

BIOLOGICAL AND HEMATOLOGICAL RESPONSES OF *BIOMPHALARIA ALEXANDRINA* TO MYCOBIOSYNTHESIS SILVER NANOPARTICLES

By

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Abstract

Silver nanoparticles (AgPNs) extracts were prepared from seven fungal isolates were evaluated through measuring their toxicity against *Biomphalaria alexandrina* snails. The effects of the two promising *Paecilomyces variotii* and *Aspergillus niger* AgNPs sub-lethal concentrations (LC₁₀ & LC₂₅) on the levels of steroid sex hormones, liver enzymes, total protein, lipids, albumin, glucose, total and differential count of hemocytes and morphology of hemocytes, oocytes and sperms were studied in this work.

The short period of snails' exposure (24h) to the two fungal AgNPs resulted in significant decrease in the levels of progesterone in *B. alexandrina*. The level of testosterone hormone showed significant increase in snails exposed to *P. variotii* AgNPs while no significant change was recorded at the exposure to *A. niger* AgNPs. Also, estradiol hormone concentration increased significantly in this investigation with the increase of the concentration of the two tested compounds. In addition, significant elevation in ALT, AST and Alkaline phosphatase was recorded. The total number of the hematocytes increased significantly by 17.4-47.8%. Snails' granulocytes were reduced by 19.1- 43.8%, while hyalinocytes increased by 63.6-354.5%. The exposure of *B. alexandrina* to LC₂₅ of both *P. variotii* and *A. niger* AgNPs showed apoptotic hemolymph cells, fragmented, vacuolated and degenerated cytoplasm, shrunken nucleus and phagocytosis in the light microscopy photographs of the hemocytes. Besides, the photographs showed also, abnormal nuclear division, degeneration and large fat vacuoles in the cytoplasm and swallowed atretic oocytes. Also, the photographs showed dead sperm head separated from its tail, other sperms showed abnormal swallowed head with severely nodded tail, dead sperms with wrinkled tails, hyperplasia and necrotic sperm heads led to overlapping of tails. In conclusion, applying the biosynthesized compounds which led to destruction of blood cells (the immune system), ova and sperms (the reproductive system) of snails is an important effective step to control schistosomiasis.

Key words: *Biomphalaria alexandrina*, *Paecilomyces variotii*, *Aspergillus niger*, AgNPs, steroid hormones, granulocytes, hyalinocytes, schistosomiasis.

Introduction

There are multiple parameters affecting the transmission of schistosomiasis as water related vector-borne disease. Among them, the life expectancy of the snail intermediate hosts, in which schistosome larvae undergo asexual reproduction and developed to the human infecting stage "cercariae" (Anderson and May, 1985). Snail control could be regarded as a rapid and efficient of re-

ducing or eliminating transmission and remains among the methods of choice for schistosomiasis control (Rizk *et al.*, 2012). A new approach to control the vector snails of schistosomiasis; *Biomphalaria alexandrina* is the using of nanoparticles (El-Hommossany and El-Sherbibni, 2011).

On the other hand, chemical and physical methods have various limitations in terms of synthesis procedures which

may involve use of drastic experimental conditions coupled with release of toxic byproducts, thus damaging the environment. An alternative approach is their production by the process of mycosynthesis, using different species of fungi which act as nanobiofactories, since they produce and secrete enzymes which help in reduction of metal ions to nanoparticles. They are further easy to culture, maintain and due to considerable biomass favor the large scale production of a wide variety of metal nanoparticles including those of silver, gold, iron, cadmium, selenium and platinum. They have been found to possess monodispersity, stability and other favorable properties which have led to their wide usage and applications. However, biological methods, which make use of fungi, bacteria and plants, are more ecofriendly and are being explored as alternatives to conventional techniques which invariably involve hazardous waste generation (Das & Thiagarajan, 2012).

The fungus *Aspergillus fumigatus* (Sastry *et al.*, 2003) fungus resulted in the rapid synthesis of silver nanoparticles and the particles formed were monodisperse which can be used in bacterial applications. *Aspergillus flavus* (Mahendra *et al.*, 2009) also resulted in the accumulation of silver nanoparticles on the surface of its cell wall when incubated with silver nitrate solution.

Circulating hemocytes represent the primary mediator of cellular defense reactions in molluscs. Hemocyte morphology is continuously being given importance and several studies have been conducted on snail species (Noda and Loker, 1989; Barraco *et al.*, 1993; Kambale and Potdar, 2010; Di *et al.*, 2011; Cavalcanti *et al.*, 2012). Schistosome vector Snail' elimination could be possible through applying safe biocontrol agents affecting its immunity (Cir-

culating hemocytes) and reproduction (oocytes and sperms).

This work was undertaken to evaluate the role of mycobiosynthesis silver nanoparticles against *B. alexandrina* snails and its effects on some snails' hematological and biological parameters. Also, the study aims to test the possibility of using these compounds as a biological control for schistosomiasis vector snails.

Materials and Methods

Laboratory bred adult *Biomphalaria alexandrina* snails (8-10 mm) were obtained from the Medical Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Seven fungal isolates: *Penicillium aurantiogresium* (IMI 89372); *P. roqueforti* (IMI 285518); *Aspergillus niger* (NRRL, 595); *Verticillium chlamydo-sporium* var. (CBS 600.88); *Trichoderma viride* (RCMB 004001); *T. longibranchiatum* (RCMB 004006) and *Paecilomyces variotii* (RCMB 004101) were kindly provided from the culture collection unit of the Regional Center for Mycology and Biotechnology (RCMB) Al-Azhar University, Cairo-Egypt. All fungal isolates were subcultured and maintained on Sabouraud's Glucose agar (SGA) medium containing (g/l); glucose-20; peptone-10; agar-20 and distilled water, 1000ml, pH of medium was adjust to 5.4±0.2 at 25±2°C.

Previously, all fungal isolates were subjected for the extracellular biosynthesis of AgNPs, studying their optimal condition and characterized using UV-Visible Spectral Analysis, FT-IR, EDX, XRD and TEM analyses in an earlier work (Helmy and Mekawey, 2013).

The seven fungal AgNPs compounds were investigated against *B. alexandrina* snails by the exposure of 10 snails to each compound for 24 h at 100 ppm concentration. Then they maintained in

dechlorinated water 24 h for recovery and the mortality rate for snails was calculated. The promising compounds were chosen to complete the experimental study.

For molluscicidal screening, a stock solution of the tested compounds (1000 ppm) was prepared on the basis of W/V using dechlorinated tap water (pH 7.0–7.5). A series of concentrations that would permit the computation of LC₅₀ and LC₉₀ values were prepared from the compound (WHO, 1965). Three replicates were used, each of 10 snails (6–8 mm)/L of each concentration. Exposure and recovery periods were 24 h each at 25±1°C. For each test, three replicates of control snails were maintained under the same experimental conditions. The effectiveness for each compound has been expressed in terms of LC₅₀ and LC₉₀.

B. alexandrina snails exposed 24 hours to the sublethal concentrations (LC₁₀ and LC₂₅) of the promising fungal silver nanoparticles compounds in all experiments.

For hemolymph collection, the snails were dried by a paper towel and placed on their left side (i.e. spiral clockwise) on the bottom of a dry Petri dish. A small portion of the shell situated directly above the heart was removed and a syringe was inserted into the heart to collect the hemolymph as described by Negm *et al.* (1995).

The levels of total protein (Domas, 1975), albumin (Gustafsson, 1976), glucose (Trinder, 1969) and lipids concentrations (Knight *et al.*, 1972) were determined in the collected hemolymph. Also, the activities of transaminases Aspartate (AST) and alanine aminotransferase (ALT) (Reitman and Frankel, 1957; White *et al.*, 1970), the phosphatases; acid phosphatase; AcP (Moss, 1984) and alkaline phosphatase; AkP

(Babson, 1965) enzymes in the hemolymph of *B. alexandrina* snails were detected. Tested biochemical parameters were evaluated spectrophotometrically by using kits from Quimica Clinica Aplicada S. A. (QCA) Ltd., Span.

The concentrations of progesterone, Testosterone and Estradiol hormones were also determined by gas-liquid chromatography according the method of La atikainen *et al.* (1973).

For total and differential counting, monolayer of hemocytes were stained with Giemsa stain for 20 minutes, according to the methods of Abdul-Salam and Michelson (1983), examined and counted by light microscopy and photographed by Toup View Camera. Another sample for each group was withdrawn from the region of the hermaphrodite gland (after gentle crushing) and stained as previously mentioned for the examination of ova and sperms.

Statistical analysis was performed by the SPSS computer program (version 20 for windows).

Results

The silver nanoparticles (AgNPs) of *Penicillium aurantiogresium* (IMI 89372); *P. roqueforti* (IMI 285518); *Aspergillus niger* (NRRL 595); *Verticillium chlamyosporium* var. (CBS 600.88); *Trichoderma viride* (RCMB 004001); *T. longibranchiatum* (RCMB 004006) and *Paecilomyces variotii* (RCMB 004101) were investigated for their molluscicidal activity against *Schistosoma mansoni* vector snails (*Biomphalaria alexandrina*). Table (1) revealed that the AgNPs of two fungi only (*Paecilomyces variotii* and *Aspergillus niger*) have molluscicidal activity (85 and 95% mortality) at concentration of 100 ppm when exposed 24 hours and recovered for another day.

Table 1: Molluscicidal effect of fungal AgNPs against *B. alexandrina* after exposure to 100 ppm for 24 hours.

Fungal AgNPs	Snails' mortality %
<i>Penicillium aurantiogresium</i>	0
<i>Penicillium roqueforti</i>	0
<i>Verticillium chlamydosporium</i>	0
<i>Trichoderma viride</i>	0
<i>Trichoderma longibranchiatum</i>	0
<i>Paecilomyces variotii</i>	85
<i>Aspergillus niger</i>	95

Molluscicidal activity of silver nanoparticles of the two promising fungal AgNPs (*Paecilomyces variotii* and *Aspergillus niger*) against *B. alexandrina* snails after 24 hours of exposure and

other 24 h recovery (Tab. 2). Data obtained indicated that *A. niger* AgNPs is more toxic to *B. alexandrina* than *P. variotii* AgNPs.

Table 2: Molluscicidal activity of *Paecilomyces variotii* and *Aspergillus niger* AgNPs against adult *B. alexandrina* (24 hours exposure).

	LC ₁₀	LC ₂₅	LC ₅₀	LC ₉₀	SLOPE
<i>Paecilomyces variotii</i> AgNPs	65.5	74.7	85.0	104.5	1.21
<i>Aspergillus niger</i> AgNPs	32.6	47.9	64.8	97.0	1.54

Regarding biochemical analysis (Tab. 4) the exposure to the tested compounds led to disturbance in the levels of the steroid hormones in *B. alexandrina* hemolymph. The progesterone levels decreased significantly, while the levels of testosterone and estradiol increased. *A. niger* AgNPs did not affect the testosterone hormone. The liver enzymes (ALT & AST) increased significantly by

the short period of exposure to both *P. variotii* and *A. niger* AgNPs. Also, the alkaline phosphatase enzyme increased significantly while the acid phosphatase showed no significant differences compared to control.

Also, the concentration of the total protein, glucose, albumin and total lipids are not affected significantly ($p > 0.05$) when compared to the control group.

Table 3: Effect of *Paecilomyces variotii* and *Aspergillus niger* AgNPs sublethal concentrations on some biochemical parameters and steroid hormones of *B. alexandrina* after 24 h exposure.

Test	Control		<i>P. variotii</i> AgNPs				<i>A. niger</i> AgNPs			
			LC ₁₀ (65.5 ppm)		LC ₂₅ (74.7 ppm)		LC ₁₀ (32.6 ppm)		LC ₂₅ (47.9 ppm)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Progesterone (Nmol/L)	0.51	0.05	0.37*	0.03	0.39 *	0.04	0.36 *	0.03	0.31 **	0.04
Testosterone (Nmol/L)	0.46	0.04	1.55***	0.07	1.33***	0.06	0.41ns	0.02	0.52ns	0.05
Estradiol (Pg/ml)	23.9	3.50	33.1*	3.81	36.1**	2.67	41.6**	4.11	42.8**	3.08
ALT (U/ml)	179	9.01	183 ^{ns}	6.23	423***	11.08	201*	9.88	280***	4.46
AST (U/ml)	80	5.31	214***	7.67	235***	8.09	212***	6.33	747***	13.04
Alkaline phosphatase(U/L)	84	7.02	108*	9.11	181***	5.32	317***	7.91	410***	9.54
Acid phosphatase (U/ml)	0.08	0.02	0.06ns	0.01	0.05	0.03	0.07 ^{ns}	0.02	0.06 ^{ns}	0.04
Total protein (mg/100 ml)	2.84	0.82	3.1ns	0.67	3.4ns	0.35	3.45 ^{ns}	0.79	2.7 ^{ns}	0.56
Glucose (mg/100 ml)	31.9	4.51	30.6ns	6.11	35.3ns	5.53	33.6ns	8.01	35.8 ^{ns}	7.09
Albumin (g/l)	1.8	0.80	1.9ns	0.09	1.8ns	0.51	1.7ns	0.46	2.0 ^{ns}	0.08
Total lipids (mg/100 ml)	352.6	15.38	375.6ns	13.44	352.1	9.78	321.1	14.11	322.7 ^{ns}	14.47

*, ** & *** = significantly different from control at $p < 0.05$, $p < 0.01$ & $p < 0.001$, ns= no significant difference ($p > 0.05$).

In hematological study, total count of the hemocytes showed significantly in-

crease by 17.4-47.8% in the snails exposed to both *A. niger* AgNPs and *P.*

variotii AgNPs sublethal concentrations 24 h compared to control ones (Tab. 3). Differential count indicated a significant reduction in the large granulocytes by 44.7% and 28.9% ($p < 0.001$) in snails

exposed to LC₂₅ from *A. niger* and *P. variotii* AgNPs, respectively. On the other hand, the hyalinocytes increased significantly in treated groups than the untreated control.

Table 4: Total and differential count of *B. alexandrina* hemocytes after exposure to *Paecilomyces variotii* and *Aspergillus niger* AgNPs for 24 hours.

AgNPs compound	Total Count		Differential count %		
	Mean	SD	LG	SG	Hy
Control	2300	101.4	76	13	11
<i>P. variotii</i> (LC ₁₀)	2700 *	165.7	62 *	10 ns	18 ns
<i>P. variotii</i> (LC ₂₅)	2900 *	202.8	42 ***	8 ns	50 ***
<i>A. niger</i> (LC ₁₀)	3200 **	300.0	51 ***	6 ns	43 ***
<i>A. niger</i> (LC ₂₅)	3400 **	265.7	54 ***	6 ns	40 ***

LG = large granulocyte, SG = small granulocyte, Hy = Hyalinocyte, *, ** & *** = significantly different from control at $p < 0.05$, $p < 0.01$ & $p < 0.001$, ns = no significant difference ($p > 0.05$)

The light microscopy photographs of *B. alexandrina* hemocytes (Fig. 1, Control) showed two morphologically distinct types of hemocytes, granulocytes and hyalinocytes. Granulocytes are distinguished to two types, large and small granulocytes with a low nucleus to cytoplasm ratio. Large granulocyte cells (LG) are variable in size with small nucleus and high cytoplasmic content due to the presence of refractile granules. Small granulocytes (SG) are intermediate cells with several micron sized granules and distinguished from LG by the central location of the nucleus and a relatively constant size. The hyalinocyte cells (Hy) are characterized by small size and they are circular shape with clear dense cell membrane, large nucleus and basophilic cytoplasm with a high nucleus to cytoplasm ratio.

B. alexandrina snails exposed to LC₁₀ of *P. variotii* AgNPs for 24 h showed some abnormalities in LG cells; eccentric nucleus (Fig. 1, A) nucleus division (Fig. 1, B) and the hyalinocytes appeared with vacuolated nucleus. When the concentration increased to LC₂₅, the LG cells showed abnormal dividing with highly vacuolated cytoplasm (Fig. 1, D), cell wall invagination (Fig. 1, E) pseudopodia (Fig. 1, F), vacuolated cy-

toplasm (Fig. 1, G) and the Hy cells appeared with cytoplasmic vacuoles (Fig. 1, H).

The exposure to LC₁₀ of *A. niger* AgNPs for 24 h showed enlarged dividing LG (Fig. 1, I), phagocytosis (Fig. 1, J) and lysed Hy (Fig. 1, N). When *B. alexandrina* exposed to LC₂₅ of *A. niger* AgNPs, the hemocytes' morphology showed obvious abnormalities. Granulocytes and hyalinocytes showed cell apoptosis with degenerated cytoplasm with released granules and shrunk nucleus (Fig. 1, L- N).

The impact of the tested compounds on the morphology of *B. alexandrina* oocytes and sperms was illustrated in figures (2 & 3). Figure (2, A) showed the normal mature oocyte and sperm in control group. *B. alexandrina* treated with LC₁₀ of *P. variotii* AgNPs showed terminal and divided nuclei (Fig 2, B & C), sperm head separated from its tail (Fig 2, D) and in Fig (2, E) the sperm showed pin head and thickened tail. The exposure to LC₂₅ led to light degeneration of the oocyte contents even in the oocyte division (Fig 2, F & G), the sperms showed swallowed head and thin tail (Fig 2, H) and other sperms showed abnormal swallowed head with severely noddled tail (Fig 2, I).

The exposure to *A. niger* AgNPs showed strong toxic effects on the oocytes and sperms of snails. At LC₁₀ exposure, the photographs showed dead aggregated ova (Fig. 3, A), sperms appeared in necklace shape (Fig. 3, B) and other showed swallowing of sperms heads. Increasing the concentration to LC₂₅ led to the appearance of swallowed, vacuolated and degenerated ova (Fig. 3, D), also other oocytes appeared with large fat vacuoles which led to degeneration of its contents (Fig. 3, E). In addition, the photographs showed atretic oocyte surrounded by dead sperms with wrinkled tails (Fig. 3, F) and hyperplasia and necrotic sperms heads led to overlapping of tails (Fig. 3, G).

Discussion

The present study results revealed that the silver nanoparticles of two fungi (*Paecilomyces variotii* and *Aspergillus niger*) from seven proved to have molluscicidal activity against *B. alexandrina* (at concentration of 100 ppm) of 85% and 95%, respectively. Ghoneimy *et al.* (2014) recorded toxic five fungal strains extracts (*Aspergillus sclerotium*; *Chaetomium globosum*; *Penicillium canescens*; *Penicillium griseofulvum* and *Trichoderma longibrachiatum*) from thirty against *B. alexandrina* and *Bulinus truncatus* snails. These results are also, in parallel with that of El-Hommosany and El-Sherbibni (2011) who recorded lethal effect of the photosensitizer Hematoporphyrin coated gold Nanoparticles (Hpd coated GNPs) against *B. alexandrina*.

The present results reported significance decrease in the levels of progesterone in snails treated with the two fungal AgNPs, which may lead to poor reproductive efficiency as a management for control snails' strategy. This agrees with the results of Mohamed and Aly (2014) who found significant low

level of progesterone in Fenitrothion treated snails. In addition, the levels of testosterone hormone increased significantly in snails treated with *P. variotii* AgNPs while no significant changes were observed at the exposure to *A. niger* AgNPs. This agreed with Rizk *et al.* (2012) who found disturbance in the level of steroid hormones in *B. alexandrina* treated with *Haplophyllum tuberculatum*.

Also, the concentration of estradiol hormone increased significantly in this investigation with the increase of the two fungal AgNPs concentration. The high concentration of estradiol in treated snails may be related to increasing rates of proliferation and protein synthesis that in turn leads to increase in total protein content as documented in the present results. On the other hand, Mohamed and Aly (2014) found decrease in estradiol hormone and total protein concentrations in Fenitrothion treated snails. The significant elevation in ALT, AST and AkP in treated snails is in agreement with Al-Sayed *et al.* (2014) who found that AkP and AcP were significantly increased by 38.5 and 48.4% in the hemolymph of *B. alexandrina* exposed to LC₂₅ of the methanol extract of *E. globulus* and *M. styphelioides*, respectively. In addition, Ghoneimy *et al.* (2014) found that all the liver enzymes (AST, ALT, AcP and AkP) in the hemolymph of the exposed snails were elevated significantly by the exposure to LC₂₅ of the *Penicillium canescens* bioactive compound for 4 weeks.

The hemocytes total count of snails exposed to sublethal concentrations of *P. variotii* and *A. niger* AgNPs showed significant increase compared to control group. The elevation of total hemocytes is the most commonly observed response in different molluscan species exposed to different stressors. Russo

and Lagadic (2004) stated that, the increase in hemocyte density might be seen as a way for the snails to maintain a constant level of global phagocytic activity, which was inhibited as a result of treatment. In agreement with this result, Mohamed (2011), found that exposure of *B. alexandrina* snails to sublethal concentration of Roundup herbicide led to significant increase in total hemocytes counts during 7 days of exposure. Consequently, the spreading hemocytes increased during the whole experimental period.

The present data recorded significant reduction in the number of granulocytes by 19.1- 43.8% and increase in the number of hyalinocytes by 63.6-354.5% in treated experimental groups. In agreement with these results, Gawish *et al.* (2008) recorded a reduction by 34.9% in granulocytes of *Bulinus truncatus* continuously exposed to LC₁₀ of the *Callistemon citrinus* extract for one week, while amoebocytes and hyalinocytes were increased by 54.4% and 21.7%, respectively. Mossallam *et al.* (2013) found a decrease in the number of *B. alexandrina* hemocytes when exposed continuously to LC₂₅ Artemether. Similar results were found by Sharaf El-Din (2003), Souza and Andrade (2006) and Kamel *et al.* (2006, 2007). The light microscopy photographs of untreated *B. alexandrina* hemolymph showed two morphologically distinct types of hemocytes; granulocytes and hyalinocytes. This was agreed with Sminia and Van der Knaap (1987), Lie *et al.* (1987), Barraco *et al.* (1993) and Serrano *et al.* (2002).

The exposure of *B. alexandrina* to LC₂₅ of both *P. variotii* and *A. niger* AgNPs showed apoptotic hemolymph cells, fragmented, vacuolated and degenerated cytoplasm, shranked nucleus and phagocytosis. Kamel *et al.* (2007) and Gawish *et al.* (2008) reported the

same results when using different plant molluscicides on hemocyte of *B. alexandrina* and *B. truncatus*. These abnormalities recorded in the hemocytes were also confirmed with the findings of Sharaf El-Din (2003), Souza and Andrade (2006), Kamel *et al.* (2006) and Mossallam *et al.* (2013).

Severe abnormalities also were detected in *B. alexandrina* oocytes and sperms due to the toxic effect of *P. variotii* and *A. niger* AgNPs in the present work. The photographs showed abnormal nuclear division, degeneration and large fat vacuoles in the cytoplasm and swallowed atretic oocytes. Also, the photographs showed dead sperm head separated from its tail, other sperms showed abnormal swallowed head with severely noddled tail, dead sperms with wrinkled tails, hyperplasia and necrotic sperm heads led to overlapping of tails. These abnormalities may be due to the inhibiting action of the fungal AgNPs on oogenesis and spermatogenesis in the ovotestis gland. These results in agreement with Abdel-Hamid *et al.* (2014) who recorded ova and sperm abnormalities as a result of *B. alexandrina* and *B. truncatus* exposure to sublethal concentrations of *P. canescens* bioactive compound. Also, Bakry *et al.* (2002) found that, *B. alexandrina* snails exposed to fungicide (Isoprothiolane) laid highly significant number of abnormal egg masses. Pagano *et al.* (2001) showed significant toxicity of R6 fungicide on sea urchins resulting in embryo developmental defects, transmissible damage from sperm to the offspring and cytogenetic abnormalities.

Conclusion

The applying the biosynthesized compounds which led to destruction of blood cells (the immune system), ova and sperms (the reproductive system) of snails is an important effective step to control schistosomiasis.

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Explanation of figures

Fig. 1: Light microscopy photographs of *Biomphalaria alexandrina* hemocytes showing two types of hematocytes in the control; granulocytes (large [LG] and small [SG]) and hyalinocytes (Hy). Photographs showing hemocytes of *B. alexandrina* exposed for 24 h to LC₁₀ of *Paecilomyces variotii* AgNPs (A-C), LC₂₅ of *P. variotii* AgNPs (D-H), LC₁₀ of *Aspergillus niger* AgNPs (I-K) and LC₂₅ of *A. niger* AgNPs (L-N) (1000X).

Fig. 2: Light microscopy photographs of *Biomphalaria alexandrina* oocytes and sperms, control showing normal mature ovum and sperm (A), oocytes and sperms of *B. alexandrina* exposed for 24 h to LC₁₀ of *Paecilomyces variotii* AgNPs (B-E) and LC₂₅ of *P. variotii* AgNPs (F-I) (1000X).

Fig. 3: Light microscopy photographs of oocytes and sperms of *Biomphalaria alexandrina* exposed for 24 h to LC₁₀ of *Aspergillus niger* AgNPs (A-C) and LC₂₅ of *A. niger* AgNPs (D-G) (1000X).



