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EFFECT OF ROUNDUP HERBICIDE ON BIOLOGICAL ACTIVITIES OF *BIOMPHALARIA ALEXANDRINA* SNAILS INFECTED WITH *SCHISTOSOMA MANSONI*

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ABSTRACT

Roundup (48% Glyphosate), the most widely used herbicide, was tested against *Biomphalaria alexandrina* snails and the free larval stages of *Schistosoma mansoni*. After 24 hrs of exposure to Roundup the LC_{50} and LC_{90} values for adult snails were 129.02 and 262.44 ppm, respectively. Larvicidal activity of the exprimental herbicide against *S. mansoni* miracidia and cercariae was tested after one hour. LC_{50} and LC_{90} were 14.54 and 16.34 ppm, for miracidia and 5.12 and 7.65 ppm, for cercariae respectively. The effect of prolonged exposure to sub-lethal concentration of Roundup was studied on biological activities of *B. alexandrina* snails. The results obtained showed an inhibitory effect on survival rate, growth rate and egg laying capacity on both non-infected and *S. mansoni* infected snails during four weeks of exposure in comparison with untreated- control group. Roundup caused high reduction in mean values of cercarial production output (255.7) cercariae/snail when compared with the control infected group (603.30) cercariae/snail. Sever damage and alterations in the histological structure of ovotestis and digestive glands of *B. alexandrina* infected and non-infected snails were observed at the 4th week post exposure. Therefore, these results revealed that Roundup may has a molluscicidal and larvicidal activities towards adult *B. alexandrina* snails and free larval stages of *S. mansoni*.

Keywords: *Biomphalaria* snails-herbicide- Roundup-*Schistosoma mansoni*.

INTRODUCTION

Schistosomiasis is a world wide public health problem in many developing countries. It affects 200 million individuals in tropical regions of Africa, Asia and South America and it is an endemic disease in 74 countries [**WHO (2002)**]. **WHO (1993)** reported that controlling the disease depends on many factors. one of them is the control of the snail intermediate host. One of the most effective methods for schistosomiasis control is using molluscicides [**Shiff (1961)**]. Schistosomes infections may affect the vital functions of their molluscan hosts and initiates a dynamic confrontation with marked effects on host metabolism, growth, survival, reproductive activites and immune system [**Bayne & Loker (1987)**].

Herbicides are a distinctive group of pesticides and are considered as selective weed killers. Glyphosate is one of the most important herbicides ever developed, which is the active substrate in the commonly used preparation Roundup^{TM} [**Cağlar & Kolankaya (2008)**]. Glyphosate can contaminate surface water either directly as a result of aquatic weed control or indirectly via spray drift run-off and soil erosion. Glyphosate must be mixed with a surfactant (a soap- like substance) that facilitates the uptake of glyphosate by the plant [**WHO (1994)**]. Toxicological properties of Glyphosate may have an impact on the aquatic environment and other aquatic organisms [**USDA (1984)**]. Therefore, the evaluation of its biological activities on aquatic organisms is very useful and important. The herbicides' effect on different snails has been studied [**Roses *et al* (1999); Tantawy (2002); Zidan *et al.*, (2002); Sakran (2004) and Tantawy (2006)**].

This investigation was designed to evaluate the effect of Roundup herbicide application on some biological activities and histological aspects of both infected and non-infected *Biomphalaria alexandrina* snails and larval stages of *Schistosoma mansoni*.

MATERIALS AND METHODS

Experimental Animals:

Laboratory-bred *B. alexandrina* snails (shell diameter 8-10 mm) and white albino mice CD1 strain were originally obtained from Theodor Bilharz Research Institute, Giza, Egypt. *S. mansoni* cercariae were obtained from laboratory infected *B. alexandrina* snails.

Experimental Materials:

Roundup: Glyphosate [(N-phosphonomethyl glycine] 48% active ingredient used in the liquid commercial formulation produced by Monsanto Company, st. Louis Mo USA was purchased from the pest control Unit Ministry of Agriculture.

Experimental Infection:

Mice Infection: CD1 mice were exposed to freshly emerged cercariae of *S. mansoni* by bathing them in dechlorinated tap water of 1 cm depth containing 80-100 cercariae for 1-2 hrs.

Snail Infection: .Miracidia of *S. mansoni* hatched under illumination from eggs were isolated from homogenized liver and intestine of 6-8 weeks infected CD1 mice **[Chernin (1970)]**. *B. alexandrina* snails were exposed individually to 4-5 miracidia in glass test tubes filled with 1 ml dechlorinated tap water for 2 hours **[Anderson *et al.*, (1982)]**.

Cercarial Count:

The infected non-treated and infected treated snails were individually isolated in glass test tubes having 1 ml decholrinated tap water and exposed to artificial light for 1 hour to stimulate cercarial shedding **[Meuleman (1972)]**. From each tube, 300 µl was withdrawn and the cercariae were counted in every 100 µl, the mean number of cercariae was calculated for each snail.

Bioassay Tests:

A stock solution of 1000 ppm based on the active ingredient of Roundup (48 % Glyphosate) was freshly prepared on the basis of w/v using decholrinated tap water (pH 7.5-7.7). A series of concentrations that would allow the computation of LC_{50} and LC_{90} values were prepared according to **WHO (1965)**. Three replicates were used, each of 10 snails being immersed in one liter of each tested concentration. The exposure period was 24 hrs at room temperature. Three replicates of control snails were also kept under the same experimental conditions in decholrinated tap water only. LC_{50} and LC_{90} were computed using Probit Proban analysis programmer (ver. 1.1).

Prolonged exposure of snails to sub-lethal concentration of Roundup (10 ppm):

Mature *B. alexandrina* snails (120 individual) were divided into four experimental groups (30 for each). 1- Non treated and non infected snails (control). 2- Treated non-infected. 3- infected snails and 4- Treated-infected snails. Snails were maintained in 1000 ml of the experimental concentration in two-liter capacity plastic containers. Roundup solution had to be replaced with new prepared one, two times a week. Fresh lettuce leaves were provided as the daily food. Observations were recorded weekly for mortality, number of egg masses laid and the shell diameter (growth rate).

Effect of Roundup on larval stages of *Schistosoma mansoni*:

The larvicidal properties of Roundup were tested against miracidia and cercariae. Three replicates of approximately 20-30 freshly hatched miracidia and cercariae were placed in petri dishes and exposed to different concentrations of Roundup. A binocular stereo microscope was used to monitor the activity of the miracidia or cercariae at intervals of 15, 30, 45, 60 minutes. To calculate LC_{50} and LC_{90} Probit proban analysis (Ver 1.1) was used.

Histological study:

Five snails were selected randomly from each experimental group at the 4^{th} week post exposure. Ovotestis and digestive glands were separated and immediately fixed in alcoholic Bouin's fluid. After dehydration, clearing and embedding, serial 5 µm thick sections were cut, mounted and stained with Ehrlich's haematoxylin and counterstained in eosin according to **Presnell *et al.*, (1997)**. Preparations were investigated using Zeiss photoresearch microscope. Micrographs using Kodak gold 200 Aza Film were prepared.

Statistical analyses

Data analyses were carried out using the computer program SPSS Inc. (2001, version 11.0 for Windows). The comparison between means and standard deviations of the biological parameters of *Biomphalaria alexandrina* snails infected with *Schistosoma mansoni* and treated with Roundup was tested for significance using two independent samples *t*-test. The differences were considered significant if $p<0.05$.

RESULTS

Biological activities:

The obtained results revealed that Roundup has a molluscicidal potency against adult *B. alexandrina* snails. The LC_{50} and LC_{90} values after 24 hrs were 129.02 and 262.44 ppm, respectively as illustrated in Table (1). The larvicidal activity was examined against *S. mansoni* miracidia and cercariae after 60 minutes as presented in Table (2). For miracidia, it was found that LC_{50} and LC_{90} values were 14.54 and 16.34 ppm whereas LC_{50} and LC_{90} values for cercariae were 5.12 and 7.65 ppm, respectively.

Table (1): Molluscicidal activity of the herbicide Roundup on adult *Biomphalaria alexandrina* snails.

Exposure Time (hours)	Concentration (ppm)		Slope function(S)
	LC_{50}	LC_{90}	
24 hrs	129.02	262.44	4.16

Table (2): The larvicidal activity of the herbicide Roundup on miracidia and cercariae of *Schistosoma mansoni.*

S. mansoni stages	LC_{50}	95%confidencial limit		LC_{90}	99%confidencial limit		Slope function (S)
		Lower	upper		Lower	upper	
miracidia	14.54	10.7	15.2	16.34	16.3	32.4	9.29
cercariae	5.12	4.3	5.8	7.65	6.7	9.3	8.39

The impact of sub-lethal concentration of Roundup (10 ppm) on the survival, growth rates and egg laying capacity on infected and non-infected *B. alexandrina* snails were studied throughout the experimental period of 4 weeks as presented in Table (3). The achieved results indicated that the survival rate of treated *B. alexandrina* snails showed a reduction during the entire experimental period when compared with the control snails Table (3). The survival rate of treated non-infected snails was found (90 %) by the end of the 4^{th} week of exposure in comparison with control (100%). However, treated-infected snails indicated (81.6 %) by the end of the 4^{th} week when compared to control-infected snails which was (93.3 %).

The growth rate (expressed as mean shell- diameter- mm) of *B. alexandrina* snails in experimental and control groups is shown in Table 3 and figure 2. The results revealed that the Roundup treated snails had a significant decrease in growth rate in comparison with the control group. The mean shell diameter at the 4^{th} week of treated and control snails showed 11.17±0.68 and 13.43±0.63 mm/snails. respectively. While treated- infected had significant decrease in growth rate than infected snails group. The shell diameter of treated-infected snails 10.87±0.84 and 11.42±0.81 for infected snails group.

Roundup treatment affected strongly the number of egg masses/snail/week during the whole duration of the experiment (4 weeks). The egg production of treated snails was significantly reduced from the first week of exposure (Table-3 figure 1). At the 4^{th} week post exposure, the mean number of egg masses and total eggs laid by treated snails was 0.84±0.26 egg-masses\snail\week and 14.25±4.07 eggs\snail\week compared to 4.90±0.46 egg masses\snail\week and 138.83±11.6 eggs\snail\week for control group. In treated-infected snails, 0.19±0.06 egg masses/snail/week and 2.41±0.83 eggs/snail/week were highly significant reduced in comparison with 2.79±0.31 egg masses\snail\week and 69.08±15.55 eggs\snail\week for control-infected snails.

Regarding the cercarial production it is obvious as illustrated in Table (4) that infected snails exposed to Roundup revealed a decrease in the total cercarial production in comparison with untreated infected group. The mean number of cercariae/snail for infected untreated snails was 603.3 while it decreased to 255.7 cercariae/snail for treated-infected snails. No significant differences were observed in the length of the prepatent period in relation to treated-infected and infected snails.

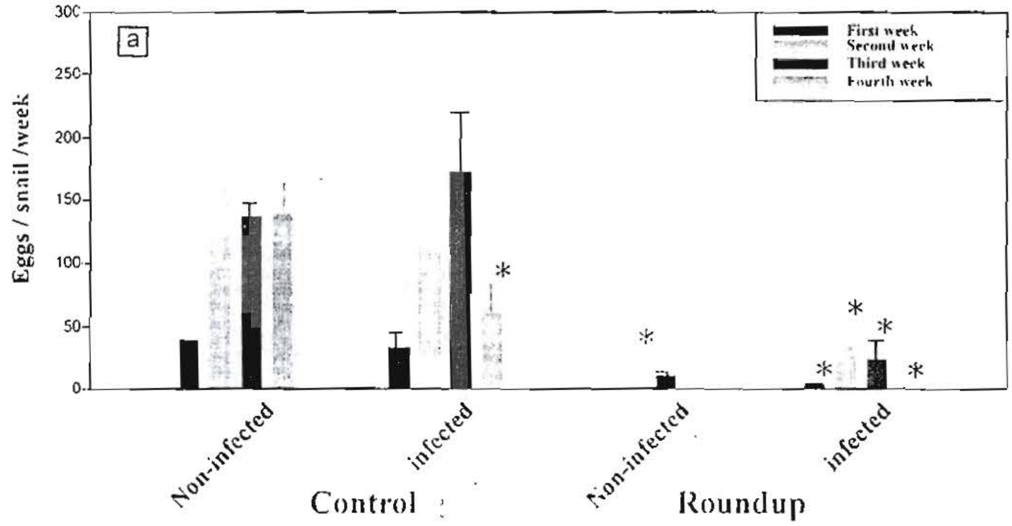
Table (3): Effect of sub-lethal concentration of Roundup on some biological parameters on non-infected and infected *Biomphalaria alexandrina* snails with *Schistosoma mansoni*

Exposure period (weeks)	Biological parameters \ Treatment	Control snails		Roundup -treated snails	
		Non-infected	Infected with *S.mansoni*	Non-infected	Infected with *S mansoni*
1	Survival rate	100	100	98 3	93 3
	Egg mass/snail/week	1 37±0 12	1.50±0 53	0*	0.07±0.06*
	Eggs /snail / week	39 03±11.68	29.23±16 48	0*	2.35±1 73*
	Shell diameter	10 63±0.54	9.75±0.82*	9.85±0 78*	9.92±0 60*
2	Survival rate	100	96.6	96.6	88 3
	Egg mass/snail/week	4 83±0 83	7.89±1 82	0.63±0 24*	1.43±0.73*
	Eggs /snail / week	121.2±22.17	200.48±45 23	22 39±21.9*	43.15±29.0*
	Shell diameter	11.70±0.50	10.4±0 69*	10.32±0.78*	10.33±0 74*
3	Survival rate	100	96 6	95	86 6
	Egg mass/snail/week	4.97±0.64	6 87±0.59*	0.94±0.29*	2.67±0.38*
	Eggs /snail / week	136 17±25.9	125.39±91 67	12 56±5 05*	31.00±14 9*
	Shell diameter	12 58±0.59	11.02±0.78*	10.68±0.74*	10.55±0 83*
4	Survival rate	100	93.3	90	81.6
	Egg mass/snail/week	4.90±0.46	2 79±0 31*	0.84±0 26*	0.19±0.06*
	Eggs /snail / week	138.83±11.6	69.08±15.55 *	14.25±4.07*	2.41±0 84*
	Shell diameter	13.43±0 63	11.42±0 81*	11.17±0 68*	10.87±0 84*

Data in the table expressed as mean ± SD

* Significant difference compared to control group at $P \leq 0.05$

Figure (1): Effect of sub-lethal concentration of Roundup on egg laying capacity of *Biomphalaria alexandrina* snails.



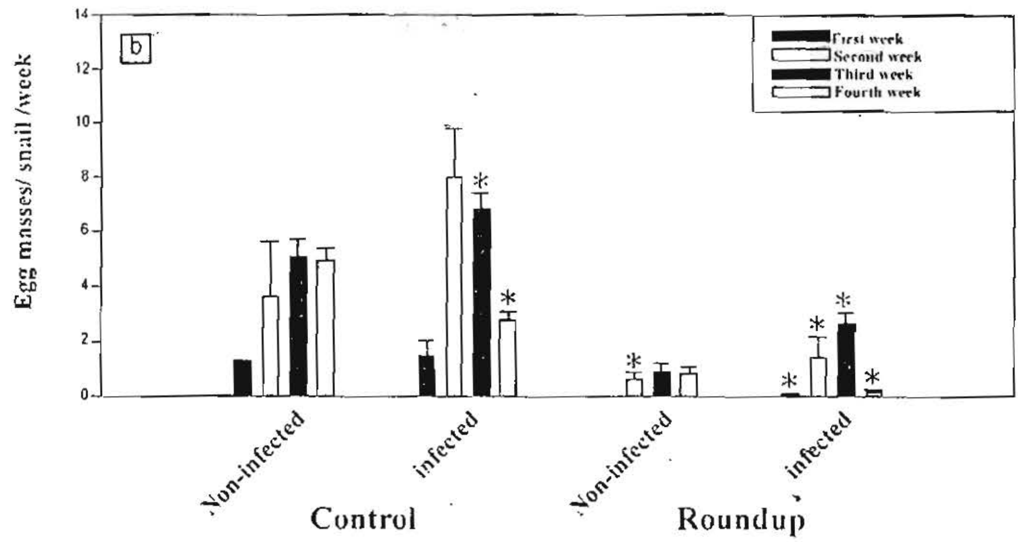
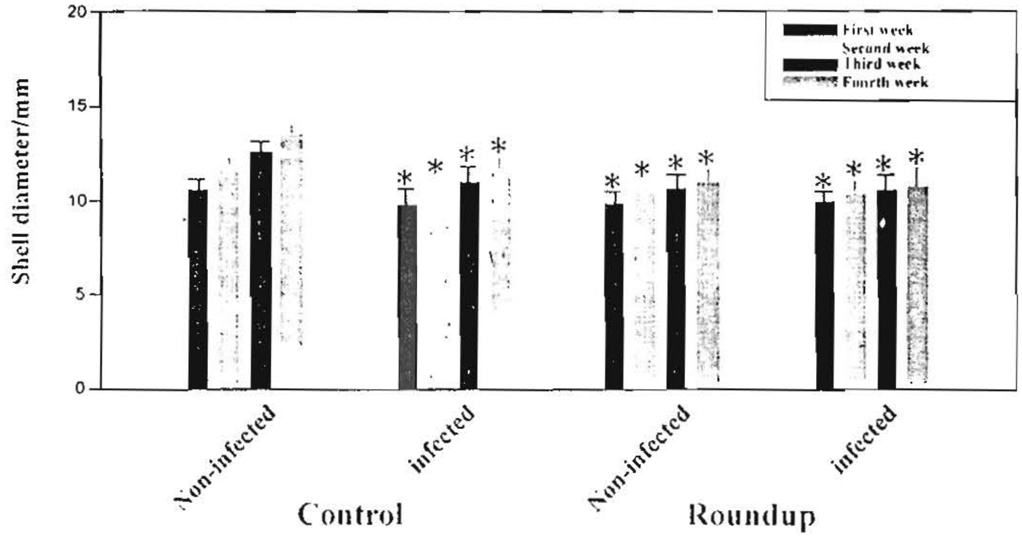


Figure (2): Effect of sub-lethal concentration of Roundup on growth rate of *Biomphalaria alexandrina* snails.



The values shown are the mean ± SD
*P<0.05 compared to control-non-infected snails(t-test)

Table (4): Effect of sub-lethal concentration of Roundup (10 ppm) on cercarial output of *Biomphalaria alexandrina* snails.

post infection (weeks)	Treatment	Total exposed snails	No. of alive snails	No. of shedding snails	Infection rate	Mean no. of cercariae/snail	Reduction %
4 week	Control	30	26	13	50%	603.30	-
	Roundup	30	19	7	36%	255.7	57.6%

Histological Investigation

Ovotestis of non-infected (control) snails is demonstrating general construction of acini incubating successive stages of oogenesis and spermatogenesis and held together by connective tissues, the sex cells originate from the germinal epithelium lining the lumen of the acini (**Plate 1-b,c**). Exposure of non-infected snails to 10 ppm of Roundup caused remarkable changes in the histological architecture of the ovotestis gland after 4 weeks of exposure. The acini lost their normal shape; an a abnormal increase of spermatozoa which filled the lumen of the acini. No other stages of oogenesis and spermatogenesis were observed (**Plate1- d, e**). The effect of *S. mansoni* infection on ovotestis of *B. alexandrina* snails caused loose of connective tissues, different degrees of degeneration and reduction in the number of sperms and oocytes. Sporocysts were remarkably observed at 4th week post infection as shown in **plate (1-a).** *S. mansoni* infection and Roundup exposure affected ovotestis of *B. alexandrina* snails after 4 weeks of exposure. The acini have deformed shape, connective tissue became loose and the oogenic and spermatogenic cells began degeneration. The sperms became short and condensed and irregular in shape. Atrophy was also detected. *S. mansoni* sporocysts were observed at the 4th week in treated - infected group (**Plate 1- f, g**).

The normal architecture of the digestive gland of *B. alexandrina* snails is composed of compact acini surrounded by connective tissue, their central lumens are lined with epithelial cell and filled with secrotory granules and secrotory cells (**Plate2, c, d**). As a result of prolonged exposure of snails to 10 ppm of Roundup, remarkable histological changes after 4 weeks of treatment were observed. Degenerative processes of connective tissue and secretory cells and loss of the tubules structure were observed. The acini were scattered, lost their normal shape, atrophy appeared in some acini and disappeared of lumen and tubules architecture (**Plate 2, e, f**). As a result of infection, snails showed several *S. mansoni* sporocystes and deformed shape of acini (Plate 2, c, d).

S. mansoni infection and Roundup exposure dramatically affected digestive gland of *B. alexandrina* snails. The acini had deformed shape also atrophy appeared in some acini. Several *S. mansoni* sporocysts were observed in treated-infected (Plate 2, g, h).

Plate (1):

Photomicrographs of T.S in ovotestis of *B. alexandrina* snails stained with E & H (at 4th week post exposure): control (b , c) (X100 & X400) ; infected with *S. mansoni* (a) (X400) ; treated with sub-lethal concentration of Roundup 10 ppm (d. e) (X100 & X400) and treated-infected with Roundup 10 ppm (f . g) (X100 & X400).

Sp (Sperms)

Spr (Sporocyst)

Ct (Connective tissue)

Ov (mature ova)

Oc (Oocyte)

Plate (2):

Photomicrographs of T.S in digestive gland of *B. alexandrina* snails stained with E & H (at 4th week post exposure): control (c . d) (X100 & X400); infected with *S. mansoni* (a . b) (X100 & X400); treated with sub-lethal concentration of Roundup 10 ppm (e . f) (X100 & X400) and treated- infected with Roundup 10 ppm (g . h) (X100 & X400).

Ac (Acini)

Ct (Connective tissue)

L (Lumen)

Sg (Secrotory granules)

Sc (Secrotory cell)

Spr (Sporocyst)

At (Atrophy)

Ep (Epithelium)

Plate (1)

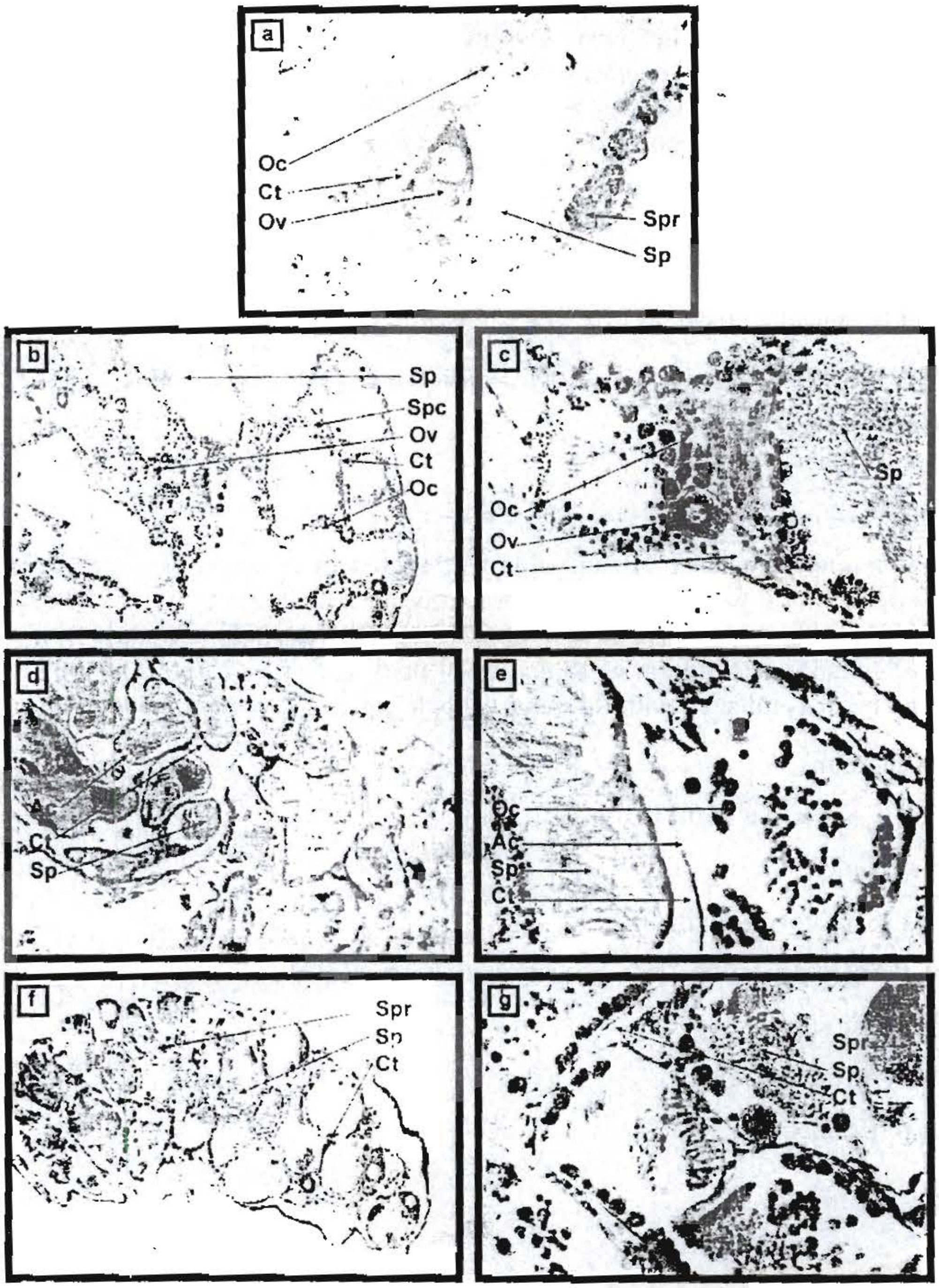

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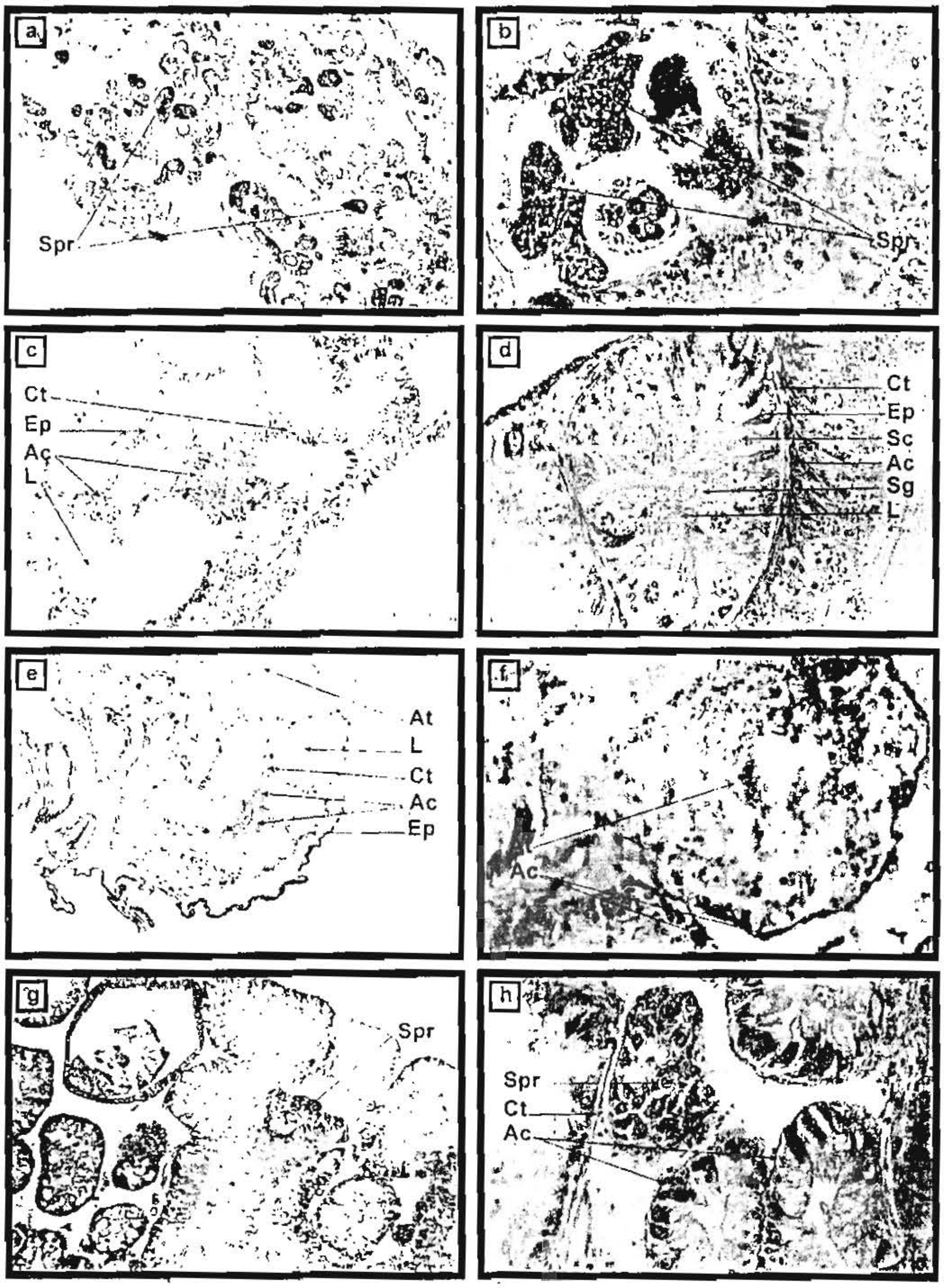
Plate (2)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DISCUSSION

Much effort has been directed by many countries to include snails control in their anti-schistosomasis programmes. The results of this study revealed that the herbicide Roundup is toxic to *B. alexandrina* snails after 24 hrs. It was found that LC_{50} and LC_{90} were 129.02 ppm and 262.44 ppm, respectively. Concerning the LC_{50} and LC_{90} values of Roundup after 60 minutes exposure on miracidia were 14.54 and 16.34 ppm while on cercariae were 5.12 and 7.65 ppm respectively. The result showed that Roundup was more toxic to the free larval stages of *S. mansoni* than to their snails. This finding agreed with **Tantawy (2006)** who found that fenitorthion (insecticide) was more toxic to miracidia and cercariae than to their snails. **Koprivnikar *et al.*, (2006)** reported that, atrazine (herbicide) affected the cercariae of 4 different species of digenetic trematodes. They also found that atrazine has more effect on cercariae. **Tantawy (2002)** also studied the effect of two herbicides (Butachlor and fluazifop-p butyl) against the miracidia and cercariae of *S. mansoni*. He stated that mortality rates of miracidia and cercariae were elevated gradually by increasing the sub-lethal concentrations of herbicides after 6 hours of exposure.

The results of the current study showed remarkable reduction in the survival rate of *B. alexandrina* snails treated with sub-lethal concentration of the Roundup. The survival rate decreased by increasing the exposure period. This finding is in agreement with that obtained by **Sakran (2004),** who showed that Butachlor and fluazifop-p-butyl (herbicides) caused reduction in the survival rate of treated *B. alexandrina*. The present results revealed that *B. alexandrina* snails exposed only to *S .mansoni* infection and \ or to both infection and herbicide Roundup exhibit high mortality rate than control snails. Different investigators came out with similar results **(Pan, 1965; Meier and Meier-Brook, 1981)**

Ford (1986) suggested that tissue damage due to cercarial production, depletion of certain metabolic substrates and/or interruption of biosynthetic pathways by *S. mansoni* may be a primary cause of death of infected snails. This may describe the reduction in survival of infected snails obtained in the present investigation.

The obtained results revealed a significant reduction of the growth rate (shell diameter) of *B. alexandrina* snails treated with sub-lethal concentration of herbicide Roundup. **Tantawy (2006)** stated that Fenitothion (insecticide) caused reduction in growth of *B. alexandrina.*

In the current study, prolonged exposure of the snails to Roundup at sub-lethal concentrations led to a remarkable reduction in egg production. Glyphosate, at sub-lethal concentration, affects the reproduction and development of *Pseudosuccinea columella* snails **[Tate *et al.*, (1997)]**. This may be due to that the herbicide Glyphosate, (which is the active substance in the commonly used preparation Roundup™), controls weeds by inhibiting a single plant enzyme, EPSPS (5-enolpyruvoylshikimate 3-phosphate synthase. It is known that EPSPS is a key enzyme in the aromatic amino acid biosynthetic pathway. The obstruction of this enzyme results in severe effects on protein synthesis **[Cole (1985) and Baylis (2000)]**. **Bacchetta *et al.*, (2002)** studied the influence of herbicide paraquat on the ovipository activity of *Physa fontinalis* and its histological effects on these snails. They reported that the number of egg masses and eggs laid decreased significantly under the effect of paraquat. The histological analysis showed that paraquat induced oocyte degeneration and altered ovipository activity in the *Physa fontinalis* snails. **Sakran (2004)** used two herbicides (Butachlor and fluazifop-p-butyl) against *B. alexandrina* which caused significant reduction in the egg laying capacity. The reduction in egg laying capaciy may arise as a result of the action of the Roundup upon the steroid sex hormones or may be due to the harmful effect on the male and female genital tract or may arise from metabolic disorders. **El-Ansary *et al.*, (2001)** elucidated that molluscicides suppressed egg laying capacity and population even in very small concentration. The results of this study revealed that *B. alexandrina* snails exposed only to *S. mansoni* infection or to infection and treated with sub-lethal concentration of Roundup exhibit high reduction in fecundity. **Pan (1965)** found that egg production by infected snails declined during 4th and 5th weeks post infection and subsequently ceased. **Looker & Etges (1979)** showed that the egg production in the snail *B. glabrata* infected with *S. mansoni* declined on day 23 post infection, and was significantly lower than uninfected control snails by day 28. The results of the current study showed remarkable reduction in the cercarial production of infected *B. alexandrina* snails treated with sub-lethal concentration of the Roundup. This agree with the results obtained by **Tantawy (2002)** who reported that continuous treatment of snails with sub-lethal concentration of

Butachlor and Fluazifop-p-butyl (herbicides) resulted in highly significant reduction of total cercarial shedding per infected snails.

Effect of prolonged exposure of *B. alexandrina* snails to sub-lethal concentration of Roundup showed obviously cellular damage of the ovotestis and digestive glands. These alterations were frequently found also in fish exposed to Roundup. **Langiano & Martinez (2008)** reported that Roundup induced several histological alterations in Neotropical fish, *Prochilodus lineatus*. They found that, short-term exposure of Roundup at sublethal concentration induced biochemical, physiological and histological alterations in *P. lineatus*. **Rosès *et al.*, (1999)** noticed that, kidney cells of *Physa acuta* displayed an important cell lysis when snails were exposed to atrazine (herbicide) for 10 days, and this effect was not reversed after a decontamination process. Also **Mantecca *et al.*, (2006)** stated that, severe lesions, such as cellular vaculation, lysis and thinness of germinative epithelial were observed in the digestive gland and testis of the zebra mussel *Dreissena polymorpha* after herbicide paraquat exposure. In the present work, the digestive and secretory cells of digestive glands became degenerate which can explain the reduction in the growth rate of treated snails. In the ovotestis of treated *B. alexandrina* snails, both oogenesis and spermatogenesis were influenced by treatment with Roundup. Thus complete destruction of gametogenic cells and sever damage of ovotestis gland can explain the reduction in egg laying capacity of treated snails and infected snails by *S. mansoni*. **Mohamed *et al.*, (2004)** concluded that *B. alexandrina* treated with Mepiquat chloride (plant growth regulator) caused noticable changes in the histological architecture of the digestive and ovotestis glands. It is known that the digestive gland of gastropod molluscs is the key organ of metabolism serving also as the main site of accumulation and biotransformation of xenobiotics. **Simkiss (1977)** and **Desouky (2006)** reported that both essential and non-essential elements in excess of physiological needs must either be rapidly excreted out or stored in an insoluble form to prevent their diffusion to tissues where they can interfere with biochemical reactions. In the current study sub-lethal concentration treatment of Roundup and/or *S. mansoni* infection caused changes in the histological organization of the *B. alexandrina* digestive gland. Various environmental stressors may affect the sizes of the mollscan digestive gland tubules, Lumina and the thickness of the epithelia **Snyman *et al.*, (2005)**.

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تأثير مبيد الحشائش الراونداب على النشاطات البيولوجيه لقواقع البيومفلاريا ألكسندرينا المعداة ببلهارسيا المستقيم

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تم في هذا البحث أختبار تأثير مبيد الحشائش الراونداب- أكثر مبيدات الحشائش استخداما - ضد قواقع البيومفلاريا ألكسندرينا والأطوار اليرقية لطفيلي بلهارسيا المستقيم. وقد وجد أن قيم التركيزات المميته ل ٥٠% و٩٠% (LC_{90} -LC_{50}) للراونداب ضد القواقع البالغه بعد ٢٤ ساعة من التعريض هى 129.02 و 262.44 جزء من المليون على التوالي. كما تم اختبار النشاط الإبادي اليرقي لمبيد الحشائش لكلا من ميرسيديا وسركاريا بلهارسيا المستقيم بعد ساعة واحدة من التعريض.و قد وجد أن قيم التركيزات المميته ل ٥٠% و٩٠% (LC_{90} - LC_{50}) للمرسيديا هى 14.54 و 16.34 جزء من المليون بينما كانت 5.12 و 7.65 جزء من المليون ضد السركاريا على التوالي.

كذلك تم دراسة تأثير التركيز تحت المميت (١٠جزء من المليون) للراونداب على النشاطات البيولوجيه لقواقع البيومفلاريا ألكسندرينا.وقد أوضحت النتائج حدوث تأثير تثبيطي علي كل من معدل البقاء ومعدل النمو وكذلك القدرة علي وضع البيض للقواقع المعداه ببلهارسيا المستقيم وكذا غير المعداه مقارنه بقواقع المجموعة الضابطة وذلك خلال أربعه أسابيع من التعريض . وقد أظهرت النتائج أن مبيد الحشائش الراونداب قد سبب إنخفاضا معنويا في أنتاج السركاريا (255.7 سركاريا / قوقع) عند مقارنتها بالمجموعة الضابطه المعداه (603.30 سركاريا / قوقع).

كما أوضحت الدراسه الهستولوجيه ظهور تغيرات وتلف كبير فى التركيب النسيجي للغده الخنثويه والغده الهاضمة لقواقع البيومفلاريا ألكسندرينا المعداه وغير المعداه بعد أربعة أسابيع من التعريض .

وهذا مايؤكد أن مبيد الحشائش الراونداب له تاثير على قواقع البيومفلاريا ألكسندرينا البالغة و وتاثيرة اشد علي الأطوار اليرقية الحرة لطفيلي بلهارسيا المستقيم.