



International Journal of  
**Zoological  
Research**

ISSN 1811-9778



Academic  
Journals Inc.

[www.academicjournals.com](http://www.academicjournals.com)

## **Comparative Study of the Molluscicidal Activity of Some Plant Extracts on the Snail Vector of *Schistosoma mansoni*, *Biomphalaria alexandrina***

<sup>1</sup>S.M. Rawi, <sup>2</sup>M. Al-Hazmi and <sup>1</sup>F. Seif Al Nassr

<sup>1</sup>Department of Zoology Faculty of Science, Cairo University, Giza, Egypt

<sup>2</sup>Department of Zoology, Faculty of Science and Arts, King Abdul-Aziz University, Khulais, Saudi Arabia

### **ABSTRACT**

Schistosomiasis is considered as one of the most important trematode disease of man. The most important goal of the present study is to use the natural plants as cheaper and available sources for snail control. The present investigation concerned with the evaluation of toxicological, biological and physiological effects of water suspension, cold water and boiled water extracts of *Agave filifera* whole plant, *Ammi majus* flowers and leaves and *Canna indica* flowers and leaves comparing with the effect of different sulphate compounds. The present work revealed that, copper sulphate as well as the water suspension of *Ammi majus* flowers and leaves disclosed the most potent effect on the cumulative mortality after 6 weeks exposure periods. Also, egg laying capacity and egg hatchability of *Biomphalaria alexandrina* snails were affected with the exposure to CuSO<sub>4</sub> and water suspension of *Ammi majus* flowers. The sublethal doses (LC<sub>10</sub>) of copper sulphate and water suspension of the tested plants reduced the total protein and total lipid contents of the hemolymph of *B. alexandrina* snails. The activity of transaminases enzymes as well as acetylcholinesterase had been affected by the exposure to the sublethal doses of copper sulphate and the water suspension of the tested plants. Also, histopathological changes were recorded in hermaphrodite gland of *B. alexandrina* snails post 6 weeks of exposure.

**Key words:** *Biomphalaria alexandrina*, Sulphate salts, *Agave filifera*, *Ammi majus*, *Canna indica*, toxicological, biological, biochemical effects

### **INTRODUCTION**

Schistosomiasis is considered on of the most fatal and drastic disease of human (Mostafa and Gad, 1997). It is also one of the most prevalent endemic diseases in tropical and subtropical regions, where it is spreading as the cultivated areas increase (Ernould *et al.*, 2004). It is estimated to infect about 207 million people worldwide and 700 million people are at risk in 74 endemic countries and more than 80% of all people infected with schistosomes are in sub-saharan Africa (WHO, 2010).

Schistosomiasis mainly affects the genito-urinary and gastrointestinal systems and produces a variety of clinical syndromes depending on the anatomic location of the adult worms and the egg released. Some symptoms of schistosomiasis include fever, arthralgias, abdominal pain, bloody diarrhea and hematuria. Ultimately, patients develop hepatosplenomegaly, ascites and lymphadenopathy (Nour, 2010). The disease is debilitating causing marked weakness, physical and mental retardation, thus it is not only affecting the health but also leads to reduction of the income of the individual, the family and the national productivity in general (Sow *et al.*, 2002; Anosike *et al.*, 2003).

In Egypt, schistosomiasis is not only a prime health problem, but it is also an economic one, as it affects millions of farmers at the early age, diminishing their productivity and exerting a serious socioeconomic problem (Yousif *et al.*, 1998).

The causative agents of human disease are trematodes of genus schistosoma among which there are five principal species; *S. haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalation*. *S. haematobium* affects both urinary and reproductive tract systems, where as the four other species impact the hepatic and gastrointestinal system (Schmitt, 2006).

The species of schistosomes have similar life cycles and develop over successive stages; egg, miracidium, sporocyst, cercaria and adult. The basic life cycle has an alteration of generations; the sexual generation of adult schistosomes in the definite vertebrate host and an asexual multiplication in a molluscan intermediate host (Behrman, 2008). After passing ova into water by the final host, the ova hatch in freshwater liberating free swimming miracidia, which penetrate the suitable snail, where they metamorphose into primary and secondary sporocytes and finally cercaria which are liberated into fresh water (Jourdan *et al.*, 1990).

In Egypt, schistosomiasis is transmitted to man by two intermediate hosts, *Biomphalaria alexandrina* for *Schistosoma mansoni* and *Bulinus truncatus* for *Schistosoma haematobium* (Badran, 1996). Farghaly (1992) confirmed the endemicity of *S. mansoni* and the low prevalence of *S. haematobium* infection in Delta region in Egypt. Also, according to Abdel-Wahab *et al.* (1990), *S. mansoni* is a series endemic disease, affecting nearly half of the population.

Schistosomiasis control has been attempted in several ways, chemotherapy, vector elimination, improved sanitation and health education (WHO, 2010). However, the successful control of this problem should be based on an integral approach including the control of intermediate host snails (Kenawy and Rizk, 2004; De S Luna *et al.*, 2005).

Snail control may be achieved by physical (Mc Cullough, 1986), chemical (Masola *et al.*, 2003) and biological methods (Kloos *et al.*, 2001; Rashed, 2002).

Chemical control by molluscicides is performed by using different compounds (Tantawy, 2006; Essawy *et al.*, 2009; Kristoff *et al.*, 2010). However, high costs of chemical molluscicides, the possible built up of snail resistance to molluscicides and their toxicity to non target organisms has drawn much attention during recent years for the use of plant molluscicides. As reported by several investigators, these represent cheap, safe, locally produced, biodegradable and effective agents in rural areas of developing countries where schistosomiasis is endemic (Bakry *et al.*, 2002; Mantawy *et al.*, 2004; Nihei *et al.*, 2005; Jaiswal and Singh, 2008; Al-Daihan, 2010).

The present study was carried out in order to search for ideal source alternative to chemotherapy and synthetic molluscicides. So we investigate the effect of some natural plants (*Agave filifera* whole, *Ammi majus* flowers and leaves and *Canna indica* flowers and leaves) as molluscicidal agents and comparing their effects with sulphate salts ( $\text{CuSO}_4$ ,  $\text{ZnSO}_4$ ,  $\text{Fe}_2(\text{SO}_4)_3$  and  $(\text{NH}_4)_2\text{SO}_4$ ) against *Biomphalaria alexandrina* snails.

## MATERIALS AND METHODS

**Date and location of research:** The research was conducted in Physiology Research lab of Prof. Dr. Sayed Mohamed Rawi in Zoology Department, Faculty of Science, Cairo University, Giza, Egypt in the period between May 2009 and Dec. 2009.

**Animals:** Snails used in the present study were adult *Biomphalaria alexandrina* (12-15 mm in diameter and 0.34-0.52 g in weight), the intermediate host of *Schistosoma mansoni* in Egypt. These

snails were collected from the irrigation and drainage networks. The collected snails were kept under laboratory conditions according to the method described by Shoeb and El-Emam (1976) and modified by El-Emam and Ebeid (1989), in dechlorinated tap water of  $\text{pH} = 7 \pm 0.4$  for one month before being used in experiments. Lettuce leaves were added daily as a food source.

### Tested molluscicides

**Chemical molluscicides:** Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), ferric sulphate ( $\text{Fe}_2(\text{SO}_4)_3 \cdot \text{H}_2\text{O}$ ), zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) and ammonium sulphate ( $(\text{NH}_4)_2\text{SO}_4$ ) are supplied by El-Nasr company for chemicals and Drugs, Egypt. Stock solution of 1000 ppm of each sulphate salts was prepared in distilled water on the basis of weight/volume and then series of concentrations were used in the experiments.

**Experimental plants:** The experimental plants used in this study were:

***Agave filifera*:** It belongs to family Agavaceae. It was collected during full growing season (March-April) from El-Fayoum Governorate.

***Ammi majus*:** It belongs to family Apiaceae. The plant was collected during the flowering stage from El-Fayoum Governorate during March and April.

***Canna indica*:** It belongs to family Cannaceae. The plant flowers and leaves were collected from certain localities around Giza Governorate during November and December.

**Experimental design:** The various extracts of the tested parts of the chosen plants; *Agave filifera* (whole plant), *Ammi majus* (flowers and leaves) and *Canna indica* (flowers and leaves) were prepared directly in a series of concentrations that permit the computation of  $\text{LC}_{50}$  and  $\text{LC}_{90}$  value. Also, stock solutions of sulphate salts were prepared according to the method described by Oteifa *et al.* (1975) and then series of concentrations were used to determine  $\text{LC}_{50}$ . The exposure period was 24 h.

Death of snails was determined by many ways (Oteifa *et al.*, 1975). The standard method in which the snails were immersed two or three at a time in 15-20% sodium hydroxide in petri-dish was used, if bubbles and blood come out of the shell, it is recorded as alive, if not it is recorded as dead snails.

The sublethal concentration ( $\text{LC}_{10}$ ) of the most effective promising materials based on the recorded  $\text{LC}_{50}$  values are used to study their long term effects (6 weeks) on different parameters including survival and cumulative mortality, egg laying capacity, egg hatchability and some biochemical parameters (Total protein, total lipid, transaminases and acetylcholinesterase) in hemolymph. Control snails were maintained under the same experimental conditions without exposure to the tested substances.

At the end of the experiment, hemolymph was pooled from 30 large snails as described by Figueiredo *et al.* (1973). The snails were wiped dry with a cloth, a small window was scratched in the shell in the vicinity of the heart, the mantle was pierced with a needle and the hemolymph collected with a pipette to which a small rubber bulb had been attached (Abdel-Kader and Tantawy, 2000). The snails were dissected and hermaphrodite glands were removed and fixed for histological examination.

## Methods

**Handling and preparation of plant materials:** The plant used *Agave filifera* (whole plant), *Ammi majus* (flowers and leaves) and *Canna indica* (flowers and leaves) were collected from the field then transferred to the laboratory. The samples left to dry in air and then in an oven at 50°C and powdered by a mixer.

For determination of plant toxicity, the following extracts were prepared:

**Water suspension:** For each plant part, weight amounts of the powdered material were added to 1000 mL of dechlorinated tap water to make up the desired of weight/volume concentrations (Hashem, 1999).

**Preparation of cold water extract:** For each plant part, a stock extract was prepared by soaking ten grams of the powdered plant part in 250 mL of dechlorinated tap water for two days at room temperature. This suspension was occasionally shaken every 24 h. The suspension was filtered through filter paper and cold dechlorinated tap water was added to adjust the filtrate at specific volume and replenish any water loss. The adjusted filtrate was used as stock extract to make series of concentrations (converted to dry weight of plant material before extraction) using appropriate dilutions (Hashem, 1999).

**Preparation of boiled water extract:** For each plant part, a stock extract was prepared by soaking ten grams of the powdered plant part in about 400 mL of dechlorinated tap water, the suspension then warmed at 100°C for one hour. Boiling tap water was continually added to replenish the evaporated part. The suspension then left to cool at room temperature and filtered through filter paper. The filtrate was used as stock to make series of concentrations (converted to dry weight of plant material before extraction) using appropriate dilutions (Hashem, 1999).

**Establishment of toxicity regression lines of the tested materials:** Ten snails were immersed in 1000 mL of the experimental concentrations. Three replicates were prepared for each concentration. The exposure period was 24 h. After each period snails were removed, washed thoroughly with dechlorinated tap water and transferred to containers with fresh dechlorinated tap water. Percentage of mortality was calculated against the concentration used. The  $LC_{50}$  and  $LC_{90}$  values of the tested plants and the sulphate salts were determined according to Litchfield and Wilcoxon (1949). The  $LC_{10}$  (sublethal concentration) was determined.

## Phytochemical screening of the tested plants

- Test for carbohydrates and/or glycosides: according to the method of Molish (1963, Monatsh Chem., 7.197)
- Test for cardenolides: according to the method of Geissman (1962) and Tadros (1979)
- Test for saponins: according to the method of *Wolf from et al.* (1940) and Wall *et al.* (1954)
- Test for sterols and/or triterpenes: according to the method of Fieser and Fieser (1949) and Wall *et al.* (1954)
- Test for tannins: according to the method of Wall *et al.* (1954) and Trease and Evans (1978)
- Test for catchin: according to the method of Trease and Evans (1978)
- Test for flavonoids: according to the method of Wall *et al.* (1954) and Geissman (1962)

**Survival and cumulative mortality:** Five replicates were prepared for each test. Ten snails were immersed in 1000 mL of sublethal concentration ( $LC_{10}$ ) of each of the tested compounds. The exposure period lasts 6 weeks at room temperature ( $22\pm 2^\circ\text{C}$ ). Renewing the experimental concentrations was done weekly to minimize the change in the nature of the tested materials (Rawi *et al.*, 1994; Abdel-Kader and Tantawy, 2000).

**Egg laying capacity:** The egg laying capacity was determined according to the method described by Oliver and Haskins (1960). Six replicates were used for each test. Ten adult snails being immersed in 1000 mL of each experimental concentration. A control group of untreated normal snails were maintained only in dechlorinated water under the same experimental conditions. The jars containing snails were provided by thin plastic sheets for egg deposition. The egg laying capacity is expressed in the form of the number of eggs per snail per week (E/S/W) and this was determined by dividing the total number of laid eggs in any week by the total number of living snails.

**Egg hatchability:** The egg clutches from the previous experiment were collected and transferred into another jars containing the same experimental concentrations of the tested materials and the control groups were maintained into dechlorinated water. The egg hatching was observed and determined as percentage difference relative to the total number of eggs.

**Biochemical analysis:** Total protein was determined according to the method of Gornall *et al.* (1949) using kits purchased from Biodiagnostic (Egypt). Total lipid was determined according to the method of Knight *et al.* (1972) using kits purchased from Biodiagnostic (Egypt).

Transaminases (AST and ALT) activities were determined in the snail hemolymph using reagent kits purchased from Biocon Chemical Company (Germany) according to the method of Reitman and Frankel (1957). Acetylcholinesterase activity was determined in hemolymph of snails by a modified method of Ellman *et al.* (1961) as described by Gorun *et al.* (1978).

**Histological examination:** For histological examination, hermaphrodite gland were removed, fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned. The sections were stained with haematoxyline and eosin (Carleton, 1976).

**Statistical analysis:** The obtained data were analyzed for the Mean  $\pm$  standard error (SE). Student (t) test was applied in the present study as reported by Campbell (1989) and Bailey (1995).

## RESULTS AND DISCUSSION

### Toxicological studies

**Influence of sulphate salts:** The influence of sulphate salts on mortality rate of normal *B. alexandrina* snails is represented in Table 1 and illustrated in Fig. 1. Data recorded showed the molluscicidal efficacy of copper sulphate against *B. alexandrina* after 24 h exposure period as compared with the other tested sulphate salts. The recorded  $LC_{50}$  values were 1.79, 54, 420 and 490 ppm for copper sulphate, zinc sulphate, ferric sulphate and ammonium sulphate, respectively.

Table 1: The influence of sulphate salts on the mortality rate of normal *B. alexandrina* snails

Metal	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)	Slope (ppm)
CuSO <sub>4</sub>	1.79	6.54	3.70
ZnSO <sub>4</sub>	54	80	1.36
Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	420	500	1.15
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	490	800	1.47

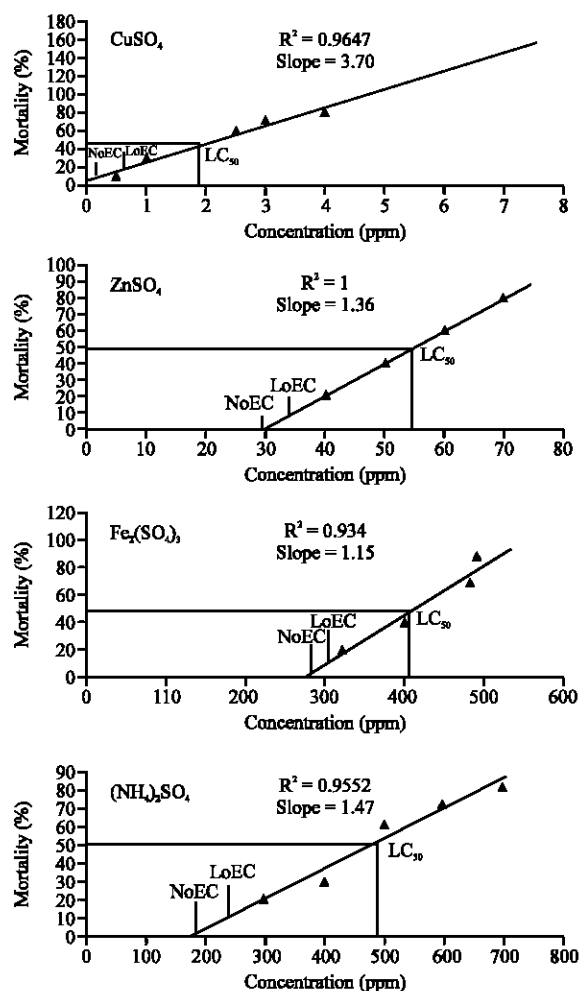


Fig. 1: The molluscicidal toxicity of tested sulphate salts against *Biomphalaria alexandrina* snails after 24 h exposure. NoEC: No observed effect; LoEC: Lowest observed effect; LC<sub>50</sub>: Median lethal concentration at 24 h

**Influence of plant extracts:** The influence of different plants extracts on mortality rate of normal *B. alexandrina* snails after 24 h is shown in Table 2 and illustrated in Fig. 2-4. The recorded data showed that water suspension has more potent effect than cold and boiled water extracts of the same plant part. The potency increased in the order of *Agave filifera* (whole plant), *Ammi majus* leaves, *Ammi majus* flowers, *Canna indica* leaves and *Canna indica* flowers with LC<sub>50</sub> values 42.30, 738.27, 747.66, 7219.60 and 7600.87 ppm, respectively.

Table 2: The influence of different plant extracts on the mortality rate of normal *B. alexandrina* snails

Plant	Plant part	Extract								
		Water-suspension			Cold water			Boiled water		
		LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)	Slope	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)	Slope	LC <sub>50</sub> (ppm)	LC <sub>90</sub> (ppm)	Slope
<i>Agave filifera</i>	Whole plant	42.30	85.62	1.4	462.96	692.71	1.4	256.99	328.91	1.2
<i>Ammi majus</i>	Flowers	747.66	1370.30	1.7	2126.51	6775.19	2.6	944.85	1394.14	1.3
	Leaves	738.27	1157.22	1.4	4731.72	10377.11	2.3	1364.92	2537.36	1.6
<i>Canna indica</i>	Flowers	7600.87	13890.93	1.6	>10,000	--	--	4172.12	5160.02	1.2
	Leaves	7219.60	14105.26	1.6	>10,000	--	--	7434.80	9071.57	1.2

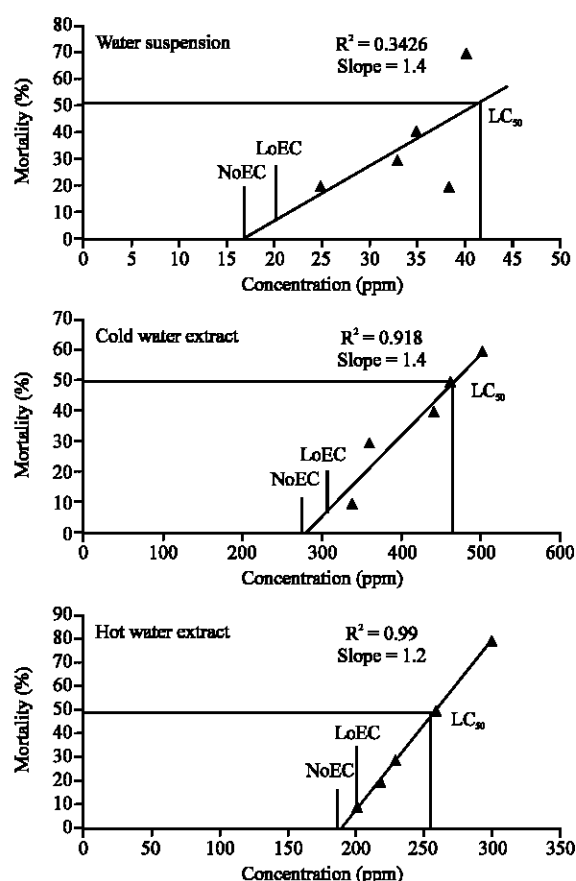


Fig. 2: The molluscicidal toxicity of *Agave filifera* whole plant against *Biomphalaria alexandrina* snails after 24 h exposure. NoEC: No observed effect; LoEC: Lowest observed effect; LC<sub>50</sub>: Median lethal concentration at 24 h

**Phytochemical screening of the tested plants:** Table 3 represents the phytochemical screening of *Agave filifera* (whole plant), *Ammi majus* (flowers and leaves) and *Canna indica* (flowers and leaves) using the plant powder. As recorded in the Table 3, the results disclose the presence of carbohydrates, tannins, catechin and saponin in all the tested plant parts. On the other hand, all the tested plant parts are free from flavonoids, sterols and cardenolides.



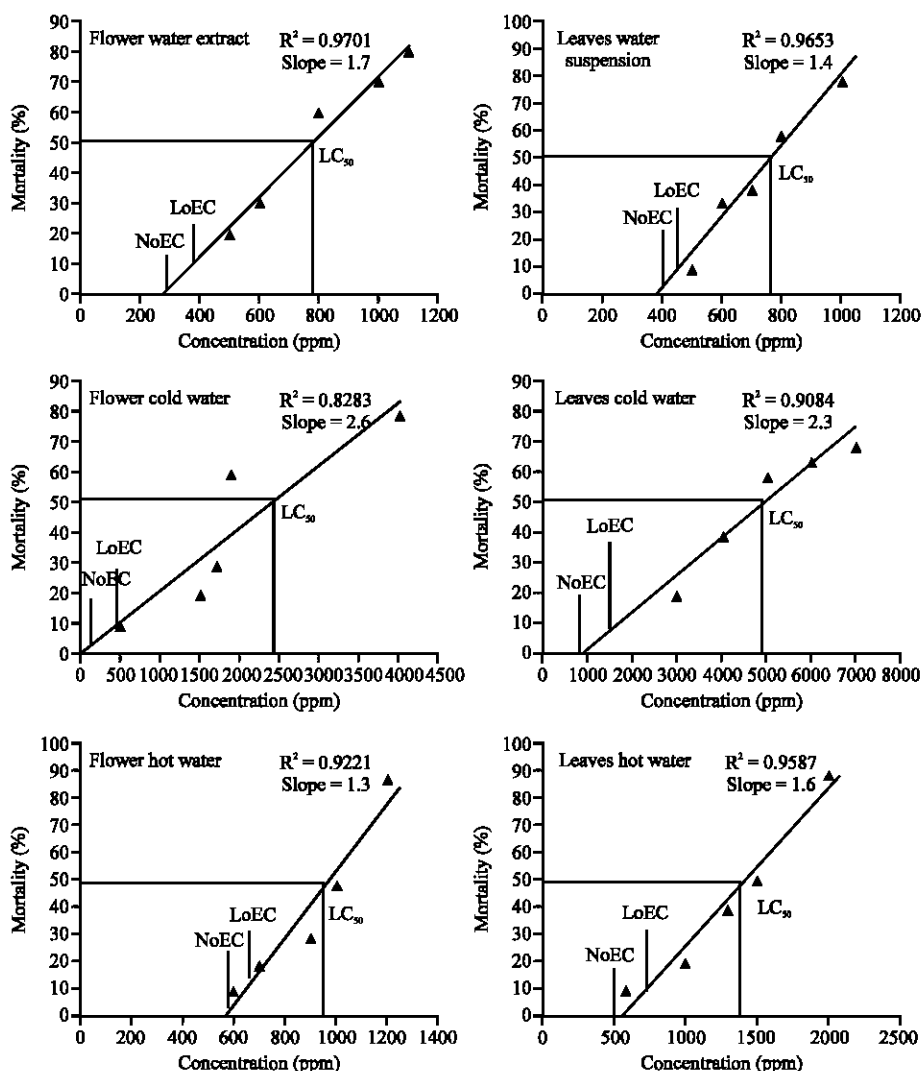


Fig. 3: The molluscicidal toxicity of *Ammi majus* flowers and leaves against *Biomphalaria alexandrina* snails after 24 h exposure. NoEC: No observed effect; LoEC: Lowest observed effect; LC<sub>50</sub>: Median lethal concentration at 24 h

**Cumulative effect of the sublethal doses:** The sublethal concentration (LC<sub>10</sub>) of the most effective materials based on the recorded LC<sub>50</sub> values including CuSO<sub>4</sub>, water suspension of *Agave filifera* (whole plant) and *Ammi majus* (flowers and leaves) are used to study their long term effects on:

**Survival and cumulative mortality:** Data recorded in Table 4 showed that the sublethal concentration of copper sulphate and water suspension of *Ammi majus* flowers and leaves disclose the most potent effect on the cumulative mortality after 6 weeks with percentage difference 19.2%, 10 and 6.8%, respectively.

**Egg laying capacity and egg hatchability:** Table 5 represents the data for the effect of prolonged exposure to sublethal concentration of the tested compounds on egg laying capacity and

Table 3: Preliminary phytochemical screening of the tested plant extracts

	Plant				
		<i>Ammi majus</i>		<i>Canna indica</i>	
Test	<i>Agave filifera</i> (whole plant)	Flowers	Leaves	Flowers	Leaves
Carbohydrates	+ve	+ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve	+ve
Catechin	+ve	+ve	+ve	+ve	+ve
Flavonoids					
(a)	-ve	-ve	-ve	-ve	-ve
(b)	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve	+ve
Sterols	-ve	-ve	-ve	-ve	-ve
Cardenolides	-ve	-ve	-ve	-ve	-ve

+ve: Presence of active principals, -ve: Absence of active principals

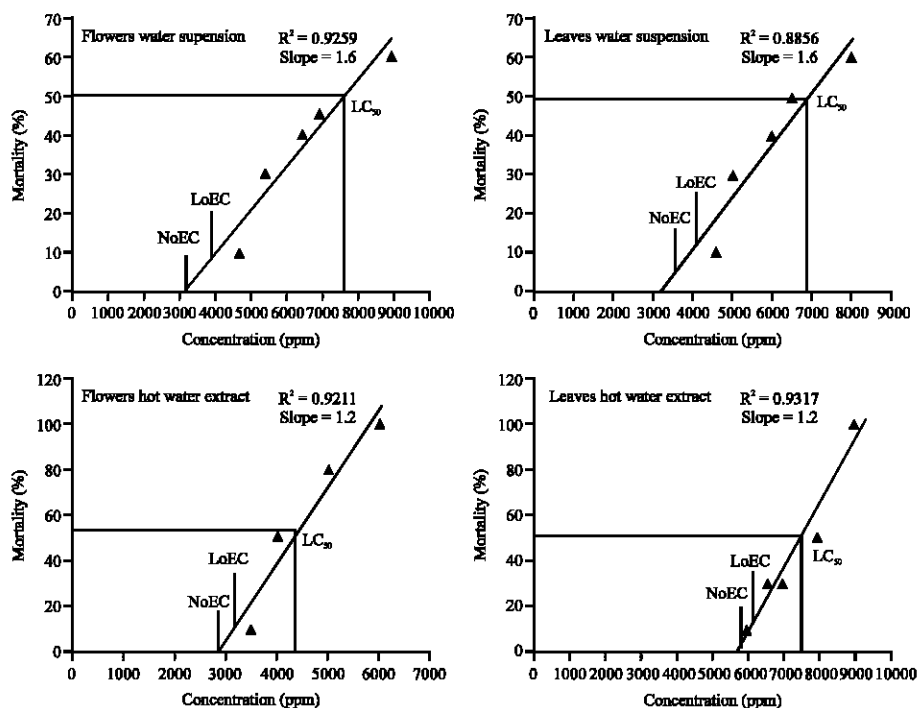


Fig. 4: The molluscicidal toxicity of *Canna indica* flowers and leaves against *Biomphalaria alexandrina* snails after 24 h exposure. NoEC: No observed effect, LoEC: Lowest observed effect;  $LC_{50}$ : Median lethal concentration at 24 h

egg hatchability. The recorded data revealed that copper sulphate and *Ammi majus* flowers were effective in reducing the egg laying capacity of the snails, while the water suspensions of *Agave filifera* whole plant and *Ammi majus* leaves promote the capacity of the snails to lay more eggs.

Regarding to the egg hatchability, the sublethal doses ( $LC_{10}$ ) of copper sulphate and water suspensions of *Agave filifera* whole plant and *Ammi majus* flowers and leaves reduced the egg hatchability of the snails.

Table 4: Effect of prolonged exposure to sublethal concentration (LC<sub>10</sub>) of copper sulphate and water suspension of *Agave filifera* (whole plant) and *Ammi majus* (flowers and leaves) on the survival and cumulative mortality of *B. alexandrina* snails

Parameters	LC <sub>10</sub> (ppm)	Exposure period (6 weeks)	
		NDS	Mortality (%)
Control	--	9.00	3.6
CuSO <sub>4</sub>	0.18	48.00	19.2
<i>Agave filifera</i> (whole plant)	4.23	8.00	3.2
<i>Ammi majus</i> (flowers)	74.77	25.00	10.0
<i>Ammi majus</i> (leaves)	73.83	17.00	6.8

NDS: No. of dead snails out of 250 in each experiment

Table 5: Effect of prolonged exposure to sublethal concentration (LC<sub>10</sub>) of copper sulphate and water suspension of *Agave filifera* (whole plant) and *Ammi majus* (flowers and leaves) on egg laying capacity and egg hatchability of *B. alexandrina* snails

Parameters	LC <sub>10</sub> (ppm)	Exposure period (6 weeks)			
		Egg laying capacity		Egg hatchability	
		E/S/W	Difference (%)	No. of egg laid	Hatchability (%)
Control	-	20.70	-	1242	624 (50.24%)
CuSO <sub>4</sub>	0.18	14.61	-29.42	877	256 (29.19%)
<i>Agave filifera</i> (whole plant)	4.23	24.60	18.84	1476	599 (40.58%)
<i>Ammi majus</i> (flowers)	74.77	17.17	-17.05	1030	481 (46.69%)
<i>Ammi majus</i> (leaves)	73.83	21.18	2.32	1271	512 (40.28%)

E/S/W: No. of egg/snail/week, % difference was calculated relative to the control, Hatchability % was calculated relative to the number of egg laid

Table 6: Effect of prolonged exposure to sublethal concentration (LC<sub>10</sub>) of copper sulphate and water suspension of *Agave filifera* (whole plant) and *Ammi majus* (flowers and leaves) on some biochemical parameters of hemolymph of *B. alexandrina* snails

Groups	LC <sub>10</sub>	Exposure period (6 weeks)				
		Biochemical parameters				
		Total protein (mg mL)	Total lipid (mg mL)	AST (U L <sup>-1</sup> )	ALT (U L <sup>-1</sup> )	AchE (μMSH/min/100 mL)
Control	-	5.18±0.11	0.95±0.02	114.20±6.73	119.60±3.01	8.98±0.3
CuSO <sub>4</sub>	0.18	4.60±0.16*	0.27±0.04*	143.40±2.04*	114.40±6.01	6.49±0.5*
		-11.20%	-71.58%	25.57%	-4.35%	-27.73%
<i>Agave filifera</i> (whole plant)	4.23	4.83±0.08*	0.78±0.04*	140.44±1.90*	127.40±8.30	7.60±0.10*
		-6.76%	-17.89%	22.97%	6.52%	-15.73%
<i>Ammi majus</i> (flowers)	74.77	3.41±0.11*	0.82±0.04*	298.00±33.82*	170.00±28.81*	6.95±0.19*
		-34.17%	-13.68%	160.95%	42.14%	-22.61%
<i>Ammi majus</i> (leaves)	73.83	4.37±0.30*	0.93±0.04*	202.00±30.72*	212.00±15.30*	7.31±0.12*
		-15.64%	-2.11%	76.88%	77.26%	-18.59%

No. of snails is 50 (5 replicates) in each experiment, Data are expressed as Mean±SE, Significant different from control; p&lt;0.05, % difference was calculated relative to the control

**Biochemical effects:** The effect of prolonged exposure to sublethal concentrations (LC<sub>10</sub>) of the tested compounds on some biochemical parameters of the hemolymph of *B. alexandrina* snails is shown in Table 6.

**Total protein:** The prolonged exposure to copper sulphate and water suspension of *Agave filifera* whole plant, *Ammi majus* flowers and leaves reduced the total protein content of the hemolymph of *B. alexandrina*. The maximal decrease (-34.17%) was noticed on using *Ammi majus* flowers.

**Total lipid:** Copper sulphate and the water suspension of both *Agave filifera* whole plant and *Ammi majus* flowers induced significant reduction in the total lipid content of the hemolymph of the snails, the maximal reduction was obtained for copper sulphate exposure (-71.58%), while the water suspension of *Ammi majus* leaves induced non significant change in total lipid content.

**Aspartate aminotransferase (AST) activity:** Copper sulphate, water suspensions of *Agave filifera* whole plant and *Ammi majus* flowers and leaves induced significant elevation in AST activity after 6 weeks of exposure, the maximal value (160.95%) was recorded after exposure to *Ammi majus* flowers.

**Alanine aminotransferase (ALT) activity:** The water suspension of both *Ammi majus* flowers and leaves induced significant elevation in ALT activity after 6 weeks of exposure. On the other hand, the prolonged exposure to copper sulphate and *Agave filifera* whole plant had non-significant effect on ALT activity.

**Acetylcholinesterase activity:** The prolonged exposure to sublethal doses of copper sulphate and the selected plant extracts induced significant reduction in AChE activity. The maximal reduction (-27.73%) was recorded post  $\text{CuSO}_4$  treatment.

**Histopathological effects:** Microscopical examination of the gonadal tissue of the control snails revealed the presence of male and female gonads surrounded with an outer capsule of fibroplastic connective tissue bound the gonadal tubule. The structure of male and female gonadal cells is illustrated in Fig. 5.

**Effect of copper sulphate:** The treatment of normal *B. alexandrina* snails with sublethal concentration ( $\text{LC}_{10}$ ) of  $\text{CuSO}_4$  for 6 weeks caused marked necrobiotic changes and shrinkage, partial destruction in the follicular membrane, the nuclei of the ova become faint in staining and in most cells, the nucleolus are disappeared (Fig. 6A).

**Effect of *Agave filifera* whole plant:** The prolonged exposure for 6 weeks to water suspension of *Agave filifera* whole plant induced higher incidence of gonadal changes. The gonadal cells lost their normal architecture. Irregular arrangement and vacuolation was partly recognized in some of the examined sections. Also, some of the oocytes are degenerated or with shrunk ovum and/or cytoplasmic vacuolation (Fig. 6B).

**Effect of *Ammi majus* flowers:** The treatment with sublethal concentration ( $\text{LC}_{10}$ ) of *Ammi majus* flowers water suspension for 6 weeks caused destruction in the inter acinar connective tissue and disappearance of follicular cavity around the mature ova and destruction in their nucleoli (Fig. 6C).

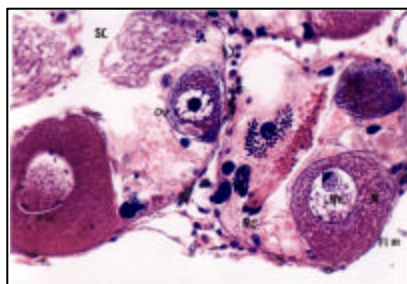


Fig. 5: A photomicrograph of the hermaphrodite gland of normal *B. alexandrina* snail showing the mature ova (Ov) with large nuclei (N), round nucleolus (Nu) and surrounded by a follicular membrane (Flm) and follicular cavity (Flc). A varying number of flattened cells called nurse cells (Nc) adhere to the follicular membrane. The male gonadal cells are also presented as sperms at different developmental stages collected around sertoli cells (Sc). (HE, X40)

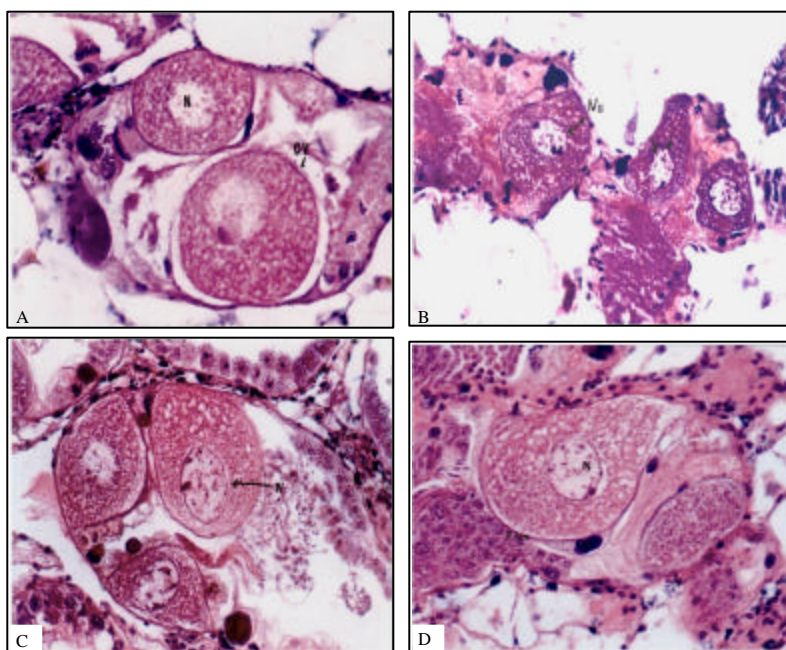


Fig. 6: Photomicrographs of hermaphrodite glands of *B. alexandrina* after 6 weeks exposure for sublethal concentrations ( $LC_{10}$ ) of the tested compounds. (A): Photomicrograph of hermaphrodite gland of *B. alexandrina* treated with  $CuSO_4$  showing destruction in the follicular memberane, mature ovum loses its nucleolus and others has faint staining nucleus (N). (HE, X40), (B): Photomicrograph of hermaphrodite gland of *B. alexandrina* treated with *Agave filifera* showing congested tubules with foci of coagulative necrosis, destroyed nucleus (arrow) and a reduction in the size of the follicular cavity (HE, X40), C: Photomicrograph of hermaphrodite gland of *B. alexandrina* treated with *Ammi majus* flowers showing a disappearance of the follicular cavity, a destruction in the nucleolus (arrow) and irregularity of the nuclear membrane (HE, X40), (D): Photomicrograph of hermaphrodite gland of *B. alexandrina* treated with *Ammi majus* leaves showing destruction in the intra follicular connective tissue, mature ova with necrotic nucleus and disappearance of the follicular cavity (HE, XS40)

**Effect of *Ammi majus* leaves:** The micrograph illustrates the effect of sublethal concentration of *Ammi majus* leaves post 6 weeks showed only little number of the gonadal cells and degeneration of acinar connective tissue. In female gonadal acini, most of the recognized ova being irregular in shape and without nuclei or nucleolus (Fig. 6D).

## DISCUSSION

The environmental and economic problems arised with the use of synthetic molluscicides encouraged the search for substituents of plant origin. At present, increasing attention is currently given to the study of plant molluscides in hope that may prove less toxic, cheaper, readily available and easily applicable by simple technique. So, the present investigation is intended to search for ideal molluscicide of plant origin on the basis of dosage response for combating *B. alexandrina* snails.

From the established regression lines of the tested sulphate salts, the molluscicidal activity was similar to that illustratd by Swaileh and Ezzughayyar (2000) and Ebenso (2004) against terrestrial land snail and Iglesias *et al.* (2000) and Reddy *et al.* (2004) against aquatic snails including *B. alexandrina*. In the present study, the tested sulphate salts are descendingly arranged based on their molluscicidal efficiency in the following order  $\text{CuSO}_4$ ,  $\text{ZnSO}_4$ ,  $\text{Fe}_2(\text{SO}_4)_3$  and finally  $(\text{NH}_4)_2\text{SO}_4$ , respectively.

Furthermore, the toxicity of the tested metal has been shown to increase as the concentration increase. The observed sensitivity of adult *B. alexandrina* towards  $\text{CuSO}_4$  and  $\text{ZnSO}_4$  is consistent with Erdely *et al.* (1998). As reported by several investigators elevated copper and zinc concentrations have been associated with an aggravated toxic response for several aquatic species.

In the present study, the observed correlation between the survival rate of *B. alexandrina* and the dose level can be explained on the basis of metals bioaccumulation, physiological alterations and histological damage caused by a particular stressor. According to Gamot and Pihan (1997), Kirichuk *et al.* (2002) and Regoli *et al.* (2006), there is great tendency of the bioaccumulation of copper and zinc in different snail types. These findings, were also, recommended by Abdel-Moati and Farag (1991) who postulated 58 times copper bioaccumulation in the freshwater gastropod, *Lanistes bolteni chemnitz* after exposure to different concentrations of copper salts. Similarly, according to the same author about 20-30% of the ingested zinc is absorbed and Zn contents in the soft parts was 34 times higher than in control and the most abundant amounts was found in viscera. The less toxicity of ammonium sulphate in the studied snail species can be explained on the basis of its deposition. Ammonia is toxic substance and because of its toxicity ammonia must be excreted as rapidly as it is formed. As reported by many investigators (Stadnichenko and Kirichuk, 2000; Mummert *et al.*, 2003), many invertebrate appear to be more tolerant of ammonia. From the present study it would appear that metal bioaccumulation could be particular in t he longevity of *B. alexandrina*. Some species, however, had a much greater tendency to accumulate one toxicant than the other (Dallinger *et al.*, 2000). Such differences are mainly related to the mode of exposure, rate of adsorption, species differences and the physiological and histopathological effects induced by the tested metals.

Exclusion and the excretory mechanisms are also important factors in the processes of bioaccumulation. George and Pirie (1980) stated that Zn excretion would be independently of the route of uptake. The bioaccumulation may decrease after long exposure due to saturation of binding capacities (Coeurdassier *et al.*, 2005). Onest of mortality can also be explained on the basis of the effect of the accumulated salts on pH, water contents and the respiratory behavior of the

exposed snails. According to Stadnichenko *et al.* (1994), exposure to heavy metals causing an acid-base disturbances, net branchial ion loss and decrease in body mass and hemolymph volume and the oxidative phosphorylation of mitochondrial respiration. Crespo and Kanaky (1983) reported that, zinc inhibited chloride transport across the isolated opercular epithelium of *Fundulus heteroclitus*, possibly as a consequence of its inhibitory effect *in vitro* upon Na<sup>+</sup>, K<sup>+</sup> and ATP.

In the present study, the molluscicidal potency of water suspension, cold and boiled water extracts of *Agave filifera*, *Ammi majus* and *Canna indica* on the longevity of *B. alexandrina* was also evaluated. From the established regression lines, the molluscicidal activity based on LC<sub>50</sub> values falls within the range of other plants that have been Judged as promising molluscicides (Abdel-Kader and Sharaf El-Din, 2000; Abdel-Hamid, 2003; De S Luna *et al.*, 2005). The recorded data showed that water suspension had more potent effect than cold and boiled water extracts of the same plant part, the potency increased in the order of *Agave filifera* (whole plant), *Ammi majus* leaves, *Ammi majus* flowers, *Canna indica* leaves and *Canna indica* flowers.

The recorded toxic effects on the longevity of the studied snails mainly attributed to several factors including plant specific differences of the extracted active ingredient, types of extracted products, differences in their mode of action, method of penetration and the behavioral characteristics of the studied animal (Mantawy *et al.*, 2004). It is now well established that in many plants including the tested plants, the activity is due to the presence of saponin contents (Rawi *et al.*, 1996; Singh and Singh, 2009), tannins compounds (Bezerra *et al.*, 2002), triterpenoid and alkaloid components (Singh *et al.*, 2010).

The present study also showed the presence of saponin, catechin and tannins in all the tested plant extracts. Such compounds, however, were responsible for their molluscicidal activity. The increased mortality rate with water suspension also can be explained on the basis of environmental changes as reported by Landis and Yu (1995) and Oliveria-Filho *et al.* (1999). Apparently, suspended material may be in the form of droplets or particles, all of these may absorbed or become in contact with the animal surface that may affect the animal respiratory rate. The suspended particles may still on the surface or may be carried down leading to faster depletion of water oxygen concentration.

Exposure time is another important determination of toxic effects, so the present investigation is gaining in popularity to establish the effect of prolonged exposure for 6 weeks to the sublethal concentration (LC<sub>10</sub>) of the tested compound on the longevity, biological activity and biochemical constituents of the studied snails.

Regarding to the survival rate and cumulative mortality after 6 weeks exposure to sublethal doses, the present work showed the highest effect after exposure to CuSO<sub>4</sub> (19.2%) followed by *Ammi majus* flowers water suspension (10%) and *Ammi majus* leaves water suspension (6.8%) in a descending order, respectively. These changes are in agreement with Abdel-Hamid and Mantawy (1999) and Tantawy (2002), as they showed a reduction in the survival rate of *B. alexandrina* after prolonged exposure to sublethal concentrations of different plants molluscicides as well as copper sulphate. The present finding can be explained on the basis of storage depots, metabolic, physiological and biochemical hazardous effects of the accumulated doses.

In the light of the encouraging results obtained on the mortality rates as a result of the prolonged exposure to sublethal doses, it seems important to study the mechanisms of action of the studied promising molluscicides on snail's fertility. At sublethal doses CuSO<sub>4</sub> and *Ammi majus* flowers water suspension proved to most effective in suppressing egg laying capacity, while exposure to *Agave filifera* whole plant and *Ammi majus* leaves water suspension positively affected

the egg laying capacity. The tested materials also decreased the egg hatchability. These findings agree with the data reported by Rawi *et al.* (1995) and El-Ansary *et al.* (2001) who showed inhibition or increased rate of egg laying production based on the type of the tested molluscicides.

Inhibition or increased rate of egg production may arise from different modes of action of the tested compounds on the steroid hormones or may be due to the harmful effects on male and female genital tract (Rawi *et al.*, 1996) or due to metabolic disorders effect (Rawi *et al.*, 1994).

The present findings can also be explained on the basis of gonadal histopathological changes post treatments. The recorded effects are not completely similar but mainly depending on the type of the tested compound. The major histopathological effects on the gonadal organs presented here are recognized as slight and severe disintegration of the gonadal cells, gonadal acini are diminished in size and decreased in number, fading of the boundaries between the reproductive units and necrosis and vacuolation in some treatments were recorded. The type of injury depends on the amount of toxicant that reaches or that is absorbed by these gonadal tissues. The problem of transport is important because permeability barrier may prevent some of the botanical molluscicides to reach to that site. As reported by Rashed (2002), lipophilic of botanical extracts will obviously affect the lipid layers of the membrane which destroy specific permeability properties and this may lead to water loss causing dehydration which might lead to the encountered abnormalities of egg laying capacity.

Results obtained from the biochemical effects of the tested compounds showed that there was significant decrease in total protein content in the hemolymph of the studied snails. The recorded values are in accordance with Abdel-Kader and Tantawy (2000) who reported that exposure to water suspension of *Agave filifera* and *Agave attenuate* caused significant decrease in total protein content of *B. alexandrina* hemolymph. Similar results were also obtained by Rawi *et al.* (1996) and El-Sayed (2006) using  $\text{CuSO}_4$  and different plant molluscicides including *Ammi majus* flowers and leaves.

Extensive work has been carried out in order to determine how various toxic agents affect total protein content. Refaie (2006) showed that a diminution of the rate of ATP synthesis and the inhibition of RNA synthesis are the main causes of the decrease total protein content. As reported by Singh and Singh (2004), the reduction in protein synthesis in infected *B. glabrata* mainly due to the proteolysis of tissue protein. Also, according to Rawi *et al.* (1995, 1996), protein leakage during intoxication may arise from conversion of the protein to amino acids, the direct effect of the tested compounds on the amino acid transport and degradation of protein to release energy.

Regarding to the total lipid content, the prolonged exposure to the tested doses of the selected compounds showed noticeable decreases of hemolymph total lipid. The present findings coincide with Rajyalakshmi *et al.* (1996) and Abdel-Megeed (1999) using different molluscicides. According to the last author, the tested compounds being moderately lipophilic, can penetrate external membrane structure and rapidly affect the function of cells. Another explanation to decreased lipid contents after treatment may be due to reduced synthesis of lipids or increased activity of lipase involved in the oxidation of lipid (El-Wakil and Radwan, 1991). Also, as recorded by the same authors, decrease in total lipid may be due to drastic decrease in glycogen content in the same tissue which induced by hypoxia, after glycogen depletion, lipid content may be used for energy production.

In the present study, the prolonged exposure to the tested doses of the selected compounds showed significant increase of AST activity. On the other hand,  $\text{CuSO}_4$  and *Agave filifera* have insignificant effect on ALT activity, while *Ammi majus* flowers and leaves caused



significant elevation in its level. These results are in accordance with Rawi *et al.* (1995) and Abdel-Kader *et al.* (2005). The recorded changes, however, seem necessary for the animal to restore amino acid balance since transaminases are necessary in regulating the concentration of keto and amino acids. As reported by Rawi *et al.* (1996) and Kandeel (2004), such effects reflect damage of parenchymal cells.

Acetylcholinesterase activity has the potential for serving as a biochemical indicator in measuring the effect of any toxic agents (Kristoff *et al.*, 2006). In the present work, the alteration in AchE activity was also investigated in hemolymph of *B. alexandrina*. A significant inhibition of the enzyme activity was recorded post prolonged exposure to sublethal concentration of the tested compounds. The results are in accordance with Tripathi *et al.* (2004) and Essawy *et al.* (2009) using different molluscicidal agents. Hassanein (2005) has postulated that the inhibitor (pesticide) serves as a pseudosubstrate and becomes attached to the active site of the enzyme. The hydrolysis of the inhibited enzyme is slow and therefore the amount of AchE becomes lesser which leads to accumulation of acetylcholine at the nerve endings. Thus toxicity depends on the affinity of the enzyme for inhibitor as well as hydrolysis rate. The relative degree of each effect may well depend upon the rate of absorption, the permeability of membrane and the resistance to attack.

From the present study, it is concluded that the water suspension of the tested plants has a highly molluscicidal effect on *B. alexandrina* snails than cold or hot water extracts comparing with CuSO<sub>4</sub> as a control molluscicide. The survival rate, fecundity and the physiological parameters were much affected by the water suspension of *Ammi majus* flowers, as it inhibited the egg laying capacity and affected the longevity; also, it reduced the total protein and total lipid contents of the hemolymph of *B. alexandrina* snails. The transaminases and acetylcholinesterase activity were also affected by the prolonged exposure to the water suspension of *Ammi majus* flowers.

In future, more attention must be paid to the efficient extraction, mode of action and application techniques for use in rural communities.

## REFERENCES

- Abdel-Hamid, A.Z. and M.M.I. Massntawy, 1999. Effect of the plants *Frenkiana hirstua* and *Withania somnifera* on biological and physiological activities of *B. alexandrina* snails. Egypt. J. Bilharz., 19: 99-113.
- Abdel-Hamid, H.F., 2003. Molluscicidal and *in vitro* schistosomicidal activities of the latex and some extracts of some plants belonging to Euphorbiaceae. J. Egypt. Soc. Parasitol., 33: 947-954.
- Abdel-Kader, A. and A. Sharaf El-Din, 2000. Potency of copper sulphate, bayluscide, *Calendula micrantha* officinalis and *Ammi majus* flowers on egg masses of *B. alexandrina* and *Bulinus truncates*. Egypt J. Schisto. End. Infec. Dis., 22: 159-173.
- Abdel-Kader, A. and A.A. Tantawy, 2000. Effect of *Agave filifera* and *Agave attenuate* on *B. alexandrina* snails, the vector of *Schistosoma mansoni* in Egypt. Egypt. J. Schisto. Infec. End. Dis., 22: 175-185.
- Abdel-Kader, A., S.A. Hamdi and S.M. Rawi, 2005. Biological and biochemical studies on *Biomphalaria alexandrina* snails, treated with low concentrations of certain molluscicides (synthetic and of plant origin). J. Egypt. Soc. Parasitol., 35: 841-858.
- Abdel-Megeed, K.N., 1999. Studies on the molluscicidal activity of *Calendula micrantha* officinalis on fascioliasis transmitting snails. J. Egypt Soc. Parasitol., 29: 183-192.
- Abdel-Moati, A.R. and E.A. Farag, 1991. Toxicological and bioaccumulation studies of Cu, Zn and Pb on the fresh water gastropod *Lanistes bolteni* Chemnitz. J. Egypt. Ger. Soc. Zool., 4: 289-299.

- Abdel-Wahab, M.F., G. Esmat, S.I. Narooz, A. Yosery, J.P. Struewing and G.T. Strickland, 1990. Sonographic studies of school children in a village endemic for *Schistosoma mansoni*. Trans. R. Soc. Trop. Med. Hyg., 84: 69-73.
- Al-Daihan, S., 2010. Effect of plant molluscicides on selected enzymes related to energy metabolism in *Biomphalaria arabica* snails molluscan hosts to *Schistosoma mansoni* in Saudi Arabia. J. Egypt. Soc. Parasitol., 40: 187-195.
- Anosike, J.C., A.N. Okere, B.E. Nwko, J.U. Chukwu and D.C. Nwosu *et al.*, 2003. Endemicity of vesical schistosomiasis in the ebonyi benue river valley, south eastern Nigeria. Int. J. Hyg. Environ. Health, 206: 205-210.
- Badran, R.A., 1996. The interactive effect of some molluscicides on the intermediate host of human bilharziasis and their associated zoospore fungi in the river Nile, Egypt. Bull. Soc. Pathol. Exot., 89: 227-231.
- Bailey, N.T.J., 1995. Statistical Methods in Biology. 3rd Edn., Cambridge University Press, Cambridge, UK.
- Bakry, F.A., A.A. Sakrane and N.M. Ismail, 2002. Molluscicidal effect of fungicide, herbicide and plant extract on some biological and physiological parameters of *Biomphalaria alexandrina*. J. Egypt Soc. Parasitol., 32: 821-835.
- Behrman, A.J., 2008. Schistosomiasis. Emedicine (Accessed March 16, 2010). <http://emedicine.medscape.com/article/788867-overview>.
- Bezerra, J.C., I.A. Silva, H.D. Ferreira, P.H. Ferri and S.C. Santos, 2002. Molluscicidal activity against *Biomphalaria glabrata* of Brazilian cerrado medicinal plants. Fitotropa, 73: 428-430.
- Campbell, R.C., 1989. Statistics for Biologists. 3rd Edn., Cambridge University Press, Cambridge, UK., pp: 446.
- Carleton, H., 1976. Carleton Histopathological Technique. 4th Edn., Oxford University Press, New York, London.
- Coeurdassier, M., A. De Vauflery, N. Crini, R. Scheifler and P.M. Badot, 2005. Assessment of whole effluent toxicity on aquatic snails: Bioaccumulation of Cu, Zn and Fe and individual effects in bioassays. Environ. Toxicol. Chem., 24: 198-204.
- Crespo, S. and K.J. Karnaky, 1983. Copper and zinc inhibit chloride transport across the oercula epithelium of seawater adapted kill-fish *Fundulus heteroclitus*. J. Exp. Biol., 102: 337-341.
- Dallinger, R., B. Berger, C. Gruber, P. Hunziker and S. Sturzenbaum, 2000. Metallothioneins in terrestrial invertebrates: Structural aspects, biological significance and implications for their use as biomarkers. Cell. Mol. Biol., 46: 331-346.
- De S Luna, J., A.F. De S Santos, M.R. De Lim, M.C. De Omenta, F.A. De Mendonca, L.W. Bieber and A.E. Sat'Ana, 2005. A study of the larvicidal and molluscicidal activities of some medicinal plants from northeast Brazil. J. Ethnopharmacol., 97: 199-206.
- Ebenso, I.E., 2004. Molluscicidal effects of neem (*Azadirachta indica*) extracts on edible tropical land snails. Pest Manage. Sci., 60: 178-182.
- El-Ansary, A., E.M. Sammour, M.S. Soliman and F.A. Gawish, 2001. *In vivo* attenuation of Schistosome cercarial development and disturbance of egg laying capacity in *B. alexandrina* using sublethal concentrations of plant molluscicides. J. Egypt Soc. Parasitol., 31: 657-669.
- El-Emam, M.A. and F.A. Ebeid, 1989. Effect of *Schistosoma mansoni* infection, starvation and molluscicides on acid phosphatase, transaminases and total protein in tissues and haemolymph of *B. glabrata*. J. Egypt Soc. Parasitol., 19: 139-147.
- El-Sayed, K.A., 2006. Effect of plant *Cupressus macro*. Carpa (Cupressaceae) on some hematological and biochemical parameters of *B. alexandrina* snails. J. Egypt Soc. Parasitol., 36: 911-924.

- El-Wakil, H.B. and M.A. Radwan, 1991. Biochemical studies on the terrestrial snail. *Eubania vermiculata* (Muller) treated with some pesticides. J. Environ. Sci. Health B., 26: 479-489.
- Ellman, G.L., K.D. Courtney, V. Andres and R.M. Feather-Stone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
- Erdelyi, L., T. Kovacs, K. Insperger and Z. Foldesi, 1998. Pharmacological actions of essential (zinc, copper) and toxic metal ions (lead, mercury) on neurons and neuronal synapses of snail *Helix pomatia* L. Neurotoxicology, 19: 565-569.
- Ernould, J.C., A. Garba, R. Labbo, A.K. Kaman, A. Sidiki, A. Djibrilla and J.P. Chipaux, 2004. Heterogeneity of *Schistosoma haematobium* transmission in irrigated field. Bull. Soc. Pathol. Exot., 97: 19-23.
- Essawy, A.E., N.E. Abdelmeguid, M.A. Radwan, S.S. Hamed and A.E. Hegazy, 2009. Neuropathological effect of carbamate molluscicides on the land snail *Eobania vermiculata*. Cell Biol. Toxicol., 25: 275-290.
- Farghaly, A.G., 1992. Cross sectional study of schistosomiasis among military recruits in Alexandria. J. Egypt Public Health Assoc., 67: 593-605.
- Fieser, I. and M. Fieser, 1949. Natural Products Related to Phenanthrene. 3rd Edn., Reinhold Publishing Co., New York.
- Figueiredo, E.A., M.V. Gomaz, I.F Henein, I.O. Santos and F.B. Hargreaves, 1973. Isolation and physiochemical properties of the haemoglobin of *Biomphalaria glabrata* (Mollusca, Planorbidae). Comp. Biochem. Physiol. Part B Comp. Biochem., 44: 781-791.
- Gamot, A. and F. Pihan, 1997. Comparison of the bioaccumulation capacities of copper and zinc in two snail subspecies (*Helix*). Ecotoxicol. Environ. Safety, 38: 85-94.
- Geissman, T.A., 1962. Chemistry of Flavonoid Compounds. Pergamon Press, Oxford.
- George, S.G. and B.J.S. Pirie, 1980. Metabolism of zinc in the mussel, *Mytilus edulis* L. A combined ultrastructural and biochemical study. J. Mar. Biol. Assoc. U. K., 60: 575-590.
- Gornall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem., 177: 751-766.
- Gorun, V., I. Proinov, V. Baltescu, G. Balaban and O. Barzu, 1978. Modified Ellman procedure for assay of cholinesterase in crude enzymatic preparation. Anal. Biochem., 86: 324-326.
- Hashem, K., 1999. Comparative study on the molluscicidal activity of some natural extracts on the snail vectors of *Schistosoma mansoni*, *B. alexandrina*. M.Sc. Thesis, Faculty Science Cairo University.
- Hassanein, H.M.A., 2005. Acetylcholinesterase Inhibition. In: Recent Trends in Acetylcholinesterase System, Parveen, M. and S. Kumar (Eds.). IOS Press, Amsterdam, New York.
- Iglesias, J., J. Castillejo, R. Parama and R. Mascato, 2000. Susceptibility of the eggs of the pest slug *Deroceras reticulatum* to contact with metal salts. J. Moll. Stud., 66: 171-176.
- Jaiswal, P. and D.K. Singh, 2008. Molluscicidal activity of *Carica papaya* and *Areca catechu* against the fresh water snail *Lymnaea acuminata*. Vet. Parasitol., 152: 264-270.
- Jourdan, J., J.B. Mokunkassa and D. Imbert-Establet, 1990. Influence of intramolluscan larval stages of *Echinostoma liei* on the infectivity of *S. mansoni* cercaria. J. Helminthol., 64: 71-74.
- Kandeel, N.M., 2004. Toxicological and metabolic studies of some molluscicides on harmful terrestrial snails. M.Sc. Thesis, Zoology Department, Faculty of Science, Cairo University.
- Kenawy, R.E. and E.S. Rizk, 2004. Polymeric controlled release formulations of niclosamide for control of *Biomphalaria alexandrina*, the vector snail of schistosomiasis. Macromol. Biosci., 4: 119-128.

- Kirichuk, G.E., A.P. Stadnichenko and I.A. Pershko, 2002. The effect of the trematode invasion and accumulation of heavy metals onto the pond snail (Mollusca: Gastropoda: Lymnaeidae). *Parazitologiya*, 36: 295-303.
- Kloos, H., C. De Souza, A. Gazzinelli, B.S. Soares Filho and T. De Costa *et al.*, 2001. The distribution of *Biomphalaria* spp. In different habitats in relation to physical, biological water contact and cognitive factors in a rural area in Minas Gerais, Brazil. *Mem. Inst. Oswaldo Cruz*, 96: 57-66.
- Knight, J.A., S. Anderson and J.M. Rawle, 1972. Chemical basis of sulfo-phospho-vanillin reaction for estimating total serum lipids. *Clin. Chem.*, 18: 199-202.
- Kristoff, G., N.V. Guerrero, A.M. de D'Angelo and A.C. Cochon, 2006. Inhibition of cholinesterase activity by azinphos- methyl in two freshwater invertebrates: *Biomphalaria glabrata* and *lumbriculus variegatus*. *Toxicology*, 222: 185-194.
- Kristoff, G., N.R.V. Guerrero and A.C. Cochon, 2010. Inhibition of cholinesterases and carboxylesterases of two invertebrate species, *Biomphalaria glabrata* and *Lumbriculus variegatus* by the carbamate pesticide carbaryl. *Aquat. Toxicol.*, 96: 115-123.
- Landis, W.G. and M.H. Yu, 1995. Factors Modifying the Activity of Toxicants. Lewis Publishers, Florida.
- Litchfield, J.T. and F. Wilcoxon, 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.*, 96: 99-133.
- Mantawy, M.M., M.A. Hamed, E.M. Sammour and M. Sanad, 2004. Influence of *Capparis spinosa* and *Acacia arabica* on certain biochemical haemolymph parameters of *Biomphalaria alexandrina*. *J. Egypt Soc. Parasitol.*, 34: 659-677.
- Masola, B., M. Chibi, Y.S. Naik, E. Kandoare and M.F. Zaranyika, 2003. Activities of glutamate dehydrogenase and alanine amino transferases in fresh water snails *Helisoma duryi* and *Lymnaea natalensis* exposed to copper. *Biomarkers*, 8: 33-42.
- Mc Cullough, F.S., 1986. Snail control in relation to a strategy for reduction of morbidity due to schistosomiasis control. *Bull. WHO*, 58: 681-689.
- Mostafa, B.B. and H.S. Gad, 1997. Effect of UV-irradiation, Gamma irradiation and praziquantel on infected *Biomphalaria alexandrina* snails. *J. Egypt Soc. Parasitol.*, 27: 35-46.
- Mummert, A.K., R.J. Neves, T.J. Newcomb and D.S. Cherry, 2003. Sensitivity of juvenile freshwater mussels (*Lampsilis fasciola*, *Villosa iris*) to the total and un-ionized ammonia. *Environ. Toxicol. Chem.*, 22: 2545-2553.
- Nihei, K., B.P. Ying, T. Murakami, H. Matsuda, M. Hashimoto and I. Kulso, 2005. Pachvelasides and novel molluscicidal triterpene saponins from *Pachyelasma tessmanni*. *J. Agric. Food Chem.*, 53: 608-613.
- Nour, N.M., 2010. Schistosomiasis: Health effects on women. *Rev. Obstet. Gynecol.*, 3: 28-32.
- Oliver, L. and W.T. Haskins, 1960. The effect of low concentrations of sodium penta chlorophenate on the fecundity and egg viability of *Australorbis globatus*. *Am. J. Trop. Med. Hyg.*, 9: 199-205.
- Oliveria-Filho, E.C., R.R. De-Carvalho and E.J. Pangmarten, 1999. The influence of environmental factors on the molluscicidal activity of *Euphorbia milii* latex. *J. Environ. Sci. Health*, 34: 289-303.
- Oteifa, B.A., A.H. Mousa, A.A. Abou-El-Hassan, A.M. Mohamed and M.A. El-Emam, 1975. Effect of certain insecticides in the control of fresh water snails *B. alexandrina* and *B. truncates*. *Egypt J. Bilh.*, 2: 221-243.

- Rajyalakshmi, T., T. Srinivas, K.V. Swamy and P.M. Mohan, 1996. Butachlor impact on protein, free amino acid and glutamic contents and on activity levels of amino acid transferases, glutamate dehydrogenase and glutamine synthetase in the fresh water snails, *Pila globosa* (Swainson). Biochem. Mol. Biol. Int., 39: 949-960.
- Rashed, A.A., 2002. Biological studies on the snail intermediate hosts of schistosomiasis with a special emphasis on using larval *Echinostomes* as biocontrol agents against larval schistosomes and snails. J. Egypt. Soc. Parasitol., 32: 775-784.
- Rawi, S.M., H.I., El-Gindy and A. Abdel-Kader, 1994. The effect of some molluscicides on total protein, albumin, globulin and total lipid of the snail *B. alexandrina*. Bull. Fac. Sci. Cairo Univ., 61: 87-101.
- Rawi, S.M., H. El-Gindy, A.M. Haggag, A. Aboul El-Hassan and A. Abdel-Kader, 1995. New possible molluscicides from *Calendula mircantha officinalis* and *Ammi majus* plants. I. Physiological effect on *B. alexandrina* and *B. truncatus*. J. Egypt. Ger. Soc. Zool., 16: 49-75.
- Rawi, S.M., H. El-Gindy and A. Abdel-Kader, 1996. New possible molluscicide from *Calendula mircantha officinalis* and *Ammi majus*. II. Molluscicidal, physiological and egg laying effects against *Biomphalaria alexandrina* and *Bulinus truncatus*. Ecotoxicol. Environ. Saf., 35: 261-267.
- Reddy, A., E.L. Ponder and B. Fried, 2004. Effect of copper sulphate toxicity on cercariae and metacercariae of *Echinostoma caproni* and *Echinostoma trivolvis* and on the survival of *Biomphalaria alexandrina* snails. J. Parasitol., 90: 1332-1337.
- Refaie, A.A., 2006. Toxicological studies on immature rats exposed to different toxicants and their mixtures. Ph.D. Thesis, Faculty Science Cairo Univ., Egypt.
- Regoli, F., S. Gorbi, D. Fattorini, S. Tedesco and A. Notti *et al.*, 2006. Use of land snail *Helix aspersa* as sentinel organism for monitoring ecotoxicologic effects of urban pollution: An integrated approach. Environ. Health Perspect., 114: 63-69.
- Reitman, S. and S. Frankel, 1957. Colormetric method for aspartate and alanine aminotransferases. Am. J. Clin. Pathol., 28: 56-63.
- Schmitt, I., 2006. Schistosomiasis-The burden of disease and trends of intervention. International Health Practice. <http://www.cwru.edu/med/epidbio/mphp439/Schistosomiasis.htm>.
- Shoeb, H.A. and M.A. El-Emam, 1976. The molluscicidal properties of natural products from *Ambrosia maritime*. Egypt J. Bilharz., 3: 157-167.
- Singh, A. and D.K. Singh, 2004. Effect of herbal molluscicides and their combinations on the reproduction of the snail *Lymnaea acuminata*. Arch. Environ. Contam. Toxicol., 46: 470-477.
- Singh, A. and V.K. Singh, 2009. Molluscicidal activity of *Saraca asoca* and *Thuja orientalis* against the fresh water snail *Lymnaea acuminata*. Vet. Parasitol., 164: 206-210.
- Singh, S.K., R.P. Yadav and A. Singh, 2010. Molluscicides from some common medicinal plants of Eastern Uttar Pradesh, India. J. Applied Toxicol., 30: 1-7.
- Sow, S., S.J. De Vlas, D. Engels and B. Gryseels, 2002. Water related disease patterns before and after the construction of the Diama dam in northern Senegal. Ann. Trop. Med. Parasitol., 96: 575-586.
- Stadnichenko, A.P. and G.E. Kirichuk, 2000. The effect of ammonium nitrate on the residual nitrogen content in the hemolymph of pulmonate snail *Planorbarius purpura* (Mollusca: Pulmonata: Bulinidae) normally and in trematode invasion. Parazitologiya, 34: 402-407.

- Stadnichenko, A.P., L.D. Ivanenko, O.F. Vasilenko, M.M. Zinich and A.E. Vishnevskaya *et al.*, 1994. Influence of different concentrations of zinc sulphate on physico-chemical properties of the hemolymph of normal and trematode infected *Planorbarius* (Mollusca: Bulinidae). *Parazitologiya*, 27: 404-409.
- Swailah, K.M. and A. Ezzughayyar, 2000. Effect of dietary Cd and co. on feeding and growth rate of the landsnail *Helix engaddensis*. *Ecotoxicol. Environ. Saf.*, 47: 253-260.
- Tadros, S.H., 1979. Pharmacognostical study of *Enterolobium cyclocarpum* griseb growing in Egypt. Ph.D. Thesis, Faculty Pharmacology Cairo University.
- Tantawy, A.A., 2002. Effects of sublethal concentrations of *Atriplex halimus* (Chenopodiaceae) on *Biomphalaria alexandrina*, the snail vector of *Schistosoma mansoni* in Egypt. *J. Egypt. Soc. Parasitol.*, 32: 297-305.
- Tantawy, A.A., 2006. Molluscicidal effect of fenitrothion and anifolol on *Lymnaea natalensis* and *Biomphalaria alexandrina* snails and on the free larval stages of *Schistosoma mansoni*. *J. Egypt Soc. Parasitol.*, 36: 629-642.
- Trease, G.E. and W.C. Evans, 1978. A Textbook of Pharmacognosy. 11th Edn., Bailliere Tindall, London pp: 530.
- Tripathi, S.M., V.K. Singh, S. Singh and D.K. Singh, 2004. Enzyme inhibition by the molluscicidal agent *Punica granatum* Linn. Bark and *Canna indica* Linn. Root. *J. Phytother. Res.*, 18: 501-506.
- Wall, M.E., M.M. Krider, C.F. Krewson, C.R. Eddy, J.J. Willaman, D.S. Corell and H.S. Gentry, 1954. Steroidal sapogenins. VII. Survey of plants for steroidal sapogenins and other constituents. *J. Am. Pharm. Assoc.*, 43: 1-7.
- WHO, 2010. Schistosomiasis. Fact Sheet No. 115, February (2010). <http://www.who.int/mediacentre/factsheets/fs115/en>.
- Wolf from, M.L., P.W. Morgan and F.L. Benton, 1940. Osage orange pigments. IV. Degree of unsaturation and flavone nature. *J. Am. Chem. Soc.*, 62: 1484-1489.
- Yousif, F., M. El-Emam, A. Abdel-Kader, A. Sharaf El-Din, K. El-Hommosany and C. Schiff, 1998. Schistosomiasis in newly reclaimed areas in Egypt. 1-Distribution and population seasonal fluctuation of intermediate host snails. *J. Egypt Soc. Parasitol.*, 28: 915-928.