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ORIGINAL ARTICLE

Factors affecting the molluscicidal activity of *Asparagus densiflorus* and *Oreopanax guatemalensis* plants and Difenoconazole fungicide on *Biomphalaria alexandrina* snails

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Abstract Many factors may enhance or suppress the molluscicidal activity regardless of their origin. The present study aims to determine the molluscicidal activity of two plants, namely *Asparagus densiflorus* and *Oreopanax guatemalensis* besides, Difenoconazole fungicide against *Biomphalaria alexandrina* snails, miracidia and cercariae. The most effective agent was Difenoconazole followed by *A. densiflorus* and *O. guatemalensis* against *B. alexandrina* snails, where the LC_{90} values after 24 h of exposure reached 2.5, 102, and 188 ppm, respectively. Toxicity of tested agents was more pronounced at high temperatures. *A. densiflorus* and Difenoconazole showed no changes in their activities after their exposure to sunlight for 6 h. Also, neutral and acidic media were favorable for maintaining their toxic action. The presence of aquatic plants, mud particles, and storage period had negative effects on the activities of the tested agents. Both *A. densiflorus* and Difenoconazole had a fast effect against miracidia and cercariae of *Schistosoma mansoni* after 3 h of the exposure. Moreover, the tested agents recorded adverse impact on the total reproductive rate of exposed snails. Such effects were supported by histopathological examinations of the hermaphrodite gland. It showed an abnormal necrosis in male and female gametogenic cells, besides a decrease in the number of ova and other gametogenic stages.

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Introduction

Schistosomiasis is not only a major health problem but also an economic one. Yousef and EI-Kassas (2013) stated that "in Egypt, the disease is not only a prime health problem, but it affects millions of farmers at the early age diminishing their productivity and exerting a serious socioeconomic problem (El-Baz et al. 2003)." Due to the very encouraging results obtained from the Endod plant in Ethiopia by Gwatirisa et al. (1999), there has been increasing interest and search for plants with molluscicidal properties (Melendez and Capriles 2002). Today, thousands of plants have been screened for molluscicidal activities using a standard WHO procedure for the comparative purpose (Adenusi and Odaibo 2008; Saad et al. 2012). Plant molluscicides are inexpensive and have a potential to be biodegradable in nature and appropriate technology for focal control of the snail vectors (Singh et al. 2010). There are other chemical compounds that may reach water sources during the agricultural activities such as herbicides, fungicides, and pesticides which may kill snails or make their environmental conditions unsuitable for their life (Mohamed et al. 2012). The environmental factors such as different water pH values, water temperature, the presence of mud particles, and aquatic vegetation may influence the distribution and abundance of snail hosts of schistosomiasis (Sayed et al. 2004). On the other hand, these factors may influence the efficiency of molluscicide (Schall et al. 2001; El-Deeb 2002). Many authors study the impact of molluscicides on miracidia and cercariae to interrupt schistosomiasis life cycle (El-Nahas and El-Deeb 2002; Abdel Raouf 2007). Thereby, the present study aimed to evaluate the role of

environmental factors in determining the molluscicidal activity of *Asparagus densiflours*, *Oreopanax guatemalensis* plants, and Difenoconazole fungicide against *Biomphalaria alexandrina* snails and the larval stages of *Schsitosoma mansoni*.

Materials and methods

Snails

B. alexandrina snails (the intermediate hosts of *S. mansoni* in Egypt) were collected from a canal in Abu-Rawash, Giza governorate.

Miracidia and cercariae

S. mansoni ova and cercariae used in this study were obtained from Schistosome Biological Supply Program (SBSP) in Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The ova were allowed to hatch in a small amount of dechlorinated water for about 15 min under direct light to give miracidia.

Plants

A. densiflorus (Family: Asparagaceae, herbarium number; 9:65(1966)) and *O. guatemalensis* (Family: Araliaceae, herbarium number; 4, 3:108(1854)) plants were collected from El-Orman and El-Zohareya gardens. Their leaves were dried and then powdered by an electric mill.

Fungicide

Difenoconazole (Score) 250 E.C. is one of azole compounds that contains a group triazole that plays a key role as antifungal in agricultural purposes.

Bioassay tests

Molluscicidal screening

Amounts of the dry powder from each plant were weighed separately and then were added to 1000 ml of dechlorinated tap water to make up the desired weight/volume concentrations. Distilled water was used for preparing a stock solution of fungicide. While dechlorinated tap water was used for subsequent series of concentrations that would permit the computation of LC_{50} and LC_{90} values (WHO 1965). Sublethal concentrations were calculated from the lethal dose probability lines utilizing the statistical program (SPSS 2001) for windows.

Effect of some environmental factors on the activity of the three tested agents

Effect of sunlight

Two solutions were separately prepared of LC_{50} and LC_{90} concentrations of each tested agents. One solution was exposed to direct sun radiation for 6 h (exposed solution). While another one was kept in the shade (dark) also for 6 h (unexposed solution). After passing 6 h, the snails were added to the exposed, unexposed, and fresh preparation of LC_{50} and LC_{90} concentrations of each tested agent used as a control for 24 h followed by another 24 h as a recovery period.

Effect of temperature

B. alexandrina snails were exposed to the tested agents' concentrations at (15, 25, and 30 °C). Exposure and recovery periods were 24 h. Three replicates, each of ten snails/L, were used for each concentration.

Effect of pH

LC_{50} and LC_{90} concentrations from each tested agents were prepared using standard reference water that was previously adjusted (using either NaOH for alkaline medium or HCL for acidic medium) to pH values of 4, 7, and 9 (Zidan et al. 2000).

Effect of river bed mud

LC_{50} and LC_{90} concentrations from each tested agents were mixed with 5000 and 10,000 ppm of mud particles, and provided with the gentle air stream to maintain continuously and thoroughly mixing. The exposure and recovery periods were 24 h for each.

Effect of some aquatic plants

LC_{50} and LC_{90} concentrations from each tested agents were prepared in plastic aquaria containing (500, 1500, and 7500 units) of *Lemma gibba*/2 L. The other plastic aquaria were used containing (5, 10, and 15 units) of *Eichhornia crassipes*/5 L and (1, 2.5, and 5 m) of *Ceratophyllum demersum*/5 L; then, 30 healthy snails were added in three replicates to each aquarium.

Effect of the storage period

LC_{50} and LC_{90} concentrations of each tested agents were stored for 1 and 3 days for plants and 1, 3, 7, 14, and 21 days for synthetic fungicide at room temperature (25 ± 2 °C). After each storage period, 30 adult snails were added in three replicates for each concentration. Exposure and recovery periods

for all the experimental tests were 24 h. Set of untreated control tests were performed using freshly prepared solutions of the three agents (Lemma 1970).

Effect of the tested agents on the larval stages of *S. mansoni*

Evaluation of miracidicidal activity

Twenty-five milliliter of water containing 100 freshly hatched miracidia or 100 freshly shed cercariae were mixed separately with another 25 ml of double the concentration of each tested agent; 50 ml of dechlorinated water containing about 100 fresh hatched miracidia or 100 freshly shed cercariae were used as a control. Observations on the movement and mortality of miracidia and mobility and survival of cercariae were recorded at different intervals of exposure under a dissecting microscope (El-Deeb 2007; Mossalem 2003).

Effect of sublethal concentrations of the three tested agents on egg laying capacity of *B. alexandrina*

Snails (9–11-mm shell diameter) were exposed to sublethal concentrations (LC_0, LC_5, LC_{10}, and LC_{25}) of each tested agents. Three replicates, each of ten snails/L were used for each concentration. The control group of snails was maintained in dechlorinated tap water. White plastic foams were added to aquaria for egg deposition; the water of each aquarium was changed once weekly. All egg masses laid by the exposed and control snails were collected and counted.

Histological study

Snails (8–10 mm in shell diameter) were exposed to LC_5 sublethal concentration of *A. densiflorus* (40 ppm), *O. guatemalensis* (125 ppm), and Difenoconazole (1.2 ppm) for two successive weeks. Thereafter, snails were washed with water and then dried. The hermaphrodite gland of each snail was separated gently from the soft parts, fixed Bouin's solution, embedded in paraffin wax, sectioned, and stained with hematoxylin and eosin. Sections were microscopically examined and photographed by a Zeiss video camera (Germany).

Statistical analysis

Percentage of the survival rate of miracidia and cercariae was analyzed by chi-square values of contingency tables (Southwood 1978).

Results

Data in Table 1 showed the calculated lethal concentrations (LC_{90} and LC_{50}) after 24 h of exposure for *A. densiflours*, *O. guatemalensis* plants, and the Difenoconazole. The LC_{50} values were 75, 160, and 1.9 ppm, respectively. This clearly indicates that the toxicity of the fungicide was much greater than that of the two tested plants used.

Factors affecting the molluscicidal activity of the tested agents

Data in Table 2 summarized the effect of sunlight, temperature, pH, and river bed mud on the activity of *A. densiflours*, *O. guatemalensis*, and Difenoconazole concentrations against *B. alexandrina* snails. In terms of figures, the mortality percentage of *B. alexandrina* snails was 40 % at 75 ppm and 80 % at 102 ppm in the case of *A. densiflours*, while in the case of the Difenoconazole, it was 50 % mortality at 1.9 ppm and 80 % at 2.5 ppm with sunlight, respectively. On the other hand, *O. guatemalensis* activity is completely lost at (160 ppm) and recorded 20 % mortality of snails at (188 ppm) under sunlight exposure. The lower temperature was the lower molluscicidal activity of the three agents and vice versa. When alkaline medium pH (9) was decreased in the activity of the three tested agents, the neutral pH (7) and acidic media pH (4) were enhanced the activity. Mud particles have a more pronounced influence on the molluscicidal activities of the three tested agents against *B. alexandrina* snails. Snails' mortality was decreased with increasing the mud concentrations.

The *Ceratophyllum demersum* had more influence on the molluscicidal activity than the floating water plants *Eichhornia crassipes* and *L*emna *gibba*. The activity of LC_{90} of Difenoconazole was completely lost in the presence of (5 m) of *C. demersum*, while, in the presence of (15 u) of *E. crassipes* and (7500 u) of *L. gibba*, mortality percent of snails were reduced to 20 and 40 %, respectively. On the other hand, the results showed that the potency of LC_{50} for *O. guatemalensis* was reduced with (7500 u) of *L. gibba* to 36.6 % kill, while, with (5 m) of *C. demersum* and (15 u) of *E. crassipes*, it is completely lost. Finally, the activity of LC_{50} and LC_{90} of *A. densiflorus* was diminished gradually with increasing the aquatic plant density (Table 3).

Data in Table 4 indicate that the efficacy of the three tested agents was gradually decreased by storage. This decrease was continued during the prolongation of the storage period. All of the stored concentrations of both the tested plants were completely diminished after 3 days of storage compared with 21 days of storage in the case of the fungicide.

Table 1 Molluscicidal activity of the tested agents on *B. alexandrina* snails after 24 h of exposure

Tested materials	LC_{50} (ppm)	Confidence limit of LC_{50} (ppm)	LC_{90} (ppm)	Slope	LC_0 (ppm)	LC_5 (ppm)	LC_{10} (ppm)	LC_{25} (ppm)
A. densiflorus	75	(65.8–81.1)	102	1.35	25	40	47	60
O. guatemalensis	160	(151–169.9)	188	1.15	109	125	133	146
Difenoconazole	1.9	(1.7–2.2)	2.5	1.26	0.96	1.2	1.4	1.7

Miracidicidal and cercaricidal activities of the tested agents

Mortalities of miracidia were 23, 100, and 100 % after half an hour of exposure to (60, 75, and 102 ppm), respectively of the *A. densiflorus* (Table 5). Cercariae exposed to the same previous concentrations of *A. densiflorus* recorded higher mortality percent than miracidia after 1 h. Treated cercariae suffered from great morphological damages as the heads were separated from the tails, and they became paralyzed and could not be free from twisty movement.

Gradual mortalities of miracidia were obtained in the case of *O. guatemalensis*; 100 % mortality rate of miracidia was observed at 188 ppm after 3 h. However, the cercaricidal effect of *O. guatemalensis* is low. Therefore, 80 % mortality at 188 ppm was recorded after 6 h (Table 6).

Mortality of miracidia was 94 % at the first interval after exposed to (2.5 ppm) of Difenoconazole. By the end of the observation period, the mortality became 93 % for miracidia exposed to (0.96 ppm) and 100 % for those exposed to (1.4, 1.6, 1.9, and 2.5 ppm). The cercaricidal mortality was more than 50 % after 1/2 h which indicates the strong cercaricidal impact of Difenoconazole (Table 7).

Fecundity of adult snails

Effect of A. densiflorus plant

All the experimental groups that exposed to *A. densiflorus* showed a reduction in the total reproductive rate compared with the control group by the end of the experiment (Fig. 1).

Effect of O. guatemalensis plant

The total reproductive rate showed a gradual decrease with increasing the sublethal concentrations (LC_5, LC_{10}, and LC_{25}) of *O. guatemalensis* when compared to that in the control (Fig. 2).

Effect of Difenoconazole

Difenoconazole had completely suppressed the egg laying capacity of adult snails. Thus, the total reproductive rate was deleteriously suppressed by exposing snails to sublethal concentrations of Difenoconazole as shown in Fig. 3.

Table 2 Effect of sunlight, temperature, pH, and river bed mud on the activity of *A. densiflorus, O. guatemalensis*, and Difenoconazole concentrations against *B. alexandrina* snails

Tested materials	Concentrations	% Mortality of *B. alexandrina* snails											
		Sunlight			Temperature degrees (°C)			pH levels			River bed mud		
		Sunlight 6 h	Shade (dark)	Control[b]	15 °C	25 °C	30 °C	4	7	9	5000 (ppm)	10,000 (ppm)	Control[b]
A. densiflorus	LC_{50} (75 ppm)	40	50	60	10	56.6	70	53.3	60	20	10	0	50
	LC_{90} (102 ppm)	80	90	100	20	90	100	90	100	50	56.6	6.6	90
O. guatemalensis	LC_{50} (160 ppm)	0	40	50	20	50	66.6	50	56.6	10	6.6	0	55
	LC_{90} (188 ppm)	20	70	90	50	86.6	90	90	96.6	30	16.6	0	90
Difenoconazole	LC_{50} (1.9 ppm)	50	50	60	10	56.6	70	50	56.6	20	33.3	26.6	50
	LC_{90} (2.5 ppm)	80	90	90	40	90	100	90	96.6	30	60	50	90
Control[a]	–-	0	0	–	0	0	0	0	0	0	0	0	–

[a] Control (water free from tested material concentrations at the same conditions)

[b] Control (water with tested materials concentrations)

Table 3 Effect of water vegetation on the activity of the lethal concentrations of tested agents against *B. alexandrina* snails

Aquatic plants		% Mortality of *B. alexandrina* snails exposed to lethal doses of the tested materials in presence of water vegetation						Control
		A. densiflorus		*O. guatemalensis*		Difenoconazole		
		LC_{50} (75 ppm)	LC_{90} (102 ppm)	LC_{50} (160 ppm)	LC_{90} (188 ppm)	LC_{50} (1.9 ppm)	LC_{90} (2.5 ppm)	
C. demersum Length/m	1 m/m^2	50	70	20	80	10	20	0
	2.5 m/m^2	30	40	10	60	0	0	0
	5 m/m^2	10	30	0	40	0	0	0
L. gibba Unit (u)	500 u/m^2	66.6	100	50	90	50	90	0
	1500 u/m^2	40	60	40	30	40	40	0
	7500 u/m^2	10	20	36.6	20	30	40	0
E. crassipes Unit (u)	5 u/m^2	50	100	20	80	16.6	50	0
	10 u/m^2	40	36.6	0	60	10	20	0
	15 u/m^2	36.6	16.6	0	30	10	20	0

Control (only water with aquatic plants without tested agents)

Histopathological examinations

Normal hermaphrodite gland

The hermaphrodite gland of the normal *B. alexandrina* snails is composed of a number of cube-shaped acini connected together by areolar connective tissue. The acinar epithelium comprises the various stages of both male and female gametogenic cells (Plate A1, 2).

Treated hermaphrodite gland

Effect of *A. densiflorus* plant *B. alexandrina* snails treated with *A. densiflorus* (Plate A3) have marked morphological changes in both male and female gonadal cells. Considerable necrotic changes including shrinkage and partial destruction in the follicular membrane and follicular cells were recognized. Also, a degenerated mature ovum, atrophy in some primary oocytes, and disfigured acini which become irregular in shape were present. Moreover, spermatogenic stages disappeared, and the degradable spermatogonia occupied all the male gonadal acini. Finally, the connective tissue between the acini was dissolved and was replaced by vacuoles.

Effect of *O. guatemalensis* plant Snails subjected to *O. guatemalensis* (Plate A4) showed a moderate degree of effects. The micrograph illustrated an increase in the number of mature ova with malformation in their shapes accompanied

Table 4 Effect of the storage period on the activity of the lethal concentrations of tested agents against *B. alexandrina* snails

Tested agents	Concentration (ppm)	% Mortality of *B. alexandrina* snails exposed to the tested agents stored to the following periods					
		Control[a]	1 day	3 days	7 days	14 days	21 days
A. densiflorus	47	10	10	0			
	60	27	20	0			
	75	50	30	0			
	102	90	90	0			
O. guatemalensis	133	10	0	0			
	146	30	20	0			
	160	50	20	0			
	188	95	80	0			
Difenoconazole	1.4	20	20	10	0	0	0
	1.6	30	26.6	10	0	0	0
	1.9	60	60	50	10	0	0
	2.5	90	90	80	36.6	10	0

[a] Control (freshly prepared).

Table 5 Miracidicidal and cercaricidal activity of *A. densiflorus* after different exposure periods

Concentration (ppm)	% Mortality of miracidia after the following intervals (h)						% Mortality of cercariae after the following intervals (h)					
	1/2	1	1:30	2	2:30	3	½	1	1:30	2	2:30	3
LC_0 (25)	0	26	68	89	93	96	0	12	20	27	52	76
LC_{10} (47)	0	45	71	91	97	100	3	91	92	94	100	–
LC_{25} (60)	23	53	78	100	–	–	18	100	–	–	–	–
LC_{50} (75)	100	–	–	–	–	–	84	100	–	–	–	–
LC_{90} (102)	100	–	–	–	–	–	100	–	–	–	–	–
Control	0	2	4	8	11	16	0	0	0	0	1	4

with degenerated nuclei. Some of the ova are still kept inside the follicular cavity. Also, acceleration in male gametogenic developmental stages occurred and seemed to fill acini, and all spermatogonia, spermatids, and sperms were distinct. Also, a little destruction in inter acinar connective tissue is recognized.

Effect of Difenoconazole The exposure to Difenoconazole induced intensive gonadal changes to *B. alexandrina*. Plate A5 revealed a great degeneration of the membranes of acini and also losing their normal architecture shape. All the mature ova lost their nuclei and identical shape, and also, their yolk layers are ruptured. Also, spermatocytes become dispersed outside acini, and mature sperm suffered from atrophy in their shape. In addition, Sertoli cells were disappeared altogether with other spermatogenic stages.

Discussion

In the present study, the most effective agent was Difenoconazole fungicide followed by *A. densiflorus* and *O. guatemalensis* plants against *B. alexandrina* snails where the LC_{90} values after 24 h of exposure were 2.5, 102, and 188 ppm, respectively. Guided by the chemotaxonomic bases, *A. densiflorus* is known to possess steroidal saponin as a major constituent (Oketch-Rabah 1998; Mohamed 2007). However, *O. guatemalensis* contains triterpenoidal saponin (Mohamed 2001) as a principal active constituent. The saponin-containing plants responsible for molluscicidal activities are of great interest as they are inexpensive and eco-friendly compared to synthetic compounds (Garai 2014).

A. densiflorus and Difenoconazole activities were not affected by sunlight. This means that their chemical constituents are not photosensitive. However, *O. guatemalensis* activity showed a serious decline against *B. alexandrina* snails after exposure to sunlight for 6 h. This finding agrees with those recorded for Damsin and Amrosin from the plant *Euphorbia sp.* (Refahy et al. 2001) and *Viburnum tinus* (Badawy 2007). While the activity of the three tested agents was greatly increased at high temperature (30 °C), it was markedly decreased at low temperature (15 °C). At high temperature, the increasing rate of snail metabolism may release more CO_2, which decreases the pH of water during summer season (Berge et al. 2006; Singh and Singh 2009). The active ingredients of molluscicide become more soluble in high temperature than those in low ones. Therefore, summer months would be favorable for snail control programs in Egypt (Ahmed et al. 2000; Khalifa 2006). In the present work, while the potency of the three tested agents was more pronounced in neutral (pH 7) and acidic media (pH 4), it is suppressed in alkaline media (pH 9). This may imply that either activation or hydrolysis of the molluscicides might have happened (Souza 1995). The activity of *A. densiflorus* and *O. guatemalensis* was more affected than that of Difenoconazole by mud particles; this may be attributed to the absorption or adsorption of the active components of each plant on the mud particles (Ahmed 2003;

Table 6 Miracidicidal and cercaricidal activity of *O. guatemalensis* after different exposure periods

Concentration (ppm)	% Mortality of miracidia after the following intervals (h)						% Mortality of cercariae after the following intervals (h)								
	1/2	1	1:30	2	2:30	3	1/2	1	1:30	2	2:30	3	4	5	6
LC_0 (109)	0	24	50	61	65	84	0	0	0	12	15	32	48	52	72
LC_{10} (133)	0	35	58	83	84	86	0	0	0	13	17	35	52	55	74
LC_{25} (146)	0	38	60	84	87	91	0	0	0	17	23	43	57	63	77
LC_{50} (160)	45	79	83	86	90	93	0	5	10	30	42	49	57	69	78
LC_{90} (188)	65	81	86	90	98	100	3	8	21	33	44	50	60	70	80
Control	0	0	0	4	8	12	0	0	0	3	9	13	16	16	38

Table 7 Miracidicidal and cercaricidal activity of Difenoconazole against after different exposure periods

Concentration (ppm)	% Mortality of miracidia after the following intervals (h)						% Mortality of cercariae after the following intervals (h)					
	1/2	1	1: 30	2	2: 30	3	1/2	1	1: 30	2	2: 30	3
LC_0 (0.96)	28	41	47	61	74	93	59	92	93	95	98	100
LC_{10} (1.4)	29	85	89	94	96	100	70	100	–	–	–	–
LC_{25} (1.6)	51	93	96	100	–	–	77	100	–	–	–	–
LC_{50} (1.9)	91	95	100	–	–	–	81	100	–	–	–	–
LC_{90} (2.5)	94	97	100	–	–	–	100	–	–	–	–	–
Control	0	3	4	6	7	8	0	0	0	3	4	6

Khalifa 2006). In the current results, the most interfering aquatic plant was *C. demersum* with all the tested agents either by gradually reducing the activity of molluscicides depending on the density of aquatic plant as in the case of *A. densiflorus* or by getting their potency completely lost as in the case of *O. guatemalensis* and Difenoconazole. This may be due to detoxification effect of this plant or the ability of this aquatic plant to adsorb or absorb these molluscicides' constituents and remove them from snail habitat than other aquatic plants *E. Carssipes* and *L. gibba*. These results are in parallel with those obtained by Zidan et al. (2000) and Mostafa et al. (2005). While the molluscicidal activity was completely lost against *B. alexandrina* snails after three days for *A. densiflorus* and *O. guatemalensis* plants, it was lost after 21 days for Difenoconazole. This loss could be attributed to the biodegradation of their active ingredients, and this finding is in agreement with Mossalem (2003).

Another approach to the interruption of schistosomiasis life cycle is by killing cercariae and miracidia, the free larval stages of schistosomiasis. The current study exhibit the effect of the three tested agents against *S. mansoni* larval stages (miracidia and cercariae), and the results showed that miracidia mortality was greater than that of cercariae during the application of *O. guatemalensis* after the same time intervals. A different result was observed in the case of using *A. densiflorus* and Difenoconazole, as miracidia were more tolerant to the effect of these agents than cercariae. The obtained results approach those obtained by Mahmoud (1993) on Kelthane that killed miracidia faster than cercariae while Hostaquick killed cercariae faster than miracidia. Tantawy (2006) stated that at 0.17 ppm of Fentirothion gave 96 % mortality of cercariae compared with 82 % of miracidia after 6 h of exposure. Also, Abdel Raouf (2007) obtained the same results where Whipsuper had more pronounced effect on the cercariae than miracidia, while Hinosan and *Tribulus terrestris* showed more miracidicidal activity than cercaicidal activity; he concluded that mortality of larval stages seem to be dependent on the chemical structure of the used tested agents and it is not dependent on the biological nature of both larvae. The presence of heavy ciliated integument in miracidia may be able to reduce the toxic effect of the tested material (Rizk et al. 2001; Ismail 2005).

The current work revealed that *A. densiflorus* and Difenoconazole had a more pronounced effect on the reproductive rate of the adult snails. This could be attributed to the ability steroidal saponin of *A. densiflorus* plant to affect the reproductive organs of snails. Steroid saponin was found to directly inhibit the genes responsible for steroidogenesis, also to suppress the proliferation of follicle-stimulating hormone-modulated granulose cells in ovarian follicle (Francis et al. 2002). Difenoconazole is one of azole compounds which are known for their ability to inhibit certain pathways of

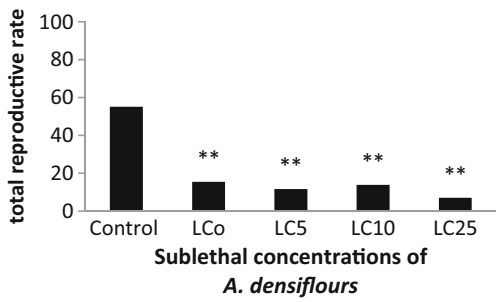

Fig. 1 Total reproductive rate of *B. alexandrina* after exposure to *A. densiflorus* sublethal concentrations for 12 weeks (*one asterisk and two asterisks* refer to significant difference between the control and each treated group at $P<0.05$ and $P<0.01$, respectively. *n.s* refers to not significant difference results)

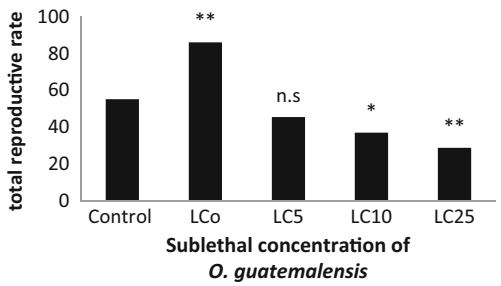

Fig. 2 Total reproductive rate of *B. alexandrina* after exposure to *O. guatemalensis* sublethal concentrations for 12 weeks (*one asterisk and two asterisks* refer to significant difference between the control and each treated group at $P<0.05$ and $P<0.01$, respectively. *n.s* refers to not significant difference results)

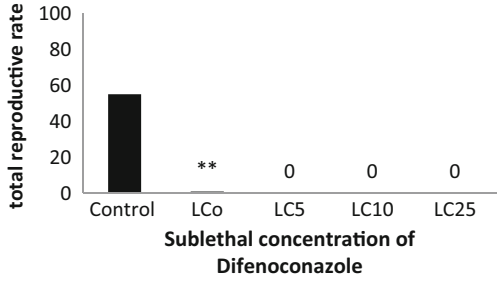

Fig. 3 Total reproductive rate of *B. alexandrina* after exposure to Difenoconazole sublethal concentrations for 12 weeks (*one asterisk and two asterisks* refer to significant difference between the control and each treated group at $P < 0.05$ and $P < 0.01$, respectively. *n.s* refers to not significant difference results)

steroidogensis by their high affinity of binding to the enzymes of sterol 14 α-Demethylase and aromatase in several species (Zarn et al. 2003). However, this suppressive effect on the egg production agrees well with previous studies (Mohamed et al. 2006; El- Emam et al. 2008; Gawish et al. 2009). On the other hand, *O. guatemalensis* application showed improvement in the reproductive ability of the treated snails. This may be due to the snails' trials to overcome the destructive effect of the plant as a defensive mechanism.

All the tested agents induced histopathological changes in the hermaphrodite gland. *A. densiflorus* causes atrophy and disappearance in some gonadal cells with severe deformed, destruction in reproductive units. Degeneration in the membrane of acini was recorded in the case of Difenoconazole treatment, and fading mature ova was seen with degenerated nuclei and yolk layer. In this respect, these observations explain the reasons for inhibition of egg laying capacity of both the snail groups that were subjected to both *A. densiflorus* and Difenoconazole; thus, no new offspring cloud be produced. These all findings are in accordance with previous studies (Atlam 2000; Mossalem 2003; Bakry 2009).

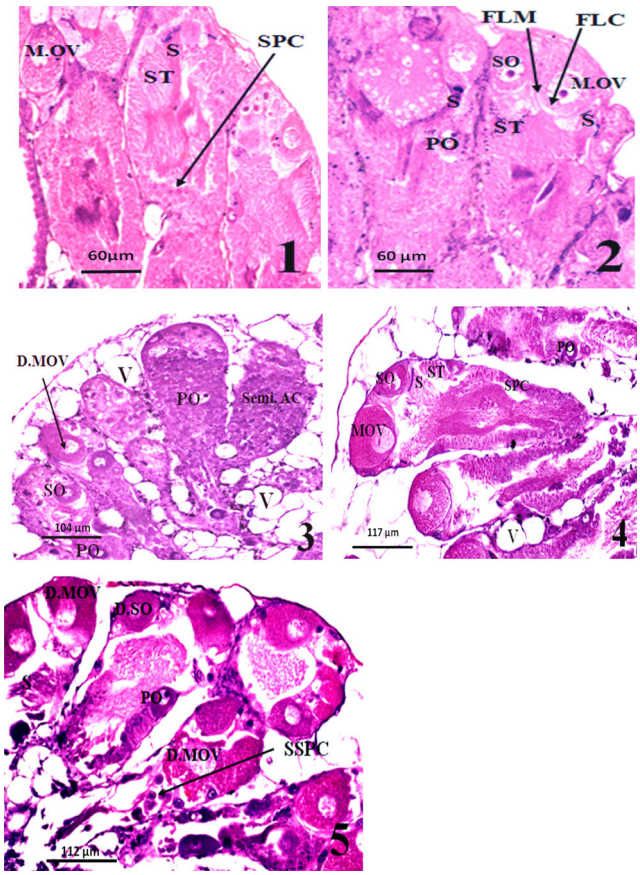

Plate A Section in the hermaphrodite gland (hematoxylin and eosin stained, 200×) of normal *B. alexandrina* snails (**1** and **2**) and treated snails with sublethal dose (LC_5) of each *A. densiflorus* (**3**), *O. guatemalensis* (**4**), and Difenoconazole (**5**), separately for two weeks. *AC* acini, *CT* connective tissue, *D.SO* degenerated secondary oocyte, *DM.OV* degenerated mature ova, *FLC* follicular cavity, *FLM* follicular membrane, *M.OV* mature ova, *PO* primary oocyte, *S* sperms, *Semi.AC* seminferous acinus, *SO* secondary oocyte, *SPC* spermatocytes, *SSPC* scattered spermatocytes, *ST* spermatids, *V* vacuole

Conclusion

Reviewing the above mentioned results, it could be concluded the important role of the studied environmental factors in determining the molluscicidal activity of the tested agents against *B. alexandrina* snails. *A. densiflorus* and Difenoconazole have promising molluscicidal materials against *B. alexandrina* snails. Moreover, miracidia were more tolerant to both *A. densiflorus* and Difenoconazole concentrations than cercariae; this observation needs further study to test the ability of the treated miracidia to infect snails.

Compliance with ethical standards

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animal were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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