The Impact of Three Herbicides on Biological and Histological Aspects of *Biomphalaria alexandrina*, Intermediate Host of *Schistosoma mansoni*

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THE IMPACT OF THREE HERBICIDES ON BIOLOGICAL AND HISTOLOGICAL ASPECTS OF *BIOMPHALARIA ALEXANDRINA*, INTERMEDIATE HOST OF *SCHISTOSOMA MANSONI*

Fathy Abdel-Ghaffar[1], Amira K. Ahmed[1], Fayez Bakry[2*], Ibrahim Rabei[3] & Amina Ibrahim[2]

ABSTRACT

Schistosomiasis remains a public health problem in the developing world. *Biomphalaria alexandrina* is the intermediate host of *Schistosoma mansoni*, with a widespread distribution in Egypt. In Egypt molluscicides have been important, but otherwise molluscicides have been of minor significance. They may, however, become important now that WHO talks about eradicating schistosomiasis in some countries and that WHO has finally accepted that snail control is important.The present study investigated the different effects of three commercial herbicidical compounds, Butralin (as Amex 48% EC), glyphosate isopropylammonium (Herphosate 48% SL) and Pendimethalin (Stomp 50% EC) on *B. alexandrina*. All three compounds were found to have a molluscicidal effect, with Pendimethalin the most toxic over the examined range of concentrations. In addition, at sublethal concentrations, these compounds reduced growth rates and reproductive output (numbers of eggs laid) in exposed *B. alexandrina,* and it reduced viability of those eggs. Interpretion of assay data was supported by histological changes in the digestive and hermaphrodite glands of snails exposed at a range of concentrations. Moreover, the three compounds were also shown to rapidly (with three hours exposure) induce a toxic effect in miracidiae and cercariae of *Schistosoma mansoni*. It can be concluded that the three herbicidical compounds have molluscicidal and antihelminth properties.

Key words: *Biomphalaria alexandrina*, *Schistosoma mansoni*, miracidiae, cercariae, Butralin, glyphosate isopropylammonium, Pendimethalin, herbicides, molluscicidal activity, antihelminth activity, schistosomiasis.

INTRODUCTION

Schistosomiasis is a group of chronic parasitic diseases affecting at least 240 million people in more than 70 countries (Larson et al., 2014). In virtually no region on the African continent are people safe from infections by *Schistosoma* species, the causative agents. About 85% of the infected populations worldwide are Africans (WHO, 2010, 2014). The freshwater snail *Biomphalaria alexandrina* (Ehrenberg, 1831) (Mollusca, Planoribidae) is the intermediate host of *Schistosoma mansoni* Sambon, 1907 (Bakry et al., 2011).

Controlling of these snails is still one of the most promising means in the battle against schistosomiasis (Bakry et al., 2012; Mahmoud et al., 2011; Mohamed et al., 2012; WHO, 2014). Some herbicidical compounds may have molluscicidal action, with effects at the cellular, physiological and molecular levels (Bakry et al., 2015).

In Egypt, various herbicidical compounds are still under investigation. Bakry et al. (2012), for example, found that atrazine and glyphosate to possess a molluscicidal activity on *B. alexandrina*. The current work aims to study the effect of three compounds commonly formulated as herbicides and widely used in Egypt on the biological and histological parameters of *B. alexandrina*.

MATERIALS AND METHODS

Snails

Biomphalaria alexandrina snails were collected from freshwater canals in Abu-Rawash (30 km from Cairo), Giza, and maintained at

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the Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), where, they were washed thoroughly with dechlorinated tap water, and maintained in plastic aquaria (16 x 23 x 9 cm). The aquaria were provided with dechlorinated aerated tap water (10 snails/L) and covered with glass plates. They were maintained in an air conditioned room at 24°C, and fluorescent light was reflected 30 cm over them during daytime. Oven-dried lettuce leaves and blue green algae (*Nostoc muscorum*) were used for feeding. Lettuce leaves were given daily, with the amount adjusted to the number and size of the snails. The algae was added weekly. *Nostoc muscorum* algae was originally obtained from Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt, and were cultivated in Medical Malacology Laboratory, according to Liang et al. (1987). Water in the aquaria was changed weekly. Snails were examined twice weekly for natural trematode infection for one month before being used in bioassay tests.

For collecting egg masses, small pieces of polyethylene sheets were introduced into the aquaria (Pellegrino & Gonçalves, 1965) and harvested daily to obtain eggs of known age.

Ova and Cercariae

Schistosoma mansoni ova and cercariae were obtained from SBSC, where ova were taken from previously infected mice and cercariae from infected snails. The ova were allowed to hatch in small amount of dechlorinated water (24°C) for about 15 min under a direct light. After hatching of ova, the liberated miracidia were used in the experimental tests.

Synthetic Herbicides

Three herbicidical compounds were used in this study: Butralin (formulated as Amex 48% EC; Registration No. 245), glyphosate isopropylammonium (Herphosate 48% SL; Registration No. 733) and Pendimethalin (Stomp 50% EC; Registration No. 200). Commercial formulations were purchased in Egypt from Neopharm, The National Company for Fertilizers & Chemicals (AGROCHEM), and BASF, respectively.

Bioassay Tests

Molluscicidal Activity

Serial dilutions were prepared from Butralin, glyphosate isopropylammonium and Pendimethalin to determine the sublethal concentration. A stock solution of 1,000 ppm was prepared from each compound on the basis of W/V using dechlorinated tap water (pH 7.0–7.5), from which a series of concentrations were prepared for various assays, again in dechlorinated tap water.

In the first assay, a range of concentrations was used to establish the lethal dose in exposed adult snails. Three replicates were used, each of ten snails (6–8 mm shell width), for each concentration. Snails were washed thoroughly with dechlorinated tap water, maintained in plastic aquaria (16 x 23 x 9 cm). The aquaria were provided with dechlorinated aerated tap water (10 snails/L) and covered with glass plates. Exposure and recovery periods were each 24 h (at 25 ± 1°C). For each test, three replicates of control snails were maintained under the same experimental conditions in dechlorinated tap water (WHO, 1965).

The second assay examined the effects of sublethal concentrations on mortality and growth rates of *B. alexandrina*. Snails were exposed to sublethal concentrations (LC_0, LC_{10}, and LC_{25} at 24 h) of each herbicidal compound and numbers alive recorded weekly, and their shell width measured with calipers. Both juvenile snails (< 3.6 mm shell width) and adult snails (6–8 mm shell width) (El-Nahas & El-Deeb, 2007), were exposed with three replicates each of ten snails/L. As in the first experiment, also, three replicates of control groups was maintained in clean water.

In the third experiment, the effects of sublethal concentrations on egg-laying capacity were examined. Snails (6–8 mm shell width) were exposed for eight weeks to the concentrations LC_0, LC_{10} and LC_{25} of each herbicidical compound for 24 h/week for four successive weeks. Three replicates, each of ten snails/L, were prepared for each concentration, another three replicates for control groups were maintained in dechlorinated water. The following parameters were recorded weekly: mortality rate Lx (proportion of the corrected for mortality among control snails), fecundity Mx (number of eggs/snail/week) and R0 (reproductive rate, as the accumulated product of LxMx) (El-Gindy & Radhawy, 1965).

Lastly, effects of concentrations lethal to adult snails were examined for effects on the viability of snail eggs. For each concentration (LC_0, LC_{10}, and LC_{25}, LC_{50} and LC_{90}), three replicates comprising about 50 eggs/100 ml) of 1, 3 and 7 days old were prepared and exposed for 24 h, then transferred to clean water to calculate the rate of hatching of eggs in each concentration. Hatchability was calculated by dividing the number of newly hatched snails by the total number of eggs at the beginning of the experiment. Other three groups of eggs (1, 3 and 7 days old) were maintained in clean dechlorinated water (25 ± 1°C) as control groups.

Miracidicidal and Cercaricidal Activity

The three compounds in a range of concentrations that had been tested against adult snails (LC_0, LC_{10}, and LC_{25}, LC_{50} and LC_{90}) were examined for their effects on *S. mansoni* miracidiae and cercariae. Five ml of water containing about 100 fresh hatched miracidiae was pipetted into a cell in a divided Petri dish, and five ml water containing 100 freshly shed *S. mansoni* cercariae pipetted in another, and to each five ml of double-strength concentration added from each compound. Another, three groups of 10 ml dechlorinated tap water containing 100 freshly shed cercariae per each group was kept as controls (Ritchie et al., 1974). Mortality after intervals of 15, 30, 45, 60, 120, 180, 240, 300 and 360 min was recorded. Observations on the movement and mortality of miracidiae and cercariae were recorded at different intervals of exposure under a dissecting microscope. Stationary miracidia and cercariae were assumed to be dead, cercariae with their heads separated from tails were considered dead, and mortality in both tested and control groups was recorded.

Histological changes in the digestive and hermaphrodite glands of treated adult snails (8–10 mm in shell diameter) were studied after exposure to LC_0, LC_{10}, and LC_{25} sublethal concentrations for 24 h/week for two successive weeks and compared with control snails, according to Mohamed & Saad (1990). Thereafter, both treated and control snails were washed with water, and then dried with ethyl alcohol (70%) (five snails from each group). The shells were gently crushed between two glass slides and the soft parts of the snails were carefully removed out of the shell. The hermaphrodite and the digestive glands of each snail were gently separated and fixed in Bouin's solution. The glands were dehydrated using ascending grades of ethanol, cleared in terpinol, embedded in paraffin wax and finally sectioned at 6 µ. Sections were stained with hematoxyline and eosin stain (H-E), then left to dry. The stained slides were examined and photographed by a germin polarized photomicroscope supplied by Zeiss Video camera.

Statistical Analysis

Lethal concentration values were computed for each assay (Litchfield & Wilcoxon, 1949). Comparison of means of experimental and control groups was carried out using Student's t-test (Spiegel, 1981). Values were expressed as mean ± S.D., and the obtained data were analyzed using the Graph Pad Prism 6.04 software for Windows (Graph Pad Software, San Diego, California, U.S.A.; 1992–2014).

RESULTS

Molluscicidal Activity

In the present study, it was observed that Pendimethalin was found to be more toxic to *B. alexandrina* snails than Butralin and glyphosate isopropyl ammonium (Table 1). On the basis of

TABLE 1. Molluscicidal activity of the tested herbicidal compounds against adult *Biomphalaria alexandrina* (24 hours exposure); LC_x = the concentration at which x percent mortality occurred.

Compound	LC_0 ppm	LC_{10} ppm	LC_{25} ppm	LC_{50} ppm	Confidence limits of $LC_{50\ PPM}$	LC_{90} ppm	Confidence limits of $LC_{90\ PPM}$	Slope of regression mortality on conc.
Butralin	0.556	2.417	3.906	5.560	(3.70–8.34)	8.703	(6.22–12.80)	1.093
Glyphosate isopropylammonium	1.506	3.875	9.174	15.062	(9.13–16.57)	26.249	(23.87–28.90)	0.335
Pendimethalin	0.2148	0.535	1.299	2.148	(1.43–3.22)	3.762	(2.40–6.02)	1.820

TABLE 2. The survival rate and growth rate of juvenile *Biomphalaria alexandrina* snails exposed to sublethal concentrations of three herbicidal compounds for exposure for eight weeks.

		Survival rate (%)									Growth rate (%)	
		Weeks										
Conc. (ppm)		0	1	2	3	4	5	6	7	8	1st four	2nd four
Butralin	LC_0 (0.556)	100	95	87	81	69	55	22.5	15.8	8.3*	19.34	31.72**
	LC_{10} (2.41)	100	87	69	45	38.5	18	5.3			18.01	13.428
	LC_{25} (3.906)	100	80	55	25	8.3					11.11	
Glyphosate isopropylam- monium	LC_0 (1.506)	100	90	80	71.5	48.3	38	26.5	18	18	19.23	33.22
	LC_{10} (3.87)	100	70	59	45	25	14	7.5			17.69	9.67
	LC_{25} (9.17)	100	66.5	41.5	21.5	6.6					16.53	
Pendimethalin	LC_0 (0.214)	100	85	80	66	45	36.5	18	15	5	24.78	14.98
	LC_{10} (0.535)	100	80	72	56.5	46.5	18				12.46	2.45
	LC_{25} (1.299)	100	56	26	16.5	5					12.55	
Control		100	98	97	95	95	91	83	75	75	61.41	66.32

* The mean of the percentage survival in the three replicates.
**The mean of the percentage Growth rate in the three replicates.

LC_{50} values, Pendimethalin toxicity to the snails was about 2.58 times that of Butralin and 7.012 times of glyphosate isopropylammonium.

The growth rates of snails exposed to LC_0, LC_{10} and LC_{25} of the three compounds over four weeks were highly significantly reduced ($p < 0.01$) compared to the control group (Table 2). Also, survival rates of juvenile snails were reduced after their exposure to the tested concentrations of these herbicides.

The reproductive rate (R_0) of snails was significantly reduced when exposed to the herbicidal compounds ($p < 0.001$) (Table 3). Also, survival rate (Lx) of adult *B. alexandrina* snails decreased with an increase in the applied concentrations. The high sublethal concentrations (LC_{25}) of the three herbicides reduced survival rate of adult snails.

Viability of *B. alexandrina* eggs were affected non significantly by their exposure to the concentrations of Butralin, Pendimethalin and glyphosate isopropylammonium that did not affect mortality in adult snails (i.e., the LC_0). However, the hatchability of eggs of age one and three days at the time of exposure to the respective compounds was markedly lower than those of seven day-old eggs at times of exposure (Table 4). At LC_{90}, no hatching of eggs occurred when exposed to butralin or pendimethalin, but with those eggs exposed to glyphosate isopropyl ammonium a low. At LC_{90}, there is 20% hatchability of eggs at age one day, 2% for seven day-old eggs, while no hatchability at three day-old eggs in snails subject to glyophosate herbicide.

Miracidicidal and Cercaricidal Activity

Mortality rate of miracidiae and cercariae were increased significantly by increasing the concentration of the herbicidal compounds and the length of the exposure period (Tables 5, 6).

TABLE 3. Survival rate and reproductive rate of *Biomphalaria alexandrina* snails exposed to sublethal concentrations of three herbicidal compounds for four weeks followed by four weeks recovery period.

		Survival rate of adult snails									Reproductive rate (%)	
		Weeks										
Conc. (ppm)		0	1	2	3	4	5	6	7	8	R_0	Reduction (%)
Butralin	LC_0 (0.556)	1.0	0.95	0.88	0.80	0.70	0.60	0.20	0.10	0.05#	7.05***	84.05
	LC_{10} (2.41)	1.0	0.88	0.76	0.50	0.30	0.20	0.07			4.52***	89.76
	LC_{25} (3.906)	1.0	0.81	0.73	0.30	0.05					4.04 ***	90.84
Glyphosate isopropylammonium	LC_0 (1.506)	1.0	0.90	0.78	0.60	0.48	0.30	0.25	0.20	0.11	4.87***	88.99
	LC_{10} (3.87)	1.0	0.81	0.61	0.50	0.16	0.10	0.05			4.20***	90.48
	LC_{25} (9.17)	1.0	0.72	0.55	0.20	0.11					4.23***	90.42
Pendimethalin	LC_0 (0.214)	1.0	0.86	0.82	0.65	0.50	0.36	0.18	0.11	0.05	5.18***	88.28
	LC_{10} (0.535)	1.0	0.83	0.76	0.72	0.35	0.14				4.75***	89.25
	LC_{25} (1.299)	1.0	0.66	0.41	0.26	0.11					4.27***	90.33
Control		1.0	0.99	0.98	0.95	0.91	0.90	0.85	0.85	0.08	44.231	

The mean of the percentage survival in the three replicates.
***Differ from control at $p < 0.001$.

Histopathological changes of the digestive and hermaphrodite glands in adults treated snails (all at x 40) were expressed as follows:

Digestive Gland

The normal digestive gland occupies a considerable part of the visceral hump and

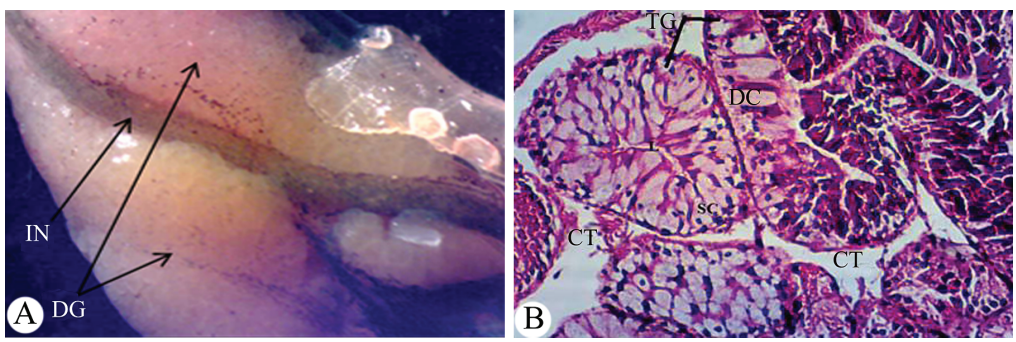

FIG. 1A: Photograph showing a dissection of a control of snails to show lobes of the digestive gland; B: Photomicrograph showing a histological section in the digestive gland of normal *B. alexandrina* snails.

TABLE 4. Effect of three herbicidal compounds (24 hours exposure) on hatchability of *Biomphalaria alexandrina* eggs of 1, 3 and 7 days old at time of exposure.

Conc. (ppm)		% Mean number of hatchability of eggs		
		1 day at exposure	3 days at exposure	7 days at exposure
Butralin	LC_0	92	95	93
	LC_{10}	82	32	90
	LC_{25}	24	18	65
	LC_{50}	6	4	30
	LC_{90}	0	0	0
Glyphosate isopropylammonium	LC_0	89	88	89
	LC_{10}	58	22	72
	LC_{25}	46	12	30
	LC_{50}	32	2	13
	LC_{90}	20	0	2
Pendimethalin	LC_0	90	84	94
	LC_{10}	30	60	74
	LC_{25}	5	10	27
	LC_{50}	0	0	8
	LC_{90}	0	0	0
Control		99	98	99

consists of two unequal lobes (Fig. 1A). The normal digestive gland consists of a number of tubular glands; each tubule is lined with one layer with two main histological types of cells; the digestive cells and the secretary cells (Fig. 1B).

Effect of the Three Herbicides on Digestive Glands

The present study (Fig. 2A) shows moderate pathological damages in the digestive gland after exposure to three herbicides, while, in intensive pathological damage, as most of the connective tissue was broken down so the intertubular space becomes distinctive around the digestive tubules (Fig. 3). The cells lost their identical shape resulting from dissolution of their cell membrane (syncytium), and the lumen inside tubule increased (in the three herbicides). While the most

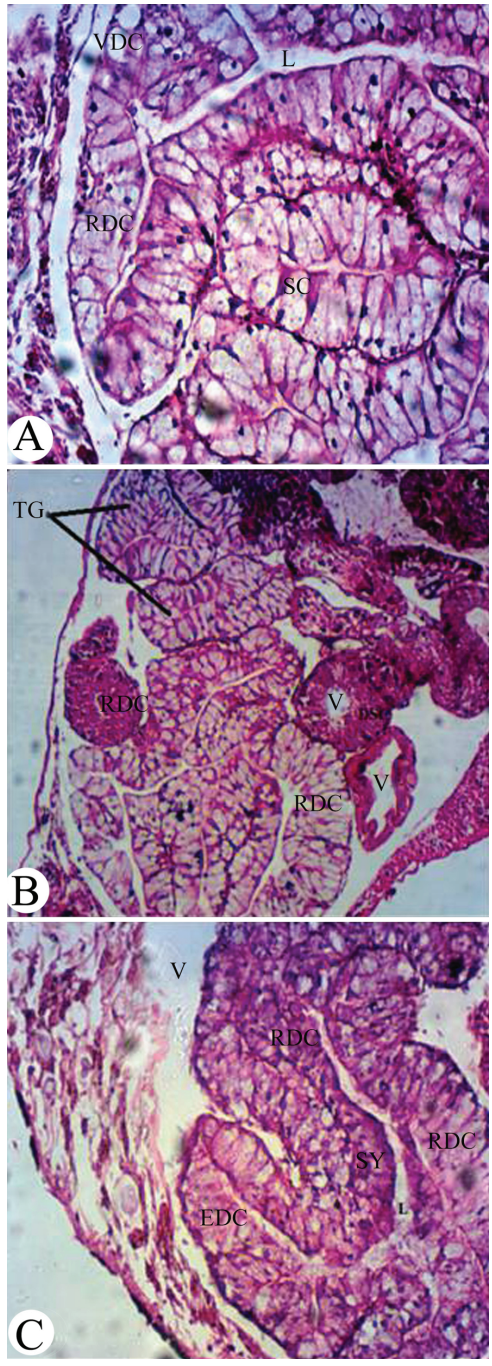

FIG. 2. Photomicrograph showing histological sections through the digestive glands of treated snails with LC_0 sublethal doses for two weeks (H & E). A: Amex 48% EC; B: Herphosate 48% SL; C: Stomp 50% EC.

TABLE 5. Effect of three herbicidal compounds on the mortality rate of *Schistosoma mansoni* miracidia.

Conc. (ppm)		% Mean of mortality							
		15	30	45	60	90	120	150	180
Butralin	LC_0	0	0	2	6	15	30	60	90
	LC_{10}	0	4	10	12	16	29	50	89
	LC_{25}	12	39	52	78	93	100		
	LC_{50}	45	73	81	100				
	LC_{90}	60	100						
Glyphosate	LC_0	0	0	14	20	25	40	70	93
isopropylam-	LC_{10}	15	23	31	43	81	100		
monium	LC_{25}	19	46	84	100				
	LC_{50}	42	65	100					
	LC_{90}	78	98	100					
Pendimethalin	LC_0	0	4	14	23	34	60	71	87
	LC_{10}	2	9	30	55	78	89	96	100
	LC_{25}	19	34	69	82	100			
	LC_{50}	32	73	100					
	LC_{90}	75	100	0					
Control		0	0	0	6	12	17	20	50

TABLE 6. Effect of three herbicidal compounds on the mortality rate of *Schistosoma mansoni* cercariae.

Conc. (ppm)		% Mean of mortality							
		15	30	45	60	90	120	150	180
Butralin	LC_0	0	4	6	9	30	58	70	99
	LC_{10}	6	20	30	60	98	100		
	LC_{25}	9	30	90	98	100			
	LC_{50}	30	66	87	100				
	LC_{90}	44	98	100					
Glyphosate	LC_0	3	10	13	15	30	40	55	89
isopropylam-	LC_{10}	15	50	60	65	85	100		
monium	LC_{25}	20	65	70	90	100			
	LC_{50}	75	87	91	100				
	LC_{90}	80	98	100					
Pendimethalin	LC_0	0	3	10	15	60	77	86	99
	LC_{10}	7	16	30	80	95	100		
	LC_{25}	15	25	53	100				
	LC_{50}	35	76	98	100				
	LC_{90}	66	100						
Control		0	0	3	10	22	45	67	80

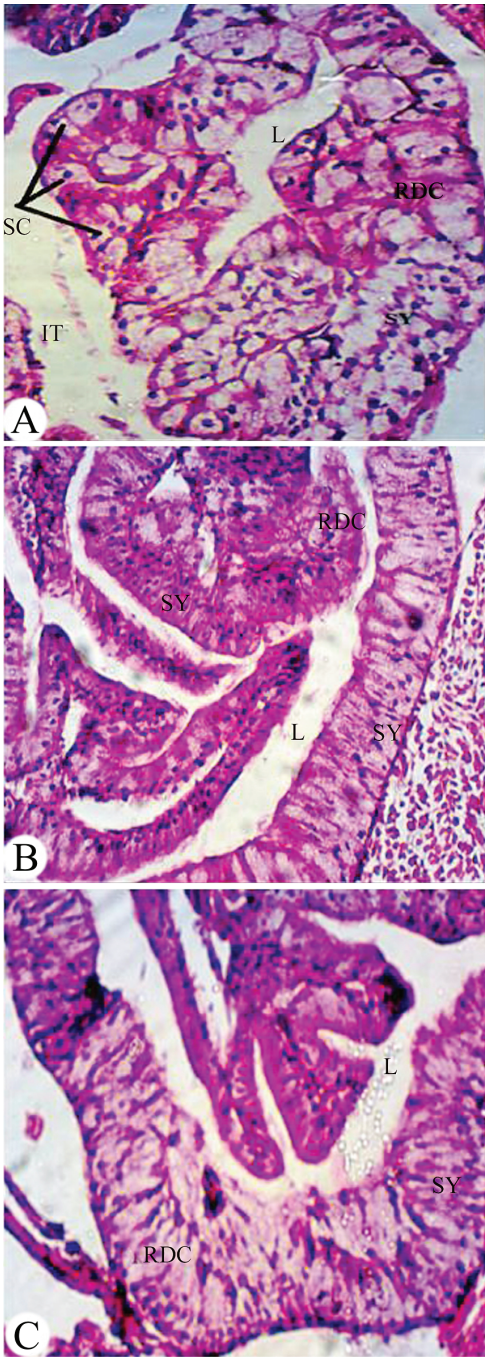

FIG. 3. Photomicrograph showing histological sections through the digestive glands of treated snails with LC_{10} sublethal doses for two weeks. A: Amex 48% EC; B: Herphosate 48% SL; C: Stomp 50% EC.

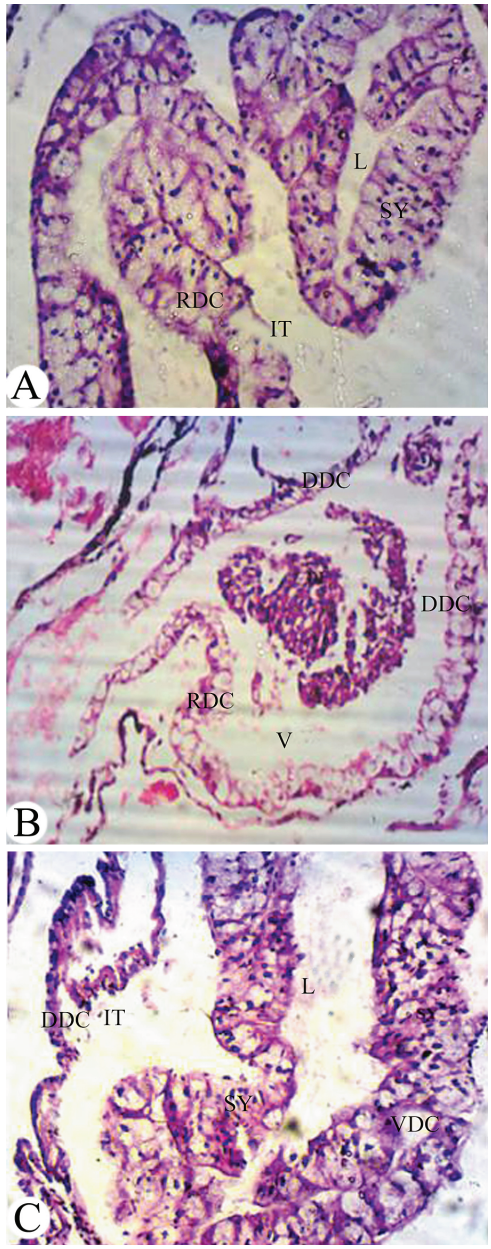

FIG. 4. Photomicrograph showing histological sections through the digestive glands of treated snails with LC_{25} sublethal doses for two weeks (H & E). A: Amex 48% EC; B: Herphosate 48% SL; C: Stomp 50% EC. DDG: Degenerated Digestive Cells, DSC: Degenerated Secretory Cells, TG: Tubular Gland, DF: Digested Food, IT: Inter Tubular Space, SC: Secretory Cells, VDC: Vacuolated Digestive Cell, RDC: Ruptured Digestive Cells, V: Vacuole, SY: Syncytium.

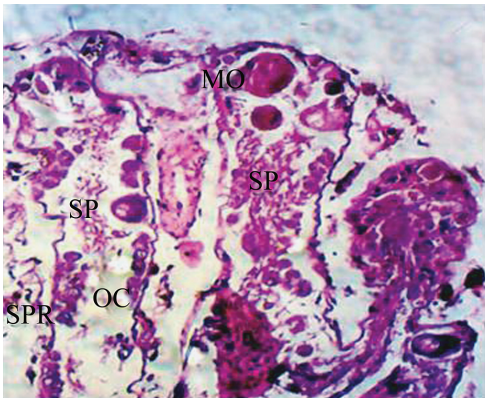

FIG. 5. Photomicrograph showing histological sections through hermaphrodite glands of normal *B. alexandrina* snails (H & E) (x 40). MO: Mature Ova, SP: Sperm, OC: Oocyte, SPR: Spermatocytes.

destructive effect in the digestive tissue was shown with snails subjected to LC_{25} (Fig. 4), where there is a great loss of identical shape of digestive cells (syncytium).

Normal Hermaphrodite Gland of the Adult B. alexandrina Snails

The hermaphrodite gland of the normal *B. alexandrina* snails is composed of a number of acini connected together by areolar connective tissue. The male reproductive cells (spermatogonia) are differentiated in clusters forming primary and secondary spermatocytes. The fully developed sperms were observed either in the lumen or attached to sertoli cell, with comparatively small heads and long tails. On the other hand, the female oogenic cells filled the acinar lumen as primary, secondary oocytes and mature ova. The mature ova contain large accumulations of yolk material (Fig. 5).

Effect of the Three Herbicides on Hermaphrodite Gland

Treatment of normal *B. alexandrina* snails with Amex at this sublethal dose for two weeks made marked morphological changes

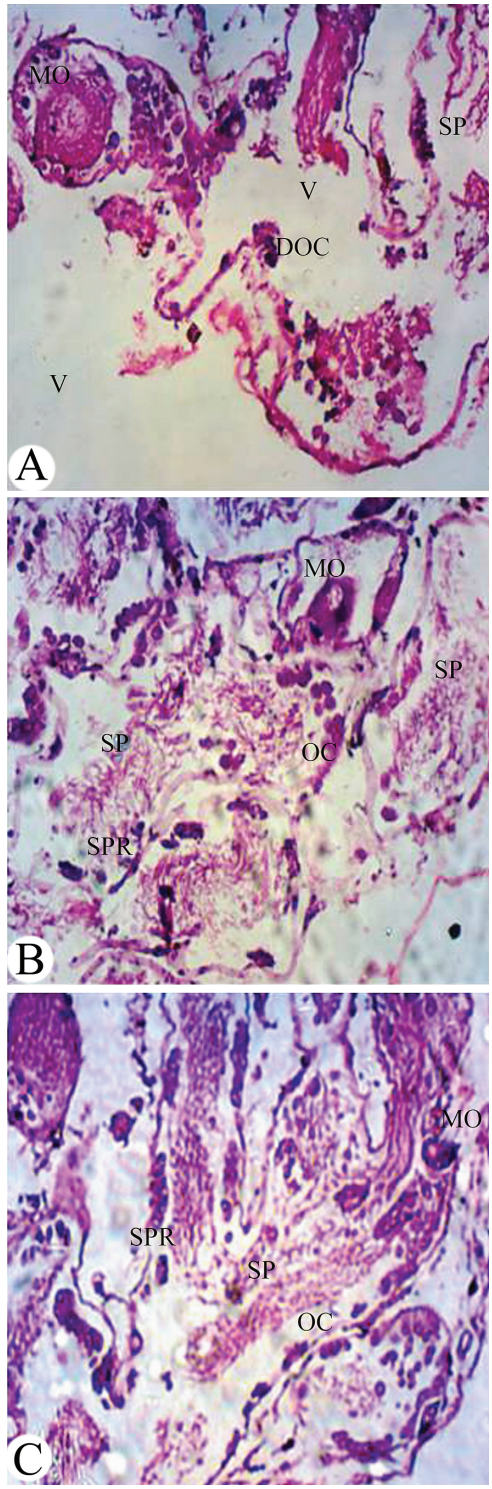

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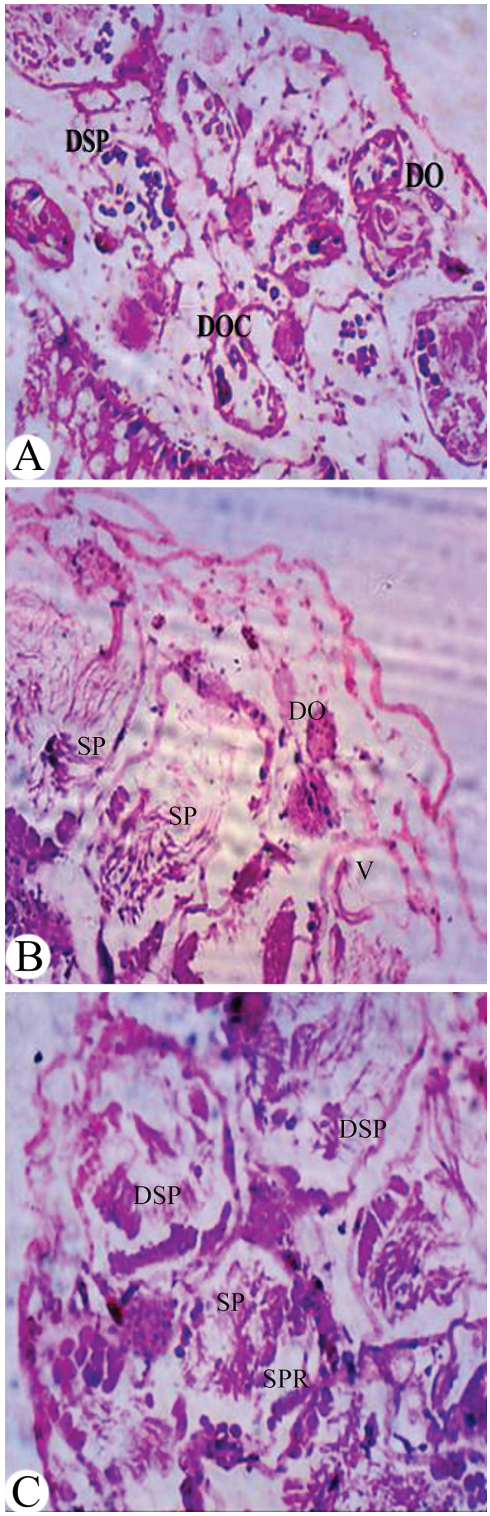
FIG. 6. Photomicrograph showing histological sections through hermaphrodite glands of treated snails with LC_0 sublethal doses for two weeks (H & E). A: Amex 48% EC; B: Herphosate 48% SL; C: Stomp 50% EC.



to gland cells in addition to several evacuations of its tubules from gametogenic stages (Fig. 6). However, they still have mature ovum and few sperm in snails, whereas in snails treated with Herphosate and Stomp, there are increases in sperm. While, as shown in Figure 7, both male and female gonadal cells are highly affected. The greatest damage to gonadal cells occurred by using the three herbicides at LC_{25} (Fig. 8), where ova lost their shape and degenerated, sperm are reduced and degenerated. Most of the spermatogenic and oogenic stages disappeared.

DISCUSSION

The present results showed that Pendimethalin (Stomp 50% EC) herbicide was more toxic to *B. alexandrina* snails than the other two herbicides. The differences in the activity of the compounds could be attributed to the differences in their pathways of penetration inside the treated organisms, as well as to the differences in their mode of action on the target organ (Etges & Guilberton, 1966), or they could be attributed to differences in the nature of their active constituents (Hasheesh et al., 2011).

The current data showed that the growth rates of *B. alexandrina* juvenile snails exposed to the tested herbicides were less than that of controls. This may be related to their physiological and biological activities being partially interrupted, or the snails may allocate their energy for maintenance (Mohamed et al., 2012).

The present data showed that, survival rates of juvenile snails were reduced after their exposure to the tested concentrations of these herbicides. It could be that these survived snails tried to overcome the residual toxic effects of the tested agents and their effort to compensate the harmful stress they suffered during the exposure period (Mohamed et al., 2012). When using Pendimethalin, it decreased again, because of the high damage to their digestive system. The same results obtained by Bakry et al. (2012), who showed that LC_{10} of the two pesticides (Atrazine and Roundup)

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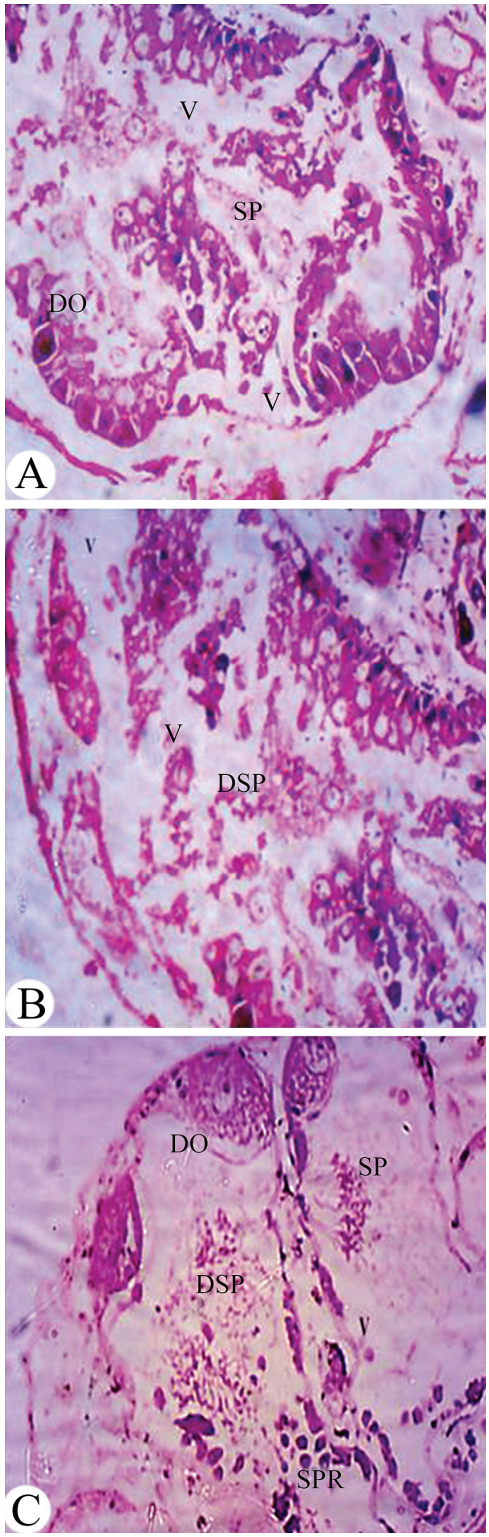
FIG. 7. Photomicrograph showing histological sections through hermaphrodite glands of treated snails with LC_{10}sublethal doses for two weeks (H & E). A Amex 48% EC; B: Herphosate 48% SL; C: Stomp 50% EC.



caused a considerable reduction in the survival rates and egg production of treated snails. Also, Hasheesh & Mohamed (2011) evaluated the molluscicidal activity of two pesticides (Chlorpyrifos and Profenophos) against *Bulinus truncatus* snails, and they found that these pesticides highly reduced the survival rate, egg production and growth rate of these snails. They confirmed this reduction by histological examinations, which showed severe damage in the digestive and hermaphrodite gland cells of the treated snails.

The reproductive rate (R_0) and fecundity (M_x) of adult *B. alexandrina* snails in the present study were significantly decreased by their exposure for four weeks to the sublethal concentrations of the tested compounds. These low values of R_0 and M_X of treated snails could be attributed partially to their high mortality rates and different periods of ceasing egg-laying during the experimental period, in comparison with the control groups. Another assumption for this reduction in R_0 of treated snails is the considerable deterioration in the activities of the enzymes AST and ALT in their tissues and hemolymph, which means damage to the snails' cells, hence interruption of their physiological activities, resulting in low or ceasing their opposition (M_x) and net reproductive rate (R_0). Also, this was confirmed by histological examination, which showed severe damage in the digestive and hermaphrodite gland cells of treated snails (Hasheesh & Mohamed, 2011). In addition, Mahmoud et al. (2011) attributed the effects partially to severe damage to the hermaphrodite gland cells of treated snails.

The present data revealed that hatchability rates of *B. alexandrina* eggs of older embryonic stages exposed for 24 hours to the sublethal concentrations LC_0, LC_{10} and LC_{25} of these herbicides were higher than those of the freshly laid eggs, in which hatchability rate increased with increasing age and decreased with increased concentration of these herbicides. However, at LC_{90} from the tested compounds no eggs (1, 3 and 7 days old) hatched. The differences in susceptibility of the tested eggs to these compounds may be due to the ability of

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FIG. 8. Photomicrograph showing histological sections through hermaphrodite glands of treated snails with LC_{25}sublethal doses for two weeks (H & E). A: Amex 48% EC; B: Herphosate 48% SL; C: Stomp 50% EC. MO: Mature Ovum, SP: Sperm, SPR: Spermatocytes, OC: Oocyte, DO: Degenerated Ovum, DSP: Degenerated Sperms, V: Vacuole.

the tested herbicides to penetrate well the thick yolk layer of fresh laid eggs and to kill most of the few developing embryos within the exposed egg masses. Hasheesh et al. (2011) observed that freshly laid eggs more susceptible than older eggs to the fungicide Score. Youssef (2010) also recorded approximately similar effects of the pesticides Vertemic and Match on hatchability of *B. alexandrina* eggs.

Regarding the effect of the tested herbicides on *S. mansoni* miracidia and cercariae, our results indicate that mortality rates of these parasite larval stages were dependant on concentration and exposure period. Thus, these rates were increased by prolongation of the exposure period up to 180 min (three h) and by increasing the sublethal concentration used. This agrees with El-Emam et al. (2008) on the pesticides Regent, Mimic, Fenitrothion, Vertimec and Match against *S. mansoni* miracidia and cercariae. A similar conclusion was reached by Hasheesh & Mohamed (2011) against *S. haematobium* miracidia and cercariae exposed to the pesticides Chorpyifos and Profenofos, and they stated that the mortality rates of the miracidia and cercariae were elevated gradually by increasing the concentration of the tested pesticides. Abdel Raouf (2007) stated that the difference in mortality rates of both larval stages seems to be dependent on the chemical structure of the tested agents and not on the biological nature of these larvae.

Because all of the three herbicides in the present study induced histopathological changes of the digestive and hermaphrodite glands and the extent of damaged increased with an increase in the concentrations, the most prominent severe damage in the digestive cells was a great loss in shape of digestive cells (syncytium). The tips of digestive cells ruptured, and most of the cells were degenerated. Vacuoles and lumens inside tubule increased, and the secretory cells became denser in color and increased in number, and the connective tissue between digestive tubules shrank.

Our results agree with El-Deeb & El-Nahas (2005) on *Euphorbia nubica* and *Sesbania sesban* plants, which caused epithelial necrosis and abnormal increase in the ratio of secretory to digestive cells. Also, Bakry (2009) reported the same observations in the digestive gland of *B. alexandrina* past two weeks of exposure to LC_{25} of *Guayacum officinalis, Atriplex stylosa* and *Euphorbia splendens* methanol extract.

In the present work, the histological examination of the hermaphrodite gland in treated snails showed loss of connective tissue, irregular sperm and mature ova appeared dense and irregular in shape after exposure to the three herbicides, and an acceleration in male gametogenic developmental stages were present in an obvious pattern after exposure to glyphosate isopropylammonium and Pendimethalin. These findings agree with the egg laying results, as the treated adult snails could lay eggs throughout the exposure period. These results agree with Al-Sharkawy et al. (1996), who observed gametogenic maturity acceleration in the hermaphrodite gland of *B. alexandrina* snails after exposure to *Ammi majus* dry plant powder. By increasing the concentrations of testing herbicides, the ova lost their normal shape, and the damage increased showing discharge, evacuation, detachment of some gonadal cells from acini and complete destruction of gametogenic cells. Moreover, the disappearance of most of the oocytes, spermatogenesis and connective tissues was evident. These observations were supported by the study of Hasheesh & Mohamed (2011), who attributed the reduction in egg laying of *Bulinus truncatus* treated with *Sesbania sesban* to severe histological damage to the snail's hermaphrodite gland cells and the evacuation of some of its tubules from various gametogenetic stages. This also agrees with a study by Mossalem et al. (2013) on the molluscicidal properties of the antihelminthic plant derivative, dihydro-artemisinin methyl ether (Artemether) against some histological and histochemical parameters of *B. alexandrina* snails, and they stated that there is a complete destruction of gametogenic cells and severe damage of hermaphrodite gland tissues, especially when the exposure period increased. From the previous results, it can be concluded that the three herbicides have a molluscicidal effect on the intermediate host of *Schistosoma mansoni.*

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