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BIOCHEMIAL CHARACTERSTICS FOR SNAILS HEMOLYMPH OF *Eobania vermiculata* AND *Monacha cartusiana* IN EGYPT

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ABSTRACT: This study was carried out in Sharkia Governorate, Egypt to compare between biochemical constituents contents, enzymes activities and elements concentrations in shell of two species of most dangerous land snail in Egypt (*Monacha cartusiana* and *Eobania vermiculata*). The obtained results showed that *M. cartusiana* hemolymph was charactarizd with high concentration of carbohydrates (2798.33 ± 59.18 ug/ml), total lipids (657.00±23.28 ug/ml), total protein (25.05 ±0.47 mg/ml), malondialdehyde (MDA) (11.43±0.33 n mol./ml) and urea content (138.33±7.04 mg/100 ml). Also enzymes activities [Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Glutathione S-transferase (GST) and phenol oxidase)] were higher in the hymolymph of *M. cartusiana* than those in hemolymph of *E. vermiculata.* Shell contents of elements in *E.vermiculata* was contained a higher concentration of P (232.14 ± 4.04 ppm), Mg (566.25±3.46 ppm), Fe (487.82 ± 6.77 ppm), Cu (18.94 ± 2.30 ppm), K (1202.50 ± 4.04 ppm), Na (1230.25±5.77 ppm) and Zn (5.98 ± 1.15 ppm) than those of *M. cartusiana*, while shell of *M. cartusiana* was characterized with higher content of Ca (503925.00±14.43 ppm) than shell of *E. vermiculata.*

Key words: Land snails, carbohydrates, protein, lipids, urea, enzymes, elements.

INTRODUCTION

Land snails are found in most governorates of Egypt as stated by Bishara *et al.* (1968), El-Okda (1984), Abd El-Aal (2001), Metwally *et al.* (2002), El-Deeb *et al.* (2003), Ramzy (2009), Shetaia *et al.* (2009), Abo-Elnaser (2013), Eshra (2013), Rady *et al.* (2014) and Abd El-Aleem *et al.* (2015). Heavy damage to cereal, maize, Egyptian clover, vegetables, potatoes, lettuce, carrots, cabbage, citrus, seed of oil plants, leaves of ornamental plants , peach, palm and bean was caused by snails (Bishara *et al.*, 1968; El-Okda, 1981; Ismail *et al*., 2003; Lokma, 2007; Shahawy *et al*, 2008). The damage by snails depends not only on their activity and population density, but also on their feeding habits (Abd El-Aleem *et al*., 2015). Crops contaminated by snail slime lose their marketability and hence their export potential in many countries as mentioned by (Baker and Hawke, 1990; Ittah and Zisman, 1992).

On the other hand, there are snails, such as *Helix pomatial*, have high demand and are highly valued in the countries of the European Union, where they are consumed as food products and for medical aims (Zymantiene *et al*., 2006).

In Sharkia Governorate, Egypt, there are two species of land snails known as brown garden snail, *Eobania vermiculata* (Muller) and the glassy clover snail, *Monacha cartusiana (*Montagu). These snails are destructive agricultural pests causing economic damage to a wide variety of plants including horticulture, field crops and vegetables (Abd El-Aal and Hamed, 2010; Abd El-Aleem *et al*., 2015).

Chemical analysis of land snails dependent on active and aestivation periods, since glucose

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was decreased in aestivation periods compared with the period of activity (Ismail *et al.*, 2013). Shell are mainly chitin and conchiolin , which consist of protein hardened with calcium carbonate as well with a variety of mineral salts (Lambert, 1973; Bowen, 1996; Etim, 2015). Also, Dominika (2008) found that shells contained 48% mass $CaCO_3$ mainly in the form of aragonite with small quantities of calcite. There maining 2% mass were probably compounds of Fe, Mg, Mn, Al, Na, K. None of the element exceeded 1% mass. The concentration of glucose and total protein in the hymolymph of the garden snails *Helix pomatia* L. were 0.8± 0.12 mmol/l and 44.18± 13.25 g/l, respectively (Bislimi *et al.*, 2013). Alanine amino transaminase (ALT) and Aspartate transaminase (AST) activities were determined by (El-Gohary and Genana, 2011) in the two land snails, *E. vermiculata* and *M.cantiana*. They found that activities of ALT and AST were 45.47± 4.32 and 586.33 ±16.50, respectively for *E. vermiculata*, while they were 221.70 ±3.06 and 923.70±25.11, respectively for *M. cantiana*. So this study was conducted to compare biochemical constituents, enzyme activities and shell element concentrations in both land snails *Eobania vermiculata* (Muller) and *Monacha cantiana* (Montagu) which are particulary the most common and serious pests in Egypt.

MATERIALS AND METHODS

This study was carried out at the Agriculture Biochemistry Department, Faculty of Agriculture, Zagazig University and Minestry of Agriculture, Egypt, to compare biochemical constituents, enzyme activities and minerals between two land snail varities, *Monacha cartusiana* and *Eobania vermiculata*.

Tested Animals

Two species of land snails, glassy clover snail, *Monacha cartusiana* and brown garden snail, *Eobania vermiculata* which were used in this study and classified according to (Godan, 1983) as follows :

Kingdom: *Animalia*

Subkingdom: *Metazoa*

Phylum: *Mollusca*

Class: *Gastropoda*

Subclass: *Pulmonata*

Order: *Stylommatophora*

Superfamily : *Helicidae*

Genus: *Eobania*

Species: *Eobania vermiculata*

Subfamily: *Monacheae*

Genus: *Monacha*

Adult snails of *Monacha cartusiana* were collected during March 2015 from fields cultivated with Egyptian clover at Alqurien, Abou Hammad Distrect, Sharkia Governorate, Egypt, while adults snails of *Eobania vermiculata* were collected from orchard fields at Moshtohor village, Toukh Distrect, Kalubia Governorate, Egypt during March, 2015. The collected snails were transferred to the laboratory, and then reared in plastic containers (40 × 30 × 30 cm) with soil base (10 snails/ jar) and fed on lettuce.

Methods

The present study was conducted to identify the activity of A spartate aminotransferase (AST), alanine aminotransferase (ALT), Glutathione S-transferase (GST), malondialdehyde (MDA), Phenoloxidase, total carbohydrates, total lipids (TL), total proteins (TP) and urea in the haemolymph of adults of *Monacha cartusiana* and *Eobania vermiculata* beside identification of element concentration of the shell (Ca , Zn , Na , K, Cu, Fe, Mg, P).

Preparation of snails for biochemical assay

The snails shell was cleaned with a paper towel and the hemolymph then collected by making a pierce just above the level of the pericardial cavity, and then a fine micropipette inserted carefully through a tiny hole and for aspiration of hemolymph according to (Bezzerra *et al.*, 1997). One ml of hemolymph was obtained from 30 snails of each two land snails. The collected hemolymph was centrifuged at 3000 (rpm) for 10 minutes in order to precipitate hemocytes and cellular debris. The resulting supernatant was used in the laboratory analyses then the shells of tested snails were removed by making a cut around the whorls in a continuous manner starting at the aperture

opening using bone scissors and the broken fragments of the shell were carefully removed to determine elements concentration.

Activity of ALT and AST

The activity of both ALT and AST were determined according to the method of Reitman and Frankle (1957).

GST determination

Glutathione S-transferase (GST) activity was estimated by the method of Habig *et al.* (1974).

Lipid peroxidation (MDA) status

Lipid peroxidation level was determined by using biodiagnostic kit No. MD2529 which is based on the spectrophotometric method of Ohkawa *et al.* (1979)

Phenoloxidase activity

Phenoloxidase activity was determined according to the method of Ishaaya (1971).

Determination of total soluble carbohydrate

Total soluble carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967). Determination was carried out according to Dubios *et al.* (1956).

Determination of total lipids

Total lipids were estimated by the method of Knight *et al.* (1972) .

Total proteins

Total proteins were determined by the method of Bradford (1976)

Urea determination

Urea was assayed using BioScope kit (BioScope diagnostics, www.betalab-eg.com) according the method of Young (1990).

Element determination

Elements in shells of tested snails were estimated by the methods of Perkin-Elmer (1964) and Walsh (1955).

Statistical Analysis

Statistical analysis was conducted on the data according to (Snedecor, 1951).

RESULTS AND DISCUSSION

The chemical constituents of land snails hemolymph were determined at active period (March), because concentration of bioconstituents dependant on both active period and aestivation period (Ismail *et al*., 2015). Also tolerance of land snails against organic or inorganic agents attributed to biochemical constituents (Bislimi *et al.*, 2013).

So results in Table 1 show the values of total soluble carbohydrates (ug/ml), total lipids (ug/ ml), total proteins (mg/ml), total malondialdyde (MDA, nM/ml) and urea content in hemolymph of two species of land snails (*E. vermiculata* and *M. cartusiana*). Results of soluble carbohydrates contents in hemolymph of *M. cartusiana* were higher than those of *E.vermiculata* (2798.33 ± 59.18 and 2340.00±40.41 ug/ml, respectively). Also *M. cartusiana* hemolymph was characterized with higher contents of lipids (657.00 ± 23.28 ug/ml), total protein (25.05 ± 0.47 mg/ml), MDA (11.43±0.33 nM/ml) and urea content (138.33±7.04 mg/100 ml), as shown in Table 1, than those of *E. vermiculata* hemolymph (476.66±28.47, 21.16±0.47 mg/ml, 7.80 ± 0.17 and 108.66±5.78 mg/100ml, respectively). These trends were conformed with those noticed by Ismail *et al.* (2013) who mentioned that, at aestivation period these parameters were increased. Table 2 elucidated results responded enzymes activities in hemolymph of *M.cartusiana* and *E.vermiculata*, where hemolymph of *M. cartusiana* has a high activities of ALT (130.33±8.95 u $x10^3$/ml), AST (795.00±17.78 u $x10^3$/ml), GST (13.93 ± 5.19 mMol.sub.conjugated/ml) and phenol oxidase (1560.66±27.03 O.D units $x10^3$/min/ml), while *E.vermiculata* has a low activities of ALT(65.66±3.96 $ux10^3$/ml), AST (149.00±6.08 $ux10^3$/ml), GST (10.80 ± 0.30 mMol. sub. conjugated/ml) and phenol oxidase (1104±58.12 O.D.units $x10^3$/min/ml). These results were in agreement with those stated by El-Gohary and Genana (2011) who stated that, *E.vermiculata* has a lower activities of ALT (45.97±4.32 $Ux10^3$/mg protein) and AST (586.33±16.50 $Ux10^3$/mg protein) than those of *M.cantiana* (221.70 ± 3.06 and 923.70 ± 25.11U x 10^3/mg protein, respectively). According to the results in Table 3 it can be noticed that element contents

Table 1. Amounts of total carbohydrates, total lipids, total proteins, MDA and urea in both land snails hemolymph *Eobania vermiculata* and *Monacha cartusiana* in active period

Parameter / Land snails	Total carbohydrate (ug/ml)	Total lipids (ug /ml)	Total proteins (mg/ml)	MDA (n mole/ml)	Urea (mg %)
Eobania vermiculata	2340.00±40.41	476.66±28.47	21.16±0.47	7.80±0.17	108.66±5.78
Monacha cartusiana	2798.33±59.18	657.00±23.28	25.05±0.47	11.43±0.33	138.33±7.04

Table 2. Enzyme activities of both land snails hemolymph *Eobania vermiculata* and *Monacha cartusiana* in active period

Enzyme / Land snails	ALT ($UX10^3$/ml)	AST ($UX10^3$/ml)	GST (m mole sub.conjugated/ml)	Phenol-oxidase (O.D.units$x10^3$/min/ml)
Eobania vermiculata	65.66±3.96	149.00±6.08	10.80±0.30	1104±58.12
Monacha cartusiana	130.33±8.95	795.00±17.78	13.93±5.19	1560.66±27.03

Table 3. Contents of some elements (ppm) in shell of both land snails *Eobania vermiculata* and *Monacha cartusiana* in active period

Element / Land snails	P	Mg	Fe	Cu	K	Na	Zn	Ca
Eobania vermiculata	232.14 ±4.04	566.25 ±3.46	487.82 ±6.77	18.94 ±2.30	1202.50 ±4.04	1230.25 ±5.77	5.98 ±1.15	124987.50 ±1.15
Monacha cartusiana	197.97 ±2.88	267.5 ±4.04	100.50 ±5.77	16.92 ±0.57	1034.25 ±8.08	584.75 ±4.61	3.61 ±0.05	503925.00 ±14.43

in the shell of *E.vermiculata* characterized with high contents of P (232.14±4.04 ppm), Mg (566.25 ± 3.46 ppm), Fe (487.82±6.77 ppm), Cu (18.94 ±2.30 ppm), K (1202-50±4.04), Na (1230.25 ±5.77 ppm), Zn (5.98±1.15 ppm), but Ca was low content (124987.50±1.15 ppm) as compared with Ca content in *M. cartusiana* shell (503925.00±14.43 ppm). These results was in agreement with those expected by Zymantiene *et al.* (2006), Abd El-Aleem *et al.* (2015) and Etim (2015) who found that the damage depends not only on the activity and density, but also, on the feeding habits of snails and shells contained 48% $CaCO_3$.

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التمييز الكيميائى الحيوى لهيموليمف نوعين من القواقع (*E.vermiculata* and *M.cartusiana*) فى مصر

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أُجريت هذه الدراسة في محافظة الشرقية – مصر – لمقارنة محتوى المواد الكيميائية الحيويه والنشاط الإنزيمى وتركيز اليوريا فى هيموليمف نوعين من القواقع الأرضية الأشد خطرا وهما *E.vermiculata* , *M.cartusiana* وقد أوضحت النتائج تميز المونـاكا عن إيوبانيا لمحتواها المرتفع من الكربوهيدرات والليبيدات والبروتين وثنائى ألدهيد المالونك ونسبة اليوريا وكذلك النشاط المرتفع لإنزيمات ALT, AST, GST وإنزيم الفينول أوكسيديز بينما تميز ال Shell فى الإيوبانيا بمحتواه المرتفع من عناصر الفسفور والماغنسيوم والحديد والنحاس والبوتاسيوم والصوديوم والزنك إلا أن الShell فى المونـاكا تميز بمحتوى عالٍ من ال Ca .

المحكمـــــون:

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