EFFECT OF TWO ALGAE; *CYSTOSEIRA BARBATA* AND *DICTYOTA DICHOTOMA* ON DIGESTIVE GLAND OF *BIOMPHALARIA ALEXANDRINA* SNAILS.

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ABSTRACT

Schistosomiasis ranks the second amongst parasitic diseases affecting human. To reduce the incidence of this disease, several strategies were applied including snail control. Chemical control methods have many drawbacks, hence biological control became the concern of many studies. In this study, eight algal species were tested against *Biomphalaria alexandrina* snails; the intermediate host of *Schistosoma mansoni*. The bioassay tests revealed that *Cystosiera barbata* and *Dictyota dichotoma* were the most effective species, where the LC_{50} values were 2200 ppm and 560 ppm consequently. Prolonged exposure to LC_{25} (280 ppm) of *D. dichotoma* resulted in death of snails after two weeks. In addition, alterations in digestive glands of treated snails with LC_5 (220 ppm) and LC_{25} (280 ppm) of *C. barbata* and *D. dichotoma*, respectively were obvious, as vacuolation of digestive cells were recorded after exposure of snails to LC_5 of *C. barbata*. Moreover, exposure of snails to LC_{25} of *D. dichotoma* resulted in noticeable vacuolation of digestive cells and degenerated secretory cells; the lumen was nearly disappeared. The maximum elevations in the activities of both aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were observed after 2 weeks of exposure to LC_5 (56 ppm) and LC_{15} (660.7 ppm) of *D. dichotoma* and *C. barbata*, respectively. The total protein contents of treated *B. alexandrina* snails were significantly different from that of control group after 2 weeks of exposure to different concentrations of both effective algae.

Testing the sublethal concentrations of both effective algae on the *Daphnia pulex* showed that LC_5 (220 ppm) of *C. barbata* and LC_{15} (168 ppm), LC_{25} (280 ppm) of *D. dichotoma* were the least harmful to these crustaceans.
Key Words*:* *ystoseira barbata*, *Dictyota dichotoma*, *Biomphalaria alexandrina*, Digestive gland, Transaminases, Total protein, biological control.

INTRODUCTION

Schistosomiasis is the most serious form of parasitism by multi-cellular organisms and still on the list of the neglected diseases prioritized by the World Health Organization. This disease affects approximately 240 million people around the world, resulting in an annual mortality of 280,000 people (WHO, 2015). Five species of schistosomiasis infect man; the most important are *Schistosoma mansoni* and *S. haematobium*. In Egypt, the intermediate host of *S. mansoni* is *Biomphalaria alexandrina,* while the intermediate host of *S. haematobium* is *Bulinus truncatus*. It is generally considered that snail control is one of the most rapid and effective means of reducing transmission of parasitic infections. The use of chemical molluscicides leads to toxicity of the non-target organisms and environmental pollution (Oliveira-Filho & Paumgartten, 2000; De Boeck *et al*., 2004; Mostafa *et al*., 2005). These drawbacks of chemical substances directed investigators to natural control, such as predators, parasites, and pathogens. These biological agents possess desirable properties than the chemical molluscicide, besides it can be safely applied (Moazami, 2008).

Some studies have focused on biological control such as the use of certain bacterial strains (Wang *et al*., 2008), extracts of some fungal species such as *Aspergillus terreus* and *Penicillium janthinellum* (Saad *et al*., 2015), and microalgae, e.g. *Spirulina platensis* (Mostafa and Gawish, 2009).

This study aims to evaluate the effect of two algal species; *C. barbata* and *D. dichotoma* on the digestive gland of *B. alexandrina* snails, and the activities of related enzymes, besides total protein contents in the haemolymph of these snails.

MATERIAL AND METHODS

1-Maintenance and rearing of snails: Adult *Biomphalaria alexandrina* snails (8-10 mm in diameter) were used in this study. They were obtained from irrigation canals at Abou Rawash area, Giza governorate. They were kept under laboratory conditions (25±2°C) and fed on fresh lettuce leaves, allowed to acclimatize in the laboratory three weeks before being used in experimental tests.

2- Collection and identification of algal species: The algae used in this study were 8 species belonging to 3 divisions and 7 families:

Ulva lactuca (Ulvaceae), *Cladophora glomerata* (Cladophoraceae) from Chlorophyta (Green algae), *Jania adhaerens* (Corallinaceae), *Digenea simplex* (Rhodomelaceae) and *Liagora farinosa* (Nemalionaceae) from Rhodophyta (Red algae) and *Sargassum dentifolium*, C*ystoseira barbata* (Sargassaceae) and *Dictyota dichotoma* (Dictyotaceae) from Phaeophyta (Brown algae). *Ulva lactuca* and *Jania adherens* were obtained from Alexandria (Cleopatra Beach in front of Qaitbay Fort), *Cystoseira barbata* from Matrouh (Mediterranean

Sea), *Cladophora glomerata* from Wadi El Rayan (Fayoum), *Liagora farinosa*, *Sargassum dentifolium*, *Digenea simplex* and *Dictyota dichotoma* from Hurghada (Red Sea).The tested algae were initially washed thoroughly with sea water to remove sand and any adhering substance, and then washed with fresh water to remove salts, then dried in shade and finely grounded. The dry powder of the experimental algae was stored in clean, dark, and dry cupboard till use. Identification of the algal species was carried out by naked eyes according to Bold and Wynne (1978) and Chapman & Gellenbeck (1983).

3. Screening and evaluation tests: The dry powder of each algal species was used in toxicity tests as aqueous suspension. A series of concentrations (500, 1000, 3000 and 5000 ppm) was prepared on basis of weight /volume using dechlorinated tap water to determine the most potent algae against *B. alexandrina* snails.

Three replicates were used, each of ten snails. The exposure period was 24 hours at room temperature. Another group of snails was maintained under the same experimental conditions as a control one. At the end of the exposure period, these snails were removed from each tested concentration, washed thoroughly with dechlorinated tap water and transferred to another clean container containing dechlorinated water for a recovery period of 24 hours. Then, dead snails were counted.

LC_{50} and LC_{90} values of the algae were computed according to the method of Litchfield and Wilcoxon (1949).

4. Effect of sublethal concentrations of algal species on survival rates of adult snails: This experiment was designed to study the effect of sublethal concentrations LC_5 (220 ppm), LC_{15} (660.7 ppm) and LC_{25} (1100 ppm) of aqueous suspension of *Cystoseira barbata* and LC_5 (56 ppm), LC_{15} (168 ppm) and LC_{25} (280 ppm) of *Dictyota dichotoma* algae on survival rates of *B. alexandrina* of 8-10 mm in diameter.

Each sublethal concentration of each tested algal species was prepared weekly in dechlorinated tap water. A group of 30 adult snails was exposed in three replicates to the tested sublethal concentrations. A control group was maintained under the same experimental conditions. These snails were fed on dried lettuce leaves twice weekly.

5. Effect of sublethal concentrations of algal species on digestive gland of snails: Adult *B. alexandrina* snails exposed to sublethal concentrations LC_5 (220 ppm), LC_{15} (660.7 ppm) and LC_{25} (1100 ppm) of aqueous suspension of *Cystoseira barbata* and LC_5 (56 ppm), LC_{15} (168 ppm) and LC_{25} (280 ppm) of *Dictyota dichotoma* algae were randomly selected after 2 weeks of exposure. The shell was gently broken, and its fragments were removed carefully using pointed forceps under a dissecting microscope. The digestive gland was carefully separated using fine scissors and immediately fixed in Bouin's solution for 24 hours. Fixed samples were dehydrated, cleared, and embedded in paraffin. Then they were serially sectioned at 5μm and stained with Haematoxylin and Eosin.

6. Effect of sublethal concentrations of algal species on some haemolymph parameters: The snails were wiped dry with a soft tissue, a small window was scratched in the shell in the vicinity of the heart, the mantle was pierced with a needle and the haemolymph was collected with a pipette to which a small rubber bulb had been attached (Abdel-Kader and Tantawy, 2000). Haemolymph was collected from the control and tested snails at the 2nd week of exposure. In each specified group, the haemolymph of 8 – 10 individual snails was pooled in 1 ml Eppendorf tube. All samples from each experimental group were centrifuged at 5000 rpm for 5 min at 4°C to pellet haemolymph and other particulate materials (Dikkeboom *et al*., 1988).

The pellet was discarded and cell - free haemolymph was mixed with sample buffer in a ratio of 4 part of haemolymph: 1 part sample buffer. Samples were boiled for 5 min at 100 ºC in a water bath. Aspartate and alanine amino transferases (AST and ALT) activities were estimated in the haemolymph of *B. alexandrina* snails according to the method of Reitman and Frankel (1957), using reagent kits purchased from Biomerieux chemicals, France. The developed colour was read on the spectrophotometer at 505 nm. All enzymes were expressed as units/liter.

Total protein was estimated in the haemolymph according to the method of Bradford (1976) using reagent kits purchased from Biomerieux chemicals, France, depending on the reaction of Bradford dye with protein forming protein Bradford complex. The developed colour was read on the spectrophotometer at 595 nm. It was expressed as g/100 ml.

RESULTS AND DISCUSSION

1. **Toxicity tests of certain algal species on *B. alexandrina* snails:** Screening tests were carried out on eight algal species to determine the most effective ones against adult *B. alexandrina* snails (Table1). It was found that only two algal species; *Dictyota dichotoma* and *Cystoseira barbata* had molluscicidal activity as they lead to 100% death of tested snails at a concentration of 1000 and 5000 ppm, respectively. The LC_{50} value of *D. dichotoma* (560 ppm) was less than that of *C. barbata* (2200 ppm) (Table 2).

Table (1): Mortality percentage of *B. alexandrina* exposed to different concentrations of algal species (after 24 hours):

Algal species	Concentrations			
	500 ppm	1000 ppm	3000 ppm	5000 ppm
Ulva lactuca	-	-	-	-
Jania adhaerens	-	-	-	-
Cladophora glomerata	-	-	-	-
Liagora farinosa	-	-	-	-
Sargassum dentifolium	-	-	-	-
Digenea simplex	-	-	-	-
Cystoseira barbata	-	-	80	100
Dictyota dichotoma	20	100	100	100

Table (2): The values of LC_{50} and LC_{90} of the effective algal species on adult *B. alexandrina* snails:

Algal species	Concentration (ppm)	
	LC_{50}	LC_{90}
Cystoseira barbata	2200	3100
Dictyota dichotoma	560	820

The effect of *D. dichotoma* in the present study could be attributed to the presence of terpenoids or alkaloids (Peres *et al*., 2012; Deyab *et al*. 2016).

Moreover, the molluscicidal effect of *Cystoseira barbata* is due to the presence of alkaloids and saponins as mentioned by Alghazeer *et al*. (2013). Francis *et al.* (2002) reported that the molluscicidal activity of saponins is due to their characteristic detergent effect on epithelial tissues of the snails.

2. Effect of sublethal concentrations of *Cystoseira barbata* and *Dictyota dichotoma* on survival rate of adult *B. alexandrina* snails:

2.1.*Cystoseira barbata:* It was observed from Figure (1) that at the 7th week of exposure, the survival rate of snails exposed to LC_5 (220 ppm) reached 52% compared to 88% for control. Also, it was found that increasing algal concentration to LC_{15} (660.7 ppm) caused more reduction in survival rate as it was gradually decreased, reaching 24% after 7 weeks of exposure. For snails exposed to 1100 ppm (LC_{25}), sudden reduction in the survival rate throughout the first 4 weeks of the experiment was recorded. The rate reached to 24% at the 4th week comparing to 88% for control group.

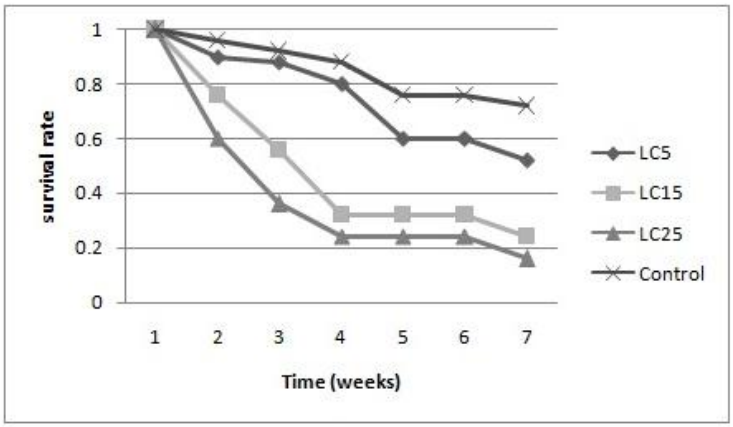


Fig. (1): Effect of sublethal concentrations of *C. barbata* on the survival rate of *B. alexandrina*.

2.2 *Dictyota dichotoma*: The survival rate of adult snails exposed to LC_{15} (168 ppm) and LC_{25} (280 ppm) decreased to 60% and 64%, respectively, while that of control was 96% at the 2nd week of the exposure. It was noticed that there was a gradual reduction in the survival rate of snails exposed to LC_5, by the 6th week, only 12% of snails were survived comparing to 88% for control group. Regarding LC_{15}, all snails have died at the 5th week of exposure. Meanwhile, the effect of LC_{25} was much more pronounced as no snails survived after 2 weeks (Fig. 2).

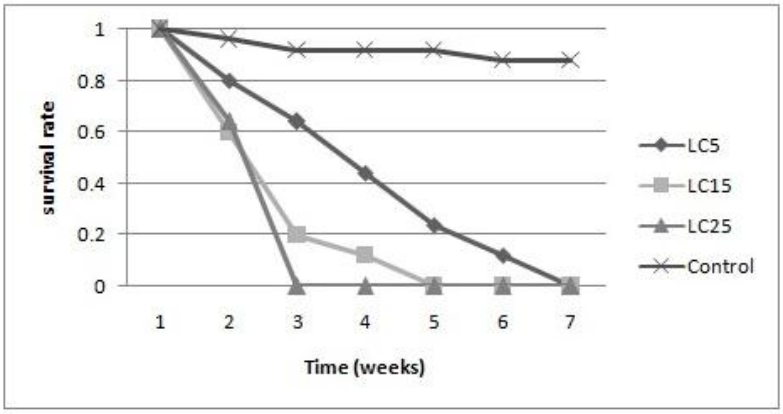


Fig. (2): Effect of sublethal concentrations of *D. dichotoma* on the survival rate of adult *B. alexandrina*.

The noticeable effect of *D. dichtoma* and *C. barbata* on the survival rate of snails in the current study may be due to the presence of phenolic compounds, saponins and alkaloids that facilitate and accelerate the rate of penetration of algal by products through snail's skin, hence increases their harmful effects (Mostafa and Gawish, 2009). Similar results were reported by Tantawy *et al*. (2000); they postulated a decrease in the survival rate of *B. alexandrina* due to continuous exposed (13 weeks) to aqueous solution of *Solanum dubium* plant.

Moreover, Abd El-Baky *et al*. (2009) indicated that the microalga *Spirulina platensis* secrets organic substances or metabolic products such as phycobiline, phenols, terponoids, steroids, polysaccharides and saponins. Low concentrations of saponin fractions increase mortality of *B. alexandrina* snails (Tadros *et al*., 2008).

3. Effect of sublethal concentrations of *Cystoseira barbata* and *Dictyota dichotoma* algae on digestive gland of adult *B. alexandrina* snails:

3.1 Control snails: The digestive gland of the snails is a main site of detoxification (wilbrink *et al*., 1990), therefore the histopathological changes in such gland after treatment of snails with molluscicidal substances are important indicators of animal toxicity (Downs *et al*., 2001). The digestive gland of *B. alexandrina* consists of a number of tubules which are connected together by connective tissue. Each tubule consists of two main cell types; the digestive and secretory cells. The digestive cells are the most numerous, they are columnar with round apices and the cytoplasm contains numerous vesicles of different sizes, their nuclei are oval and lie in basal region. In between the digestive cells, the secretory cells are distributed in smaller numbers. They are pyramidal in shape; their cytoplasm is highly basophilic and contains large number of chromatin granules (Fig.3); this agrees with Lutfy and Demian (1967).

3.2. Treated snails:

3.2.1 *Cystoseira barbata*: For snails treated with LC_5 (220 ppm) of *C. barbata* for two weeks, secretory cells were degenerated. Moreover, vacuolation of digestive cells were recorded (Fig.4). These alterations are attributed to the

presence of alkaloids and saponins. These finding agree with those recorded by Brackenbury (1999), who found that a graded series of cellular injuries to the epithelial layer was observed along the length of the digestive tract of *Bulinus africanus* treated with *Agave attenuata* plant. This included the loss of cilia and brush border, disruption of the epithelial layer, cellular vacuolation, swelling and rupture, and the discharge of secretory products from mucous gland cells. Recently, Saad *et al*. (2015) revealed that exposure of *B. alexandrina* snails to LC_5 of the fungus *Penicillium janthinellum* resulted in vacuolation of digestive cells and degeneration of secretory ones. This effect was more pronounced as the concentaration increased to LC_{15} and LC_{25}, as hydropic degeneration was observed in the latter concentration.

3.2.2. *Dictyota dichotoma*: Exposure of snails to LC_{25} (280 ppm) of *D. dichotoma* resulted in noticeable vacuolation of digestive cells and degeneration of secretory cells, the lumen was nearly disappeared (Fig.5). These findings were in agreement with Bakry (2009), who found that the exposure of snails for 2 weeks to LC_{25} of extract from the tested plants *Guayacum officinalis , Atriplex stylosa* and *Euphorbia splendens* caused great damages in the digestive gland of *B. alexandrina*. Thus, epithelial cells lost their regular shape and appeared empty from cytoplasm, having many vacuolations, secretory cell nearly degenerated from the digestive tubules and connective tissues between shrinked tubules were damaged.

Moreover, Saad *et al*. (2015) found that *B. alexandrina* snails exposed to LC_{25} of *Aspergillus terreus* filtrate showed clear necrotic changes of the digestive tubule and degeneration of the cytoplasm of digestive cells.

4. Effect of sublethal concentrations of *Cystoseira barbata* and *Dictyota dichotoma* algae on some haemolymph parameters of *B. alexandrina* snails after two weeks:

4.1. ***Cystoseira barbata:*** Table (3) reveales that exposure of snails to LC_5 (220 ppm) of this alga resulted in significant increase ($P < 0.05$) in alanine amino transferase (ALT) activity to 54 U/L comparing to 30.33U/L for control snails. By increasing the concentration to LC_{15} (660.7 ppm), the enzyme activity was significantly increased to 120.33 U/L. On contrary, elevation of the algal concentration to LC_{25} (1100 ppm) caused a significant reduction of enzyme activity to 59 U/L.

It was demonstrated that the maximum elevation of aspartate amino transferase activity (AST) was recorded in snail group exposed to LC_{15} (660.7 ppm) as it was 237.33 U/L compared to 61.16 U/L for control group. While raising the algal concentration to LC_{25} (1100 ppm) caused a reduction in the enzyme activity.

The observed elevation of both enzymes is due to tissue damage (Lebsack *et al*., 1980) or due to increased synthesis or decreased catabolism of such enzymes (Dinman *et al.*, 1963). On the other hand, the decreased activity of ALT and AST is due to either leakage of the enzyme into extracellular compartments or actual enzyme inhibition. Thus, biochemical impairment led to the deviation of both enzymes activities out of the normal range which finally resulted in lesions of the tissues and disruption of cellular functions (Radwan *et al*., 1992).Similar elevation of both ALT and AST was recorded by

Saad *et al*. (2012) after exposure of *B. alexandrina* to LC_{15} and LC_{25} of *Casimiroa edulis* plant for 24 hrs.

Exposure of snails to LC_5 (220 ppm) and LC_{15} (660.7 ppm) caused a significant increase of total protein concentrations; their values were 1.1 and 1.43g/dl comparing to 0.54 g/dl for control snails. This is due to the response to the stressful condition induced by the molluscicide (Hoek *et al*., 1997). Other investigators showed similar results; marked elevation of total protein was recorded after exposure of *B. alexandrina* to 20 ppm of *Euphorbia peplus* plant (El-khodary, 2001) and LC_{25} of *Capparis spinosa* and *Acacia arabica* plants (Mantawy *et al*., 2004). Moreover, the concentration of total protein has been reduced at LC_{25}; its value was 0.43g/dl. This result is in accordance with that reported by Rawi e*t al.* (1995). They stated that there was a continuous decrease of the total protein throughout four weeks of *B. alexandrina* exposure to LC_0 of *Calendula micrantha* and *Ammi majus* aqueous suspensions (13.5 and 610 ppm respectively). Hence, they reported that toxic agents of these tested plants might affect protein synthesis by decreasing the rate of ATP synthesis and inhibition of RNA synthesis.

Table (3): Effect of sublethal concentrations of *C. barbata* on AST, ALT and protein contents of *B. alexandrina* haemolymph after 2 weeks of exposure:

Concentrations / Biochemical parameters	Control	$LC_{5(220\ ppm)}$	$LC_{15(660.7\ ppm)}$	$LC_{25(1100\ ppm)}$
Alanine aminotransferase (ALT)	30.33 ± 1.52	54 ± 1.00*	120.33 ± 0.57*	59 ± 1.00*
Aspartate aminotransferase(AST)	61.16 ± 1.04	122.33 ± 2.51*	237.33 ± 2.08*	126.33 ± 1.52*
Total protein	0.54 ± 0.04	1.1± 0.10*	1.43 ± 0.15*	0.43 ± 0.03

4.2. *Dictyota dichotoma:* Table (4) showes that lower activities of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were observed after two weeks of snails' exposure to LC_{15} (168 ppm) of *D. dichotoma*. These findings agree with the previous results, as dried *Capparis spinosa* and *Acacia arabica* leaves significantly alter AST and ALT in haemolymph of *B. alexandrina* after one week of exposure (Mantawy *et al*., 2004). The decrease in transaminases activities can be attributed to the destructive effect of the tested materials on the enzyme itself (El-Gindy *et al*., 1991). Also, decreased energy gained from the diet caused a decrease in the activity of both ALT and AST (Truchli´nski and Grela, 1987).Similarly, a noticeable decrease in the activities of ALT and AST was stated after exposure of *B. alexandrina* snails to LC_{15} and LC_{25}, respectively of *Cestrum diurnum* plant (Saad *et al*., 2012).

On the other hand, total protein showed a significant elevation as it reached 2.03 g/dl after snails' exposure to LC_{15} (168 ppm) comparing to 0.54 g/dl for control group. Some investigators showed similar results; marked elevation of total protein was recorded after exposure of *B. alexandrina* to 20 ppm of *Euphorbia peplus* plant (El-khodary, 2001) and LC_{25} of *Capparis spinosa* and *Acacia arabica* plants (Mantawy *et al*., 2004). This rise in the content of total proteins could be related to the acceleration in the development of gluconeogenesis with the progressive level of intoxication caused by higher concentrations of the molluscicide (Mello-Silva *et al*., 2006). They recorded a gradual increase in protein content in the haemolymph of *Biomphalaria glabrata* with increasing the concentration of *Euphorbia splendens* latex.

Table (4): Effect of sublethal concentrations of *D. dichotoma* on AST, ALT and protein contents of *B. alexandrina* haemolymph after 2 weeks of exposure:

Concentrations / Biochemical parameters	Control	$LC_{5(56\ ppm)}$	$LC_{15(168\ ppm)}$	$LC_{25(280\ ppm)}$
Alanine aminotransferase (ALT)	30.33 ± 1.52	79.66 ± 1.52*	11 ± 1.00*	63.33 ± 2.08*
Aspartate aminotransferase(AST)	61.16 ± 1.04	138.33 ± 1.52*	21 ± 1.00*	134.33 ± 0.57*
Total protein	0.54 ± 0.04	1.73 ± 0.15*	2.03 ± 0.05*	0.46±0.05

5. Effect of sublethal concentrations of *Cystoseira barbata* and *Dictyota dichotoma* on *Daphnia pulex*: Cladocerans are ecologically very important members of freshwater Crustacea and amongst them *Daphnia* spp. have been often utilized as test organisms for the ecotoxicological monitoring of aquatic ecosystems (Lagadic *et al*., 1994). In the present study, LC_5 (220 ppm) of *Cystoseira barbata* and LC_{15} (168 ppm), LC_{25} (280 ppm) of *Dictyota dichotoma* didn't affect survivorship of *Daphnia pulex* individuals, while LC_{25} of *C. barbata* and LC_5 of *D. dichotoma* resulted in 20% mortality (Table 5). Some studies were carried out to evaluate the toxicity of some chemical and plant molluscicides against *Daphnia*. Brackenbury and Appleton (1997) demonstrated that the toxicity of *Agave attenuata* plant to *Daphnia* sp. was lacking or low. Recently, Saad *et al*. (2015) revealed that exposure of *Daphnia pulex* to LC_{25} of *Penicillium janthinellum* fungal filtrate resulted in only 10% death. In addition, no mortality was observed in LC_5.

Table (5): Effect of sublethal concentrations of *C. barbata* and *D. dichotoma* algae on mortality percentages of *Daphnia pulex*:

Algae / *Daphnia pulex*	Control	*Cystoseira barbata*			*Dictyota dichotoma*		
		LC_5 (220 ppm)	LC_{15} (660.7 ppm)	LC_{25} (1100 ppm)	LC_5 (56 ppm)	LC_{15} (168 ppm)	LC_{25} (280ppm)
Number of animals tested	10	10	10	10	10	10	10
Number of dead animals	0	0	1	2	2	0	0
Percentage of mortality	0	0	10	20	20	0	0

CONCLUSION

It is concluded from the present study that *Dictyota dichotoma* is more effective than *Cystosiera barbata* on *Biomphalaria alexandrina* snails as its LC_{50} value was 560 ppm.

RECOMMENDATION

We hope that the present results will provide a starting point of investigations aimed at exploiting more studies on the schistosomiasis control by using other algal species as they contain effective compounds as saponins, alkaloids and terpenoids.

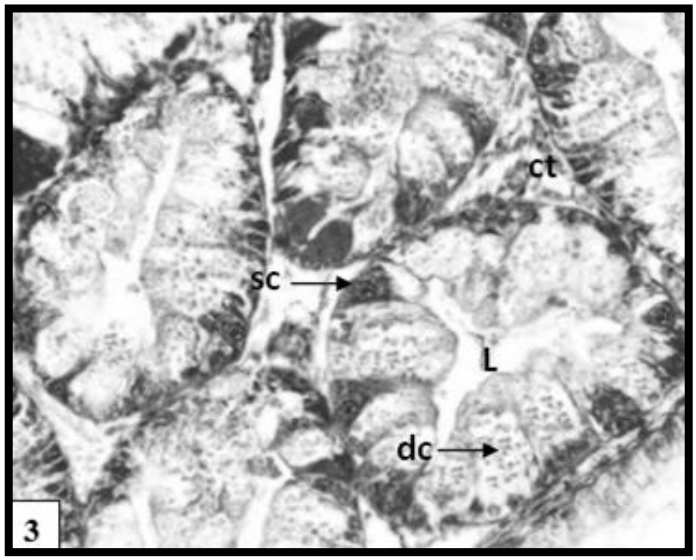
Explanation Of Figures:

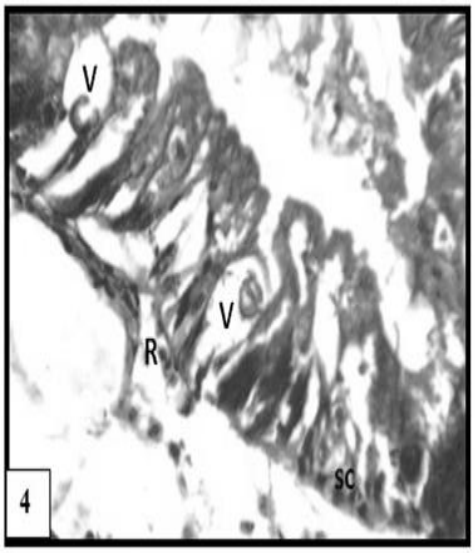
Figs.from (3 to 5): Light photomicrographs of transverse sections in the digestive gland of *Biomphalaria alexandrina* snails

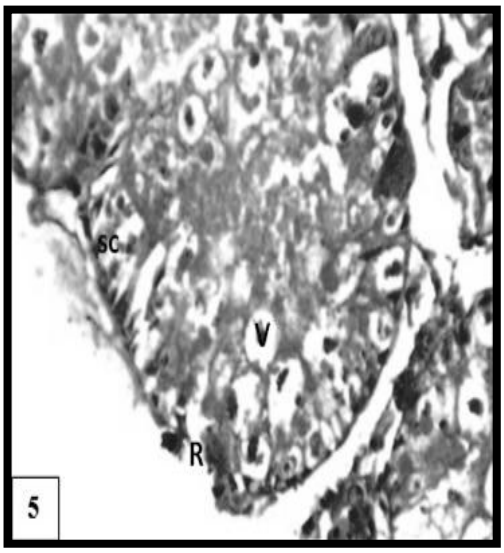
Fig. 3: Control gland showing tubules with the two main types of cells, the digestive cells (dc) and the secretory cells (sc), note the lumen (L), and the connective tissue (ct) between tubules (X100).

Fig. 4: Snails treated with LC_5 of *C.barbata* showing vacuolation of digestive cells (V) and degeneration of secretory cells (sc), rupture of the tubular membrane (R) (X400).

Fig. 5: Snails treated with LC_{25} of *D.dichotoma* showing noticeable vacuolation of digestive cells (V) and degeneration of secretory cells (sc) , the lumen was nearly disappeared (L) and rupture of the tubular membrane (X 400).







REFERENCE:

Abd El-Baky, H.H.; El Baz, F.k. and El-Baroty, G.S. (2009): Phenolics from *Spirulina maxima*: Overproduction and in vitro protective effect of its phenolics on CCl_4 induced hepatotoxicity. J. Med. Plant Res., 3(1): 24-30.

Abdel-Kader, A. and Tantawy, A. (2000) : Effect of *Agave filifera* and *Agave attenuata* on *Biomphalaria alexandrina* snails, the vector of *Schistosoma mansoni* in Egypt. Egypt. J. Schist. Infect. Endem. Dis., 22:175-185.

Alghazeer, R.; whida, F.; Abduelrhman , E.; Gammoudi, F. and Naili, M. (2013) : In vitro antibacterial activity of alkaloid extracts from green, red and brown macroalgae from western coast of Libya, Afr. J.Biotechnol., 12(51):7086-7091.

Bakry, F.A. (2009): Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. Pest. Biochem. Physiol.,95:159–165.

Bold, H.C. and Wynne, M.J. (1978): Introduction to the algae: Structure and reproduction.Englewood Cliffs, NewJersey, USA. 706. pp.

Brackenbury, T.D.(1999) : Gross histopathological effects of an extract of *Agave attenuata* on the epithelium of the digestive tract of *Bulinus africanus*. Ann. Trop. Med. & Parasitol.,93(5): 519-526.

Brackenbury, T.D. and Appleton, C.C. (1997): A comprehensive evaluation of *Agave attenuata*, a candidate plant molluscicide in South Africa. Acta Tropica., 68(2): 201–213.

Bradford, M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.Annal.Biochem.,72:248-254.

Chapman, D.J. and Gellenbeck, K.W. (1983): An historical perspective of algal biotechnology. In: Cresswell,R.C.; Ress, T.A.V. and Shah, V. (eds.). Algae and cyanobacterial biotechnology. Longman group, U.K. pp. 1- 27.

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

Deyab, M.; Elkatony, T. and Ward, F. (2016): Qualitative and quantitative analysis of phytochemical studies on brown seaweed, *Dictyota dichotoma*. Int. J. Eng. Develop. & Res.,2(4): 674- 678.

Dikkeboom, R.; Bayne, C.J.; Van DerKnaap, W.P.W. and Tijnajel, J.M.G.H. (1988): Possible role of reactive forms of oxygen in vitro killing *Schistosoma mansoni* sporocysts by hemocytes of *Lymnaea stagnalis*. Parasitol.Res.,75:148-154.

Dinman, B.D.; Hamdi, E.A.; Fox, C.F. and Frajola, W.J. (1963): Carbon tetrachloride toxicity. Hepatostructural and enzymatic changes.Arch. Environ. Health .,7: 630-646.

Downs, C. A.; Dillon, R. T.; Fauth, J. E. and Woodly,C. M. A. (2001): molecular biomarker system for assessingthe health of gastropods

(*Llyanassa obsolete*) exposedto natural and anthropogenic stressors. J. Exp. Mar.Biol. Ecol., 259: 189-214.

El-Gindy, H.I.; Rawi, S.M.; Abdel-Kader, A. and Ebeid, F. (1991): A comparative effect of different pesticides on the transaminases activities in haemolymph of *Biomphalaria alexandrina* snails. J. Egypt. Ger. Soc. Zool.,6(A):131-138.

El-Khodary, G.M. (2001): Comparative ultrastructural and immunological studies on the effect of factors of variable nature on *Schistosoma* and intermediate hosts. Ph.D. Thesis, Facul. Sci, Tanta Univ., Egypt.

Francis, G.; Kerem, Z.; Makkar, H.P.S. and Becker, K. (2002): The biological action of saponins in animal systems: A review. Brit. J. Nutr., 88:587-605.

Hoek, P.M.; Van Kesteren, R.E.; Smit, A.B.; DeJong-Brink, M. and Geraerts, W.P. (1997): Altered gene expression in the host brain caused by a trematodes parasite: neuropeptide genes are preferentially affected during parasitosis. Proc. Natl. Acad. Sci., USA, 140:72-76.

Lagadic, L.; Caquet, T. and Ramade, F.(1994) : The role of biomarkers in environmental assessment.5.Invertebrate populations and communities. Ecotoxicol., 3:193-208.

Lebsack, M.E.; Anderson, A.D.; Nelson, K.F. and Farrier, D.S. (1980): Sublethal effects of an in-situ oil shale retort water on rainbow trout Toxicol. Appl. Pharmacol., 54: 462-468.

Litchfield, J.T. and Wilcoxon , F. (1949) : A simplified method of evaluating dose effect experiments J. Pharmacol . Exp.Ther., 96 :99-113.

Lutfy, R.G. and Demian, E.S. (1967): The histology of the alimentary system of Marisa cornuarietis (Mesogastropoda: Ampullariidae). Malucologia 5 (3): 375-422.

Mantawy, M.M.; Hamed, M.A.; Sammour, E.M. and Sanad, M. (2004): Influence of *Capparis spinosa* and *Acacia arabica* on certain biochemical haemolymph parameters of *Biomphalaria alexandrina*. J. Egypt. Soc. Parasitol., 34(2): 659-677.

Mello-Silva, C.C.; Vasconcellos, M.C.; Pinheiro, J.; and Rodrigues, M.L.A. (2006): Physiological changes in *Biomphalaria glabrata* Say,1818 (Pulmonata:Planorbidae) caused by sublethal concentrations of the latex *Euphorbia splendens* var. *hislopii*. N.E.B. (Euphorbiaceae). Mem. Inst. Oswaldo Cruz.,10(1):1-6.

Moazami, N. (2008): Biopesticide production. Biotechnology. Encyclopedia of Life Support Systems (EOLSS). 6 : 1-10.

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

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

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

Peres, J.C.; Carvalho, L.R.; Goncalez, E.; Berian, L.O.S. and Felicio, J.D. (2012): Evaluation of antifungal activity of seaweed extracts. Ciên. Agrotec., 36:294–299.

Radwan, M.A.; El- Wakil, H.B. and Osman, K.A. (1992): Toxicity and biochemical impact of certain oxime carbamate pesticides against terrestrial snail,*Theba pisana* (Müller), J. Environ. Sci. Health, B., 27(6):759-773.

Rawi, S.M.; El-Gindy, H.; Haggag, A.M.; Abou El-Hassan, A. and Abdel Kader, A.(1995) : New possible molluscicides from *Calendula micrantha officinalis* and *Ammi majus*Plants.I. Physiological effects on *Biomphalaria alexandrina* and *Bulinus truncatus.* J. Egypt. Ger. Soc. Zool., 16(D): 49-75.

Reitman, S. and Frankel, S. (1957): A colourimetric method for the determination of serum glutamic oxaloacetic glutamic pyruvic transaminases. Amer. Clin. Pathol., 28:56.

Saad, A. A.; Aly, R.H.; Ragab, F. M. A. and Abdel-Wareth, M. T. A. (2012): Effect of *Cestrum diurnum* and *Casimiroa edulis* plants on the digestive gland and its related enzymes in the freshwater snail *Biomphalaria alexandrina*. Toxicol. Environ. Health Sci., 4: 109-114.

Saad, A.A.; Khalil, M.T.; Ragab, F.M.A.; Mekawey, A.A.I. and Abdel-Wareth, M.T.A. (2015): Separation of a compound effective against *Biomphalaria alexandrina* snails from the filtrate of *Penicillium janthinellum*. Int. J. Environ. Stud., 73(1): 1-17.

Tadros, M.M; Ghaly, N.S. and Moharib, M.N. (2008): Molluscicidal and schistosomicidal activities of a steroidal saponin containing fraction from *Dracaena fragrans* (L.).J. Egypt. Soc. Parasitol.,38 : 585–598.

Tantawy, A.; Sharaf El-Din, A.T. and Bakry, F.A. (2000): Laboratory evaluation of the molluscicide activity of *Solanum dubium* (Solanaceae) against *Biomphalaria alexandrina* snails.J.1st inter.Cong.Biol.Sci.(ICBS), Tanta Univ.,1(2):307-318.

Truchli´nski, J. and Grela, E. (1987): Alanine and aspartate transaminase and acid and alkaline phosphatase activities in the blood serum in relation to the protein and energylevels in the pigs' feeds. Pol. Arch. Weter., 27: 129-136.

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

WHO (2015): Neglected Tropical Disease, http://http://www.who.int/schistosomiasis /en/ (accessed March 2015).

Wilbrink, M.; Treskes, M.; Vlieger, T.A. and Vermeulen, N.P.E. (1990): Comparative toxicokinetics of 2,2′-and 4,4′ - dichlorobiphenyls in the pond snail *Lymnaea stagnalis*. Arch. Environ. Contam. Toxicol., 9:565-571.

تأثير طحلبى سيستوسيرا بارباتا و ديكتيوتا دايكوتوما على الغدة الهاضمة لقواقع بيومفلاريا ألكسندرينا

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المستخلص

تحتل البلهاريسيا المرتبة الثانية من بين الامراض الطفيلية التي تؤثر على حياة الإنسان، لذلك تم تطبيق عدة طرق للحد من إنتشار هذا المرض ومنها مكافحة القواقع. وأظهرت المكافحة الكيميائية العديد من السلبيات لذلك اتجهت الدراسات إلى المكافحة البيولوجية. في هذه الدراسة تم تقييم التأثير الابادى لثمانية أنواع من الطحالب على قواقع بيومفلاريا ألكسندرينا وقد وجد أن طحلبى ديكتيوتا ديكتوما وسيستوسيرا بارباتا أكثر فاعلية على إبادة هذه القواقع.

أوضحت النتائج أن تعريض القواقع لتركيز LC_{25} (٢٨٠ جزء فى المليون) من طحلب ديكتيوتا دايكوتوما أدى إلى حدوث الوفاة بعد الأسبوع الثانى . بالإضافة إلى حدوث تغييرات فى الغدة الهاضمة فى مجموعة القواقع المعاملة بتركيزات LC_5 (٢٢٠ جزء فى المليون) و LC_{25} من كل من سيستوسيرا بارباتا و ديكتيوتا ديكتوما على الترتيب، حيث لوحظ وجود العديد من الفجوات مختلفه الحجم في الخلايا الهاضمة لمجموعة القواقع المعرضة لتركيز LC_5 من طحلب سيستوسيرا بارباتا. كما لوحظ ايضا وجود فجوات فى الخلايا الهاضمة و موت الخلايا الافرازية فى حالة مجموعة القواقع المعرضة لتركيز LC_{25} من طحلب ديكتيوتا ديكتوما. كذلك إتضح من الدراسة حدوث ارتفاع ملحوظ فى كل من اسبرتات امينو ترانسفيز وألالانين أمينو ترانسفيرز في مجموعة القواقع المعرضة لمدة أسبوعين لكل من LC_5 و LC_{15} (١٦٨ جزء فى المليون) من طحلبى سيستوسيرا بارباتا و ديكتيوتا ديكتوما على الترتيب. كما أدى تعرض القواقع للطحالب الفعالة إلى تغيير فى المحتوى الكلى للبروتين فى هيموليمف القواقع المعاملة.

بإختبار التركيزات تحت المميتة من الطحلبين الفعالين على دافنيا بوليكس إتضح أن التركيزات التالية LC_5 من طحلب سيستوسيرا بارباتا و LC_{15} ، LC_{25} من طحلب ديكتيوتا ديكتوما هما الأقل تأثيرا على معدل موت الدافنيا. لذلك نأمل من نتائج الدراسة الحالية اجراء العديد من الدراسات لمكافحة البلهاريسيا بإستخدام أنواع طحالب أخرى حيث أنها تحتوى على مواد فعالة مثل الصابونين، القلويدات والتربينات.