

Some biochemical changes in *Eobania vermiculata* snail treated with some plant extracts under laboratory conditions

Mohammed, Gh. R. and Elshewy, A. M.

Plant Protection Department, Faculty of Agriculture, Benha University, Egypt.

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ABSTRACT

Laboratory experiments were carried out to spot light on the effect of some plant extract treatments on levels of total protein, carbohydrates, lipids and glucose, as well as on the activity of peroxide, catalase and alkaline phosphatase (ALP) enzymes in *E. vermiculata* snail. Results revealed that the least level of total protein was recorded among animals treated with 10% *Nerium oleander* plant extract (6.30mg/ml), while level of carbohydrate was the least by *Eucalyptus melliodora* plant extract treatment at 10% concentration (1.41 mg/ ml). On the other hand, the highest reduction of total lipids and glucose levels were recorded after treatment by *Azadirachta indica* plant extract at 10% concentration. The present data showed also the highest activity of peroxidase and catalase enzymes after *Eucalyptus melliodora* plant extract 20% treatment and the least activity for the same two enzymes occurred by *Nerium oleander* treatment. In addition, the highest reduction of (ALP) activity was registered after treatments by extracts of *Nerium oleander* and *Azadirachta indica* extracts.

Key words: Land snails, plant extracts, biochemical and enzymes

Introduction

Terrestrial gastropod molluscs species are currently considered of the most significant threats to sustainable agriculture in many parts of the world (Barker, 2002).

Land snails have been steadily increased as agricultural pests and became real threat to agricultural production in different localities in Egypt. Those attack numerous orchard trees, field and vegetable crops as well as ornamental plants and cause great damage to all plant parts. Furthermore, damage was manifested in chewing soft vegetative growth, flowers and fruits beside feeding on seeds, roots and tubers after sowing or during ripening. On the other hand, land molluscs leave viscous liquids on the invaded parts of plants giving bright trace films (Ghamry *et al.* 1993).

The harmful land snail *Eobania vermiculata* is one of the most spreading snails on many plant species at several governorates in Egypt. Research on plant molluscicides has increased in recent years hoping to prove to be cheaper, more readily available, highly toxic against target organisms, low or nontoxic against non-target organisms at molluscicidal concentrations and more aligned to self-reliant control strategy, than the use of imported synthetic molluscicides (McCullough, 1992).

Biochemical studies on snails proved very important to find out whether there are any pathways, enzymes or system peculiar to snails that open them up to specific control (Duncan, 1983).

Therefore, the present study was planned to study the molluscicidal activity of *Nerium oleander*., *Eucalyptus melliodora* and *Azadirachta indica* plant extracts on total protein, total carbohydrates, total lipids and glucose, in addition to three enzymes; peroxidase, catalase (CAT) and acid alkaline phosphatase in the brown garden snail *E. vermiculata* under laboratory conditions.

Materials and Methods

Tested Snails:-

Adults of the land snail *E. vermiculata* were collected by hand from *Trifolium alexandrinum* (egyptian clover) fields during spring 2015, and transported in white cloth bags to Zoology laboratory of Plant Protection Department, Faculty of Agriculture, Benha University. Healthy individuals were kept in

Corresponding Author: Mohammed, Gh. R., Plant Protection Department, Faculty of Agriculture, Benha University, Egypt.
E-mail: ghada.mohamed@fagr.bu.edu.eg

round plastic boxed (15 cm diameter) contained moistened soil. The snails were kept under laboratory conditions and were feeding daily on fresh leaves of *Lactuca sativa* (lettuce plant) for 15 days.

Plant materials:-

Fruits of the Neem plant, *Azadirachta indica*; Leaves of Eucalyptus plant, *Eucalyptus melliodora* and Leaves of Nerium plant, *Nerium oleander*.

Extraction method:-

Dried 200g powder of the tested plant parts were successively extracted with ethanol solvent according to Freedman *et al.* (1979) method.

Biochemical studies:-

In this experiment, the concentration of 10% and 20% of the tested compounds were selected to study the biochemical effects of these crude plant extracts against *E. vermiculata* snails. The animals were fed on treated *L. sativa* leaves beside the control. The surviving snails were collected 1,3,5 and 7 days after feeding for biochemical measurements.

Snail tissues were dissected out directly after transfer all tissues were homogenized in 0.1 M phosphate buffer pH 7.4 using a polytron homogenizer. The homogenates were centrifuged at 5000 rpm for 20 min at 4°C. The procedure of Bergmenyer, (1963) was followed. Total protein was assayed by the method of Bradford, (1976), while total lipids was assayed by the method of Knight *et al.* (1972). The method of Crompton and Birt, (1967) was used to assay total carbohydrates, while glucose was assayed by the method of Dubois *et al.* (1956) using stanbiokit (stanbio laboratory, Inc. 2930 East Houston Street, San Antonio, Texas 78202). Glucose oxidase catalyses the oxidation of alpha- D- glucose to D- glucono - 1.5 lacyone lguconic acid with the formation of hydrogen peroxide. The oxygen liberated from hydrogen peroxide by peroxidase reacts with the Q dianisidine and oxidizes to a red chromosphere product that was read at 500nm by a spectrophotometer, and the optical density was compared by standard (Conc100 mg%) to obtain the results. The method of Aebi, (1984) was used to assay catalase. Peroxidase was determined according to Better, (1958), while alkaline phosphatase was determined according to the method described by Powell and smith, (1954).

Statistical analysis: -

The Statistical analysis of data was carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS (ver.22). Data were treated as complete randomization design according to Steel and Dickey, (1997). Multiple comparisons were carried out applying LSD.

Results and Discussion

It was important to study the influence of some plant extracts on the biochemical systems in the target species of snails.

Effect of some plant extracts on levels of total protein, carbohydrate, lipid and glucose

Data in tables (1 and 2) present the comparative effect of *N. oleander*, *E. melliodora* and *A. indica* extracts on the levels of total protein, total carbohydrate, total lipid and glucose in *E. vermiculata* snail adults under laboratory conditions.

As shown in table (1) data indicated that the levels of total protein decreased after treatment by either of the three plant extracts in comparison with control. As shown in the same table, the highest mean total protein recorded 15.78 mg/ml for *E. melliodora*, while the lowest mean was 9.50 mg/ml recorded for *N. oleander* compared with the other plants treatments. On the other hand, considering the effect of two different concentrations (10 and 20%) of the three plant extracts, results indicated that the highest inhibitory effect against total protein level of treated animals occurred with 10% concentration for the three tested plant extracts with reductions 6.30, 12.27 and 10.50 mg/ml for *N. oleander*, *E. melliodora* and *A. indica*, respectively. Hussein *et al.* (2007) reported the reduction of molluscs protein levels as a result of toxicities by synthetic and natural toxicants.

Data in table (1) revealed also that levels of total carbohydrates decreased by plant extract treatments in comparison with control with means 1.85, 1.55 and 2.80 mg/ml for *N. oleander*, *E. melliodora* and *A. indica* respectively. Besides, data in the same table cleared that, there was nearness in total carbohydrates levels by using the two different concentrations (10 and 20%) it was 1.78 and 1.91 mg/ml for *N. oleander*, while these levels, slightly, increased to 1.68 and 3.00 mg/ml at 20% concentration for *E. melliodora* and *A. indica*. Generally, the least level of total proteins was assayed in animals treated with 10% *N. oleander* extract, being 6.30 mg/ml and the lowest level of total carbohydrates (1.41 mg/ml) was recorded after treatment by *E. melliodora* extract at 10% concentration (Mohammed, *et al.* 1981).

Table 1: Effect of some plant extracts on the levels of total protein and total carbohydrates in *E. vermiculata* snail.

Plant extract Parameter	<i>N. oleander</i>			<i>E. melliodora</i>			<i>A. indica</i>			Control	L.S.D 0.05
	Conc.%		Mean	Conc.%		Mean	Conc.%		Mean		
	10	20		10	20		10	20			
Total protein (mg/ml)	6.30 ± 0.40	12.70 ± 0.15	9.50 ± 0.32	12.27 ± 0.32	19.30 ± 1.01	15.78 ± 1.64	10.50 ± 0.25	10.93 ± 0.18	10.72 ± 0.17	17.07 ± 0.74	1.31
Total carbohydrates (mg/ml)	1.78 ± 0.07	1.91 ± 0.07	1.85 ± 0.05	1.41 ± 0.07	1.68 ± 0.08	1.55 ± 0.08	2.41 ± 0.10	3.00 ± 0.05	2.80 ± 0.10	3.01 ± 0.11	0.25

Table (2) shows the data of biochemical analysis of total lipid and glucose. Results cleared that total lipid levels were increased to 575, 574 and 309 ug/ml with *N. oleander*, *E. melliodora* and *A. indica* extracts compared to control. On the other hand, glucose level increased by *N. oleander* and *E. melliodora* extract treatments to 387 and 454 ug/ml, while decreased to 193 ug/ml in case of *A. indica*, being lower than control. Results showed also that the treatment of *A. indica* extract was the most effective on level of total lipids and glucose which decreased to 309 and 193 ug/ml, respectively in comparison with *N. oleander* and *E. melliodora*. It is also clearly obvious that the concentration of 20% was the most activating on total lipids and glucose which were decreased to (379 and 252) and (325 and 408) ug/ml for *N. oleander* and *E. melliodora*, respectively, while for *A. indica*, total lipids and glucose were decreased to 115 and 173 ug/ml, respectively at 10% concentration.

Table 2: Effect of some plant extracts on the levels of total lipid and glucose in *E. vermiculata* snail.

Plant extract Parameter	<i>N. oleander</i>			<i>E. melliodora</i>			<i>A. indica</i>			Control	L.S.D 0.05
	Conc.%		Mean	Conc.%		Mean	Conc.%		Mean		
	10	20		10	20		10	20			
Total lipids (ug/ml)	770 ± 26.21	379 ± 17.37	575 ± 88.7	823 ± 28.48	325 ± 18.03	574 ± 112.45	115 ± 7.42	503 ± 14.54	309 ± 87.14	267 ± 11.61	57.64
Glucose (ug/ml)	522 ± 7.00	252 ± 6.12	387 ± 60.59	499 ± 4.93	408 ± 7.36	454 ± 20.66	173 ± 3.51	212 ± 1.45	193 ± 8.96	211 ± 4.48	16.19

Generally, the highest level of total lipids (823 ug/ml) was recorded for *E. melliodora* extract treatment at 10% concentration, and the highest level of glucose was (522 ug/ml) recorded for 10% *N. oleander* extract treatment, while the highest reduction of total lipids and glucose was recorded for *A. indica* at 10% concentration. Al-Akraa and Mohammed, (2015) tested the effect of some chemical compounds on total protein in some land snails species, they recorded that kuik business addition total protein up to 12.34 after 1st day from request, while it decrease this price to be -5.55 and -14.81 post 3rd and 7th day, successively in comparison to control.

Effect of plant extracts on the activity of peroxidase, catalase and ALP enzymes:

The oxidoreductase enzymes peroxidase and catalase play an important role in the conversion of hydrogen peroxidase H₂O₂ to H₂O. The importance of this reaction is attributed to the toxic effect of H₂O₂ on the life cells. H₂O₂ attack the unsaturated fatty acids of the cell membrane causing its oxidation and subsequently injury occurs.

The results presented in table (3) showed variable differences in peroxidase activities in the snails treated with *N. oleander*, *E. melliodora* and *A. indica* extracts in comparison with control. The highest

activity was 745 min/ml recorded for *E. melliodora* extract. Whereas, the activity of peroxidase enzyme was reduced to 418 and 423 min/ml when animals were treated with 10% concentration of *N. oleander* and *E. melliodora* extracts, respectively, while *A. indica* treatments at 10 and 20% resulted activities approximately near the control. Also, it is clear that catalase enzyme activity was increased in snails treated with *E. melliodora* and *A. indica* extracts, those reached to 16.25 and 16.89 U/ml, respectively compared with control, while *N. oleander* caused results near the control. Besides, the present findings explained that the highest activity of catalase enzyme (21.95 U/ml) was measured due to treatment with 20% *E. melliodora* extract, while, the least activity (10.54 U/ml) was recorded after treatment by 10% *E. melliodora* extract, compared to the other tested plant extracts. *Khidr et al. (2011)* reported the effect of some chemical compounds on the activity of peroxidase enzyme in *Monacha obstructa* land snail, they found that peroxidase activity gradually decreased with increasing the period after treatment with Theophylline and Furosemide.

Table 3: Effect of some plant extracts on the activity of some enzymes in *E. vermiculata* snail.

Plant extract Enzyme	<i>N. oleander</i>			<i>E. melliodora</i>			<i>A. indica</i>			Control	L.S.D 0.05
	Conc.%		Mean	Conc.%		Mean	Conc.%		Mean		
	10	20		10	20		10	20			
Peroxidase (min/ml)	418 ± 5.77	745 ± 6.64	546 ± 57.53	423 ± 6.39	767 ± 34.26	745 ± 44.99	680 ± 5.03	679 ± 1.45	680 ± 2.35	684 ± 5.84	42.18
Catalase (U/ml)	11.68 ± 0.33	10.64 ± 0.23	11.16 ± 0.29	10.54 ± 0.91	21.95 ± 1.13	16.25 ± 2.63	14.59 ± 0.56	19.20 ± 0.85	16.89 ± 1.13	11.17 ± 0.56	2.18
ALP (U/ml)	69.67 ± 2.03	47.33 ± 2.73	58.50 ± 5.22	86.33 ± 1.86	86.00 ± 2.52	86.17 ± 1.70	53.00 ± 1.73	60.67 ± 1.20	56.83 ± 1.96	94.67 ± 3.18	6.87

Data in table (3) indicated that the maximum reduction of acid and alkaline phosphatase enzyme (ALP) activity occurred after *N. oleander* and *A. indica* extract treatments which reached 58.50 and 56.83 U/ml, respectively in compared with control. On contrary, the lowest activity of (ALP) enzyme was reported for snails treated with 20% concentration of *N. oleander* extract (47.33 U/ml) in comparison with the other tested plants. *Abd El-Halim, et al. (2006)* tested the effect of Indoxacarb, Lufenuron, *Bacillus thuringiensis kurstaki* (Bt.) and Methomyl on the activity of alanine aminotransferases (ALT) in *Monacha cartusiana* snail; the authors found that all treatments decreased (ALT) enzyme activity.

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