EFFECT OF SOME ALGAL SPECIES ON THE SNAIL INTERMEDIATE HOSTS OF SCHISTOSOMIASIS IN EGYPT I. SURVIVAL, FECUNDITY AND NET REPRODUCTIVE RATES

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Abstract

Planktonic samples were collected from two habitats in Giza Governorate, Kafr Hakem (+ snails) and Sadek canals (- snails). Oscillatoria accuminata and Lyngbya perelegans were collected from the first location, while Phormidium valderianum, Spirogyra sp and Lyngbya perelegans were separated from the second one. Nostoc muscorum was obtained from Theodor Bilharz Research Institute (TBRI) and used in some experiments as control because it is non toxic. The effect of the previously mentioned algal species on Biomphalaria alexandrina and Bulinus truncatus snails, the intermediate hosts of schistosomiasis in Egypt, was studied. Investigations were carried out on the survivorship (L_x), fecundity (M_x) and reproduction (R_0) of the snails. The obtained results indicate that O. accuminata, Spirogyra sp and L. perelegans were non toxic, while P. valderianum was toxic to the two snail species (sublethal concentration LC_0 was used in the experiments).

Concerning the survivorship, both snail species fed on *O. accuminata* had a higher survival rate than snails fed on other algal species or lettuce. While low survival rate was recorded in both snail species fed on *Spirogyra* sp. Fecundity of snails fed on lettuce (control) showed the highest value, followed by *P. valderianum* and *L. perelegans*, while the lower fecundity value was recorded in snails fed on *O. accuminata*. Concerning the net reproductive rate, snails nourished on lettuce recorded the highest value, while those fed on *L. perelegans* showed the lowest one.

Chromatographic analysis of *P. valderianum* extract (using GC/MS) revealed the presence of toxic and molluscicidaly active components.

Key words: Biomphalaria, Bulinus, cyanobacteria, green algae, schistosomiasis, snails.

Introduction

Schistosomiasis is considered to be an endemic disease in Egypt. The latest WHO report (1999) estimated that 652 million are at risk of infection with

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the five schistosome species infecting humans. About 85% of this number is on the African continent. In Egypt, close to 20 million cases have been reported (Cheng, 1973; Ayad, 1974). This number is certainly increasing with increasing population.

The snail intermediate hosts of this endemic disease in Egypt, *B. alexandrina* and *B. truncatus*, have received great attention because of their essential role in the transmission of schistosomiasis. Thus, the control of the snail host is fundamental in controlling the disease and this is based essentially on the concept of the transmission control, i.e. removal of the vector, thus stopping transmission of the parasite, and the disease would disappear slowly (Davis, 2000).

The different groups of phytoplankton (Chlorophyceae, Cyanophyceae and Bacillariophyceae) are the commonest diet component for the snail vectors which influence its reproduction and population dynamics (Rizk *et al.*, 1999). Cyanobacteria are the principal food source for these snails (Lee *et al.*, 1994; Ferreira *et al.*, 2000). Blooms of cyanobacteria are widely known to produce toxic substances which often cause, not only illness and death of animals (ducks, cows, wildlife, domestic animals) and humans (Dillenberg and Dehnel, 1960; Halderen *et al.*, 1995; Negri *et al.*, 1995; Frazier *et al.*, 1998; Cronberg *et al.*, 1999; Kabzinski *et al.*, 2000), but also have poisoning effects on aquatic organisms including snail vectors (mosquito larvae, copepods, cladocerans and fish (Turell and Middlebrook, 1988; De Bernardi and Giussani, 1990; Gilbert, 1990; Penaloza *et al.*, 1999; Zimba *et al.*, 2001).

Certain algal species exhibit potent molluscicidal activity against the snail vectors of schistosomiasis. Renno (1972) demonstrated that the green alga *Chara vulgaris* induce high snail mortality in aquaria. *Microcystis forlowiana* and *Pseudanabaena forqueti* showed a molluscicidal activity against *Lymnaea* sp causing the death of these snails at high concentrations (Gevery *et al.*, 1976). In addition, Falch *et al.* (1992) and Mohamed *et al.* (1992) reported that the cyanobacteria *Tolypothrix tjipanasenses* and *Oscillatoria agardhii* had a potent molluscicidal activity against *Biomphalaria glabrata* and *Melanoides tuberculata*, respectively.

The toxic effects of cyanobacterial endotoxin from *Oscillatoria agardhii* were investigated using the crude bloom, the crude bloom extract and pure cell free extract of the alga against young and mature snails of *Melanoides tuberculata* (Mohamed *et al.*, 1992). Prolonged exposure of snails to the sublethal concentration of pure cell free extract (1 mg/L) caused 100% mortality, one week after exposure, while young snails tolerated the toxic effect of the three tested extracts.

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This study was performed to investigate the possible influence of four freshwater cyanobacterial species (*O. accuminata, L. perelegans, N. muscorum* and *P. valderianum*) and the green alga *Spirogyra* sp which were separated from habitats where snails are (or not) recorded, on the survivorship, fecundity and reproduction of *B. alexandrina* and *B. truncatus* snails, the intermediate hosts of schistosomiasis in Egypt. This may help in combating the snail vectors in their habitats. Some of the previously mentioned species may be used also as food to maintain the life cycle of the parasite, in order to provide biological material for the different experimental studies.

Material and Methods

Collection, isolation and identification of cyanobacteria

Samples of plankton were collected using plankton nets with mesh diameter of 55 μ m. from two habitats in Giza Governorate, one harboring the snail vectors of schistosomiasis (Kafr Hakem canal) and the other free of these snails (Sadek canal). Samples were then transported to the laboratory.

The mixed algal species in the collected water samples were separated on solid BG₁₁ medium as described by Stainer *et al.* (1971) and Bolch and Blackburn (1996). Unialgal species were obtained by repeated sub-culturing on solid and then liquid BG₁₁ media. Cyanobacterial species were identified according to Desikachary (1959), Bourrelly (1970) and Prescott (1978).

The three isolated cyanobacterial species (*Lyngbya perelegans*, *Oscillatoria accuminata* and *Phormidium valderianum*) and the green alga *Spirogyra* sp were used in the experiments. The non toxic *Nostoc muscorum* was obtained from the Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI), Egypt and used as control.

Preparation of algal inocula

Cyanobacterial species were cultivated on sterilized soil according to the method of Liang *et al.* (1987). About 40 g of wet sterilized soil was placed in the center of a petri dish and stroked to form a smooth mud. Suitable amount of unialgal cyanobacterial species was homogenized and 60 ml of distilled water were added to make a homogenous algal suspension. Aliquots of 0.5 ml of this suspension were placed in the wet sterilized soil. Petri dishes were then incubated under continuous illumination (40 Watt white fluorescent lamp) at temperature of $25^{\circ}C \pm 2$, until algae were grown and ready to be used. After two weeks, the algal mats were washed with sterile distilled water and then used in the experiments.

Maintenance of Snails

The field snails, *B. alexandrina* and *B. truncates*, were kept in aquaria ($35 \times 25 \times 10 \text{ Cm}$) filled with 5 liters of dechlorinated tap water and fed on fresh leaves of lettuce. The water was changed weekly and died snails were removed daily. Lettuce was added twice weekly and removed before decomposition and

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replaced by fresh lettuce. Snails were maintained as such for about one month before use in the experiments.

Determination of the toxic algal species

To investigate algal toxicity within the four isolated algal species on both snail types, algae were used in three forms: fresh, dried and crude extract (sonicated and filtered). A wide range of algal concentrations (50- 10,000 mg/L) were tested on *B. alexandrina* and *B. truncatus* snails. Ten snails of each type were immersed in 1L of each algal concentration for 24 hours (exposure time) then transferred to dechlorinated tap water for 24 hours (recovery time). In controls, the snails were maintained in dechlorinated tap water. Experiments were carried out in triplicates. Percentage of snail mortality was recorded, and accordingly non-toxic or toxic algal species were determined.

Determination of LC_{90} , LC_{50} and LC_{0} of P. valderianum

An additional experiment with the same previous strategy was carried out using concentrations 1000-10,000 mg/L of *P. valderianum*. Determination of the algal concentration which induce the death of 90% and 50% (LC₉₀ & LC₅₀) of snails were established and LC₀ (sub-lethal concentration) was calculated as 1/10 of LC₅₀ which was used in all experiments dealing with the effect of this alga on the different snail vital activities.

Effect of low sublethal concentrations (LC_0) of the toxic P. valderianum and the non toxic species L. perelegans, O. accuminata and Spirogyra sp on the survival, fecundity and net reproductive rates

300 adult laboratories bred *B. alexandrina* (8 - 10 mm in diameter) and 300 *B. truncatus* (8 -10 mm in height) snails were used in this experiment. Each 10 snails were transferred to a plastic aquarium (20 x 9.5 x 7 Cm) containing 1 liter of dechlorinated tap water. The toxic *P. valderianum* LC₀ (sublethal concentration) was added and *N. muscorum* was used as control. In other experiments, 0.5 g of each of the non toxic algal species was added to the aquaria twice weekly and lettuce was used as control. For the collection of egg masses, polyethylene sheets were placed on the surface of each aquarium. Dead snails were removed from the aquaria every day and their number in each aquaria every three days and counted using a light microscope and the water was changed weekly. The total number of survived snails at the beginning of the week and eggs laid by exposed and control snails were calculated and recorded at the end of the week, according to Chaudhry and Morgan (1987), El-Hawary (1990) and Ragab (1996).

The survival rate (L_x) was recorded weekly and calculated by dividing the number of living snails in any week by the original number of snails as a fraction of 1.0 (100% survival).

The fecundity (M_x) (egg laying capacity) expressed as number of eggs/snail/week (E/S/W), was determined by dividing the total number of laid

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eggs every week by the total number of living snails at the beginning of each week.

The net reproductive rate (R_o) is the summation of L_xM_x (the product for each week) of the exposed snails throughout a definite period and was determined according to Bakry and Sharaf El-Din (2000).

Chromatographic analysis

A known weight of lyophilized *P. valderianum* was extracted with 90% methanol, evaporated till dryness and the residue was redissolved in 2 ml of the solvent. Silylation was carried out using Trimethylsilyl-ether and 2 μ l of the extract was injected in gas chromatography / mass spectrometer (GC/MS). Total ion chromatogram as well as the isolated components, at definite retention times, were recorded and their biological activies were determined.

Statistics

All data are means of at least 3 replicates. ANOVA analysis was performed to calculate standard deviation (S.D.) and significance at $P \ge 0.05$, using SPSS version 10.0 for windows (SPSS Inc., 1999). Mortality regression lines were established by probit analysis program according to Lauren-Maatta *et al.* (1995) and Giovanelli *et al.* (2001).

Results

The obtained results from the algal toxicity experiments revealed that all algal species in all forms are not toxic (no snail mortality was recorded), except for *P. valderianum* crude extract which induced snail mortality (percentage of toxicity increased with concentrations). Snails of *B. truncatus* were more sensitive than those of *B. alexandrina*. *L. perelegans*, *O. accuminata* and *Spirogyra* sp were shown to be non-toxic to both snail species. LC_{90} and LC_{50} were determined (Fig. 1) and LC_0 was calculated and used for further experiments.

Survival rate (L_x) of *B. alexandrina* and *B. truncatus* snails are shown in Fig. 1, which indicates that snail species fed on *O. accuminata* had a higher survival rate than snails fed on other types of cyanobacteria, green alga or lettuce. Low survival rate was observed in both snails species fed on *Spirogyra* sp (green alga).

B. alexandrina fed on lettuce (control) showed the highest fecundity value (M_x) (77.2 E/S/W), at the 4th experimental week (Fig. 2A), followed by snails fed on the cyanobacterium, *P. valderianum* (55.4 egg/snail/week), at the 10th week and *L. perelegans* (36.8 E/S/W), at the 9th week, then those fed on *Spirogyra* sp (green alga) (34.9 E/S/W), at the same experimental period. The lowest fecundity value was recorded in snails fed on *N. muscorum* (30.6 E/S/W), at the 8th week, and *O. accuminata* (30.0 E/S/W), at the 10th experimental week (Fig. 2A).

In *B. truncatus* snails, the highest fecundity value (21.1 E/S/W) (Fig. 2B) was observed in snails fed on *L. perelegans*, at the 4^{th} week, followed by snails in

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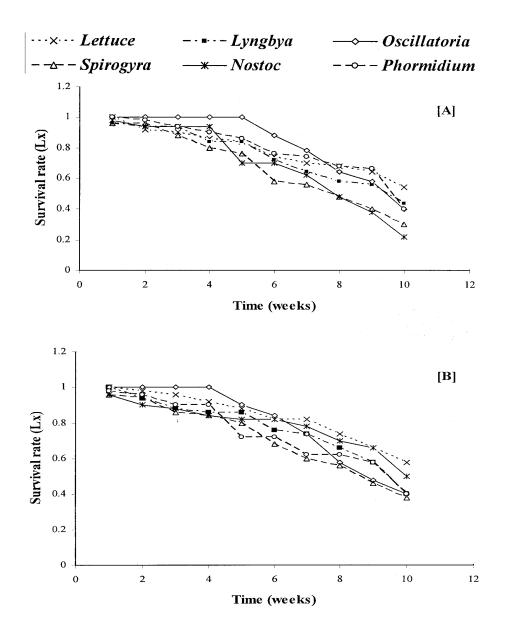


Figure (1): Survivoship of *Biomphalaria alexandrina* [A] and *Bulinus truncatus* [B] snails fed on different algal species for 10 weeks.

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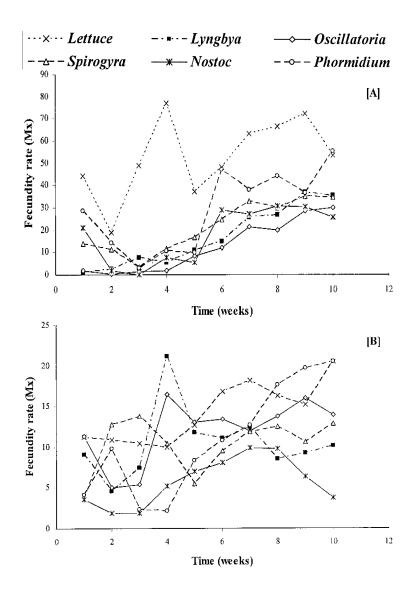


Figure (2): Fecundity of *Biomphalaria alexandrina* [A] and *Bulinus truncatus* [B]snails fed on different algal species for 10 weeks.

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the control group, fed on lettuce, (20.5 E/S/W), at the 10^{th} week and that of *Ph.* valderianum at 10^{th} week (20.0 E/S/W) followed by *O. accuminata* (16.5 E/S/W) at the 4^{th} week. While, the lowest fecundity was recorded in snails fed on *Spirogyra* sp (13.8 E/S/W) at the 3^{rd} week and on *N. muscorum* (9.9 E/S/W) at the 7^{th} week of the experiment.

The highest value of the total number of *B. alexandrina* eggs was observed in snails fed on lettuce (21313 eggs) (Fig. 3A), while the lowest value was recorded in snails fed on *O. accuminata* (4786 eggs). However, the mean number of eggs/egg mass was not significant in the two groups of snails. Meanwhile, snails fed on *P. valderianum* showed moderate number of eggs (11364 eggs), while *Spirogyra* sp or *N. muscorum* showed a lower value of the total number of eggs (6833 and 5871 eggs, respectively).

The total number of *B. truncatus* eggs laid by the control snails fed on lettuce was significantly higher (6026 eggs) than that of snails fed on the other types of food (Fig. 3B). On the other hand, the total number of eggs was higher in snails fed on *O. accuminata* (4940 eggs) when compared to other groups fed on other cyanobacteria species. Meanwhile, the value of the total number of eggs in snails fed on *N. muscorum* was lower (2347 eggs), while, in snails fed on the sublethal concentration (LC₀) of the toxic *P. valderianum*, the total number of eggs did not decrease as much (3484 eggs). Moreover, in snails fed on *Spirogyra* sp (green alga), the total number of eggs (3762 eggs) was higher than that of snails fed on *Nostoc* or *Phormidium*.

The pattern of the net reproductive rate (R_o) of *B. alexandrina* is shown in (Fig. 4A). The highest value was recorded in snails fed on lettuce (403.01), followed by that fed on *P. valderianum* (202.90), but in case of snails fed on *Spirogyra* sp and *L. perelegans* the value of (R_o) was in between (120 and 103.2, respectively). Comparable results was obtained in snails fed on *N. muscorum* (102.1), used as control, while the lowest value was observed in snails fed on *O. accuminata* (82.2).

Regarding the net reproductive rate (R_o) of the control and treated *B*. *truncatus* snails (Fig. 4B), the results revealed that the control fed on lettuce exhibited the highest net reproductive rate (114.93), followed by snails fed on *O*. *accuminata* (91.28). On the other hand, the net reproductive rate decreased in snails fed on *N. muscorum* (44.29) used as control compared to the group fed on *P. valderianum* (69.71).

Chromatographic analysis of *P. valderianum* extract (using GC/MS) revealed the presence of different toxic and molluscicidaly active components (pyridine, pyrazine, octadecenoic and hexadecanoic acids) in the extract (Table 2 and Figs. 5 and 6) which may be the cause of the previously recorded toxic effects (Table 1).

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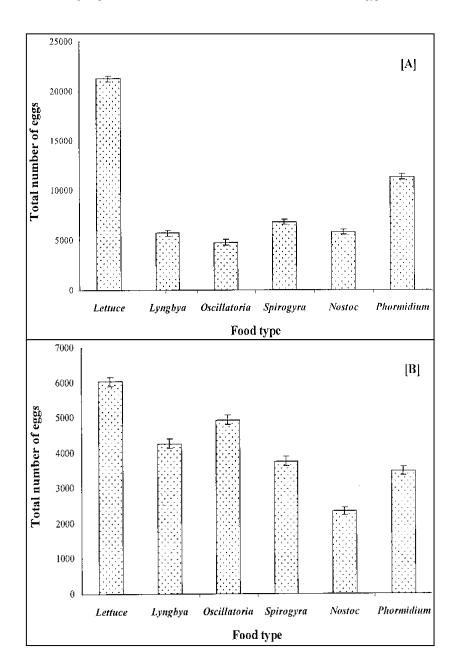


Figure (3): Total number of eggs laid by *Biomphalaria alexandrina* [A] and *Bulinus truncatus* [B] snails fed on different algal species for 10 weeks.

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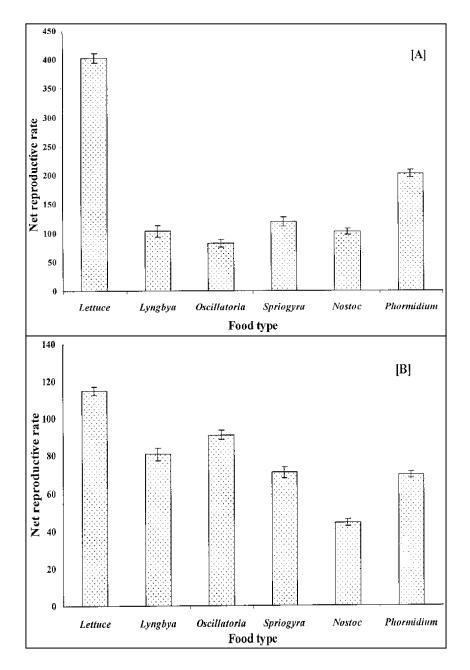


Figure (4): Net reproductive rate of *Biomphalaria alexandrina* [A] and *Bulinus truncatus* [B] snails fed on different algal species for10 weeks.

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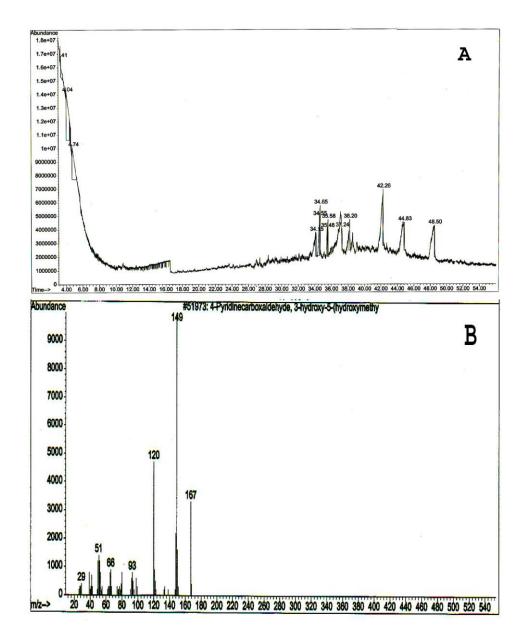


Figure (5): Chromatographic analysis using GC/MS of *Phormidiu valderianum* methanol extract. (A) Total ion chromatogram, (B) Fragmentation pattern of pyridine derivative.

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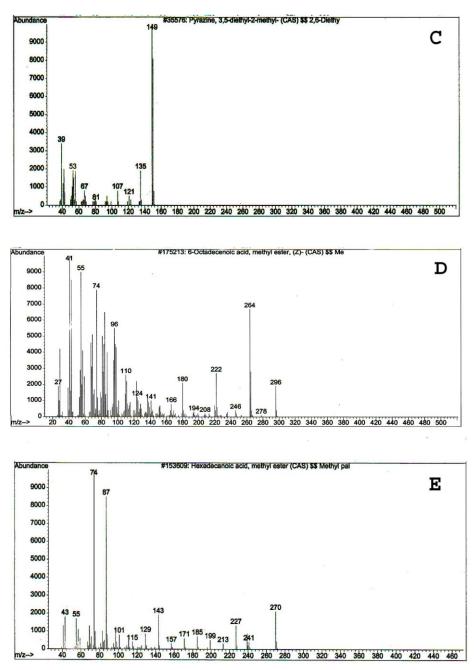


Figure (6): Chromatographic analysis using GC/MS of *Phormidium Valderianum* methanol extract. (C) pyrazine derivative,(D) 6-octadecenoic acid, (E) Hexadecanoic acid.

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Table (1): Determination of LC ₅₀ and LC ₉₀ of <i>Phormidium valderianum</i> on (A)
Biomphalaria alexandrina and (B) Bulinus truncatus snails

	Concentration			
Iortality*	Number of Survived snails	Number of Dead snails	(mg/L)	
0	30	0	500	
0	30	0	1000	
23.3	23	7	3000	
23.3	23	7	4000	
50.0	15	15	5000	
53.3	14	16	6000	
60.0	12	18	7000	
70.0	9	21	8000	
100	0	30	9000	
	I	11	(B)	
	0 Experimental sna	30		

Concentration	Experimental snails			
(mg/L)	Number of Dead snails	Number of Survived snails	% Mortality*	
500	1	29	3.3	
1000	2	28	6.6	
3000	8	22	26.66	
5000	16	14	53.3	
6000	19	11	63.3	
7000	20	10	66.6	
8000	22	8	73.3	
9000	30	0	100	

* % mortality of control snails = 13.3%

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Peak	Retention	Quantity	Separated	Biological	References
No	time	(%)	compounds	Activity*	
1	3.41	3.33	dithiane-carboxylic	Т	Abderrabi et al.
			acid		(1996)
2	4.04	29.76	Crotonic acid	Т	Solovskii et al.
			derivative		(2000)
4	34.15	2.56	Dihydroxy-	Т	Saunders et al.
			cyclopentene		(1985)
5	34.55	9.19	Hexadecanoic acid	Т	Choo et al.
6	34.65	3.65			(2001)
9	37.24	2.65	Pyridine,	T, M	Nawwar <i>et al</i> .
			piperidinyl-ether		(1994) and
					Puder et al.
					(2000)
10	38.20	7.06	Octadecenoic acid	Т	Choo et al
					(2001)
12	44.83	3.96	Pyridine	T, M & A	Fathalla et al.
			carboxaldehyde		(2000) and
			, i i i i i i i i i i i i i i i i i i i		Hirano et al.
					(2000)
13	48.50	2.54	Pyrazine derivative	Т	Dolezal et al.
			-		(2003)

Table (2): Separated biologically active components of Phormidium valderianum methanol extract by GC/MS

*T = toxic effect, M = molluscicidal activity, A = Anthelmintic activity

Discussion

The majority of investigators, studying the effect of food on snails, pointed out that the type of food on which snails are maintained has a great influence on their survival rate, egg production and growth. Ferreira *et al.* (2000) demonstrated that the survival rate as well as the number of generations, per year, of *B. truncatus* snails, fed on *O. formosa,* increased significantly. This agrees with the present result which demonstrates that *B. truncatus* and *B. alexandrina* snails fed on *O. accuminata* had the highest survival rate among snails fed on the different types of food used in this study, allover the course of the experiments. Lettuce, also, supported a good survival rate of both snail species. This is in agreement with the findings of Claugher (1960) who claimed that the most useful food for all freshwater snails is dried lettuce.

In the present work, it was observed that *B. alexandrina* and *B. truncatus* snails had a high egg laying capacity and net reproductive rate when fed on lettuce. This coincides with the findings of El Assal *et al.* (1992) who found that *B. truncatus* snails, fed on lettuce only, showed a higher egg production and a higher life span than snails fed on other types of food. Also, it concurs with the results of Thompson (1984) who demonstrated that fresh lettuce leaves are the most satisfactory food source for maintaining stock colonies of *B. glabrata*. Egyptian J. of Phycol. Vol. **6**, 2005 – 86 –

However, Rizk *et al.* (1999) found that the market food, rabbit pellets, was the most suitable food for the laboratory maintenance of *B. alexandrina* and exhibited an increase in egg production, growth rate, hatchability and survival rates of snails than other foods. Also, Madsen and Frandsen (1980) claimed that the breeding and maintenance of *B. glabrata* and *B. truncatus* snails fed on a diet rich in protein produced about three times more eggs and more clutches per snail than those fed on lettuce only. Similar observations were reported by El-Emam and Madsen (1982) and Belfaiza *et al.* (2004) who stated that egg laying and shedding of cercariae were relatively low in *B. alexandrina, B. truncatus* and *Galba truncatus* snails fed on lettuce only.

The cyanobacteria L. prelegenus, O. accuminata, N. muscorum and P. valderianum have been used as food for B. truncatus and B. alexandrina snails, in the present study. N. muscorum gave the lowest value of total egg number produced by snails and net reproductive rate, when used as food for both snail species. This is in contradiction with the findings of Rizk et al. (1999) who stated that the reproduction of adult B. alexandrina snails fed on N. muscorum was better than that of snails fed on lettuce and confirmed that alga is considered as a useful diet of high nutritional value for snail fecundity. Moreover, O. accuminata and P. valderianum afforded, in this study, a great value for egg laying capacity of snails and net reproductive rate than other species of cyanobacteria. This corresponds with the results of Ferreira et al. (2000) that have shown that B. truncatus snails fed on O. formosa reached sexual maturity earlier and had more egg masses per snail. It, also, agrees with the findings of Thompson (1984) who demonstrated that *B. glabrata* snails maintained on *Spirulina* sp (cyanobacterium) became more reproductively active and laid more eggs during the ten weeks of the experimental period. The present results are, also, in accordance with the results recorded by Ismail and Haroun (2001) who demonstrated that both snail species fed on cyanobacteria, for 16 weeks, had a higher egg laying capacity and survival rate than those fed on other foods (fish food, rat food and dried lettuce leaves).

In the water body, different groups of phytoplankton (Chlorophyceae, Cyanophyceae and Bacillariophyceae) and zooplankton are the main source of food for snail vectors. Algae seem to be the commonest diet component of the snail vector of schistosomiasis.

Certain cyanobacterial species may produce toxic substances (molluscicidal activity) which often cause poisoning or death not only to animals and humans but also to aquatic organisms including snail vectors. Some species of the same genera may exert beneficial effect or have nutritive value for snails while other species have potent molluscicidal effect causing the death of these snails. The overall effect of algal community of a water body depends on the dominant species (which depend on the ecological and seasonal variation) together with the allelopathic effects of other aquatic organisms of the water flora.

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Chromatographic analysis of *P. valderianum* extract by GC/MS separated different components including short (Crotonic acid), median (Nonanoic acid) and long chain saturated (Hexadecanoic acid C_{16} : 0) and unsaturated (Octadecenoic acid C_{18} : 1) fatty acids which have toxic activity (Solovskii *et al*, 2000; Choo *et al*, 2001) together with nitrogen containing organic compounds (Pyridine, Piperidine and Pyrazine) which have demonstrated anthelmintic and moluscicidal activities (Nawwar *et al.*, 1994; Puder *et al.*, 2000; Tarafder *et al.*, 2002; Dolezal *et al.*, 2003).

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تأثير بعض أنواع الطحالب على القواقع الناقلة لمرض البلهارسيا في مصر ا- معدلات الابقاء على الحياة، القدرة على وضع البيض و التكاثر

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يشكل مرض البلهار سبا مشكلة اقتصادية هامة في مصر، و ينتقل هذا المرض عن طريق العوائل الوسيطة لهذا الطفيل و هي قواقع بيومفلاريا الكسندرينا و بولينس ترنكاتس.

تم جمع العينات الطحلَّبية المستخدمة في هذا البحث من قناتين في محافظة الجيزة إحداهما بها. القواقع الناقلة لمرض البلهارسيا و هي قناة كفر حكيم و الثانية قناة صادق و هي خالية من القواقع.

تم فصل طحلبي الاوسيلاتوريا اكيوميناتا و اللنبيا بريليجنس من قناة كفر حكيم بينما تم فصل طحالب الفورميديم فالدريانم واللنبيا بريليجنس والاسبير وجيرا من قناة صادق ويهدف هذا البحث إلى دراسة تأثير هذه الطحالب على نوعى القواقع نـاقلي مرض البلهارسيا من حيث ابقائهما على قيد الحياة و معدل و ضع البيض و التكاثر فيهما.

تمّت در اسة تأثير الأنواع الطحلبيه المعزولة على نوعي القواقع في صورتها الطبيعية و الجافة و في صورة مستخلص. كما تم تعيين الأنواع الغير سامه (الاوسيلاتوريا و اللنبيا و الاسبيروجيرا) و الأنواع السامه (الفورميديم) و عين التركيز تحت المميت منه LC و تم استخدامه في التجارب، و استخدمت أوراق الخس و طحلب النوستك في المجموعات الضابطه.

أثبتت النتائج أن نوعى القواقع التي تغذت على الاوسيلاتوريا اكيوميناتا أظهرا أعلى معدل في البقاء على الحياة عن القواقع التي تغذت على الانواع الطحلبية الاخرى و كانت أقل قيمة لمعدل البقاء على الحياة في نوعي القواقع التي تغذت على السبير وجيرًا كما أظهرت النتائج ان أعلى معدل للتكاثر يوجد في القواقع التي تغذت على الخس بينما أقل معدل كان في القواقع التي تغذت على الاوسيلاتوريا (في حالة البيومفلاريا الكسندرينا) و على اللنبيا (في حالة البولينس ترنكاتس).

اظهر التحليل الكروماتوجر أفي لمستخلص طحلب الفورميديم فالدريانم باستخدام (GC/MS) وجود مكونات ذات نشاط سام و مضاد للقو اقع

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