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www.eajbs.eg.net

Egypt. Acad. J. Biolog. Sci., 9(1): 21 –32 (2017)

Egyptian Academic Journal of Biological Sciences
E. Medical Entom. & Parasitology

ISSN: 2090 – 0783
www.eajbs.eg.net

The Effect of A bleaching Solution on *Schistosoma mansoni* Eggs: A scanning Ultrastructural Study

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ARTICLE INFO

Article History
Received: 20/1/2016
Accepted: 15/3/2017/1/2017

Key words:
Schistosoma mansoni Eggs, A scanning Ultrastructural Study

ABSTRACT

Schistosoma mansoni is one of the parasites causing schistosomiasis, a disease that threatens millions of people worldwide. The treatment of schistosomiasis is a critical point in research and includes fighting either adult worms or any of the different life cycle stages. Sodium hypochlorite (NaOCl) is the main constituent of the bleaching solution, in the present study different concentrations of the bleaching solution were used to recognise their effects on the surface topography of *Schistosoma mansoni* eggs; 1, 5, 10 and 30 ppm of were used at time scale of 15, 30 and 60 minutes for each separate treatment. All of the concentrations experimented in this study affected the eggs if compared to the control ones. The surface topography of the eggs was greatly changed with increasing concentration and time, since, at higher concentration of the bleaching solution, some eggs were shrunk, deformed and egg shells were cracked. So, the bleaching solution may therefore be a useful treatment for *Schistosomiasis mansoni*.

INTRODUCTION

Schistosomiasis is one of the most common human parasitic diseases in the world, and second most important parasitic infection after malaria in terms of public health and economic impact (Brooker *et al*., 2009). Schistosomiasis is a major global health problem and principal among a number of neglected tropical diseases for the chronic impairment of both personal and group productivity (Gryseels *et al*., 2006; Van der Werf *et al*. 2003). Schistosomiasis affects 207 million people in the developing countries, with approximately 800 million, mostly children, at risk of the infection in more than 70 tropical and subtropical countries, particularly in Africa, Asia and Latin America. *Schistosoma mansoni* (one type of intestinal schistosomiasis) represents the dominant species of Schistosoma (Christensen, 1984; Chitsulo *et al*. 2000; Grevelding 2004; Steinmann *et al*., 2006; Andrade, 2009; El Ridi *et al*., 2010; and El Ridi & Tallima 2013). There are five species of schistosomes that can infect humans, of which *Schistosoma mansoni* is the most important one. A disease related with schistosomiasis is not directly due to the adult worms but rather to the large numbers of eggs that trapped in tissues (Pearce and MacDonald 2002).

Generally, the *Schistosoma* life cycle is complex and needs a snail intermediate host and a mammalian definitive host. Human infection commences with cursorial penetration of the skin.

After entrance into the circulatory system, parasites migrate through the lungs and eventually rest in the liver where they grow, differentiate, mate and then migrate to the urogenital system or mesenteric veins of the hepatoportal system to start egg production (Rai *et al*. 2009).

Eggs of *Schistosoma mansoni* can be targeted to stop the whole life cycle of this important human parasite; the surface topography of *Schistosoma* eggs was studied by Ford and Blankespoor (1979) who found that numerous microspines covering the surface of *S. mansoni* eggs and the density of these microspines varied somewhat among eggs. A similar observation was noticed by Eissa *et al*., (2011) on the egg surface of *S. mansoni*, but they described these microspines as microspicules like chitinous projections.

Many studies used different compounds to investigate their effect on the *Schistosoma* eggs such as; calcium cyanamide synthetic drug which had an effect on the eggs of *Schistosoma japonicum* as well as on their miracidia (Zhou *et al*., 2015). The herbal extract of *Pulsatilla chinensis* had a suppressing effect on the hatching rates of eggs of *Schistosoma japonicum* (Chen et. al, 2013). Many studies also followed the effect of different compounds on the other different stages of *Schistosoma* such as miracidia, cercaria and adult stages (Magalhães *et al*., 2009; Ahmed and Rifaat, 2005 and Chen *et al*. 2013)

Sodium hypochlorite (NaOCl), which is derived from sodium chloride solutions is a convenient source of active oxygen. It is safely used in drinking water (Khan *et al*., 2008). NaOCl has low molecular weight, so it can be easily penetrating the cellular membranes (Eventov *et al*., 1998). NaOCl is a potent disinfectant destroying a variety of viruses, bacteria and fungi and can oxidize toxins that are present not only in blood, but also in tissues (Eventov *et al*., 1998; Gerba and Kennedy, 2007; Abadias *et al*., 2008; Peeters *et al*., 2010).

Sodium hypochlorite is also used by Taha *et al*., (2014) to follow its effect on the eggs of *Fasciola gigantica* and on the intermediate host snail; NaOCl has an obvious deleterious effect on the surface of eggs and has also increased mortality rate in host snail, which is concentration and time dependent. Concerning schistosomiasis, NaOCl was found to immediately suppress the infectivity of *S. mansoni* cercariae (Al-Sharkawi, 1997), it also affected the cercarial surface integrity (El-Shaikh, 2001) and caused severe tegumental damage of *S. mansoni* adult worms (Attia, 2009).

As the sodium hypochlorite is used as a disinfectant destroying a variety of viruses, bacteria and fungi, so the aim of the present study is to evaluate its effect on the eggs of *Schistosoma mansoni* as a strategy to targeting an important stage of the life cycle of *S. mansoni* in a step of toward fighting schistosomaisis.

MATERIALS AND METHODS

***Obtaining Eggs*:** Eggs of *Schistosoma mansoni* were obtained from Theodor Bilaharz Institute, Giza, Egypt

Treatment of eggs with a bleaching solution: A commercial bleaching solution, Clorox®, with 5.25% sodium hypochlorite (active ingredient) was used in the present study. The test solutions were prepared from a stock solution of 100 ppm diluted to 1, 5, 10 and 30 ppm. Eggs were divided into five groups; the first group was the control group (untreated eggs) in which eggs were kept in 0.85% saline in dark at 4°C, the other four groups (treated groups) of eggs were kept in dark at 4°C at specific concentrations (1, 5, 10 or 30 ppm) of the bleaching solution. Each of the four eggs' groups was subdivided into three subgroups; the first subgroup was kept for 15 minutes, the second subgroup was kept for 30 minutes and the third subgroup was kept for 60 minutes.

Examination of eggs using Scanning electron microscopy (SEM): All egg groups (control and treated) were fixed in 2.5%

glutaraldehyde in phosphate buffer (0.1 mol/L, pH 7.4) at 4°C for 24 hours and post-fixed in 1% osmium tetroxide in the same buffer for 1 hour. Eggs were then washed in the same buffer then dehydrated in graded series of ethanol (70%, 80% 95% and 100%) and dried using liquid carbon dioxide as a transitional medium. They were transferred on a specimen-holder and coated with a layer of gold in vacuum evaporator to prevent charging of the specimens. Eggs were examined and photographed with a Camscan DV4-scanning electron microscope, Grindel Biocenter, University of Hamburg, Hamburg, Germany.

RESULTS

The control group: Eggs showed normal shape, their surface is covered with tiny projections without any marks, wrinkles or fissures, the spine in its normal position (Fig. 1).

The first treated group: The eggs treated with 1 ppm bleaching solution, for 15 minutes, had slightly wrinkled surfaces beside the spine. There was no change in the spine's appearance (Fig. 2A, B and C)., for longer treatment (30 and 60 minutes) eggs were marked with more shrinks and wrinkles (Fig. 2D and E).

The second treated group: The incubation of eggs in 5 ppm bleaching solution for 15 minutes, egg shell was slightly folded (Fig. 3A). Wrinkles and cracks were observed on the egg shells (Figs. 3B and C) after 30 minutes' treatment. Eggs exposed to 5 ppm for 60 minutes, were damaged, as many eggs had deformities in their shape and collapsed as well; some eggs were opened through big cracks (Figs. D, E and F). It is also observed that some areas of the eggs were devoid from the microprojections especially at the side near to the spine (3C, E and F).

The third treated group: Eggs incubated in 10 ppm bleaching solution for 15 minutes, showed much more wrinkles and shrinks on their surfaces (Fig. 4A), these wrinkles transformed into elevations and depressions (Fig. 4B) after 30 minutes' treatment. Some eggs were devoid from the microprojections that cover the egg surface (Fig. 4B). Deformed miracidium was also extruded from the egg. The eggs treated for 60 minutes were opened, as the egg shells were destroyed and extruded miracidia were affected with the bleaching solution, as some regions of the miracidium were devoid of cilia (Fig. 4C)

The fourth treated group: The largest concentration used in this study was 30 ppm; eggs treated with this concentration for 15 minutes had surfaces with great elevations and depressions, their shape changed and many deformities appeared to these eggs especially at the end opposite to the spin's end which was also devoid of microprojections, egg shells were cracked as well (Fig. 5A). Eggs treated with 30 ppm bleaching solution for 30 minutes were extremely deformed and highly collapsed (Fig. 5B). The last group of eggs which treated for 60 minutes were opened and the miracidium was emerged from the opened egg shell (Fig. 5C).

DISCUSSION

In the present investigation, the eggs of *Schistosoma mansoni* were exposed to 1, 5, 10 and 30 ppm of the bleaching solution, for 15, 30 and 60 minutes. The current study showed that the effect of the bleaching solution was a dose and a time dependant. The surface of the egg shell changed gradually with increasing concentration and time within the same treatment. Many studies were performed to recognise the effect of various chemical compounds on different stages of trematodes. Concerning the direct effect of chemicals on the *S. mansoni* eggs; Zhou *et al*., (2015) followed the effect of calcium cyanamide (synthetic drug) on eggs of *S. mansoni*, and found a darkening of eggs colour gradually and the miracidia inside were atrophied as well as thickening in their egg shells. From the severe effects of this synthetic drug, the miracidial embryonic membrane stopped development 3-days after treatment; and the miracidia were severely deformed 7-days after treatment. In the present work,

wrinkles, shrinks were found on the egg shell surface and these changes may be due to increasing rigidity of the egg shell with increasing concentration and time of treatment. The Bleaching solution also affects the miracidium due to its penetration inside the eggs as some areas of the miracidium were devoid from cilia and this effect happened with higher concentrations (10 and 30 ppm) at longer exposure (60 minutes). The extrusion of deformed miracidium is due to the much hardness of the egg shell; the deformation of miracidium may be occurred due to the penetration of the bleaching solution inside the egg.

Eissa *et al*., (2011) studied the effect of miltefosine on different stages of *S. mansoni*, and they reported changes on the surface of the eggs, as some microspicules like chitinous projections were devoid from the egg surfaces due to the treatment of miltefosine. In the present work, a similar effect was observed, as the microprojections were devoid from the eggs treated with the bleaching solution at 5ppm for 30 and 60 minutes, at 10 ppm for 30 minutes and at 30 ppm for 15 minutes. Neves *et al*., (2010) concluded that the imidazolidines derivatives could have a role in destroying eggs through production of nitric oxide. In the present study, the bleaching solution/NaOCl is a source of active oxygen which could possibly damage the eggs of *Schistosoma* especially at higher concentrations.

Chen *et al*., (2013) studied the effect of herbal extract of *Pulsatilla chinensis* on some stages of *S. japonicum*, and they reported a suppression of hatching rate of eggs at lower concentration (4 microg/ml), They also observed an increase in miracidial death rate with increasing concentration of the extract; Similar results were also observed for the cercarial death rate. In the present work, the hatching of the eggs was observed after 60 minutes' exposure to 10 and 30 ppm of the bleaching solution, morphologically eggs were hatched and miracidia were deformed and emerged and this hatching may be due to the dryness and hardness of egg shells which may lead to cracking of egg shells and their abnormal hatching.

Ahmed and Rifaat, (2005) studied the effect of the water extract of *Solanum nigrum* leaves on *S. mansoni* eggs, the number of eggs in hepatic tissues decreased significantly in treated mice. In another study by Magalhães *et al*., (2009), they found that, the effect of 5 and 10 μM, curcumin reduced the egg production of *S. mansoni* by 50% compared to control. In another study by Hegazi *et al*., 2007, they showed the effect of Egyptian propolis from three localities against *Fasciola gigantica* eggs; in their study, the propolis at specific concentrations lead to a complete failure of development and death of all immature eggs. In the present study, the number and death of eggs were not taken into account as the ultra-structural surface of eggs was the main criterion

El-Shaik *et al*., (2010) studied the ultra-structural surface of *S. mansoni* miracidia after their exposure to NaOCl; they reported obvious alteration to the dimensions and shapes of the miracidia and observed excessive damage of the miracidial body cilia. This damage to the miracidial body cilia was increasing while increasing concentration of NaOCl. In the present work, the loss of cilia from some regions on the miracidial body was observed; this loss of cilia may be due to the activity of free oxygen species produced from NaOCl. The same explanation was also mentioned by El-Shaik and his colleagues. NaOCl may also cause biosynthetic alteration in cellular metabolism and phospholipid destruction as suggested by Guida (2006). Hypochlorite is a powerful oxidant agent of biomolecules and NaOCl may cause an oxidative action with irreversible enzymatic inactivation, and degradation for lipid and fatty acid constituents (Krokosz, 2003, Cortezzo *et al.,* 2004; Small *et al.,* 2007). It is also noticed that, exposure of low-density lipoprotein (LDL) to NaOCl resulted in immediate and preferential oxidation of amino acid residues (Hazell and Stocker, 1993). NaOCl, produced by the mammalian host defence to

kill invading bacteria, targeted a select group of redox-regulated proteins, the thiol group (Leichert *et al*., 2008). The transcription of some bacterial genes is altered upon exposure of cells to high levels of the reactive oxygen species NaOCl (Peeters *et al*., 2010). Sodium hypochlorite has an effect on cercariae and adult worms of *S. mansoni*, as NaOCl showed deleterious effects against *S. mansoni* cercariae (Fripp *et al.,* 1972) as well as an effect on the survival rate of cercariae which varied with its concentrations and the times of exposure (Al-Sharkawi, 1997).

An ultrastructural investigation on *S. mansoni* cercariae showed that NaOCl caused loss of tail region, vacuolization of the tegument with subsequent disruption of the apical syncytial tegument layer, as well as changes in the head region (El-Shaikh, 2001). NaOCl also decreased the *S. mansoni* worm motility and caused an extensive destruction of the tegument (Attia, 2009). In a study by Gray *et al*., (2006), they considered the effect of NaOCl on the diapausing of invertebrate eggs in the sediment introduced with transoceanic vessels and from Lake Erie (North America); as the sediments treated with NaOCl at concentrations 0 - 10,000 mg/l for 24 h, and they found that about 90% reduction in the hatching process at at1000 mg/l; This study was performed to prevent species introduction from foreign habitats. These results confirmed the effect of NaOCl on destruction of eggs which coincide with its present destructive effect on the eggs of *S. mansoni*. Egg shell of some trematode worms are affected by the treatment with concentrated acids such as sulphuric and hydrochloric acids that weaken the junction between the eggshell and operculum, while the same diluted acids and some alkalis such as potassium and sodium hydroxides do not affect this junction Madhavi (1968) and Balasubramanian *et al*., (2010)

CONCLUSION

In conclusion, this study showed that exposure of eggs of *S. mansoni* to different concentrations of the bleaching solution for different periods resulted in concentration-dependent changes in their egg shell surfaces. Ultrastructural observations revealed that the bleaching solution markedly altered the topography of the surfaces and even collapsing and cracking the egg shells especially at high concentrations with prolonged treatment time. The alterations appeared on *S. mansoni* eggs may be linked to oxidative properties of NaOCl, the active ingredient of the bleaching solution. These results may indicate that the bleaching solution/NaOCl could become a cheap and effective molecule that can be used in schistosomiasis control. More work is needed to explore the exact mechanism of how NaOCl affect the biological materials.

ACKNOWLEDGMENT

Authors would like to thank Prof. Dr. Kamal El-Shaikh, for his advice and help in photographing the eggs by scanning electron microscope.

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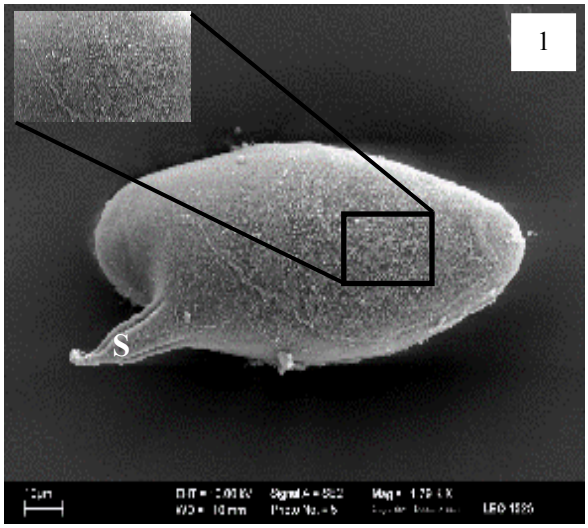
LIST OF FIGURES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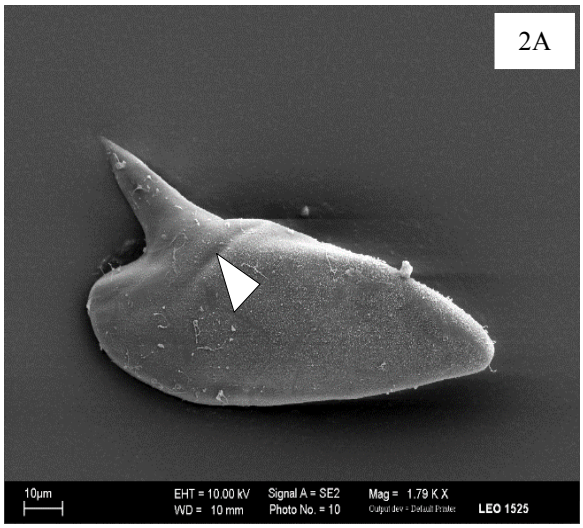
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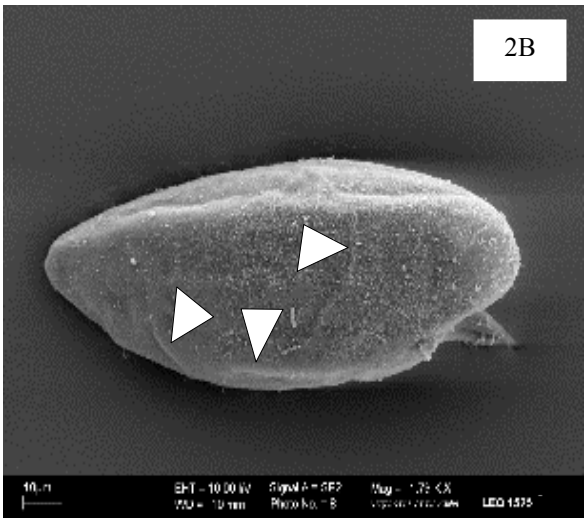
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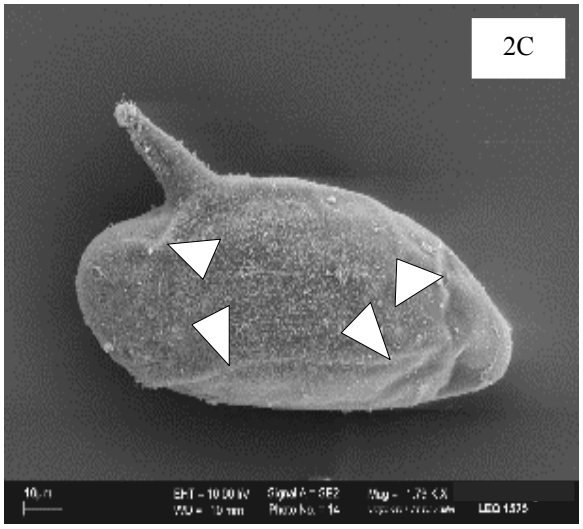
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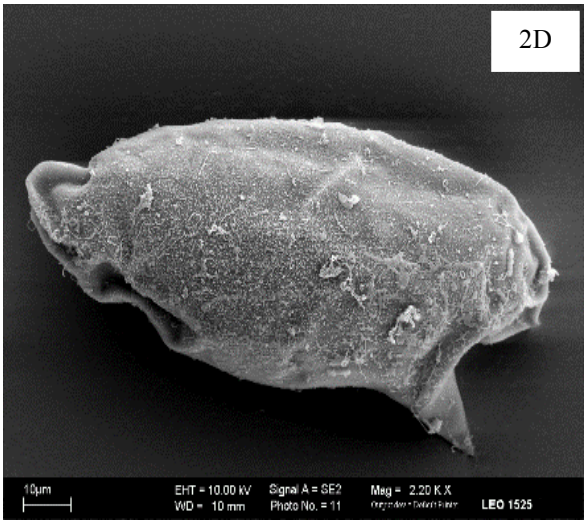
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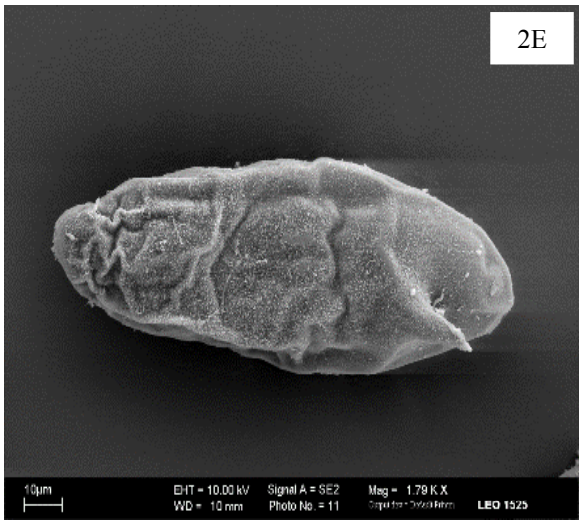
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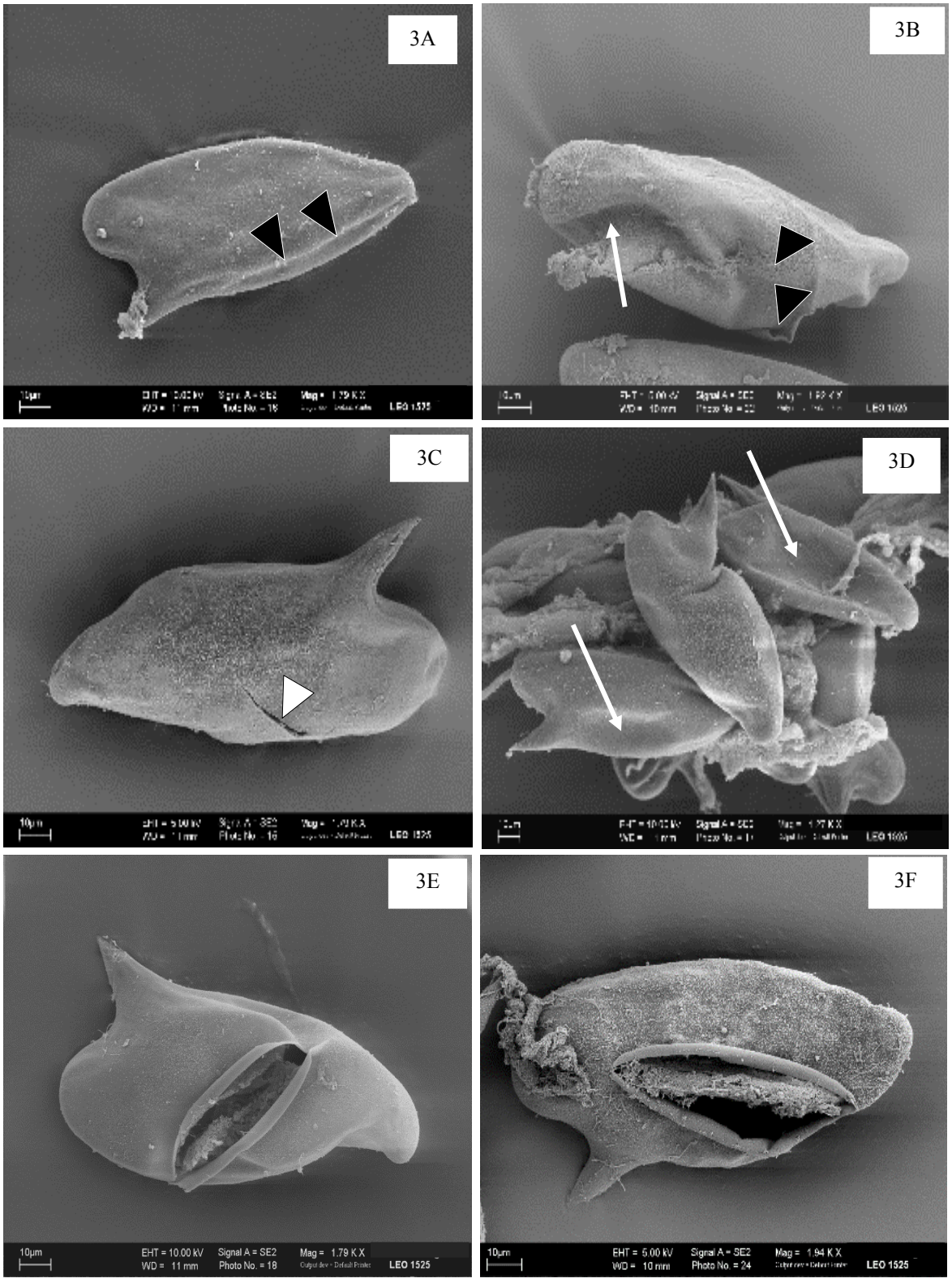
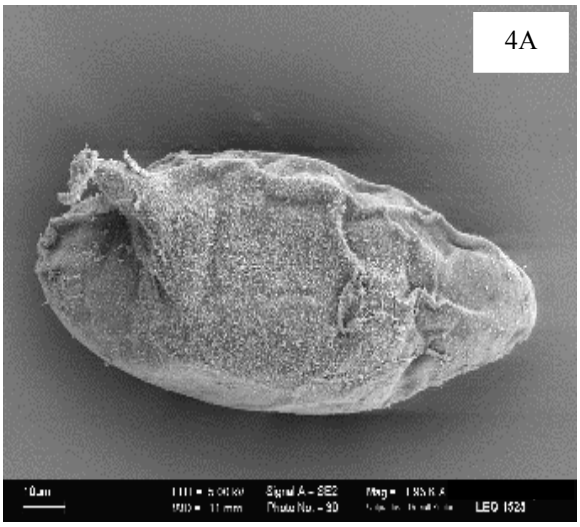
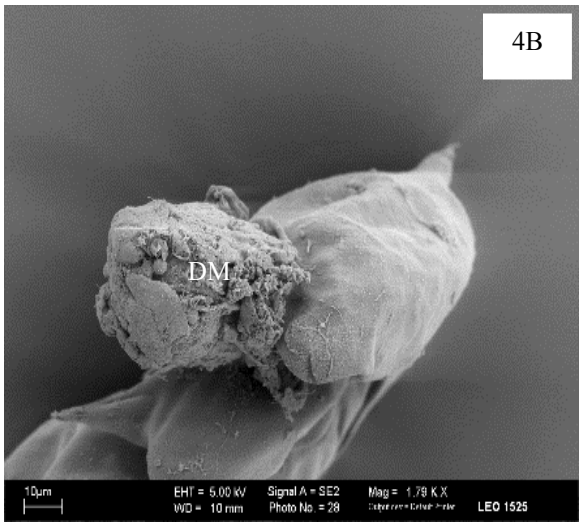
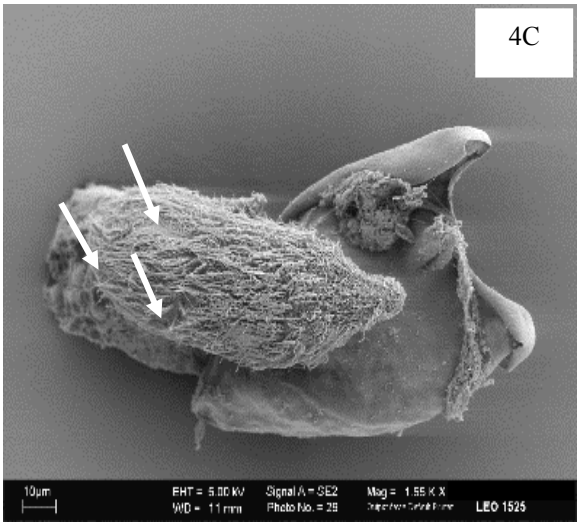
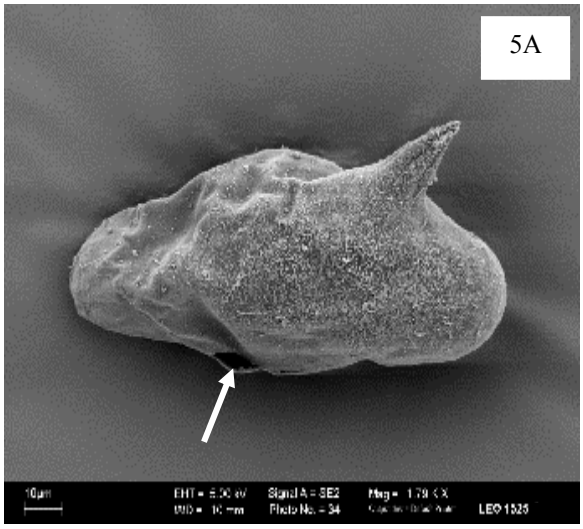
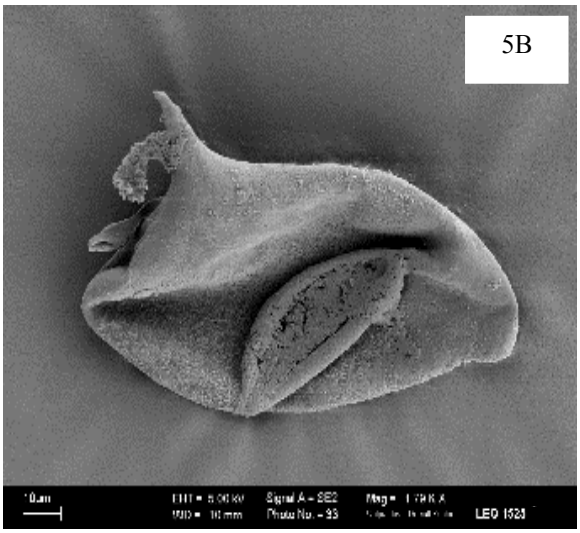

3A
3B
3C
3D
3E
3F
10µm
EHT = 10.00 kV
WD = 11 mm
Signal A = SE2
Photo No. = 18
Mag = 1.79 K X
LEO 1525
10µm
EHT = 5.00 kV
WD = 10 mm
Signal A = SE2
Photo No. = 24
Mag = 1.94 K X
LEO 1525

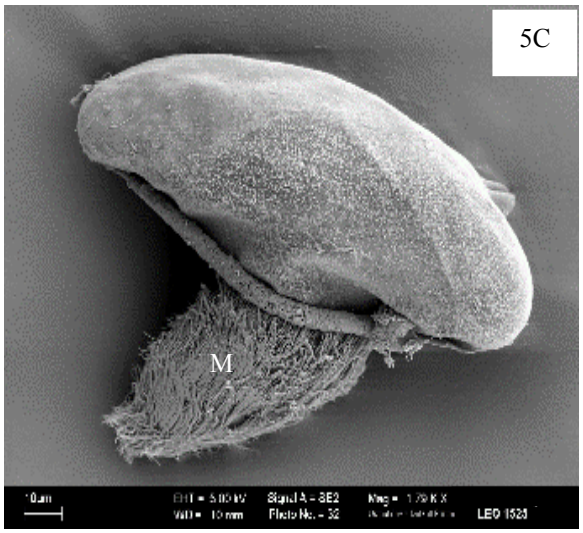

4A


4B
DM


4C


5A


5B


5C
M

ABIC SUMMERY

تأثير محلول مبيض على بيض *شيستوسوما مانسوني* في المختبر: دراسة ماسحة للتراكيب الدقيقة

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دودة البلهارسيا المعوية (*شيستوسوما مانسوني*) هي واحدة من الطفيليات التي تسبب مرض البلهارسيا، وهو مرض يهدد ملايين البشر في جميع أنحاء العالم. ويمثل علاج مرض البلهارسيا تحدي للباحثين وقد يكون العلاج إما عن طريق التخلص من الديدان البالغة أو أي من مراحل دورة حياتها المختلفة. وفي هذه الدراسة تم استخدام محلول مبيض - هيبوكلوريت الصوديوم - لمكافحة البلهارسيا من خلال التأثير على بيض البلهارسيا المعوية. وقد أُستخدمت تركيزات مختلفة من هيبوكلوريت الصوديوم للتعرف على تأثيره على سطح البيض. وتم استخدام ١، ٥، ١٠، ٣٠ جزء في المليون في هذه الدراسة على مقياس زمني قدره ١٥، ٣٠، ٦٠ دقيقة لكل معاملة منفصلة. وقد لُوحظ إن جميع التراكيز التي جُربت في هذه الدراسة أثرت على البيض إذا ما قورنت بالمجموعة الضابطة. كما أن سطح البيض قد تغير بشكل ملحوظ مع زيادة التركيز والوقت، حيث أنه عند التركيزات العالية لهيبوكلوريت الصوديوم، أنكمش بعض البيض وتشوهت قشرته. لذلك، فإن هيبوكلوريت الصوديوم قد يكون علاج مفيد للبلهارسيا المعوية.