INTERNATIONAL JOURNAL OF ENVIRONMENTAL SCIENCE AND ENGINEERING (IJESE)
Vol. 5: 25- 37 (2014)
http://www.elac.pvamu.edu/pages/5779.asp,
Prairie View A&M University, Texas, USA

Efficacy of the fungi *Aspergillus terreus* and *Penicillium janthinellum* as biological control agents against *Biomphalaria alexandrina* snails

Abd El-Halim A. Saad [1]; Magdy T. Khalil [1]; Fawzy M. A. Ragab [2]; Amal A. I. Mekawey[3] and Marwa T. A. Abd El-Wareth[2]
1-Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt
2-Environmental Researches Department, Theodor Bilharz Research Institute, Egypt
3- Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt

ARTICLE INFO
Article History
Received: March 2014
Accepted: June 2014
Available online: Dec. 2014

Keywords:
Aspergillus terreus
Penicillium janthinellum
Biological control
Biomphalaria alexandrina

ABSTRACT

Two fungal species; *Aspergillus terreus* and *Penicillium janthinellum* were tested as filtrates against *B. alexandrina* snails. The LC_{50} values were 1.05% and 1.03% respectively. Prolonged exposure to sublethal concentrations of the two fungal filtrates caused adverse effects on snails reproduction. The minimum reproduction values were observed at LC_5 and LC_{25} of *A. terreus* and *P. janthinellum*, respectively. The most pronounced effects on hermaphrodite gland of treated *B. alexandrina* snails were detected at LC_5 of *A. terreus* and LC_{25} of *P. janthinellum*. Mature ova were either degenerated or deformed and spermatocytes were also degenerated at LC_5 of *A. terreus*. Acini appeared vacant and lost their architecture at LC_{25} of *P. janthinellum*.

1. INTRODUCTION

Schistosomiasis is one of the most widespread endemic diseases in tropical and subtropical countries. This parasitic disease is of particular importance as a world health problem since it infects about 200 million and threatens about 500 million of world population in about 74 countries (Borch *et al*., 2009).

Five species of schistosomiasis infect man, the most important are *Schistosoma mansoni* and *S. haematobium*. The life cycle of this parasite necessitates the presence of a freshwater snail as an intermediate host that differs according to the parasite species. In Egypt, the intermediate host of *S. mansoni* is *Biomphalaria alexandrina* while the intermediate host of *S. haematobium* is *Bulinus truncatus*.

It is generally considered that snail control is one of the most rapid and effective means of reducing transmission of parasitic infections. These snail vectors can be controlled by mechanical, chemical, and biological methods.

The use of chemical molluscicides leads to toxicity of the non-target organisms, contamination of human food and environmental pollution (Oliveira-Filho & Paumgartten, 2000; De Boeck *et al*., 2004; Mostafa *et al*., 2005). These drawbacks of chemical substances directed investigators to natural enemies, such as predators, parasites, and pathogens.

ISSN 2156-7530

These biological agents possess desirable properties of a chemical molluscicide making it highly toxic to the target organism, besides it can be safely applied (Moazami, 2008). Many studies focused on biological control methods such as the use of certain algae (Mostafa and Gawish, 2009), bacterial strains (Wang *et al*., 2008) and extracts of some fungal strains such as *Trichoderma harzianum*, *Trichoderma viride*, and *Phanerochaete chrysosporium* (Ragab and Ismail, 2001).

The objective of the present study is to investigate the efficacy of the fungi *Aspergillus terreus* and *Penicillium janthinellum* as biological control agents against *Biomphalaria alexandrina* snails.

2. MATERIALS AND METHODS

2.1 Screening and toxicity tests:

2.1.1 Preparation of fungal filtrates:

Fungal cultures were prepared by inoculating conical flasks (250 ml capacity) containing 50 ml of potato dextrose broth medium with fungal disks (5 mm diameter) which were cut from 7 days old cultures (Ragab and Ismail, 2001). The inoculated flasks were incubated on rotary shaker (150 rpm) at 28°C for 10 days. The mycelia then were separated by filtration, using filter paper (Whatman no.3) (Umecharuba and Nwachukwa, 1997).

2.1.2Toxicity of fungal filtrates against *Biomphalaria alexandrina* snails:

A series of concentrations was prepared using dechlorinated tap water at 22±2°C to determine LC_{50} and LC_{90} values. Three replicates were used; each of ten snails (8-10 mm in diameter) for each one. The exposure period was 24 hours at room temperature. Another group of snails was maintained under the same experimental conditions as a control group (WHO, 1965). At the end of exposure period, these snails were removed from each tested concentration, washed thoroughly with dechlorinated tap water and transferred to another container for a recovery period for 24 hours. Then, dead snails were counted and LC_{50} and LC_{90} values were computed (Litchfield and Wilcoxon, 1949).

2.2 Effect of prolonged exposure to fungal filtrates on survival rate and egg-laying capacity of adult snails:

Adult *B. alexandrina* (8-10 mm in diameter) were used in this study. This experiment was designed to explore the effect of prolonged exposure to sublethal concentrations (LC_5, LC_{15} and LC_{25}) of *Aspergillus terreus* and *Penicillium janthinellum* as filtrates on the survival rate and fecundity of adult snails.

Each sublethal concentration of each tested fungal species was prepared weekly in dechlorinated tap water. A group of 20 adult snails was exposed in four replicates, each of 5 snails/250 ml dechlorinated tap water in a white glass container. A control group was maintained in clean dechlorinated tap water under the same experimental conditions. These snails were fed on dried lettuce leaves twice weekly. Each container was provided with a substrate (foam pieces) for ovipostion. Dead snails were removed from the containers every day, and their number from each single container was recorded. The egg masses laid by the snails were collected every day and recorded weekly. The total number of eggs/ egg mass was counted under a stereomicroscope. The total number of survived snails at the beginning of a week and the total number of eggs laid by the exposed and control snails were recorded and calculated at the end of the week according to Oliver and Haskins (1960) and Khalaf (1983) as following:

$$E/S/W = \frac{T_n}{S_s}$$

$$R = Sr \times E/S/W$$

Where:
E/S/W: Eggs/snail/week (egg laying capacity)
T_n: Total number of eggs at the end of a week
S_s: Survived snails at the beginning of the week
R: Reproduction Sr: survival rate

2.3 Effect of fungal filtrates on histology of hermaphrodite gland of snails:

Adult *B. alexandrina* snails exposed for one month to sublethal concentrations (LC_5, LC_{15} and LC_{25} of *Aspergillus terreus* and *Penicillium janthinellum*) were randomly selected. The shell was gently broken, and then its fragments were removed carefully using pointed forceps under a dissecting microscope. The hermaphrodite gland was carefully separated using fine scissors and immediately fixed in Bouin's solution for 24 hours. The fixed samples were dehydrated, cleared, and embedded in paraffin. Then they were sectioned serially at 5µm and stained with Haematoxylin and Eosin.

3. RESULTS AND DISCUSSION

3.1 Toxicity of fungal filtrates against *Biomphalaria alexandrina* snails:

The values of LC_{50} for *A. terreus* and *P. Janthinellum* were 1.05% and 1.03%, respectively (Table 1). Some authors investigated the effects of other fungal species on snails; Ragab and Ismail (2001) found that the LC_{50} values of *Trichoderma viride*, *T. harzianum*, and *Phanerochaete chrysosporium* filtrates were 5.50, 22.50 and 24 %, respectively on *B. alexandrina*. While, the LC_{50} value of alcoholic extract from certain strain of *Aspergillus niger* aginst *Oncomelania hupensis* snails was 284.3 mg/L (Chen *et al*., 2009). Moreover, Guo *et al*. (2011) and Osman *et al*. (2013) found that LC_{50} values of *Aspergillus fumigatus* extract was 0.101 mg/L and 0.56 ppm on *Oncomelania hupensis* and *B. alexandrina*, respectively.

Table 1: Molluscicidal activity of tested fungal filtrates against adult *B. alexandrina*

Concentration / Fungal species	*Biomphalaria alexandrina*		
	LC_{50} (%)	**LC_{90} (%)**	**slope**
Aspergillus terreus	**1.05**	**2.08**	**1.55**
Penicillium janthinellum	**1.03**	**4.09**	**2.6**

The effect of *A. terreus* filtrate in the present study could be attributed to secondary metabolites of that fungus. Fungi belonging to the *Aspergillus* genera are among the major contributors to secondary metabolites of fungal origin (Parvatcar *et al*., 2009). *Aspergillus terreus* is a ubiquitous fungus isolated from both marine and terrestrial environments (Balajee, 2009; Parvatcar *et al*., 2009). This microorganism produces a variety of secondary metabolites that are economically significant, such as the antihypercholesterolemic drug lovastatin and several other metabolites including aspulvinones (Ojima *et al*., 1976), terreic acid (Yamamoto *et al*., 1980), terreulactone A (Kim *et al*., 2002) terreineol, sulochrin and terrain, which have antibiotic activity (Macedo *et al*., 2004).

Three new compounds; terrelactone A, terremides A and B were isolated and identified from the fermentation broth of *A. terreus* by Wang *et al*. (2011). These compounds were shown to have antibacterial activities. Moreover, Dewi *et al*. (2012) demonstrated that terreic acid and terremutin from ethyl acetate extract of *A. terreus* exhibited significant antioxidant activity. Four isocoumarin derivatives were isolated from ethyl acetate extract of *A. terreus* (Choudhary *et al*., 2004). It was reported that the molluscicidal properties of *Ethulia conyzoides* plant was due to two types of coumarins which caused mortality of 90% of *Biomphalaria glabrata* and *Bulinus truncatus* at 23.5 and 15 ppm, respectively (Kady *et al*., 1992). Furthermore, Rizk and Hassan (2000) isolated eight coumarins from *Ammi majus* extract, four of them were molluscicidally active against *B. alexandrina* snails.

Molluscicidal activity of *P. janthinellum* filtrate observed in the present work could be attributed to its secondary

metabolites. *Penicillium* genus has been recognized as a rich source of bioactive compounds (Fill *et al*., 2007). Recent examples include the anticancer berkelic acid from *Penicillium* sp., polyketides with HIV integrate inhibitory activity from *P. chrysogenum* and the insecticidal paraherquamides H and I from *P. cluniae* (Singh, 2003; Stierle *et al*., 2006). *P. janthinellum* produced polyketides, basically hydroxyanthraquinones, ergosterol, poliols (Marinho *et al*., 2005) and phenolic compounds (Nakakita *et al*., 1984). It was declared that the toxicity of *Anacardium occidentale* plant extract against the golden snail (*Pomacea canaliculata*) and *Biomphalaria glabrata* was due to the presence of several phenolic compounds (Micor *et al*., 2004; Dos Santos *et al*., 2011).

3.2 Effect of prolonged exposure to sublethal concentrations of *Aspergillus terreus* and *Penicillium janthinellum* on survival rate and egg-laying capacity of adult snails:

The values of sublethal concentrations of *A. terreus* and *P. janthinellum* were the same; LC_5 = 0.1%, LC_{15}=0.3% and LC_{25}= 0.5%.

3.2.1 Survival rate:

It is observed from Fig. (1) that after 4 weeks of exposure to LC_5 of *A. terreus* filtrate, the survival rate of snails decreased to 55%, while that of snails treated with LC_{15} and LC_{25} reached 50% as compared with 100% for control snails. These values continued to decrease as the time extended, and by the 19th week, only 10% of each tested group of snails survived as compared with 90% in the control group.

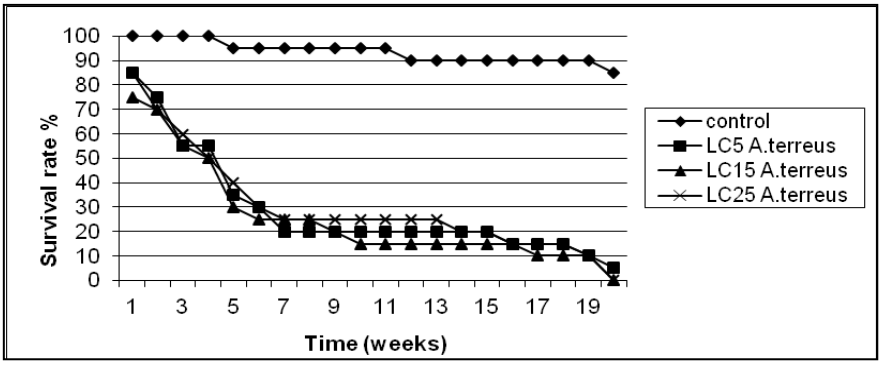


Fig. 1: Effect of sublethal concentrations of *A. terreus* on the survival rate of adult *B. alexandrina*.

Concerning *P. janthinellum* filtrate, the survival rate of snails exposed to LC_{25} of this fungus decreased sharply to 45%, while that of control was 100%. Moreover, it was found that prolonged exposure (15 weeks) resulted in the death of all snails exposed to LC_{25}, while 90, 15 and 10 % of snails survived in control, LC_5 and LC_{15} groups, respectively. By the 20th week, all the snails in LC_{15} treatment died, while the survival rate of control group was 85% (Fig. 2).

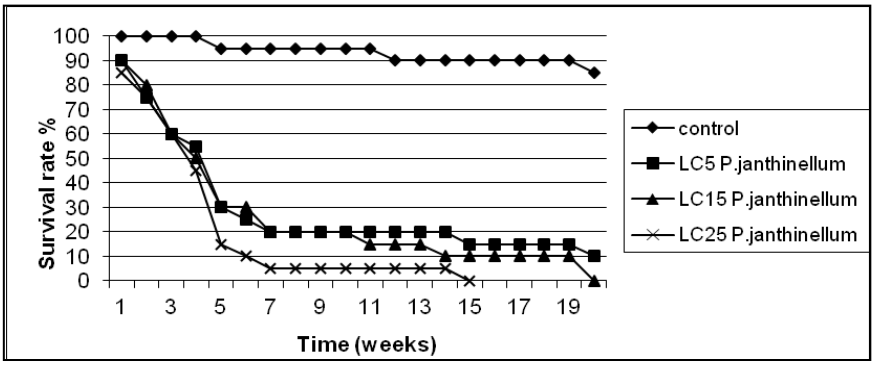


Fig. 2: Effect of sublethal concentrations of *P. janthinellum* on the survival rate of adult *B. alexandrina*

These findings are in line with the results demonstrated by Ragab and Ismail (2001), where *B. alexandrina* snails exposed to 1/10 LC_{50} of *Trichoderma harzianum* and *T. viridae* died by the 5^{th} and 12^{th} weeks, respectively. Osman *et al*. (2013) showed that the survival rate of the same snails treated with *Aspergillus fumigatus* extract was reversely proportional to the concentration; the snails died at the 10^{th}, 11^{th} and 12^{th} weeks in LC_0, LC_{10} and LC_{25}, respectively.

3.2.2 Egg-laying capacity:

3.2.2.1 *Aspergillus terreus*:

Featuring the variation in reproduction along the five months of exposure declared that during the first month, there was a positive relationship between sublethal concentrations and reproduction. As the concentration increased from LC_5, LC_{15} till LC_{25}, the reproduction increased from 5.94, 7.44 to 14.48 respectively, while that of control group was the highest (22). During the second month, the values of reproduction for snails maintained at LC_5 and LC_{15} were so close (10.2 and 9.26 respectively), while reproduction of snails treated with LC_{25} continued to be higher than both mentioned concentrations as it was 16.29. By the third month of exposure, the reproduction noticeably decreased to 1.75 in snails treated with LC_{25} as compared with 43.09, 4.5 and 5.2 for control, LC_5 and LC_{15} groups respectively. At the 4^{th} and 5^{th} months, the least reproduction values were observed in snail groups maintained at LC_{25} (2.25 and 2.1 respectively) (Fig. 3).

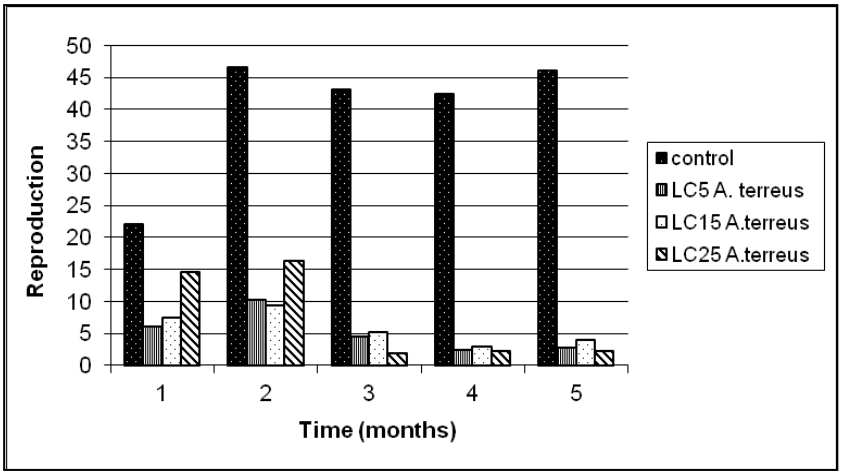


Fig. 3: Effect of sublethal concentrations of *A. terreus* on the reproduction of *B. alexandrina*

This initial increase in snails' fecundity might be later suppressed by possible re-allocation of energy for processes involved in detoxifying xenobiotics or synthesizing functional or structural proteins in response to toxicity (Mazurova *et al*., 2008). Our experiments with *A. terreus* filtrate thus seemed to suggest the presence of organic compounds that may stimulate fecundity, but such effects may be later masked by the toxicity of other compounds in the filtrate mixture. Another study carried out by Suliman and Mohammed (2012) on *A. terreus* showed different results as they found that as the concentration of *A. terreus* spore suspension increased from $5x10^6$ to $4x10^8$ spore/ ml, the inhibition of oviposition in ticks increased. On the other hand, Osman *et al*. (2013) demonstrated that among three tested concentrations (LC_0, LC_{10} and LC_{25}) of *Aspergillus fumigatus* extract, the minimum reproductive rate was observed in *B. alexandrina* snails maintained at LC_{10} after 12 weeks of exposure.

3.2.2.2 *Penicillium janthinellum*:

It was observed from Fig. (4) that along the five months of exposure to the

three tested sublethal concentrations, the snails in the control group recorded the highest reproduction value. During the first month, the maximum reproduction appeared in the group of snails maintained in LC_5, followed by LC_{25}. In the second month, there was a gradual decrease in the reproduction value as the concentration increased; 6.05, 5.1 and 1.49 for LC_5, LC_{15} and LC_{25} groups, respectively. Moreover, the reproduction decreased as the exposure time extended to three months where its value reached 1.2 for snails treated with $LC_{25.}$ At the 4th month, the values of reproduction were so close in the three tested concentrations with slight increase in LC_{15} group. This is in agreement with Ragab and Ismail (2001) who indicated that the reproduction values of *B. alexandrina* exposed to 0.55, 2.25 and 2.4% of *Trichoderma viridae*, *T. harzianum* and *Phanerochaete chrysosporium* were noticeably less than that of control, Moreover, the percentage of egg laying reduction reached 97.21% for the last fungus. Latifian and Rad (2012) also showed that exposure of certain pest to *Beauveria bassiana, Beauveria brongniartii and Metarhizium anisopliae* which are entomopathogenic fungi reduced the number of laid eggs. They declared that the effect was more pronounced as the concentration increased from 10^4 to 10^6conidia/ml and also as the exposure period extended to 14 days.

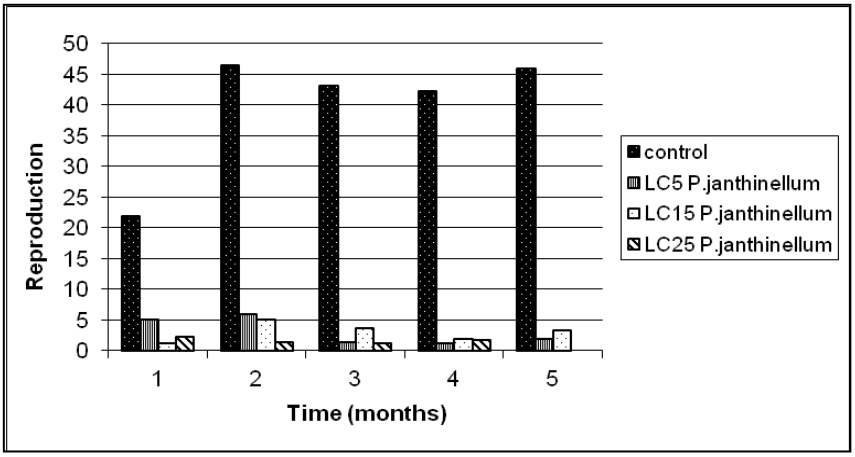


Fig. 4: Effect of sublethal concentrations of *P. janthinellum* on the reproduction of *B. alexandrina*

3.3 Effect of sublethal concentrations of *Aspergillus terreus* and *Penicillium janthinellum* on histology of hermaphrodite gland of snails:

3.3.1 Control snails:

The hermaphrodite gland of *B. alexandrina* is composed of simple branched acini which are connected to each other by a thin layer of connective tissue. The acini contain different stages of spermatogenesis; primary spermatocytes, secondary spermatocytes, and sperms. The stages of oogenesis are also represented in most of the acini as primary oocytes, secondary oocytes, and mature ova (Fig. 5).

3.3.2 Treated snails:

3.3.2.1 Snails treated with *Aspergillus terreus*:

The snails treated with LC_5 of *A. terreus* were characterized by noticeable effects on mature ova as some of them were degenerated and others were deformed. Also, early stages of oogenesis were not represented. On the other hand, primary spermatocytes were degenerated, while secondary ones were scattered in the acinus without differentiation between their cytoplasmic inclusions and nuclei (Figs. 6 & 7).

The effect of LC_{15} of *A. terreus* was weaker than that of LC_5; some acini were disrupted, moreover the acinar membrane was ruptured, there was degeneration of mature ova besides the presence of vacuoles inside the acinus, and primary spermatocytes were densely stained (Figs. 8 & 9).

Concerning the effect of LC_{25}, it was observed that the acini were more or less similar to that of control as all stages of oogenesis and spermatogenesis were represented, but the connective tissue between acini was degenerated (Figs. 10 & 11).

3.3.2.2 Snails treated with *Penicillium janthinellum*:

For snails treated with LC_5 of *P. janthinellum*, the degeneration of some ova and the deformation of others were the most obvious effects (Figs. 12 & 13). As the concentration increased to LC_{15}, more adverse effects were noticed as shrinkage and disruption of acini took place, vacuolation inside the acinus was more severe, besides the degeneration and deformation of mature ova (Figs. 14 & 15).

In case of snails exposure to LC_{25} of *P. janthinellum*, acini appeared almost vacant, only few mature ova and primary spermatocytes were represented in certain acini. In general, acini lost their architecture and acinar membrane was ruptured (Figs. 16 & 17). These findings were in agreement with El-Bolkiny *et al*. (2000) as they showed that hermaphrodite gland of treated *B. alexandrina* snails with diethyldithio-carbamate exhibited destruction of germinal epithelium and oocytes. Moreover, mature ova appeared to be necrotized and few sperms were represented. Furthermore, Mossalem *et al*. (2013) recorded complete destruction of gametogenic cells and severe damage of ovotestis gland when *B. alexandrina* snails were exposed to Artemether (dihydro-artemisinin methyl ether) for 21 days. Also, disappearance of the oocytes, spermatogenic stages and connective tissue was noticed.

4. CONCLUSION

It is demonstrated from the present study that LC_5 and LC_{25} of the fungi *Aspergilus terreus* and *Penicillium janthinellum* filtrates, respectively reduced survival rate and reproduction of *B. alexandrina* snails. Also, these two sublethal concentrations adversely affected hermaphrodite gland. Moreover, in another study by the same authors (unpublished data), it was shown that these concentrations can be applied safely for snail control without exerting harmful effects on other freshwater fauna such as the zooplankton *Daphnia pulex*.

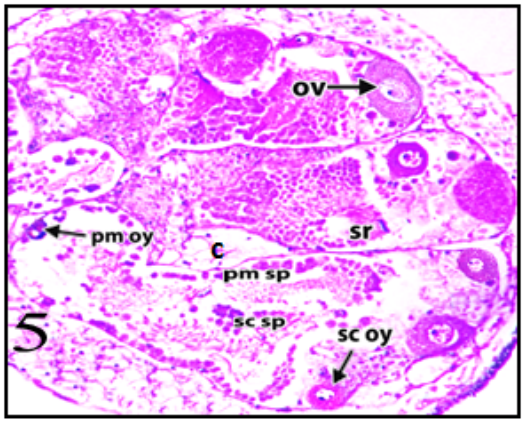
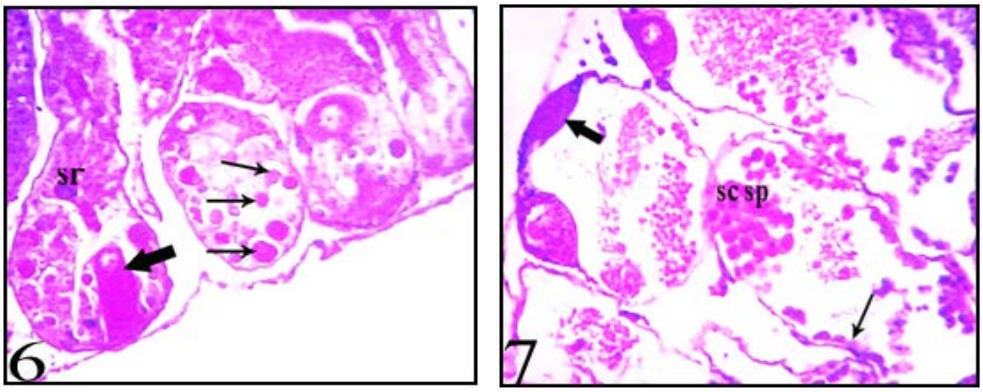
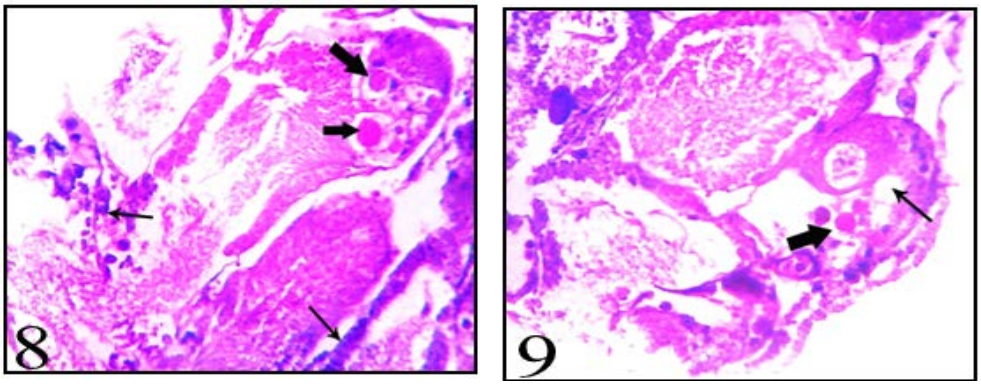


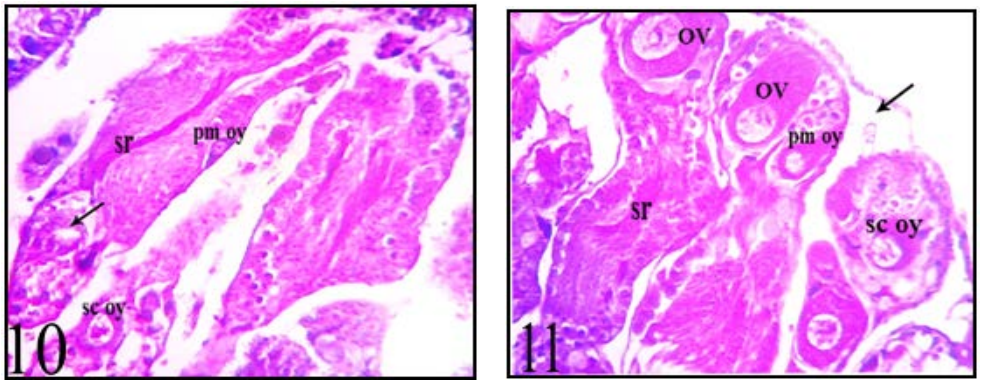
Fig. 5: Light photomicrographs of transverse sections in the hermaphrodite gland of B. alexandrina showing the control gland in which acini are represented with all stages of oogenesis; primary oocytes (pm oy), secondary oocyte (sc oy) and the mature ovum (ov), and stages of spermatogenesis; primary spermatocytes (pm sp), secondary spermatocytes (sc sp), and sperms (sr). Note, the connective tissue (ct) (X100).



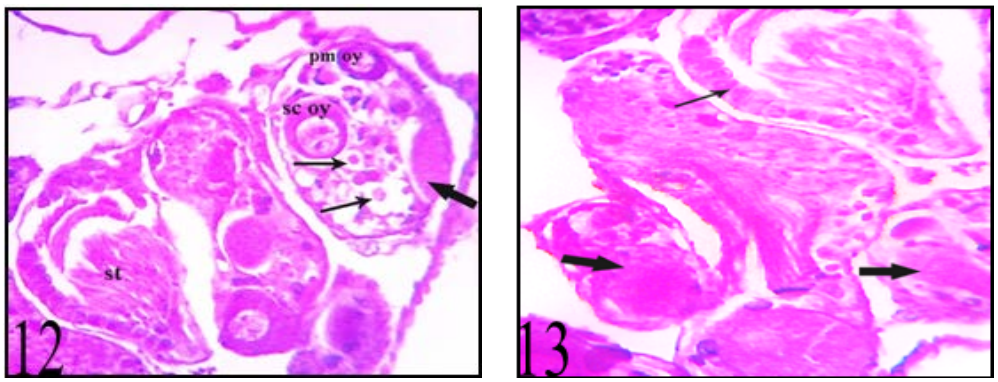
Figs. 6&7: Light photomicrographs of transverse sections in the hermaphrodite gland of B. alexandrina representing the snails treated with LC5 of A. terreus. Fig. 6 showing: absence of early stages of oogenesis, degenerated ova (thin arrows), deformed ovum (thick arrow), clearly represented sperms and degeneration of connective tissue. Fig.7 showing: deformed ovum (thick arrow), degenerated primary spermatocytes (thin arrow), secondary spermatocytes are scattered in the acinus without differentiation between their cytoplasmic inclusions and nuclei, absence of late stages of spermatogenesis (X 100).



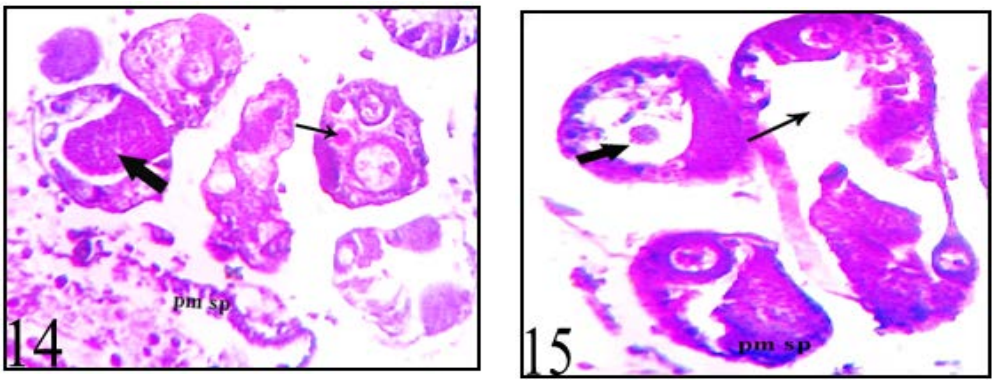
Figs. 8&9: Light photomicrographs of transverse sections in the hermaphrodite gland of B. alexandrina representing the snails treated with LC15 of A. terreus. Fig. 8 showing: absence of early stages of oogenesis, degenerated ova (thick arrows), densely stained primary spermatocytes without differentiation between their cytoplasmic inclusions and nuclei (thin arrows).Fig. 9 showing: disruption of the acini, degenerated ova (thick arrow), vacuole in the acinus (thin arrow) and rupture of the acinar membrane (X100).



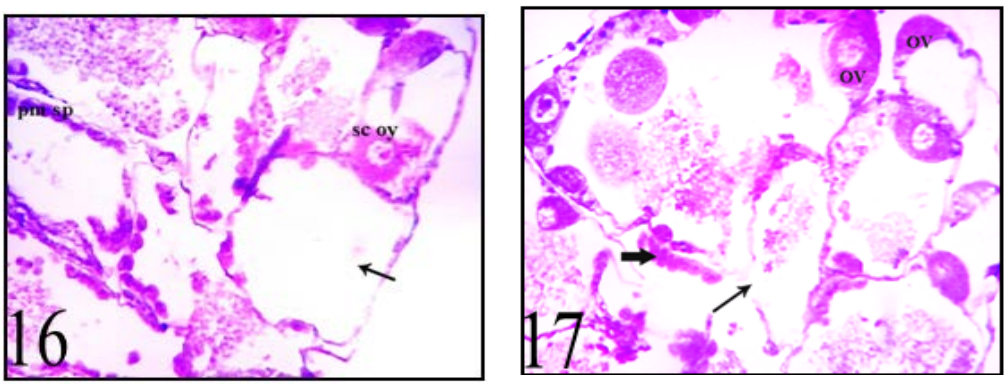
Figs. 10&11: Light photomicrographs of transverse sections in the hermaphrodite gland of B. alexandrina representing the snails treated with LC25 of A. terreus. Fig. 10 showing: primary oocyte (pm oy) and secondary oocyte (sc oy) are represented, distortion in the shape of ova (thin arrow), all stages of spermatogenesis are clearly distinguished specially sperms (sr). Fig.11 showing: degeneration of connective tissue (thin arrow), all stages of oogenesis; primary oocyte (pm oy), secondary oocyte (sc oy) and mature ova (ov) are represented. All stages of spermatogenesis are also represented (X 100).



Figs.12&13: Light photomicrographs of transverse sections in the hermaphrodite gland of *B. alexandrina* representing the snails treated with LC_5 of *P. janthinellum*. Fig.12 showing: primary and secondary oocytes are represented (pm oy) and (sc oy), some ova are degenerated (thin arrows), others are deformed (thick arrow), all stages of spermatogenesis are represented even spermatids (st). Fig.13 showing: deformed ova (thick arrows), primary spermatocytes are without differentiation between cytoplasmic inclusions and nuclei (thin arrow) and connective tissue is degenerated (X 100).



Figs. 14&15: Light photomicrographs of transverse sections in the hermaphrodite gland of B. alexandrina representing the snails treated with LC15 of P. janthinellum Fig.14 showing: clearly degenerated connective tissue, shrinkage and disruption of acini, degenerated ovum (thin arrow), deformed ovum (thick arrow), and degeneration of primary spermatocytes (pm sp). Fig. 15 showing: degenerated ovum (thick arrow), vacuolation in the acinus (thin arrow), densely stained primary spermatocytes (pm sp) without differentiation between nuclei and cytoplasmic inclusions (X 100).



Figs. 16&17: Light photomicrographs of transverse sections in the hermaphrodite gland of *B. alexandrina* representing the snails treated with LC_{25} of *P. janthinellu*m. Fig.16 showing: some acini contain only primary spermatocytes (pm sp), others contain only secondary oocytes (sc oy) while some acini are completely vacant (thin arrow) Fig.17 showing: rupture of the acinar membrane (thin arrow), loss of the architecture of acini, few mature ova (ov) are represented without clearly distinguished nucleus, few primary spermatocytes are found without differentiation between their nuclei and cytoplasmic inclusions (thick arrow) , sperms are absent (X 100).

5. REFERENCES

Balajee, S. A. (2009). *Aspergillus terreus* complex. Med. Mycol., 47: 42-46.

Borch, M.; Kiernan, M.; Rust, K.; Baron, B.; Simmons, B.; Hattala, P.; Davey, A.; Yovanovich, J.; Shayder, D.; Wasilewski, A. and Lafaro, V. E. (2009). Schistosomiasis: a case study. Urol. Nurs., 29 (1): 26- 29.

Chen, J.; Han, B.; Guo, S.; Wang, Y.; He, J.; Zhou, X.; Yang, X.; Han, F. (2009). Molluscicidal activity against *Oncomelania hupensis* of endophyte JJ18 from *Pseudolarix kaempferi* Gord. Pharmac. Res., 1(6): 421-427.

Choudhary, M. I.; Musharraf, S. G.; Mukhmoor, T.; Shaheen, F.; Ali, S. and Atta-ur-Rahman, H. E. (2004). Isolation of bioactive compounds from *Aspergillus terreus*. J. Research Institute of Chemistry, International Center for Chemical Sciences, Z. Naturforsch, 59b: 324 – 328.

De Boeck, G.; Meeus, W.; De Coen, W. and Blust, R. (2004). Tissue-specific copper bioaccumulation patterns and differences in sensitivity to waterborne copper in three freshwater fish: rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*), and gibel carp (*Carassius auratus gibelio*). Aquat. Toxicol., 70(3): 179-188.

Dewi, R. T.; Tachibana, S.; Itoh, K.; Ilyas, M. (2012): Isolation of antioxidant compounds from *Aspergillus terreus* LS01. J. Microbial. Biochem. Technol., 4: 10-14.

Dos Santos, G. H. F.; da Silva, E. B.; Amorim, E. L. C.; Sobrinho, T.J.S.P.; Melo, A. M. D. and Lima, C.S.D. (2011). Interaction of gamma radiation on the functionality of the molluscicidal extract of *Anacardium occidentale* Linn. 12 p; INAC 2011: International nuclear atlantic conference. Nuclear energy: new jobs for a better life; 17. ENFIR: Meeting on nuclear reactor physics and thermal hydraulics; 10. ENAN: Meeting on nuclear applications; 2. ENIN: Meeting on nuclear industry; Belo Horizonte, MG (Brazil).

El-Bolkiny,Y. E.; Rizk, E. T. and EI-Ansary, A. A. (2000). Effect of Diethyl dithiocarbamate on some biological and physiological parameters of *Biomphalaria alexandrina* snails. Egypt. J. Aquat. Biol. & Fish., 4 (2):157 -181.

Fill, T. P.; Pereira G. K.; Santos R. M. G. and Rodrigues- Filho, E. (2007). Four additional meroterpenes produced by *Penicillium* sp. found in association with *Melia azedarach*. Possible biosynthetic intermediates to austin. Z. Naturforsch, 62b: 1035 – 1044.

Guo, D.; Chen, J.; Liu, Y.; Yao, H.; Han, F. and Pan, J. (2011). A high-performance molluscicidal ingredient against *Oncomelania hupensis* produced by a rhizospheric strain from *Phytolacca acinosa* Roxb. Pharmacogn. Mag., 7(28): 277–283.

Kady, M. M.; Brimer, L.; Furu, P.; Lemmich, E., Nielsen, H.M.; Thiilborg, S.T.; Thastrup, O.; Christensen, S.B. (1992). The molluscicidal activity of coumarins from *Ethulia conyzoides* and of dicumarol. Planta Med. 58(4):334-337.

Khalaf, S.K. (1983). Biological and toxicological studies in certain tetranychid mites. M.Sc. Thesis Zool., Dept of plant protection. Facul. of Agric. Ain Shams Univ.

Kim, W.; Cho, K.; Lee, C. and Yoo, I. D. (2002). Terreulactone A, a novel meroterpenoid with anti-acetylcholinesterase activity from *Aspergillus terreus*. Tetrahed. Lett., 43: 3197–3198.

Latifian, M. and Rad, B. (2012). Pathogenicity of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillmin, *Beauveria brongniartii* Saccardo and *Metarhizium anisopliae* Metsch to adult *Oryctes elegans* Prell and effects on feeding and fecundity. Internat. J. Agric. & Crop Sci., 4 (14): 1026-1032.

Litchfield, J. T. and Wilcoxon, F. (1949). A simplified method of evaluating dose-

effect experiments. J. Pharmacol. Exp. Ther., 96: 99-113.

Macedo, C. F.; Porto, A. L. M. and Marsaioli, A. J. (2004). Terreinol, a novel metabolite from *Aspergillus terreus*: Structure and 13C labeling. Tetrahed. Lett., 45 (1): 53-55.

Marinhoa, A. M. d.; Rodrigues-Filho, A.; Moitinho, M. D. L. R. and Santos, L. (2005). Biologically active polyketides produced by *Penicillium janthinellum* isolated as an endophytic fungus from fruits of *Melia azedarach*. J. Braz. Chem. Soc., 16 (2): 280-283.

Mazurova, E.; Hilscherova, K.; Jalova, V.; Kohler, H. R.; Triebskorn, R.; Giesy, J. P. and Blaha, L. (2008). Endocrine effects of contaminated sediments on the freshwater snail *Potamopyrgus antipodarum* in vivo and in the cell bioassays in vitro. Aquat. Toxicol., 89: 172–179.

Micor, J. R. L.; Mojica, E. E.; Leyson, G. P. M.; Petrache, C. A. and Deocaris, C. C. (2004). Preliminary study on the characterization and toxicity of cashew nut shell liquid in *Pomacea canalicula* and *Artemia salina*. Asia Life sci., 3 (1&2): 71-80.

Moazami, N. (2008). Biopesticide production. Encyclopedia of Life Support Systems (EOLSS).

Mossalem, H. S.; Abdel-Hamid, H. and El-Shinnawy, N. A. (2013). Impact of artemether on some histological and histochemical parameters in *Biomphalaria alexandrina*. Afr. J. Pharm. & Pharmacol., 7(31):2220-2230.

Mostafa, B.B.; El-Deeb, F.A.; Ismail, N.M. and El-Said, K.M. (2005). Impact of certain plants and synthetic molluscicides on some fresh water snails and fish. J. Egypt. Soc. Parasitol., 35(3): 989-1007.

Mostafa, S. S. M. and Gawish, F.A. (2009). Towards to control *Biomphalaria alexandrina* snails and the free living larval stages of *Schistosoma mansoni* using the microalga *Spirulina platensis*. Aust. J. Bas. Appl. Sci., 3(4): 4112-4119.

Nakakita, Y.; Yomosa, K.; Hirota, A. and Sakai, H. (1984). Isolation of a novel phenolic compound from *Penicillium janthinellum* Biourge. Agric. Biol. Chem., 48 (1): 239-240.

Ojima, N.; Takahashi, I.; Ogura, K. and Seto, S. (1976). New metabolites from *Aspergillus terreus* related to the biosynthesis of aspulvinones. Tetrahed. Lett., 13: 1013–1014.

Oliver, L. and Haskins, W.T. (1960). The effect of low concentration of sodium pentachlorophenate on the fecundity and egg viability of *Australorbis glabrata*. Amer. J. Trop. Med. Hyg., 9: 199-203.

Oliveria-Filho, E. C. and Paumgartten, F. J. (2000). Toxicity of *Euphorbia milii* latex and Niclosamide to snails and non-target aquatic species. Ecotoxicol. Environ. Saf., 46: 342-350.

Osman, G. Y.; Mohamed, A. M.; Abdel Kader, A. and Mohamed, A. A. (2013). Biological and biochemical impacts of the fungal extract of *Aspergillus fumigatus* on *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni*. Biosci., 7 (12):473-485.

Parvatcar, R. R.; D'Souza, C.; Tripathi, A. and Naik, C.G. (2009). Aspernolides A and b butenolides from marine-derived fungus *Aspergillus terreus*. Phytochem., 70: 128-132.

Ragab, F.M.A. and Ismail, H. (2001). Biological activity of certain fungal extracts against *Biomphalaria alexandrina* snails. Safe Altern. Pest. for Pest Manag. Assiut Univ., 28-29: 273-284.

Rizk, E. T. and Hassan, S. M. M. (2000). Molluscicidal activity of furanocoumarins isolated from *Ammi majus* against *Biomphalaria alexandrina* snails. Egypt. J. Pharm. Sci., 40 (1): 61-71.

Singh, S. B. (2003). Isolation, structure, and HIV-1 integrase inhibitory activity of xanthoviridicatin E and F, two novel fungal metabolites produced by *Penicillium chrysogenum*. Helv. Chim. Acta, 86: 3380 – 3385.

Stierle, A. A.; Stierle, D. B. and Kelly, K. (2006). Berkelic acid, a novel spiroketal with selective anticancer activity from an acid mine waste fungal extremophile. J. Org. Chem., 71: 5357 – 5360.

Suliman, E. A. and Mohammed, Y. O. (2012). The activity of *Aspergillus terreus* as entomopathogenic fungi on different stages of *Hyalomma anatolicum* under experimental conditions. J. Entom., 9(6): 343-351.

Umecharuba, G.I.; and Nwachukwa, E.O. (1997). The effect of filtrates of seed-borne fungi of African yam bean on seed germination and seedling development. Global J. Pure & Appl. Sci., 3: 165-176.

Wang, X.; Yu, R.; Luo, X.; Zhou, M.; Shen, J. and Gu, Z. (2008). Toxicity screening and identification of bacteria isolated from snails *Nassarius semiplicatus* and their habitat. Wei Sheng Wu Xue Bao, 48(7): 911-916.

Wang, Y.; Zheng, J.; Liu, P.; Wang, W. and Zhu, W. (2011). Three New Compounds from *Aspergillus terreus* PT06-2 grown in a high salt medium. Mar. Drug., 9: 1368-1378.

WHO (1965). Molluscicide screening and evaluation. Bull. Hlth. Org. Monogr. Ser., 50: 124-138.

Yamamoto, H.; Takahashi, S.; Moriyama, K.; Jinnouchi, H.; Takahashi, N. and Yagishita, K. (1980). Terreic acid (5, 6-epoxy-3-hydroxy-p-toluquinone) produced by a strain in *Aspergillus terreus* group. Production, extraction, isolation, physicochemical properties, chemical structure, biological properties, and antitumor effects. Nihon Daigaku Nojuigakubu Gijutsu Kenkyu Hokoku., 37: 9-19.