

Toxicity of *Aspergillus niger* Fungus Against *Biomphalaria alexandrina* Snails and *Schistosoma mansoni* Free Larval Stages

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ABSTRACT

Aspergillus niger is a one-of-a-kind fungus with benefits for both terrestrial and aquatic species, although research on the effects of *A. niger* on aquatic animal species is limited. The present study investigated the impact of *Aspergillus niger*, *A. flavus*, and *Alternaria alternata* filtrates on *Biomphalaria alexandrina* snails (the main intermediate host of *Schistosoma mansoni* in Egypt). Moreover, the effect of *A. niger* (the most toxic fungus) filtrate was assessed against infected *B. alexandrina* snails with *S. mansoni*, in addition to its efficacy against *S. mansoni* free larval stages. *Aspergillus niger* had more toxicity (100 % mortality) than *A. flavus* (40% mortality), while *Alternaria alternata* did not affect *B. alexandrina* snails. Chronic exposure to sub-lethal concentrations of *A. niger* caused histopathological changes in the head-foot, digestive gland, kidney, and hermaphrodite gland of *B. alexandrina* snails. Furthermore, after exposure to the determined sub-lethal concentrations of *A. niger*, both uninfected and infected snails with *S. mansoni* showed low survival rates. In addition, the lethal time for miracidia and cercariae after exposure to LC₂₅ of *A. niger* was 130 and 200 minutes, respectively. Additionally, exposure to sub-lethal *A. niger* concentrations resulted in a significant decrease in the infection rate and mean total count of cercariae shed per snail, as well as the shed of aberrant cercariae. In conclusion, *Aspergillus niger* filtrate had a potent molluscicidal activity against *B. alexandrina*, and a damaging effect on cercariae, hence it can play a role in reducing schistosomiasis.

INTRODUCTION

Biomphalaria alexandrina snails are the intermediate host of *Schistosoma mansoni*, where it is endemic in Egypt and causes approximately 35% of children to have liver diseases and 70% of adults to have chronic liver diseases (El-Khoby *et al.*, 2000). Adult trematode and cestode worms have typically been treated with praziquantel. However, it is less effective against free larval stages (Faust *et al.*, 2019). As a result, numerous tactics including mechanical, chemical, and biological procedures have been employed to manage snail

populations and disrupt the *Schistosoma* species life cycle (**Omobhude *et al.*, 2017; Mansour *et al.*, 2021**). Chemical molluscicides have several negative effects, including being hazardous to non-target animals, potentially accumulating in the environment and being high-cost (**Hamed, 2010**). Due to the disadvantages of chemical compounds, the researchers turned to natural adversaries such as pathogens, predators, and parasites.

Some bio-agents are extremely poisonous to the target organism since they have the same desirable characteristics of a chemical molluscicide, moreover they can be safely applied, and are low in cost (**Abd El Ghaffar *et al.*, 2018**). Numerous researches examined biological control methods including bacteria (**Wang *et al.*, 2008**), some algae (**Mostafa & Gawish, 2009; Mansour *et al.*, 2023**), and extracts of specific fungal species (**Osman *et al.*, 2013**). It was discovered that between 30 and 40 percent of the popular fungi can produce toxic substances of different degrees of gravity, such as toxins produced by the following fungi: *Aspergillus fumigatus*, *A. niger*, *A. clavatus*, *A. terreus*, *A. giganteus*, *Penicillium terreus*, *P. expansum*, *P. urticae*, *P. griseofulvum* *ægriseofulvum*, *P. expansum*, and *P. urticae* (**Bayman & Baker, 2006**). Mycotoxin is a naturally harmful secondary metabolite produced by fungal organisms. Aflatoxins are a form of mycotoxin generated by various species of *Aspergillus*, such as *A. flavus*, *A. parasiticus* and *A. fumigatus* (**Martins *et al.*, 2001**). Only ochratoxin A, a mycotoxin, is produced by *A. niger* strains (**Schuster *et al.*, 2002**). There have been several *Aspergilli* isolated from different parts of the world. An aerobic filamentous fungus called *A. niger* thrives on organic compounds. It occurs naturally in soil, compost, decomposing plant waste, and litter (**Schuster *et al.*, 2002**). This fungus is one of the most important microorganisms used in biotechnology. It is produced secondary metabolites, which is classified into five kinds: pyranone, alkaloid, cyclopentapeptide, polyketide, and sterol based on their chemical structures (**Yu *et al.*, 2021**). There is a long history of safe usage of some members of the *A. niger* species in the industry of fermentation. It is used significantly in the production of citric acid and several enzymes such as amylases, pectinases, and proteases (**Godfrey & West, 1996**). Furthermore, using seed husk *Jatropha curcas* and its endophyte *A. niger* for nickel and manganese bio-sorption from wastewater resulted in 100% removal of nickel and manganese (**Tamim *et al.*, 2023**).

Alternaria alternata is a widespread fungus isolated from plants as an endophyte and a pathogen, as well as from soil, food, and indoor air. It is, however, a rare source of human infection (**Loveless *et al.*, 1981**). *Alternaria* fungus metabolites are classified into numerous groups, including nitrogen-containing metabolites, steroids, terpenoids, pyranones, quinones, and phenolics (**Lou *et al.*, 2013**). Certain *Alternaria* fungal metabolites are hazardous to plants and animals, and are referred to as phytotoxins and mycotoxins, respectively. Certain metabolites from *Alternaria*, such as tenuazonic acid, maculosin, and tentoxin, have been investigated as herbicide candidates (**Stierle *et al.*, 1988; Liebermann *et al.*, 1996; Sanodiya *et al.*, 2010**).

The current investigation was designed to look into the molluscicides activity of *A. niger*, *A. flavus*, and *Alternaria alternate* filtrates against *B. alexandrina* snails, as well as the toxicity of *A. niger* against *S. mansoni* free larval stages.

MATERIALS AND METHODS

1. Experimental animals

The larval stages of *S. mansoni* (miracidia and cercariae) and *B. alexandrina* snails were both utilized in the current study. These were obtained from Schistosomiasis Biological Supply Centre (SBSC), Theodor Bilharz Research Institute, Giza, Egypt. The ova of *S. mansoni* were taken from previously infected mice and cercariae from infected *B. alexandrina* snails. The ova were allowed to hatch in small amount of dechlorinated water (25 °C) for about 15 minutes under a direct light. Then, the hatched miracidia were used in the experimental tests. The described procedure by **Boissier & Mone (2000)** was used to keep the experimental snails in a laboratory setting at a temperature of 25°C.

2. Preparation of fungal filtrations

Aspergillus niger, *A. flavus*, and *Alternaria alternate* fungi were utilized in the current study. The pure cultures of fungi were inoculated on PDA plates and incubated at 28±1°C for biomass preparation. Fungal discs of 0.5 mm in diameter that were cut from cultures that had been developed for 7 days were put into conical flasks with 50 ml of potato dextrose broth media and, incubated in triplicate for another 7 days (**Umecharuba & Nwachukwa, 1997**). After filtration by Whatman No. 1 filter paper, the filtrates were collected to measure their influence on *B. alexandrina*.

3. Fungal filtration toxicity against *B. alexandrina* snails

To determine the toxicity of *Aspergillus niger*, *A. flavus*, and *Alternaria alternate*, ten *B. alexandrina* snails (8–10 mm in diameter) were exposed to their filtrate concentrations (100%) for 24 hours. These snails were then taken out of each filtrate following the exposure period, properly cleaned with dechlorinated tap water, and then placed in a different container as a recovery period of 24 hours, and the dead snails were counted. To determine the LC₅₀ and LC₉₀ values of *A. niger* (the most toxic fungus), a series of fungal concentrations (100, 75, 50, and 25%) were created using dechlorinated tap water at 22±2°C. Ten snails were used in three replicates (**El-Mahdy et al., 2021**). The exposure time at room temperature was 24 hours, followed by a recovery period of 24 hours. The same experimental circumstances were used to maintain a different group of snails as a control (**WHO, 1965**). Then, LC₅₀ and LC₉₀ values were calculated after counting the dead snails (**Litchfield & Wilcoxon, 1949**).

4. The impact of *Aspergillus niger* filtration on snail survival rates

The goal of the present study was to determine how long-term exposure to sub-lethal concentrations (LC₁₀ and LC₂₅) of the most potent fungus filtration affected the probability of

B. alexandrina snails surviving. A sub-lethal dosage of *A. niger* was prepared weekly (for both LC₁₀ and LC₂₅) and applied to three replicates of 20 mature snails. A control group was retained in the identical experimental conditions with pure, dechlorinated tap water. Dry lettuce leaves were used as food for these snails. Dead snails were taken out of the containers, and a count of them was made.

5. Histological studies

The sub-lethal concentrations of *A. niger* were applied on *B. alexandrina* snails (8–10 mm in diameter) for 3 weeks. The soft tissues of the snails were extracted from the shell using a forceps and then fixed in Bouin's solution for 24 h, subsequently the soft tissues were put in gradually increasing concentrations of ethanol, cleared with xylol, then embedded in paraffin wax, before being cut into sections at 6 µm, and stained with hematoxylin and eosin (Mohamed & Saad, 1990).

6. Miracidicidal and cercaricidal effect of *Aspergillus niger*

A group of twenty newly hatched miracidia and another group of fifty cercariae in 4 ml dechlorinated tap water were prepared. The two groups were exposed to double concentrations of the LC₁₀ and LC₂₅ values of *A. niger* filtration. Another two groups of control test were prepared, the 1st one for miracidia (20 newly hatched) and the 2nd group containing 50 cercariae each in 4 ml dechlorinated water (Tantawy, 2008). The movement's microscopic observations and demise of cercariae and miracidia during treating time were reported at time periods of 15, 30, 60, 120, 130, 180 and 240 minutes.

7. Effect of *Aspergillus niger* filtrate on infectivity of *B. alexandrina* with *S. mansoni*

Seven groups of *B. alexandrina* snails were exposed to both two sub-lethal concentrations (LC₁₀ and LC₂₅) of *A. niger* for 24 hours either pre- (groups 1, 2), during (groups 3,4) and post-infection (groups 5,6) of snails to *S. mansoni* miracidia. To expose large numbers of snails to miracidia, three replicates with each containing 20 snails/250 ml in a glass container, were exposed to newly hatched miracidia at a dose of 10 miracidia per snail. As a control, a separate group was exposed to miracidia but not delivered the test concentrations. The infected snails that had endured exposure were evaluated for cercarial shedding individually in various dishes with 2 ml of dechlorinated water per snail to promote cercarial shedding at 21 days following exposure. The infection rate, the average number of cercariae, percent of up-normal movement cercariae were calculated for each snail. The cercariae that showed abnormal movement were first counted. Following the addition of a few drops of iodine solution to each well containing cercariae, each snail's cercariae were counted and documented using a stereomicroscope. To prevent snails from being worn out, this examination was done once a week (Badawy, 2007).

8. Statistical analysis

Using chi-square values from contingency tables, data on survival, infection rates, and percentages of aberrant cercariae were displayed as percentages and analyzed to identify the significant differences between the control and experimental groups. The t-test was used to analyze the median cercariae count. The SPSS computer programme (version 20 for Windows) carried out all statistical analyses.

RESULTS

1. The molluscicidal effect of experimental fungi on *Biomphalaria alexandrina* snails

The molluscicidal activity of *Aspergillus niger*, *A. flavus*, and *Alternaria alternate* filtrates against *B. alexandrina* was examined. Table (1) shows that *A. niger* had more molluscicidal activity (100% snails mortality) than *A. flavus* (40% snails mortality) after being exposed to 100% concentrations for 24 hours, followed by another 24 hours recovering. *Alternaria alternate* did not have an effect on *B. alexandrina* snails (Table 1). The molluscicidal activity of the *A. niger* on the adult snails are presented in Table (2). The data revealed that the lethal concentrations (LC₅₀ and LC₉₀) of *A. niger* were 66.5 and 97.8 %, respectively, after 24 hrs (Table 2).

Table 1. The molluscicidal effect of fungi against *B. alexandrina* after being exposed to 100% of filtrates for 24 hours

Fungi	Snails' mortality %
<i>Aspergillus niger</i>	100
<i>Aspergillus flavus</i>	40
<i>Alternaria alternate</i>	0

Table 2. Molluscicidal activity of *A. niger* filtrate on mature *B. alexandrina* (exposure for 24 hours)

<i>A. niger</i>	LC ₁₀	LC ₂₅	LC ₅₀	LC ₉₀	Slope
Concentrations %	35.34	50.1	66.5	97.8	1.49

2. Effect of prolonged exposure of *Aspergillus niger* filtrate on *B. alexandrina* snail survival rates

Fig. (1) illustrates the effect of extended exposure of snails to sub-lethal concentrations of *A. niger* (LC₁₀ and LC₂₅). Snails exposed to *A. niger* had a lower survival rate than control snails. As time passed, their levels continued to decrease. At the fourth week of exposure, the

snails exposed to *A. niger* filtrate LC₂₅ had 40% survival rate compared to 90% for untreated control snails.

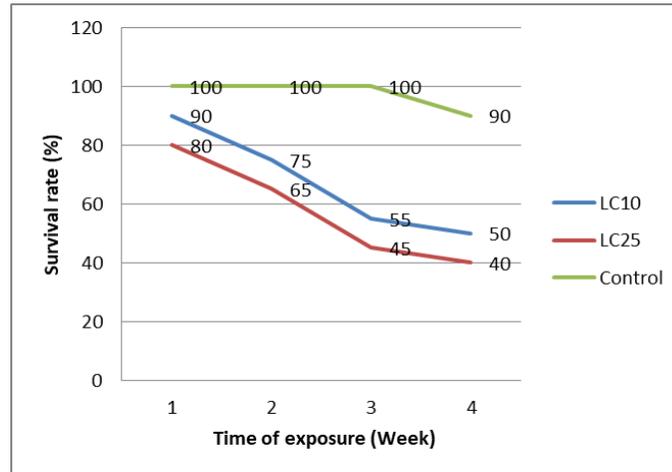


Fig. 1. Effect of prolonged exposure of *B. alexandrina* snails to sub-lethal concentrations (LC₁₀ and LC₂₅) of *A. niger* filtrate

3. Effect of *Aspergillus niger* filtrate on head-foot, digestive gland, kidney and hermaphrodite gland of *Biomphalaria alexandrina* snails

B. alexandrina's head-foot area has a layer of ciliated cylindrical epithelial cells forming an epithelium and unicellular mucous glands, and it is supported by strong connective tissue and muscular tissue (Fig. 2a). The chronic exposure of the snails to *A. niger* filtrate at LC₁₀ caused histopathological alterations such as damaged cilia, focal destruction of the epithelial covering and muscular fiber, degeneration of connective tissue, vacuoles, and edema. Moreover, some brown toxic agents was observed (Fig. 2b). Snails exposure to LC₂₅ of *A. niger* promoted vacuolation and necrotic change, resulting in muscle atrophy and increased accumulation of brown toxic substances (Fig. 2c).

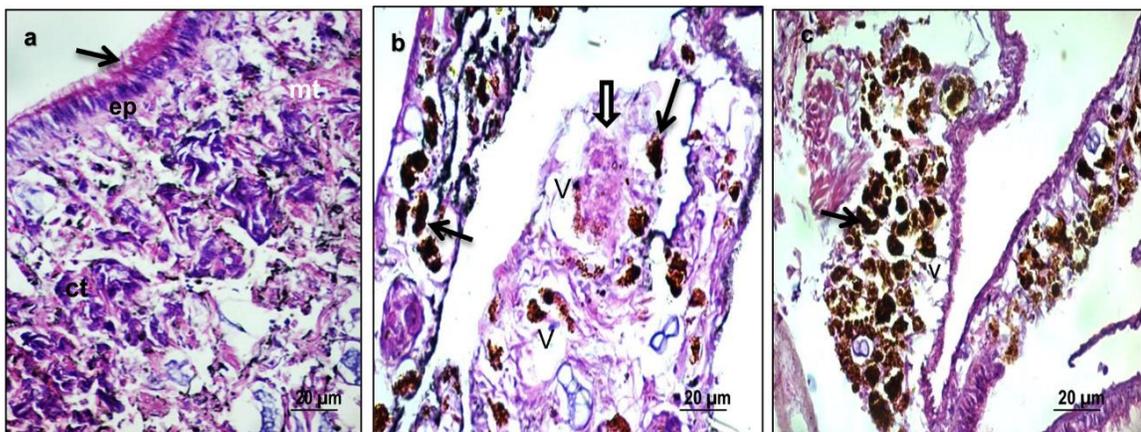


Fig. 2. Photomicrographs of sections through the head-foot of *B. alexandrina* showing: (a) Normal ciliated (arrow) epithelial cells (ep) and dense connective tissue (ct) that in turn was supported by muscular tissue (mt) in control snails, (b) The snails exposed to LC₁₀ of *A. niger* show damaged cilia, focal destruction of the epithelial covering and the muscular fiber, degeneration of connective tissue, vacuoles (v), edema (thick arrow), some brown toxic agent (thin arrow), and (c) The snails exposed to LC₂₅ of *A. niger* increased vacuolation (v) and atrophy within the muscles of the snail and more accumulation of brown toxic agents (arrow)

Biomphalaria alexandrina's digestive glands were made up of several tubes connected together by connective tissue and lined with a single layer of two different types of cells: digestive cells (the most frequent cell type) and secretory cells (Fig. 3a, b). When snails were exposed to LC₁₀ of *A. niger* filtrate, the majority of digestive cells were vacuolated, degenerated and burst, particular secretions were found in certain vacuoles, and the lumen was reduced. Secretory cells as well as connective tissue were destroyed (Fig. 3c-f). When the snails were exposed to LC₂₅ of *A. niger*, they showed severe damage, the tubular glands lost their typical shape, and the majority of cells lost their walls, vacuolated, and degenerated (Fig. 3g-j).

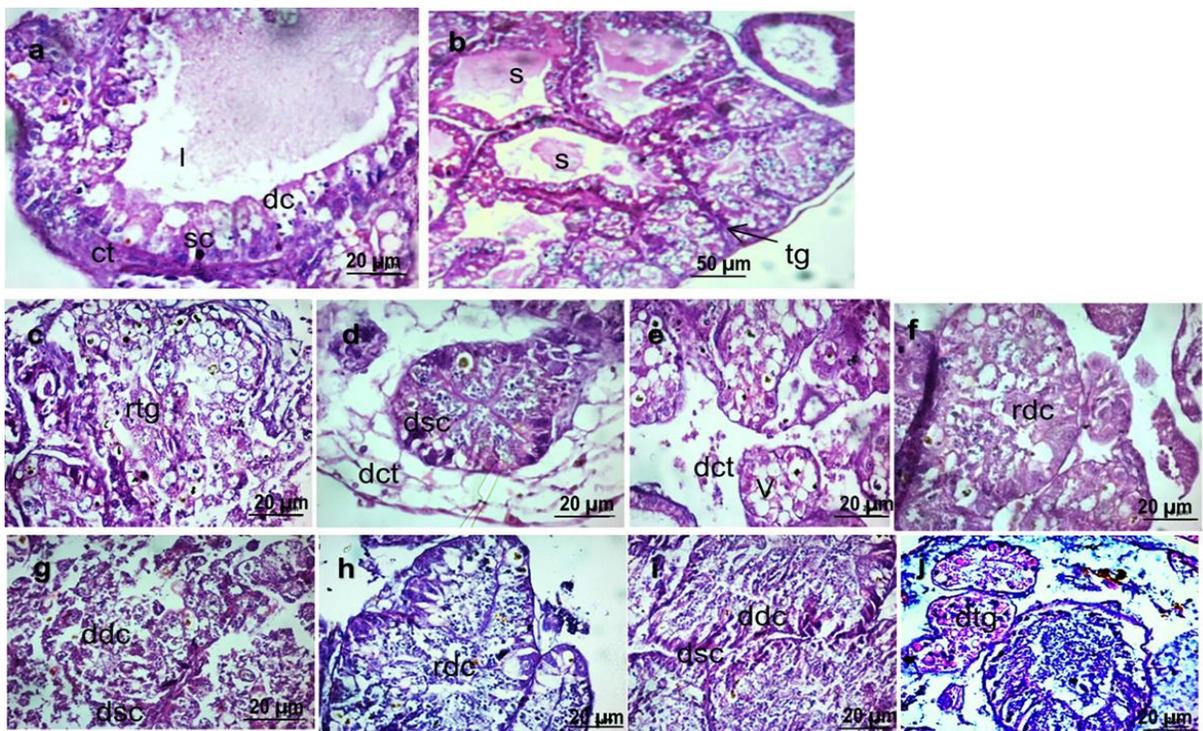


Fig. 3. Photomicrographs of sections through the digestive glands of *B. alexandrina* showing: (a, b) Normal snails, the tubules of digestive gland (tg) containing digestive cells (dc) and secretory cells (sc), secretions (s) connective tissue (ct) between tubules, (c-f) The snails exposed to LC₁₀ of *A. niger*, and (g-j) The snails exposed to LC₂₅ of *A. niger*. Rupture tubular glands (rtg), degenerative connective tissue (dct), rupture digestive cell (rdc), degenerative

digestive cell (ddc), degenerative secretory cell (dsc), vacuoles with certain secretion (v), degenerative tubules of digestive gland (dtg)

A light microscopic examination of a normal *B. alexandrina* snail kidney revealed two distinct types of epithelial cells, both of which have central nuclei. Large apical vacuoles are present in some epithelial cells, while others lack vacuoles and have deeply colored cytoplasm and granules (Fig. 4a). The kidneys of the snails exposed to LC₁₀ of *A. niger* filtrate displayed numerous pyknotic peripheral nuclei and cysts (Fig. 4b). Snails exposed to LC₂₅ of *A. niger* filtrate developed thicker kidneys, some cell degeneration, and a large number of cysts. (Fig. 4c).

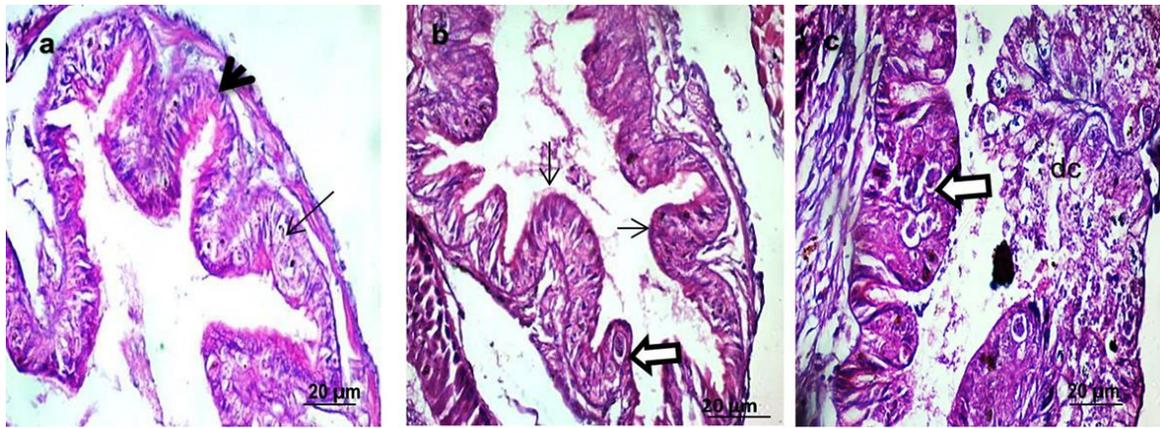


Fig. 4. Photomicrographs of sections through the kidney showing: (a) A normal *B. alexandrina* kidney with two types of epithelial cells, type 1 (arrow) with a large apical vacuole and type 2 (arrowhead) with no vacuoles, and their cytoplasm and granules are deeply stained, (b) The kidneys of the snails exposed to LC₁₀ of *A. niger* exhibited a cyst (arrowhead) and numerous pyknotic peripheral nuclei (arrows), and (c) The kidneys of snails exposed to LC₂₅ of *A. niger* showed many cysts (arrowhead), and degenerating cells (dc)

The hermaphrodite gland of normal *B. alexandrina* is made up of a few cub-shaped acini that are connected by a connective tissue. Primary, secondary spermatocytes and sperm were produced by the differentiation of male reproductive cells. Fully mature sperm have been seen attached to or in the lumen of Sertoli cells. The acinar lumen of the female oogenic cells was loaded with primary and secondary oocytes and mature ova (Fig. 5a). The snails exposed to LC₁₀ of *A. niger* filtrate were characterized by noticeable impacts on mature ova, with degraded aggregated ova detected. Early stages of oogenesis were not represented (Fig. 5b, c). When the concentration was increased to LC₂₅, swallowed ova with large fat vacuoles appeared, resulting in the degeneration of its contents. Primary spermatocytes had deteriorated, whereas secondary spermatocytes were dispersed throughout the acinus, without distinguishing between their nuclei and cytoplasmic inclusions. The connective tissue between acini has degenerated, and vacuolation inside the acinus was more severe (Fig. 5d-f).

4. Larvicidal activity of *Aspergillus niger* filtrate against *Schistosoma mansoni* free larval stages

According to Tables (3, 4), *A. niger* filtrate has larvicidal action against miracidia and cercariae of *S. mansoni*. Miracidia died faster than cercariae within the first 240 minutes of *A. niger* exposure. The mortality of miracidia and cercariae are linearly related to the testing period and concentrations of filtrate. The lethal time for miracidia and cercariae after exposure to *A. niger* filtrate (LC₂₅) was 130 and 200 minutes, respectively (Tables 3, 4).

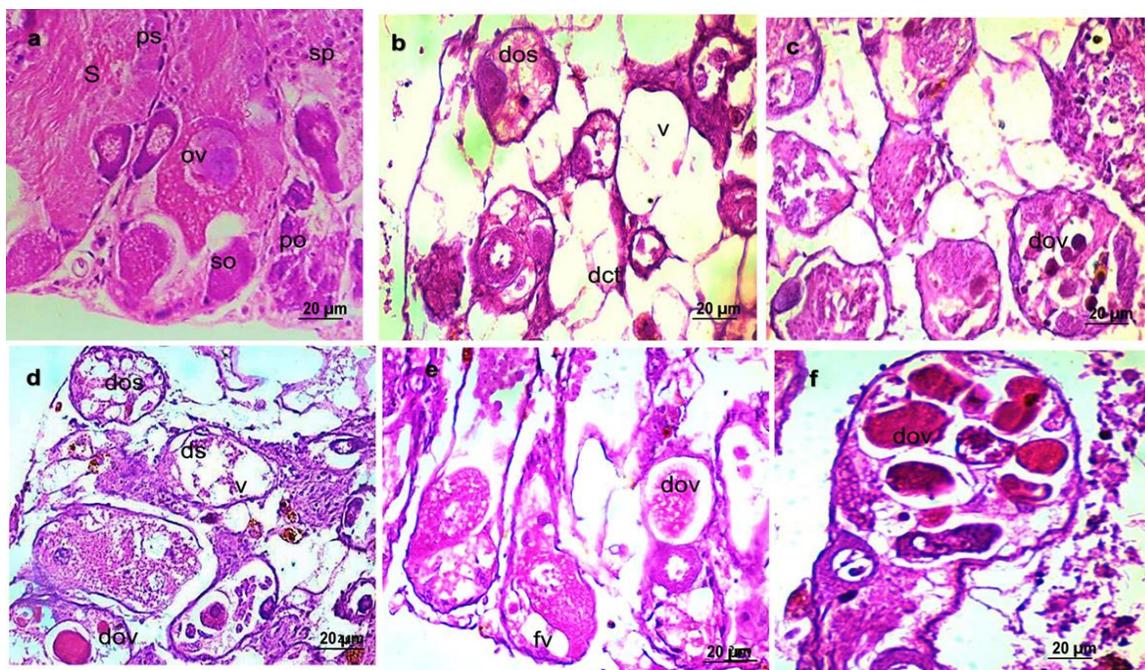


Fig. 5. Photomicrographs of sections through the hermaphrodite glands of adult *B. alexandrina* showing: (a) Normal snail in which acini are represented with all stages of oogenesis; primary oocytes (po), secondary oocytes (so) and the mature ovum (ov), and stages of spermatogenesis; primary spermatocytes (ps), secondary spermatocytes (sp) and sperms (s), and connective tissue (ct), (b, c) The snails were exposed to LC₁₀ of *A. niger*, and (d-f) The snails exposed to LC₂₅ of *A. niger*, sperms (s), degenerated ovum (dov), degenerated oocyte (dos), degenerated spermatocyte (ds), degenerated connective tissue (dct), vacuole (v), fate vacuole (fv)

Table 3. Miracidicidal effect of *A. niger* filtrate against *S. mansoni* miracidia

Time (mins) Concentration	%Mortality of miracidia after the following time interval (minute)						
	15	30	60	120	130	180	240
LC ₁₀ (35.34)	10	15	35	50	60	70	85
LC ₂₅ (50.1)	20	25	50	70	100	100	100
control	0	0	0	0	0	5	10

Table 4. Cercaricidal effect of *A. niger* filtrate against *S. mansoni* cercariae

Time (mins)	Cercariae mortality % after the following time (minute)						
	15	30	60	120	180	200	240
Concentration							
LC ₁₀ (35.34)	4	10	26	40	60	70	80
LC ₂₅ (50.1)	10	16	36	60	90	100	100
control	0	0	0	0	0	4	8

5. Effect of sublethal concentrations of *Aspergillus niger* filtrate on infectivity of *Schistosoma mansoni* miracidia to *Biomphalaria alexandrina* snails and movement of released cercariae

Table (5) displays a significant decrease in the survival rate, infection rate and the mean total number of shedding cercariae/snail in comparison with control groups when infected snails were treated for 24 hours with sub-lethal concentrations (LC₁₀ and LC₂₅) of *A. niger* filtrate before (groups 1, 2), during (groups 3, 4) or after (groups 5, 6) exposure to *S. mansoni* miracidia. The percentage of cercariae from treated snails that are unable to move is shown in Table (5). Groups 3 and 4 presented the highest percentage of abnormal cercariae (Table 5). Infected *B. alexandrina* hosts release *S. mansoni* cercariae, which gather below the water's surface to increase their chances of finding a final host (Fig. 6a). The cercariae are around 75 µm long and consist of an anterior body (head), a thin tail with forked end known as the furca (Fig. 6b), cercariae display an intermittent swimming behavior. Cercariae swim against gravity by furca make T-swimmer gait. Cercariae move in this manner, beating their tails at intervals while retaining enhanced flexibility near their rear and anterior ends. This flexibility allows an interaction between fluid drag and bending resistance, known as an elasto-hydrodynamic coupling, by naturally breaking time-reversal symmetry and allowing movement at minuscule length scales. While some *S. mansoni* cercariae released from treated infected snails showed a variety of deviations from normality in motility and shape (Fig. 6c-h). Where they appear unable to swim but only they simply crept down crawled along the container's bottom while curled their tails; meanwhile, the most of normal cercariae were still swimming near the surface. Some cercariae fixed the anterior body and fork but twisted their slender tails while other cercariae fix the anterior body and move the tail and fork. Moreover, some cercariae made incomplete movement of all structures at the same point. Furthermore, some cercariae appeared to be abnormally short, measuring only 60µm in length although otherwise they appeared normal (Fig. 6h). Additionally, some cercariae appear striated with no movement all the time at the bottom not backed to surface at 6 week post exposure (WPE) (Fig. 7a). At 7 WPE, most of the cercariae have heads detached from the tails, where cercariae became sticky, probably as a result of gland contents discharged from suckers or released at the head-tail junction after separation. The heads of the dead cercariae were opaque and bloated. After a few minutes of shedding, the tails floated slightly above the heads, quickly detached, and becoming entirely relaxed (Fig. 7b, c).

Table 5. Effect of sub-lethal concentrations of *A. niger* filtrate on infectivity of *S. mansoni* miracidia to *B. alexandrina* snails and percent of abnormal cercariae

Treatment related to miracidial exposure	Concentration (%)	Total exposed snails	Survival rate (%)	Infection rate (%)	Mean no. of cercariae / snail	% of abnormal cercariae
One day Pre-infection	LC ₁₀ (35.34) (g 1)	20	50***	70***	508.7±188.2***	40***
	LC ₂₅ (50.1) (g 2)	20	45***	66.6***	475±168.9.8***	55***
	control	20	85	94.1	1120.4±391.7	0
During infection	LC ₁₀ (35.34) (g 3)	20	55***	72.7***	430±161***	66.6***
	LC ₂₅ (50.1) (g 4)	20	45***	77.7***	515±197.7***	70***
	Control	20	85	94.1	1120.4±391.7	0
One day Post-infection	LC ₁₀ (35.34) (g 5)	20	60***	75***	372.75±136.3***	35.7***
	LC ₂₅ (50.1) (g 6)	20	65**	69.2***	415±150.8***	39***
	control	20	80	93.7	1071.3±386	0

** = Significantly different at $P < 0.01$, *** = $P < 0.001$

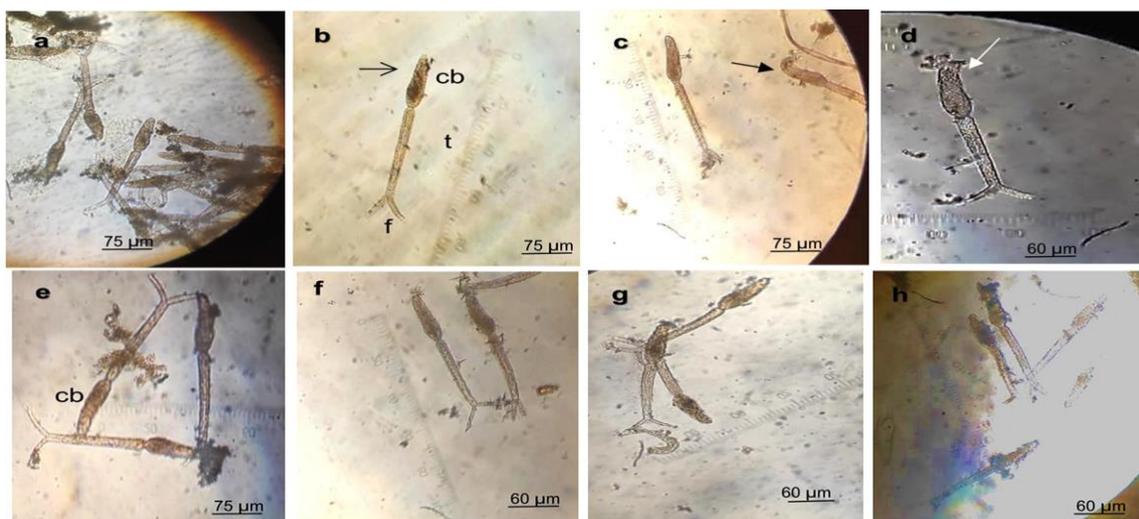


Fig. 6. Photomicrographs of *S. mansoni* cercariae in 70% alcohol showing: (a, b) Normal cercariae from infected snail consist of cercarial body (cb) with oral sucker (arrow), tail (t) and furca (f), (c-h) Cercariae from snails at 6 weeks post exposure to sub-lethal concentration (LC₂₅) of *A. niger* (g 4) with degenerated oral sucker (arrow). (e) Elongated cercarial body (cb), and (f-h) Short cercariae 60 μ

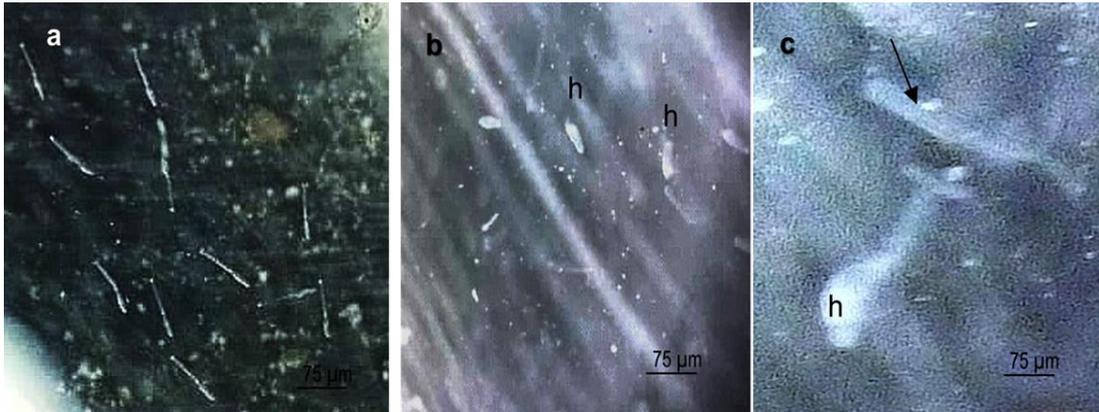


Fig. 7. Photomicrographs showing: (a) Alive and damaged *S. mansoni* cercaria from infected snails at 6 weeks post-exposure to LC₂₅ of *A. niger* (group 4) with a rigid tails, tightly curled fork, contraction and stretching of the heads, unable to movement, (b) Cercariae from group 4 at 7 weeks post-exposure to LC₂₅ of *A. niger* with the heads (h) separated from the tails, and (c) Cercariae appears here in random motion with head (h), and another cercariae without a head (arrow)

DISCUSSION

Over 90% of Africans are afflicted by the contagious parasite disease schistosomiasis (WHO, 2016). *Biomphalaria alexandrina* snails (the main intermediate host of *Schistosoma mansoni* in Egypt) are necessary to complete the parasite's life cycle. One of the main goals of current control efforts using chemical molluscicides is to decrease the population of these snails or impair their ability to breed (WHO, 2008). To stop the damaging impacts of chemical molluscicides on both the environment and human health, it is vitally necessary to look for molluscicidal substances of biological origin (Oliveria-Filho & Paumgarten, 2000). The findings of this study showed that *Asperigullus niger* filtrate exhibits a substantial higher toxicity against *B. alexandrina* snails and drastically reduces schistosomiasis. This is similar to prior research that found some fungi are harmful to *B. alexandrina*, including *Aspergillus terreus*, *Aspergillus janthinellum* and *Penicillium fumigatus* (Osman *et al.*, 2013; Saad *et al.*, 2014). Chen *et al.* (2009) found that after 24 hours of exposure to 400 mg/l of *A. niger* alcoholic extract, 46.7% of *Oncomelania hupensis* snails died. Moreover, Guo *et al.* (2011) showed that the compound diethyl ether polar fraction from *A. fumigatus* has the greatest molluscicidal efficacy on *O. hupensis* snails (100% mortality). The current data showed that *A.*

flavus filtrate caused only 40% snails mortality at a concentration of 100 %. **El-Sabbagh et al. (2013)** reported that *A. flavus* produced the highest mortality rate (30%) of the snail *Monacha cartusiana* among five fungal genera, *Penicillium*, *Trichoderma*, *Acremonium*, *Fusarium* and *Aspergillus*. According to **Demain (2014)**, fungi create a wide range of hazardous metabolites, ranging in molecular weight from low-weight (secondary metabolites) to complex proteolytic enzymes and complex peptides. The mechanism by which the harmful fungi affect the living organism may be related to cuticle penetration (**Singh & Prakash, 2011**).

The survival rate of the snails that were exposed to LC₂₅ of *A. niger* filtrate decreased over time and reached 40% during the fourth week of treatment, compared to 90% for untreated control snails. These outcomes were in agreement with **Ragab and Ismail (2001)** findings, which showed that *B. alexandrina* exposed to 1/10 LC₅₀ *Trichoderma harzianum* and *T. viridae* were dead after five and twelve weeks, respectively. Additionally, **Osman et al. (2013)** demonstrated that the survival rate of *B. alexandrina* snails treated with *A. fumigatus* extract was adversely correlated with fungus concentration. Furthermore, **Saad et al. (2014)** discovered that after 4 weeks of *B. alexandrina* exposure to LC₁₅ and LC₂₅ of *A. terreus* filtrate, their survival rate dropped to 50%, meanwhile snails subjected to LC₂₅ of *Penicillium janthinellum* filtrate showed a dramatic drop to 45%.

The present results showed some histological disturbances in the head-foot region when the snails were exposed to *A. niger* filtrate such as damaged cilia, focal destruction of the epithelial covering and the muscular fiber, degeneration of connective tissue, vacuoles and edema, and the presence of some brown toxic agent. Histopathology is an important endpoint in evaluation of the pathological changes in aquatic organisms exposed to contaminants (**Lajtner et al., 1996**). The present obtained results support those observed by **El-Khayat et al. (2018)**, who detected histological alterations in the head-foot region of *B. alexandrina* snails following exposure to Pb and Fe in Manzala Lake water samples. These changes included mucous-secreting unicellular gland shrinkage, breaking fiber tissues, increasing empty spaces, and atrophy. Additionally, **Wang et al. (2018)** showed how the molluscicides 1-(4-Bromophenyl)-3-(pyridine-3-yl) urea and 1-(4-Chlorophenyl)-3-(pyridine-3-yl) urea (LC₅₀ of 0.50 and 0.51 mg L⁻¹, respectively) caused an extensive ultrastructural destruction of the mantle tissues, foot plantaris and tentacle of mature *B. straminea* snails. They suggested that the snail might perish as a result of a tentacle, foot plantaris, and mantle injuries.

Examining the histopathological abnormalities that occur in digestive gland cells under xenobiotic stress can reveal information about the organism's toxicity (**Viarengo & Canesi, 1991**). In the current study, the snails were exposed to the *A. niger* filtrate, which caused the majority of the digestive cells to vacuolated, degenerated and rupture, some vacuoles contained certain secretions and the lumen decreased. Moreover, secretory cells and connective tissue have degenerated. **Lajtner et al. (1996)** studied the impact of phenol on the digestive system of *Amphimelania holandrii* snails and determined that digestion and absorption-related processes were more closely tied to the vacuolation of digestive cells (**Ibrahim, 2006**). The degree of

cytoplasmic vacuolation rises as a result of these processes in snails that have previously been exposed to molluscicides. The effects of two carbamate molluscicides on the digestive gland of the snail *Eobania vermiculata* were explored by **Hamed *et al.* (2007)**, who found considerable cytoplasmic vacuolization. Furthermore, exposure to Mirazid caused the necrosis of connective tissue in *B. alexandrina* snails, loss of the usual form of the digestive tubules, and detection of significant vacuoles in both digestive and secretory cells (**Osman *et al.*, 2014**).

A light microscopic examination of a normal kidney of *B. alexandrina* snails revealed two types of epithelial cells, both of which have central nuclei. The two types of molluscan kidney epithelial cells play a significant role in the accumulation of metals, excretion, and reabsorption (**Reynolds, 1990**). The present observation indicated that snail exposure to LC₂₅ of *A. niger* filtrate caused cell thickness, degeneration of certain cells, and the presence of numerous cysts, which were similarly found in *Mya arenaria* obtained from polluted sediments (**Seiler & Morse, 1988**). Moreover, in agreement with the present data, the study that assessed the effects of sub-lethal cadmium exposure on the kidney of the *Littorina littorea*, showed a modification in the kidney structure (**Marigomez *et al.*, 1990**). Oxalic acid, a metabolite of *A. niger*, is thought to be the substance causing the harmful impact according to **Moreau (1979)**.

In the present study, the observed deformation in the histology of hermaphrodite gland of *B. alexandrina* after exposure to LC₁₀ of *A. niger* was distinguished by notable impacts on mature ova where degraded aggregated ova were detected. The early phases of oogenesis were not depicted. These defects could be caused by *A. niger*'s inhibition of spermatogenesis and oogenesis process in the ovotestis gland. These results are in line with those of **Abdel-Hamid *et al.* (2014)** who found that exposure to sub-lethal quantities of the bioactive chemical of *P. canescens* caused abnormalities in sperm and ova in *B. alexandrina* and *B. truncatus* snails. Similar histological variations were showed in the hermaphrodite glands of *B. alexandrina* snails treated with copper oxide nano-composite (CuO NC), where the ova and sperms deteriorated and connective structures between acini were lost (**Saad *et al.*, 2019**). Additionally, **Bakry *et al.* (2002)** discovered that *B. alexandrina* snails treated with the fungicide isoprothiolane produced aberrant egg masses in substantial numbers. Moreover, **Pagano *et al.* (2001)** reported that the R6 fungicide was very toxic to sea urchins, causing embryo developmental defects and chromosomal abnormalities.

The current data showed that during the same time intervals, miracidial mortalities are higher than cercariae. Furthermore, 100% mortality of miracidia and cercariae following exposure to LC₂₅ of *A. niger* filtrate for 130 and 200 minutes, respectively. **Osman *et al.* (2013)** demonstrated that after 5 hours of exposure to LC₂₅ of *A. fungimatus*, miracidia and cercariae were completely dead.

The present data indicated a noticeably lower survival rate for both infected and uninfected *B. alexandrina*. Such a decrease in survival rate may result from metabolic problems as detailed by **Mohamed *et al.* (1981)** who investigated the efficacy of certain organometallic substances at low doses. According to **Ragab & Ismail (2001)**, *Saccharomyces*

cerevisiae, *Trichoderma viride*, *Phanerochaete chrysosporium*, and *Coriolus versicolor* free cell extracts at sub-lethal quantities had a negative impact on the growth and survival of *B. alexandrina* snails. Additional demands, such as cercarial emergence, further reduce the energy of the *B. alexandrina* snails, leaving low amounts for survival, reproduction, development and detoxification (Ibrahim, 2006).

The current data demonstrated that the snails exposed to *A. niger* filtrate for 24 hours, either before, during, and after miracidial exposure, had lower infection rates and a lower average total number of shedding cercariae/snail than their control groups. Bakry *et al.* (2001, 2007) and Massoud *et al.* (2004) found the same results for *S. mansoni* infectivity to *B. alexandrina* snails under the influence of different plants at different times of exposure.

The transmission cycle of *S. mansoni* required the importance of motility in parasite infecting their hosts (Haas, 1992). To maximize their chances of infecting people, cercariae are discharged from snail intermediate hosts and collected beneath the water surface (Haas, 1992; 2003). Since cercariae do not feed and have a lifespan of around 12 hours (Lawson & Wilson, 1980; Whitfield *et al.*, 2003), an effective motility is crucial for schistosomiasis transmission. The fork is an odd appendage that hasn't been seen in any thoroughly investigated swimming microorganisms like bacteria or ciliates (Lauga & Powers, 2009; Subramanian & Nott, 2012). Moreover, some cercariae, in the current study, were unable to move and showed abnormal forms. Previous studies on the presence of aberrant schistosome and other trematode cercariae have been reviewed by Kuntz (1948) and Hussey & Stahle (1961). Diconza & Basch (1974) discovered branching *Schistosoma mansoni* daughter sporocysts. Thus, the behavioral and qualitative characteristics of cercarial motility have been focused in the current research. Van Wyk & Van Resnburg (2003) observed a wide range of aberrant cercariae shapes and kinds. These included deformities of the tail, appendages of the tail or head, and the size of the cercariae being shorter or taller than normal, as well as exposing Ox mice to abnormal cercariae that are not infectious. They pointed out that since *S. matthei's* cercariae cannot swim, they are unlikely to infect animals in the wild or even an ox whose tail is submerged in a measuring cylinder holding a cercarial suspension since the animal in this situation appears agitated and has a tendency to move its tail continuously. Knutz (1948) proposed that in addition to hereditary factors, damage or physiological changes in the host at a critical point in the development of a trematode could play a role in abnormalities. Coat *et al.* (1960a, b) described the anomalies of cercariae caused by snail hyper-infection with microsporidian. Similarly to the present finding, Hazell *et al.* (2021) reported that cercariae were visibly damaged by exposure to ultraviolet fluencies. In this study, abnormal cercariae shed from treated snails may be due to a toxic effect of *A. niger* on the tissues of snails and also its effect on the development of sporocysts inside the snails lead to abnormal cercariae.

CONCLUSION

The current study recommends a longer-term sustainable biological control approach that limits the use of harmful molluscicides to reduce the population of snails while utilizing non-lethal fungi treatments. *Aspergillus niger* filtrate showed a higher toxicity level (mortality) than *A. flavus*. LC_{10} and LC_{25} of *A. niger* reduced the survival and infection rates of *B. alexandrina*. Moreover, it caused damage to the hermaphrodite gland, resulting in a decrease in the fecundity of *B. alexandrina*, and the treated snails shed aberrant cercariae, which could reduce schistosomiasis transmission.

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