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Biological and biochemical impacts of the fungal extract of *Aspergillus fumigatus* on *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni*

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ABSTRACT

Effect of the fungal extract of *Aspergillus fumigatus* was evaluated against non-infected and *Schistosoma mansoni*-infected *Biomphalaria alexandrina* snails as well as it's efficacy against the free larval stages of *S.mansoni*. The fungal extract of *A. fumigatus* has molluscicidal and larvicidal activities against *S. mansoni* larvae (miracidia and cercariae). The results revealed that the tested sub-lethal concentrations reduced the survival, growth rates and egg laying capacity of both non-infected and *S. mansoni*-infected snails.The tested concentrations of the fungal extract of *A. fumigatus* entirely stopped egg hatching of one, three and six days ages. Exposing *B. alexandrina* snails to sub-lethal concentrations of the fungal extract for 24 hours either pre-, during or post exposure of snails to *S. mansoni* miracidia caused a marked reduction in the infection rate and decreased the mean total number of shedding cercariae/snail. Protein analysis showed qualitative and quantitative differences in protein profile with differences in the similarity coefficient "S" values. The quantitative analysis by HPTLC of free amino acids in ovotestis and hemolymph of non-infected and infected snails treated with fungal extract showed significant differences in the density of most free amino acids. Finally, it can be concluded that the fungal extract has high molluscicidal activity against *B. alexandrina* snails as they affect the snail's life, biological and physiological activities.

KEYWORDS

Biomphalaria snails;
Biocontrol agents;
Aspergillus fumigatus;
Free amino acids;
SDS-PAGE.

INTRODUCTION

Schistosomiasis remains one of the most prevalent parasitic infections in the world. It has been estimated that more than 200 million people in 76 countries are infected and approximately 500 - 600 million people at risk of infection[8]. Snail's control is an essential part in the combat against schistosomiasis and the biological control of snail populations offers an expensive and environmentally acceptable alternative to chemical mol-

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luscicides[2]. It was found that 30 to 40% of the known fungi are capable of producing toxic products with varying degrees of gravity, such as the toxin produced by fungi: *Aspergillus clavatus, A.fumigatus, A.giganteus, A.terreus, Penicillium expansum, P. terreus, Penicillium expansum, P.urticae, P. urticae, P.griseofulvum ægriseofulvum* and others[6]. Mycotoxin is a naturally toxic secondary metabolite produced by an organism of the fungus kingdom. Aflatoxins: are a type of mycotoxin produced by many species of *A.spergillus*, most notably *A.flavus, A. parasiticus* and *A. fumigatus*[24].

This study aimed to evaluate the effect of the fungal extract of *A. fumigatus* against non-infected and *S. mansoni*-infected *Biomphalaria alexandrina* snails as well as it's efficancy against the free larval stages of *S.mansoni*, total protein and free amino acids.

MATERIALS AND METHODS

Experimental animals

Experimental animals used in the present study were *Biomphalaria alexandrina* snails; *Schistosoma mansoni* miracidia and cercariae and male white albino mice (CD1) brought from Schistosomiasis Biological Supply Center (SBSC) Theodor Bilharz Research Institute, Giza, Egypt. The experimental snails were maintained under experimental laboratory conditions (25± 2ºC) according to the method described by[7] .

The fungal extract

The fungal extract of *Aspergillus fumigatus* (320 ppm/ml). It was prepared according to Ragab and Ismail[34]. A series of concentrations that would allow the computation of LC_{50} and LC_{90} values were prepared, while the sub-lethal concentration (LC_o) was calculated as it equals 1/10 LC_{50} [37].

Experimental infection

Mice infection

CD1 mice were individually exposed to 80-100 freshly emerged *S. mansoni* cercariae by paddling method, in dechlorinated tap water for 1-2 hrs.

Snail infection

Infected mice 6-8 weeks post infections were dissected, the infected livers and intestines were homogenized and eggs were extracted washed in saline. *S. mansoni* miracidia hatched under illumination from the isolated eggs[7]. *B. alexandrina* snails were individually infected each with 4-5 miracidia in glass test tubes filled with 1 ml dechlorinated tap water for 2 hours.

Cercaricidal and miracidicidal effect of the fungal extract of *A. fumigatus*

Twenty five ml of dechlorinated tap water containing 100 freshly hatched miracidia or cercariae were mixed with 25 ml double concentrations of LC_o, LC_{10}, LC_{25}, LC_{50} & LC_{90} values of the fungal extract. Fifty ml of dechlorinated water containing 100 freshly hatched miracidia were used as a control[37]. During treatment period, microscopical observations on the movement and mortality of the miracidia and cercariae were recorded at time intervals of 1/4, 1/2, 3/4, 1, 2, 3, 4, 5 & 6 hrs.

Prolonged exposure of snails to sub-lethal concentrations (LC_o, LC_{10} and LC_{25}) of the fungal extract of *A.fumigatus*

Sets of 120 mature *B. alexandrina* snails with 8-10 mm shell diameter were divided into four groups. The 1st one was kept as non-treated and non-infected group (control). The 2nd group was treated with the fungal extract, whereas the 3rd group was exposed to *S. mansoni* infection (control infected). The 4th group was exposed to both the fungal extract and *S. mansoni* infection (treated-infected). Snails were maintained in 1000 ml of the experimental concentration in two-liter capacity plastic containers. For 12 weeks the concentrations were changed with freshly prepared ones every week. Fresh lettuce leaves were provided as the daily food. Observations were recorded weekly for mortality, number of egg masses laid and the shell diameter (growth rate).

Effect on hatchability

For studying the effect of the fungal extract of *A.fumigatus* on the hatchability of *B.alexandrina* eggs, three replicates of egg masses, each of about 60 eggs of one, three and six days old were used. Egg masses were obtained from healthy *B. alexandrina* snails, which were laid on foam pieces, maintained in the laboratory. Egg masses were continuously exposed to 100 ml of LC_o, LC_{10}, LC_{25}, LC_{50} & LC_{90} concentrations of the

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fungal extract in Petri dishes until hatching. Another group of about 60 eggs was maintained in dechlorinated tap water as a control. Egg masses were examined daily during the experimental period under a steromicroscope and the number of normal viable eggs and hatched embryos were recorded[29]. At the end of the experiment, the percentage of hatchability was calculated.

Effect on infectivity

B. alexandrina snails were exposed to sub-lethal concentrations (LC_o, LC_{10} & LC_{25}) of the fungal extract of *A. fumigatus* for 24 hours either pre-, during or post exposure of snails to *S. mansoni* miracidia[3]. Snail exposure to miracidia was carried out in mass, i.e. for each experimental concentration three replicates, each of 10 snails/ L in glass container, were exposed to miracidia freshly hatched from ova at a dose of 10 miracidia/snail, either with or without the experimental concentrations. Another group untreated with the tested concentrations, but exposed to miracidia was maintained as a control. After 25 days of miracidial exposure, surviving snails were individualy examined for cercarial shedding in multi-dishes under artificial light for 1 hr and 2 ml dechlorinated water /snail to stimulate cercarial shedding, the mean number of cercariae, the incubation period (prepatent period), duration of cercarial shedding (patent period) and the infection were calculated for each snail.

Biochemical analysis

At the 4th week post exposure to the sub-lethal concentration LC_{10} value of the fungal extract of *A. fumigatus* (0.16 ppm), total protein of non-infected and *S. mansoni* infected *B. alexandrina* snails was determined using Sodium Dodecyle Sulfate–Polyacrylamide Gel Electrophorsis (SDS-PAGE) which was performed under reducing conditions according to the protocol of Boswell *et al*.[9]. Hemolymph was collected from the four experimental groups.

Free amino acid was determined using High Performance Thin Layer Chromatography (HPTLC) that was performed according to the protocol of Das and Sawant[11].

Statistical analysis

Student t-test was carried out to determine the significance between control and experimental groups.

RESULTS

Molluscicidal activity of the fungal extract of *A. fumigatus* against adult *B.alexandrina* snails is presented in TABLE 1. The data revealed that the LC_{50} and LC_{90} values were 0.56 and 0.96 ppm, respectively after 24hrs.

TABLE 1 : Molluscicidal activity of the fungal extract of *A. fumigatus* on adult *Biomphalaria alexandrina* after 24 hours of exposure.

	LC_{50} (ppm)	LC_{90} (ppm)	Slope function	Sublethal Concentrations (ppm)		
				LC_o	LC_{10}	LC_{25}
The fungal extract of *A.fumigatus*	0.56	0.96	1.99	0.056	0.16	0.35

The results also showed that the fungal extract displayed a larvicidal activity against *S. mansoni* miracidia and cercariae as shown in TABLES (2 and 3).

TABLE 2 : Miracidicidal effect of the fungal extract of *A.fumigatus* on *S.mansoni* miracidia.

Concentrations (ppm)	%Mortality of miracidia after the following intervals (hours)								
	1/4	1/2	3/4	1	2	3	4	5	6
LC_o(0.056)	2	15	19	24	27	54	68	88	100
LC_{10}(0.16)	18	20	22	25	29	62	82	94	100
LC_{25}(0.35)	25	27	28	30	34	81	96	100	100
LC_{50}(0.56)	28	34	38	43	50	89	98	100	100
LC_{90}(0.96)	36	39	41	48	53	96	100	100	100
Control	0	0	0	0	0	0	2	4	5

TABLE 3 : Cercaricidal effect of the fungal extract of *A.fumigatus* on *S.mansoni* cercariae.

Concentrations (ppm)	%Mortality of cercariae after the following intervals (hours)								
	1/4	1/2	3/4	1	2	3	4	5	6
LC_o(0.056)	2	15	19	24	27	54	68	88	100
LC_{10}(0.16)	18	20	22	25	29	62	82	94	100
LC_{25}(0.35)	25	27	28	30	34	81	96	100	100
LC_{50}(0.56)	28	34	38	43	50	89	98	100	100
LC_{90}(0.96)	36	39	41	48	53	96	100	100	100
Control	0	0	0	0	0	0	2	4	5

The miracidia are more sensitive towards the toxic action of the tested agent than cercariae during 6 hours of exposure and the mortality percent of miracidia and cercariae is directly proportional to the time and the tested

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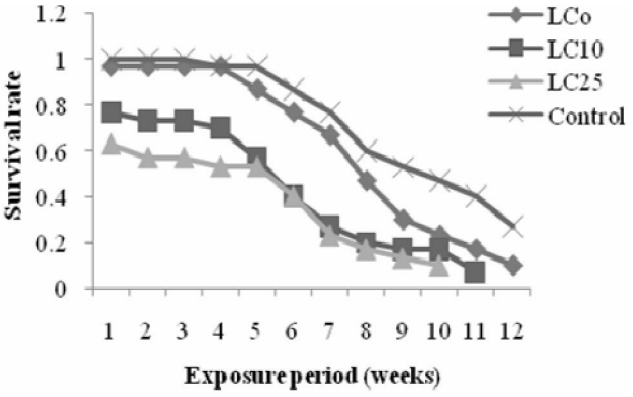
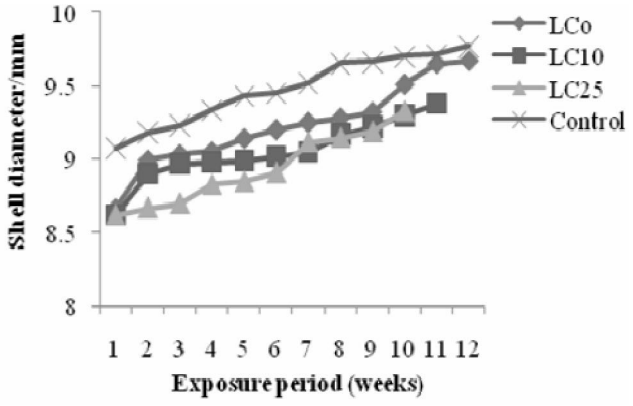
concentrations.

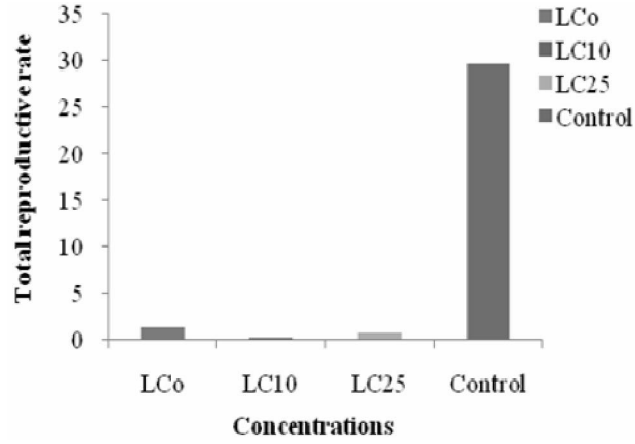
Figures (1 and 2) illustrate the effect of prolonged exposure of snails to sub-lethal concentrations (LC_o, LC_{10} and LC_{25}) of the fungal extract of *A.fumigatus*.



(A) Survival rate



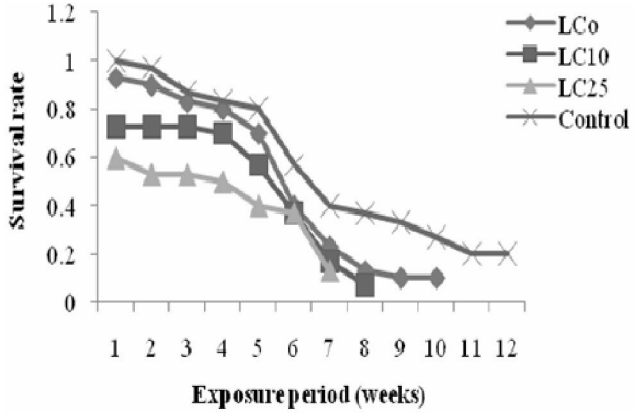
(B) Growth rate



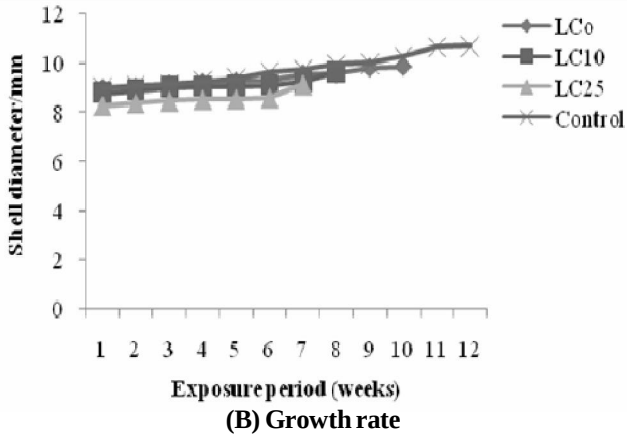
(C) Reproductive rate

Figure 1 : Effect of sub-lethal concentrations of the fungal extract of *A. fumigatus* on survival rate (A), growth rate (B) and reproductive rate (C) of adult non-infected *B. alexandrina* snails.

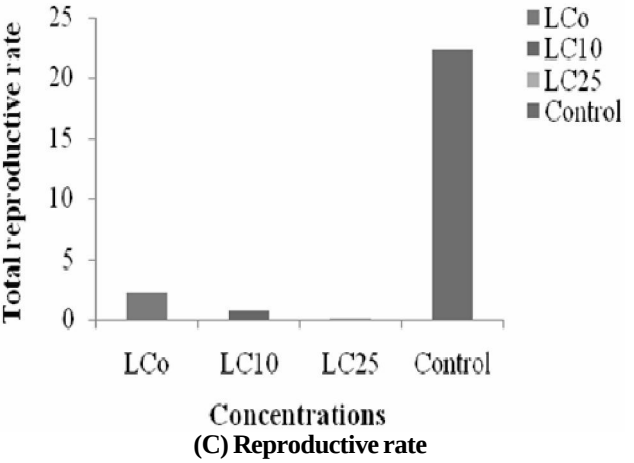
The obtained results showed a significant decrease on survival rate, growth rate and egg laying capacity in both non-infected and *S. mansoni*-infected snails during 12



(A) Survival rate



(B) Growth rate



(C) Reproductive rate

Figure 2 : Effect of sub-lethal concentrations of the fungal extract of *A. fumigatus* on survival rate (A), growth rate (B) and reproductive rate (C) of adult *S. mansoni* infected *B. alexandrina* snails.

weeks of exposure compared to untreated control group.

Regarding to the hatchability of *B. alexandrina* eggs, the obtained results indicated that the concentrations (LC_o, LC_{10}, LC_{25}, LC_{50} and LC_{90}) of the fungal extract of *A. fumigatus* entirely stopped egg hatching of one, three and six days old, except eggs at three

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TABLE 4 : Effect of the fungal extract of *A. fumigatus* on hatchability of *B. alexandrina* eggs of different ages.

Conc. (ppm)	% of hatchability of *B.alexandrina* eggs		
	1 day old	3 days old	6 days old
LC_o (0.056)	0	10.34	9.02
LC_{10} (0.16)	0	0	0
LC_{25} (0.35)	0	0	0
LC_{50} (0.56)	0	0	0
LC_{90} (0.96)	0	0	0
Control	95.45	96.36	96.89

and six days ages exposed to LC_o, their hatchability percent were 10.34 and 9.02% compared to 96.36 and 96.89% for control groups, respectively (TABLE 4).

The results illustrated in (TABLE 5) revealed that the treatment of infected *B. alexandrina* snails with sublethal concentrations (LC_o, LC_{10} & LC_{25}) of the fungal extract of *A. fumigatus* for 24 hours either pre-, during or post exposure of snails to *S. mansoni* miracidia caused a marked reduction in the infection rate and the mean total number of shedding cercariae/snail. Also, elongated their prepatent period (cercarial incubation period) and shortened the duration of cercarial shedding in comparison with their control group.

The total ovotestis protein patterns of non-infected and infected *B. alexandrina* snails treated with LC_{10} value of the fungal extract of *A.fumigatus* (0.16 ppm) was studied by using SDS-PAGE at the 4th week post exposure. The results illustrated in Figure 3 and TABLE 6 showed qualitative and quantitative differences in protein profile with differences in the similarity coefficient "S" values between treated, and control snail groups. Electrophoretic analysis of *B.alexandrina* snail's ovotestis homogenate revealed 13 protein fractions of molecular masses between12.804 –230.95 kDa in fungal extract treated snails and 9 bands between17.38 – 179.3 kDa in control ones. It was also noticed that appearance of certain bands may occur as a result of fungal extract treatment. The bands of 230.95, 156.28, 124.65, 77.028, 73.712, 52.465, 22.246 and 12.804 kDa appeared in treated non-infected snails compared to the control group. The "S" value was (0.455), as the treated non-infected snails shared 5 protien bands of 97.4, 85.356, 65.527, 36.22 and 17.836 kDa with the non-infected control group.

SDS-PAGE analysis of protein profile in the ovotestis of *B. alexandrina* snails treated with fungal extract and infected with *S. mansoni* produce 12 complex patterns of polypeptides ranging in molecular weight between 15.621 - 230.95 compared to 9 protein bands between 18.248 - 191.47 kDa in control ones (Figure 3 and TABLE 6). The results indicated quantitative differences between fungal extract treated-infected and control-infected groups. The treated-infected snails had

TABLE 5 : Effect of sublethal concentrations of the fungal extract of *A.fumigatus* on infectivity of *S.mansoni* miracidia to *B.alexandrina* snails.

Treatment related to miracidial exposure	Concentration (ppm)	Total Exposed snails	No. of alive snails	No. of shedding snails	Infection rate (%)	Prepatent period (day)	Duration of shedding (day)	Mean no. of cercariae/snail
One day pre-exposure	LC_o (0.056)	30	15	13	86.67*	32.32***±0.30	29.00***±3.80	599.80*±182.51
	LC_{10} (0.16)	30	18	14	77.78***	35.00*±5.33	29.20***±4.41	423.98***±161.22
	LC_{25} (0.35)	30	13	8	61.54***	34.35***±0.92	21.91***±1.23	354.31***±191.68
	Control	30	24	23	95.83	32.0±0.00	37.00±0.91	795.70±379.91
During exposure	LC_o (0.056)	30	25	25	100	28.00±0.00	14.00***±7.00	488.44±263.08
	LC_{10} (0.16)	30	24	22	91.67**	28.67±2.08	10.50***±4.95	283.50***±99.78
	LC_{25} (0.35)	30	17	14	82.35***	29.33**±1.53	10.50***±4.95	145.30***±108.13
	Control	30	16	16	100	28.0±0.00	42.40±0.41	635.00±345.92
One day post-exposure	LC_o (0.056)	30	20	13	65***	37.00±5.71	24.91***±2.52	494.12±149.45
	LC_{10} (0.16)	30	17	10	58.82***	40.00***±0.90	22.82***±3.50	311.80***±149.83
	LC_{25} (0.35)	30	21	11	52.38***	42.41***±0.40	19.70***±2.23	316.54***±35.64
	Control	30	12	12	100	36.82±0.95	46.22±4.73	601.00±163.19

Significant difference compared to control group at $p < 0.05$,:Non Significant ($p > 0.05$), *:Significant ($p < 0.05$), **:Highly Significant ($p < 0.01$), *: More highly Significant ($p < 0.001$).**

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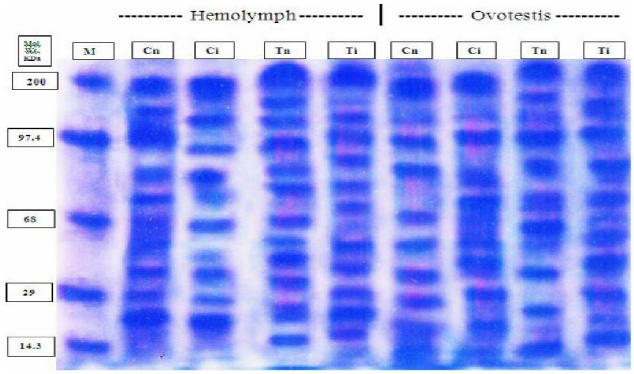

M: marker; Cn: control non-infected; Ci: control infected
Tn: treated non-infected; Ti: treated infected

Figure 3 : Electrophoretic patterns of hemolymph and ovotestis protein extracted from non–infected and *S.mansoni* infected *B.alexandrina* snails treated with sub-lethal concentration of the fungal extract of *A.fumigatus* at the 4th week post.

10 bands 230.95, 153.1, 127.24, 101.49, 86.617, 80.493, 74.801, 68.5, 54.445 and 15.621 kDa if compared to the control ones. It was also found that the 42.792 and 27.746 kDa in treated - infected snails shared with the bands of infected control group and the "S" value between the two groups was 0.190.

Representative SDS-PAGE profile of *B. alexandrina* hemolymph protein treated with fungal extract was illustrated in Figure 3. The results demonstrated that the fungal extract treatment led to quantitative differences in total protein patterns as shown in TABLE 7. Electrophoresis of hemolymph protein gave up to 13 complex patterns of polypeptide ranging in molecular weight between15.28 –235.75 kDa in fungal extract treated snails and 10 bands between 19.89 –188.36 kDa in control snails. SDS-PAGE analysis

TABLE 6 : Electrophoretic analysis of ovotestis protein extracted from non–infected and *S. mansoni* infected *B. alexandrina* snails treated with sub-lethal concentration of the fungal extract of *A. fumigatus* at the 4th week post exposure.

Band	Marker		Control snails				Fungal extract-treated snails			
			Non -infected		Infected with *S.mansoni*		Non -infected		Infected with *S.mansoni*	
	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%
1							230.95	11.08	230.95	7.164
2	200	19.2								
3					191.47	10.549				
4			179.3	10.05						
5							156.28	5.26		
6									153.1	6.274
7									127.24	7.39
8							124.65	6.89		
9					121.13	9.14				
10			118.5	7.939						
11									101.49	19.40
12	97.4	12.85	96.06	9.285	97.4	16.585	97.4	5.242		
13			84.83	16.6			85.356	6.183	86.617	8.461
14									80.493	4.17
15					78.078	8.21	77.028	13.3		
16							73.712	4.159	74.801	5.4
17					71.865	16.389				
18	68	18.8							68.5	4.54
19			66.72	10.8			65.527	5.084		
20									54.445	9.04
21							52.465	18.5		
22			49.28	11.7	50.224	6.27				
23					41.559	20.004			42.792	9.11
24			35.72	11.2			36.22	9.19		
25	29	22.62								

(TABLE 6 continue)

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Band	Marker		Control snails				Fungal extract-treated snails			
			Non -infected		Infected with *S.mansoni*		Non -infected		Infected with *S.mansoni*	
	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%
26					27.62	4.9			27.746	10.285
27			25.67	9.82						
28							22.246	4.2		
29			17.38	12.38	18.248	7.94	17.836	4.82		
30	14.3	26.5							15.621	9.271
31							12.804	5.824		
No. of bands			9		9		13		12	
Similarity index					0.333		0.455		0.190	

showed the presence of several bands in the group treated with LC_{10} value of fungal extract snail's hemolymph in comparison to control snail's hemolymph. The treated non-infected snails had 10 protien bands of 235.75, 156.28, 122.11, 77.595, 68.5, 53.446, 43.592, 24.844, 22.246 and 15.28 kDa. The fungal extract treated non-infected snails shared the protein bands of MWs 95.281, 85.356 and 34.262 kDa with the control and the similarity index was (0.261) between the two groups.

Electrophoretic analysis of hemolymph protein from fungal extract treated-infected snails revealed 13 complex patterns of polypeptides ranging in molecular weight between 16.692 -221.65kDa in treated-infected and 10 bands between 18.532- 180.98 kDa in control-infected group (Figure 3). The results in TABLE 7 indicated quantitative differences between treated-infected and infected group. The "S" value was 0.087 and the only 65.527 kDa protein band in treated-infected snails shared with control- infected ones. It was also noticed that the other 12 bands presented in fungal extract treated-infected snail's hemolymph not found in control- infected group.

The identified amino acids of ovotestis and hemolymph of *B.alexandrina* snails non-infected; infected with *S. mansoni* and treated with the fungal extract of *A.fumigatus* at the 4th week post exposure are namely Aspartic, Glutamic, Serine, Glycine, Histidine, Arginin, Theronine, Alanine, Proline, Tyrosine, Valine, Methionine, Cystine, Isoleucine, Leucine, Phenylalanine and Lysine amino acids, on the basis of the migration of standard 17 amino acids. The results of the qualitative and quantitative analysis for the 17 amino acids in the ovotestis and hemolymph of *B. alexandrina* snails groups are shown in (Figures 4A & B and 5A & B).

The quantitative analysis of amino acids in ovotestis of fungal extract-treated non-infected *B. alexandrina* snails showed a significant decrease in density of Glutamic acid, Leucine, Lysine, Alanine, Cystine, Histidine, Proline and Valine. Fungal extract treatment and *S. mansoni* infection caused a significant decrease in density of Aspartic acid, Tyrosine, Isoleucine, Leucine, Lysine, Methionine and Proline in comparison with their control group.

TABLE 7 : Electrophoretic analysis of hemolymph protein extracted from non–infected and *S. mansoni* infected *B.alexandrina* snails treated with sub-lethal concentration of the fungal extract of *A.fumigatus* at the 4th week post exposure.

Band	Marker		Control snails				Fungal extract-treated snails			
			Non -infected		Infected with *S. mansoni*		Non -infected		Infected with *S. mansoni*	
	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%
1							235.75	12.37		
2	200	19.2								
3									221.65	10.28
4			188.36	4.87						
5					180.98	9.741				
6							156.28	5.32	156.28	3.254
7			139.57	12.57						
8									132.58	6.96

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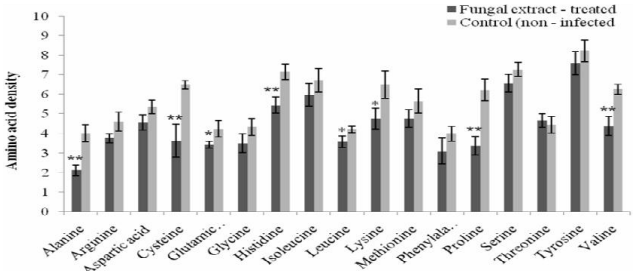
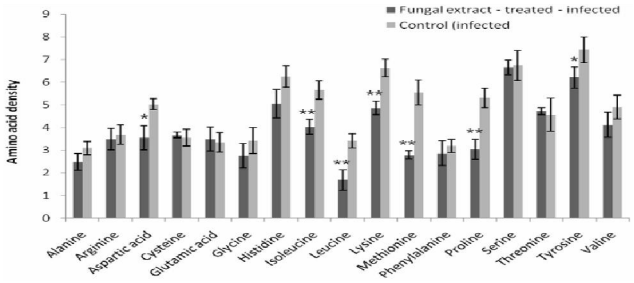
Band	Marker		Control snails				Fungal extract-treated snails			
			Non -infected		Infected with *S. mansoni*		Non -infected		Infected with *S. mansoni*	
	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%	Mol. Wt.	%
9					121.35	9.36	122.11	9.55		
10	97.4	12.85	96.01	5.13			95.281	4.258	96.688	6.89
11					92.622	4.91				
12									89.852	6.211
13			83.157	23.087	84.36	14.4	85.356	5.57		
14							77.595	14.7	78.7411	5.99
15			74.659	12.14	75.74	11.28				
16									71.582	4.284
17	68	18.8					68.5	3.651		
18			64.244	8.69	64.244	9.84			65.527	12.35
19							53.446	14		
20			51.185	10.5					51.50	5.241
21					45.687	9.72				
22							43.592	5.674		
23									40.478	7.28
24					37.095	10.092				
25			35.717	6.84			34.262	8.88		
26	29	22.62	29	10.8					30.657	14.2
27					26.391	6.35				
28							24.844	9.11		
29							22.246	4.259	22.246	10.26
30			19.89	5.38	18.532	14.28				
31	14.3	26.5					15.28	3.156	16.692	6.78
No. of bands			10		10		13		13	
Similarity index					0.40		0.261		0.087	



(A) Non-infected snails



(B) Infected snails

Figure 4 : Effect of sub-lethal concentration of the fungal extract of *A. fumigatus* on free amino acids in the ovotestis of non-infected (A) and *S.mansoni* infected (B) *B.alexandrina* snails at the 4th week post exposure.

Hemolymph of treated non-infected snails revealed a significant decrease in the density of Alanine, while the other amino acids were non-significantly decreased except Glycine level showed non- significant increase ($p>0.05$) if compared to the control group. Treated-infected group showed a significant decrease in density of Alanine, Histidine, Phenylalanine and Proline when compared to control group.

The present study provides information on the biological and physiological responses of *B. alexandrina* snails exposed to abiotic stressor (the fungal extract *A.fumigatus*) accompanied with a biotic stressor (*S.mansoni*). So, further investigations are required to study for the effective control programs of schistosomiasis under field conditions.

DISSCUSSION

The fungal extract of *A. fumigatus* showed high molluscicidal effect against *B. alexandrina* snails and

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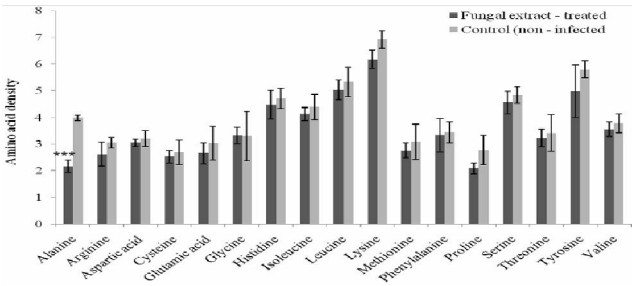
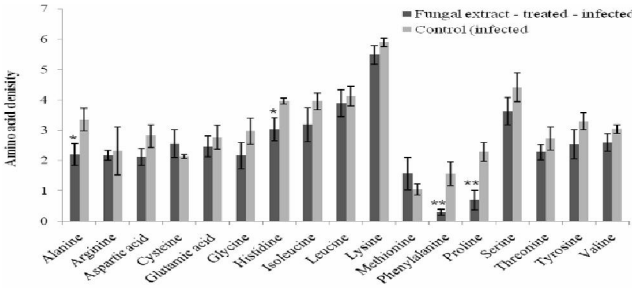


(A) Non-infected snails



(B) Infected snails

Figure 5 : Effect of sub-lethal concentration of the fungal extract of *A. fumigatus* on free amino acids in the hemolymph of non-infected (A) and *S.mansoni* infected (B) *B.alexandrina* snails at the 4th week post exposure.

Significant difference compared to control group at $p < 0.05$, :Non Significant ($p > 0.05$), *:Significant ($p < 0.05$), **:Highly Significant ($p < 0.01$), *: More highly Significant ($p < 0.001$).**

this toxic effect of the fungal extract may be due to the toxins produced by the fungus *A.fumigatus* in its culture filterate which had lethal effect.

Results of the current study also revealed that the miracidial mortalities are greater than that of cercariae after the same time intervals. This observation is in agreement with the study of Mahmoud[23] on Kelthane that killed miracidia faster than cercariae. Also, Ibrahim *et al*.[20] showed that miracidial mortality were greater than that of cercariae during application of Hinsan and the plant *T.terrestris* after the same time intervals.

The fungal extract showed a significant reduction on the survival rate, growth rate and egg laying capacity of both adult non-infected and *S. mansoni*-infected *B.alexandrina* snails. Such reduction of snail's survival and fecundity may arise as a result of the action of the tested fungal extract upon the steroid hormones, the harmful effect on the male and female genital tract, or may arise from metabolic disorders as has been described by Mohamed *et al*.[27] who tested the efficacy of low concentrations of some organometallic compounds that may alter the reproduction of *B. alexandrina* snails as a result of reduction of the growth of the male and female organs of the genital tract and endocrine disruption, which reduce or stop their oviposition. Ragab and Ismail[34] found that the growth and survival rates, egg laying capacity of *B. alexandrina* snails exposed to sublethal concentrations of the free cell extract of some fungal strains namely *Trichoderma viride*, *Saccharomyces cerevisiae*, *Coriolus versicolor* and *Phanerochaete chrysosporium* were decreased in comparison with that of their control ones. Ibrahim[19] stated that, at 4th week post infection, additional demonds consume the energy of the *B. alexandrina* snails, i.e. cercarial emergence, thus leaving low amounts for survival, growth, detoxification and reproduction.

The current study also revealed that the tested material entirely stopped *B. alexandrina* egg hatching of one, three and six days ages. Several authors recorded a similar harmful and remarkable reduction in hatchability of *B. alexandrina* eggs treated with different molluscicides[1,14,35].

The present results indicated that 24 hours of snails exposure to the fungal extract either pre-, during and post miracidial exposure reduced their infection rates, the mean total number of shedding cercariae/snail. Also, elongated their prepatent period and shortened the duration of cercarial shedding in comparison with their control group. The same results were observed by Mostafa and Tantawy[30]; Bakry *et al*.[5,4]; El-Ansary *et al*.[13]; Massoud *et al*.[25] and El-Sayed *et al*.[15] on different plants at different periods of *B. alexandrina* snail's exposure to *S.mansoni* miracidia.

The results revealed that the smallest "S" value (similarity coefficient) was observed between the bands of fungal extract treated-infected snail's hemolymph and *S. mansoni* infected snails. Higher "S" value was observed between the fungal extract treated non-infected snail's ovotestis and control non-infected group. This may indicate that some factors were increased or decreased depending on the compound and concentration used leading to disturbance in functions of the internal organs which may lead to alterations in protein fractions and metabolic processes[38]. In addition, many investigators reported that trematode infection caused obvious changes in the protein metabolism of the snails[36]. Mohamed and El-Fiki[26] demonstrated similar results in *S. mansoni* in *B. alexandrina* snails. El-Ansary *et al*.[12] revealed that *S. mansoni*-infected *B. alexandrina* tissue has a completely different protein

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pattern compared to control with low similarity coefficient "S" value (0.3). They explained the correlation between separation pattern and metabolic redirection of the snail host by the developing sporocysts. Mostafa *et al*.[28] reported that, the similarity coefficient between non-infected and *S. mansoni* infected *B. alexandrina* was 0.29. Moreover, Coustau *et al*.[10] stated that, *S. mansoni* excretory-secretory products could reflect both a stimulatory effect on metabolism and a response to the presence of toxic compounds, which regulate protein transcription of *Biomphalaria* snails.

The analysis (using HPTLC) of free pool amino acids of the ovotestis and hemolymph extracted from non-infected and *S. mansoni*-infected *B.alexandrina* snails at the 4th week of exposure to sublethal concentration of the fungal extract of *A.fumigatus*, showed a marked differences, increase or decrease, in amino acid levels of different treatments when compared to control groups. The interactions of several acting factors lead to specific qualitative and quantitative changes of free amino acids composition[22]. Free amino acids in aquatic invertebrate were involved in a variety of other environmental and developmental processes and can also be affected by reproduction[21], parasitism[32] and pollutants[18]. The present results are in harmony with that obtained with El-Halim *et al*.[16] who found that The most levels of the free amino acids and total proteins were lower in infected snails than non-infected ones, which may indicated that the parasite obtains part of its amino acid and protein requirements from the host. Faddah and El-Ansary[17] reported that the *B. alexandrina* snails under the influence of larval parasitic infection showed an increase in most of the tested amino acids except Serine, Arginine and Threonine. Cercarial-shedding snails exhibited depletion of most of the studied amino acids, accompanied by an increase in Tyrosine, Cystine, phenylalanine and Leucine. They suggested that amino acid profile of *B. alexandrina* snails is of critical importance for the development of *S. mansoni* larvae. Studies by Pachuski *et al*.[31] revealed a significant reduction in the amino acid Lysine of the digestive gland-gonado complex (DGG) of *B. glabrata* infected snails. Lysine is probably an essential amino acid for the developing intramolluscan stages, i.e. sporocysts and cercariae of the *S. mansoni* parasite can not synthesize the lysine, thus, it must be provided by the *B. glabrata*. Also Ponder *et al*.[33] reported that, qualitative analysis revealed the presence of Histidine, Lysine, Serine, Alanine, Valine, and Isoleucine or Leucine in all tested samples. Finally, it can be concluded that the fungal extract has high molluscicidal activity against *B. alexandrina* snails as they affect the snail's life, biological and physiological activities.

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