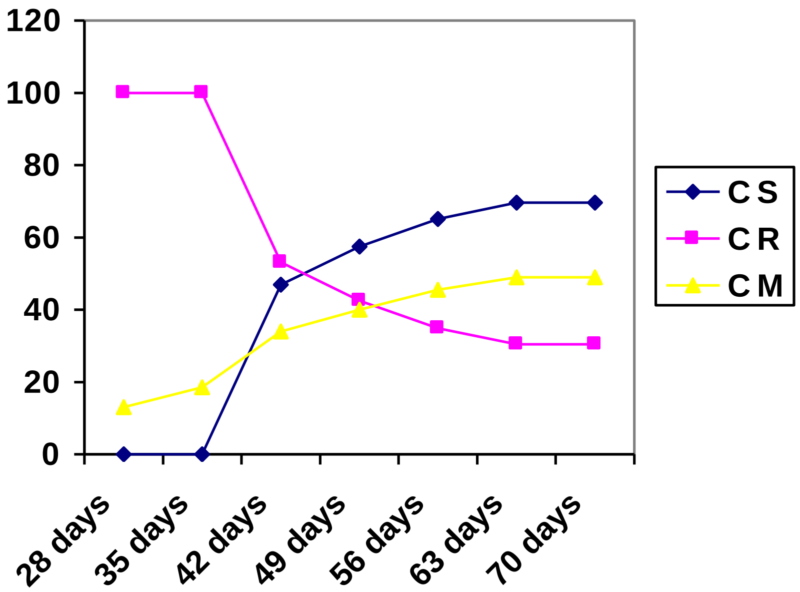
Snail phenotype	Time of Examination / week															
	B. alexandrina								***B. glabrata***							
	1^{st} (M±SD)	**2^{nd} (M±SD)**	**3^{rd} (M±SD)**	**4^{th} (M±SD)**	**5^{th} (M±SD)**	**6^{th} (M±SD)**	**7^{th} (M±SD)**	**8^{th} (M±SD)**	**1^{st} (M±SD)**	**2^{nd} (M±SD)**	**3^{rd} (M±SD)**	**4^{th} (M±SD)**	**5^{th} (M±SD)**	**6^{th} (M±SD)**	**7^{th} (M±SD)**	**8^{th} (M±SD)**
AdS& AdR	1.25 ± 0.13	2.27 ± 0.10	2.70 ± 0.10	3.50 ± 0.15	4.00 ± 0.15	4.70 ± 0.30	5.70 ± 0.30	6.80 ± 0.30	1.35 ± 0.13	2.28 ± 0.10	2.90 ± 0.10	3.67 ± 0.15	4.63 ± 0.15	5.70 ± 0.30	7.00 ± 0.30	8.10 ± 0.20
Ad_S & Se_R	1.22 ± 0.15	2.20 ± 0.20	2.68 ± 0.15	3.30 ± 0.20	3.90 ± 0.10	4.63 ± 0.15	5.60 ± 0.30	6.70 ± 0.30	1.30 ± 0.10	2.80 ± 0.15	2.89 ± 0.10	3.53 ± 0.15	4.53 ± 0.15	5.67 ± 0.25	7.01 ± 0.25	8.01 ± 0.20
Ad_R & Se_S	1.13 ± 0.15	2.33 ± 0.20	2.70 ± 0.10	3.40 ± 0.10	3.90 ± 0.10	4.63 ± 0.20	5.67 ± 0.25	6.73 ± 0.25	1.28 ± 0.10	2.85 ± 0.20	2.90 ± 0.10	3.60 ± 0.10	4.57 ± 0.20	5.60 ± 0.30	7.02 ± 0.25	8.03 ± 0.15
SeS & SeR	1.10 ± 0.09	2.20 ± 0.20	2.70 ± 0.15	3.40 ± 0.10	3.90 ± 0.10	4.65 ± 0.27	5.63 ± 0.30	6.70 ± 0.30	1.25 ± 0.20	2.13 ± 0.10	2.85 ± 0.15	3.83 ± 0.15	4.53 ± 0.30	5.57 ± 0.25	6.98 ± 0.30	8.30 ± 0.15
Anova	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.: Non-significant

B. alexandrina



B. glabrata



CS. : Cumulative percentage of susceptible strain .
CR: Cumulative percentage of resistant strain .
CM : Cumulative percentage of mortality rate .

Fig (1): Cumulative susceptibility / resistance and mortality rate percentage in *B. alexandrina or B. glabrata* after miracidial expousure .

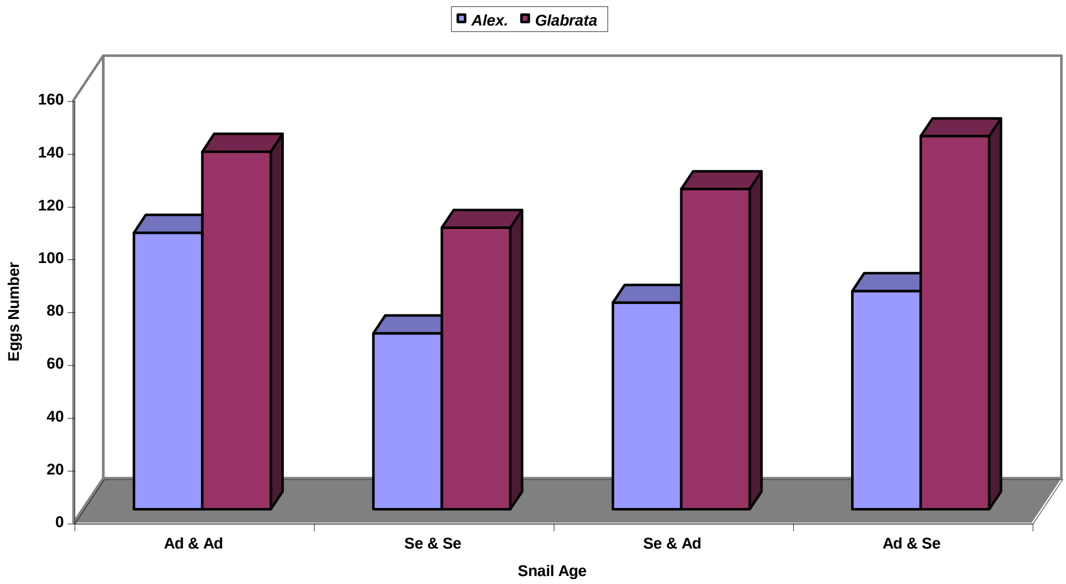


Fig (2): Total egg number produced by cross fertilization between susceptible and resistant strain of both *B. alexandrina* and *B. glabrata* at different ages.

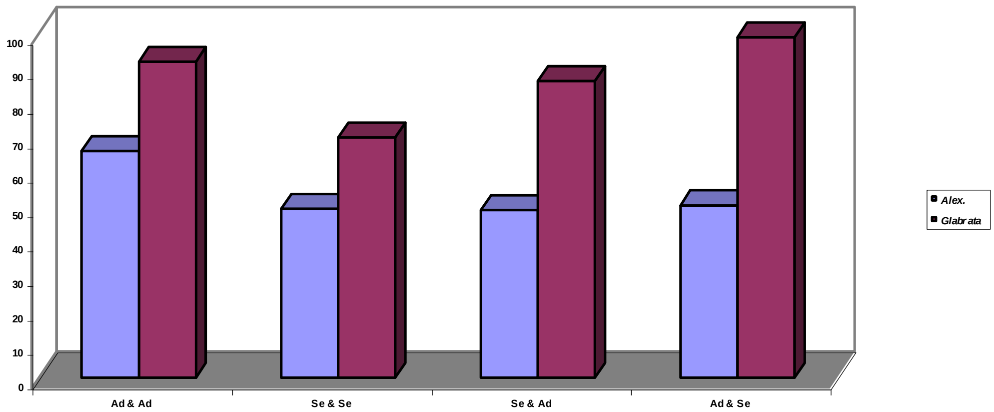


Fig (3): Cumulative number of hatching snails produced by cross fertilization between susceptible and resistant strain of both *B. alexandrina* and *B. glabrata* at different ages .

Discussion

It is well acknowledged that the distribution of schistosomiasis is focal (Sturrock, 1993) , and that transmission only takes place in area where intermediate host snails are present and where the human population is frequently in contact with infected water .

The results of the present study obtained by experimental infection with a high dose of 10 miracidia exhibits 36.7 % and 30.4 % of both *B. alexandrina* and *B. glabrata* are resistant to *S. mansoni* infection comparing with 63.3 % and 69.6 % of their snails which yielded high infection frequencies , respectively . These results confirmed that *B. alexandrina* and *B. glabrata* are important schistosomiasis vector in Egypt . Moreover , the finding of

Yousif *et al*. (1996) gave some support to this suggestion , by reporting that *B. glabrata* forms a new threat for schistosomiasis transmission in Egypt . The collected *B. glabrata* snails from natural water courses and proved them to be susceptible to a local strain of *S. mansoni* with infection rates 52 % versus 75 % for *B. alexandrina* under the same laboratory conditions . In the same respect , Haroun (1996) examined 53.9 – 60 % infection rate of *B. alexandrina* . Similar results were recorded by Spada *et al*. (2002) who examined 63 % of *B. glabrata* snails yielded high infection frequencies in contrast to 37 % of these snails were refractory .

In the current study , the variability in susceptibility / resistance of *Biomphalaria* species to S*chistosoma* infection are in agreement with the results obtained by Souza *et al*. (1995) who demonstrated considerable variations in susceptibility in different *Biomphalaria* species . Such variability can even be observed in snails belonging to the same species , from different geographical areas (Richards , 1984) . Thus, resistance or susceptibility is genetically dependent rather than acquired immunity (Richards , 1976) which may ultimately be related to genetic background (Lemos and Andrade , 2001) .

Although both *B. alexandrina* and *B. glabrata* snails are hermaphroditic and can self-fertilize , the obtained results support the phenomenon that the snails prefer to reproduce by cross-fertilization when paired . These biological characteristics are evolutionary very significant , providing the snail with the ability to establish or re-establish colonies from an individual organism with maintaining the genetic characters through sexual reproduction .

A number of molluscan intermediate host species is hermaphrodite and can self - fertilize but prefer to reproduce by cross-fertilization when paired (Vianey-Liaud and Dussart , 2002 ; Trouve *et al*., 2003) . The present study was further extended to compare the mean frequencies of egg production as reflected by EM , hatching offspring and hatchlings reaching maturity among progeny snail groups of susceptible and resistant *Biomphalaria* snails either by self-or cross- fertilization at different ages .

The present results indicated that the level of egg production in any given group of both susceptible and resistant either selfed or crossed snail stocks at different ages ranged from 216 – 256 egg / month / snail and from 322 – 412 egg / month / snail of both *B. alexandrina* and *B. glabrata*, respectively . Such results agree with the results obtained by Lewis *et al*. (1986) who demonstrated that a single *B. glabrata* snail can produce 200 – 500 eggs / month . They also reported that 50 – 100 egg-laying snails should provide more than enough eggs to meet the demands of a large life cycle production .

In the present study , the patterns of oviposition showed a high significant reduction in the actual reproductive output of susceptible snails , within fewer embryos being produced per week than resistant ones . These results agree with previous results obtained by some authors who demonstrated that , upon infection (generally in susceptible snails) of schistosome with their molluscan hosts , reproduction is reduced but never completely repressed (Schrag and Rollinson , 1994 ; Jong-Brink , 1995 ; Cooper *et al*., 1996 ; Johston *et al* ., 1997) .

During the course of studies on the reproductive biology of *B. alexandrina* and *B. glabrata* , the mean number of eggs produced by cross-fertilization was significantly higher than those corresponding in self-fertilization . Similar results were obtained by Vernon (1995) , who found that , isolated self-fertilizing individuals of *B. glabrata* had reduced reproductive output compared with paired predominantly cross-fertilizing snails. However , the reproductive output of control snails (kept in pairs , but prevented from cross-fertilizing) was similar to that of paired cross-fertilizing snails , suggesting that the low reproductive output of snails in isolation was not simply due to inbreeding depression with self-fertilization .

It has been demonstrated from the present results that , the egg masses produced from an inbreeding of both susceptible of either self or cross-fertilization develop and hatch normally with many embryos per-clutches , but with lower rate than in resistant ones . These results are in agreement with the finding of Etges and Gresso (1965) .

In current study , the number and percentage of newly haching snails and snail reaching maturity per selfing is lower than those of crossing and slightly higher in resistant strain than in susceptible ones . These observations tend to confirm those of Cooper *et al*.(1994) on the effect of self and cross fertilization on the egg laying capacity of molluscan host , *B. glabrata* , as well as De Freitase *et al*. (1997) , who reported that , the production of viable embryos by cross – fertilization was more than by self – fertilization performance .

Maturity was determined by onest of egg laying in our laboratory-bred *Biomphalaria* snails of F1 progeny at shell diameter approximately 5 – 7 mm. , but the growth rate in *B. glabrata* was faster than *B. alexandrina* . This result was supported by Malek (1958) who reported that , *B. glabrata* shell diameter ranged between 15 to 30 mm. .On the other hand , *B. alexandrina* was reported to be maximally 19 mm. in diameter by Meier and Meier – Brook (1981) .

Considering the results by age of snails and its effect on the output of reproductive system , it was shown that egg production is inversely proportional to the snail age and significantly higher in adult parental snails than in senescent ones . Similar results were obtained by Richards and Meritt (1972) which indicated that the onest of egg laying has been observed in *B. glabrata* around 5 mm. in diameter .

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دراسات بيولوجية على قواقع الماء العذب المستهدفة لطفيلى البلهارسيا

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من قسم الكيمياء العلاجية بالمركز القومي للبحوث وقسم علم الحيوان بكلية علوم * بنات الأزهر بالقاهرة

تلعب قواقع الماء العذب دوراً حيوياً في إنتشار مرض البلهارسيا حيث أنها تمثل العائل الوسيط اللازم والضروري لإتمام دورة حياة الطفيلى .ففي مصر ظلت ولفترات زمنية طويلة قواقع البيومفلاريا الكسندرينا تمثل العائل الوسيط لطفيل الشستوسوما مانسونى الى أن تم إكتشاف نوع جديد من القواقع في منطقة الدلتا تم تصنيفه على أنه بيومفلاريا جلابراتا. وفي هذه الدراسة تم تحديد وفصل نوعين من القواقع داخل كل جنس من قواقع البيومفلاريا الكسندرينا وكذلك البيومفلاريا جلابراتا . هذين النوعين هما قواقع قابلة للعدوى وحضانة الطفيلى وأخرى غير قابلة للعدوى ومقاومة لحضانة الطفيلى بهدف إلقاء الضوء على الإختلافات البيولوجية بين نوعى القواقع مثل دراسة معدلات وضع البيض ونسب فقسه وقياس معدلات النمو للقواقع حديثة الفقس بين السلالتين المختلفتين في نوعى القواقع قيد الدراسة وذلك بهدف مقارنة القواقع القابلة للعدوى بتلك المقاومة لها في المراحل العمرية المختلفة . وتشير نتائج البحث في الجيل الأول إلى أن هناك علاقة قوية بين معدلات إنتاج البيض وقابلية القواقع للعدوى . حيث وجد أن معدل إنتاج وضع البيض بين القواقع القابلة للعدوى أقل من معدلاتها في تلك المقاومة لها ، وكذلك لوحظ إنخفاض معدلات وضع البيض بين القواقع ذات التكاثر اللاتزاوجي عند مقارنتها بمثيلتها من القواقع ذات التكاثر التزاوجي . كما ان معدلات وضع البيض في القواقع البالغة كان أعلى من مثيلاتها في القواقع المتقدمة في العمر (المسنة) ، أما من ناحية أعداد ونسب فقس البيض فقد أظهرت النتائج إختلافاً واضحاً بين المجموعات محل الدراسة . و بالنسبة إلى مقارنة معدلات النمو بين القواقع حديثي الفقس فلم يوجد إختلاف ملحوظ بين المجموعات ولكن كان معدل نمو قواقع البيومفلاريا جلابراتا أعلى من معدل نمو قواقع البيومفلاريا الكسندرينا وذلك بداية من الأسبوع الرابع وحتى الأسبوع الثامن.