# BIOCHEMICAL EFFECTS OF TWO NATURAL PESTICIDES ON THE BROWN GARDEN SNAIL *EOBANIA VERMICULATA* MULLER

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***Abstract***

The biochemical effects of the two natural products i.e. Neemix 4.5% (plant extract) and Vertemic 1.8% (biocide abamectin) were studied on the brown garden snail *Eobania vermiculata* Muller Animals were treated with sub-lethal concentration ($^1/_4$ $LC_{50}$) of each tested compound using contact (thin film) technique. Some enzymes activities i. e. liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein and total lipid were determined. Also, respiratory enzymes (peroxidase and catalase) were measured after 1, 2, 3 and 4 days post-treatment. Results showed that the two tested compounds reduced the activity of AST gradually with the progression of periods after treatment while the contrary occurred with ALT as both compounds enhanced its activity proportionally than control. Regarding total lipid, both compound had the same effects as they raised total lipid percentage while their effect differed on total protein specially in the third and fourth days of treatment whereas Neemix induced a markedly reduction in total protein percentage comparatively with increase in case of Vertemic. Concerning peroxidase enzyme, its activity decreased gradually with the elongation of the period after treatment for the two tested compounds. Catalase enzyme took adverse way as its activity increased in the $1^{st}$, $2^{nd}$, $3^{rd}$ days, then it decreased in the $4^{th}$ day for both compounds. Generally, Neemix compound was more effective than Vertemic against *E. vermiculata*.

## INTRODUCTION

During the last few years, the terrestrial snails became an economic serious pest in Egypt. Land snails attack the different kinds of plants e.g. cereal, vegetables, orchards and ornamental plants at the different stages. They attract the attention because of the great economic damage they do in agriculture, horticulture and ornamental plants (Godan, 1983). These animal pests were controlled by chemical compounds which cause health and environmental pollution in addition to the toxic effects to non-target organisms. Some natural compounds e.g. Neemix (crude plant extract of Neem) and Vertemic biocide (abamectin) proved promising efficiency for control of land snail species (Gabr *et al.*, 2005).

Therefore, the present work aims to study the biochemical effects of the two natural compounds i.e. Neemix and Vertemic against the brown garden snail species, *Eobania vermiculata* one of the most common and harmful species in Egypt.

## MATERIALS AND METHODS

**1- Tested Compounds:**

- Neemix 4.5% : Ready made crude plant extract of Neem *Azadirachta indica* It was supplied by Agri Dyne technologies INC.
- Vertemic 1.8% E.C. (Abamectin): Biocide produced by the soil microorganism, *Streptomyces avermitilis*. It was supplied by Syngenta Co.

**2- Tested Animals :**

Adult individuals of the brown garden snail *Eobania vermiculata* were collected from the infested ornamental plants at Giza Governorate. Animals were transferred to laboratory, kept in glass boxes and fed on fresh lettuce leaves (El-Deeb *et al.*, 2003). For each treatment, 40 healthy animals were allocated and divided into four replicates, each of 10 individuals.

**3- Contact Treatment:**

Animals were treated with sub-lethal concentration ($^1/_4$ $LC_{50}$) of each compound using thin layer film technique according to Ascher and Mirian (1981), as the tested concentration i.e. 0.7% for Neemix and 0.5% for Vertemic (Gabr *et al.*, 2005) was applied in Petri-dishes using water. Two ml of each compound concentration were spread on inner surface of a Petri-dish by moving the dish gently in circles. Water was evaporated under room conditions in a few minutes leaving a thin layer film of the tested compounds. Animals were exposed to the candidate concentration of both tested compounds for 72h. A parallel control test was conducted using plain water.

**4- Biochemical Studies:**

Effect of sub-lethal concentration ($^1/_4$ $LC_{50}$) of each tested compound on some enzymes activities was studied to clarify the physiological response in the land snail *Eobania vermiculata*. Aspartate aminotransferase (AST), Alanine amino transferase (ALT), total protein and total lipid were determined. Also, the oxidoreductase enzymes activities (peroxidase and catalase) were measured at different periods 1, 2, 3 and 4 days post-treatment. A parallel control test was conducted.

**4-1- Samples Preparation:** Samples were prepared according to Bergmeyer (1963). Ten animals were homogenized in homogenizer for 3 minutes with 10 ml of 0.006 M phosphate buffer, pH 7 at 1-4 °C and centrifuged at 3500 r.p.m for 10 minutes. The whole extract sample was cleaned through centrifugation several times. The extraction process takes not more than 24h under cooling.

**4-2- Determination of AST and ALT Enzymes:** The activity of Aspartate aminotranferase (AST) and Alanine aminotranferase (ALT) was determined according to the method of Reitman and Frankel (1957) using commercial reagents. This method depends on photometric determination of the concentration of oxaloacetate hydrazone

in case of AST or pyruvate hydrazone in case of ALT formed with 2, 4-dinitrophenyl hydrazine.

**4-3- Determination of Total Protein:** Colorimetric determination of total protein was done using commercial reagents according to the method of Gornall *et al.*, (1968) that based on the principle of Biuret reagent mixture consists of color reagent (copper sulphate) in alkaline medium (potassium sodium tartarate).

**4-4- Determination of Total Lipid:** Colorimetric determination of soluble lipid was carried out using commercial reagents according to the method of Zollner and Kirsch (1962).

**4-5- Determination of Peroxidase:** A direct colorimetric of guaiacol oxidation was used according to Ponting and Joslyn (1948). Absorbencies at 530 nm were read at 30 second intervals at 20 °C. The solution tested containing 2ml tissue extract, 5ml acetate buffer pH 5.4 (0.02 M), 1 ml guaiacul (0.1 M), 1 ml hydrogen peroxide (0.5 M) and 1 ml distilled water. A blank was conducted containing distilled water instead of hydrogen peroxide.

**4-6- Determination of Catalase:** A direct colorimetric was used according to the method of Saunders (1964). The solution tested containing 1 ml tissue extract, 1 ml hydrogen peroxide 0.01 M (substrate) and 5 ml phosphate buffer pH 6.8 (0.0067 M) were cooled to 0 °C, 1 ml of 2N sulphuric acid (this arrests peroxide decomposition) was added with vigorous stirring. The mixture was nitrated with 0.0005N permanganate to the first persistent pink color. Absorbencies at 530 nm were read at 30 second intervals at 20 °C. The enzyme activity was calculated as follows:

$$\text{Activity \%} = \frac{\text{Optical density of treated}}{\text{Optical density of control}} \times 100$$

## RESULTS AND DISCUSSION

**1- Effect on AST and ALT Enzymes:**

Alterations in the activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are known to be helpful in the diagnosis of hepatic disease and infracts of the heart. Data in Table 1 show the effect of sub-lethal concentration ($^1/_4$ $LC_{50}$) of the two natural compounds Neemix 4.5% (plant extract) and Vertemic 1.8% biocide on the activity of AST and ALT enzymes in the land snail *Eobania vermiculata*. Results indicate that Neemix treatment reduced the AST activity gradually with prolongation the period after treatment as the enzyme activity decreased than control with -11.8, -39.1, 48.3 and -59.3% after 1, 2, 3 and 4 days post-treatment, respectively. Concerning their effect on the ALT enzyme, the contrary occurred as the same treatment raised the enzyme activity proportionally to 16.7 and 66.7% post 1 and 2 days, then it decreased to 50 and 33.3% after 3 and 4 days, respectively than

control. Regarding Vertemic biocide, data took the same trend as AST activity reduced to -18.9, -54.6, -58.0 and -64.3% than control after 1, 2, 3 and 4 days of treatment, consecutively. On the other hand, the activity of ALT enzyme enhanced than control with 33.3 and 66.7% post 1 and 2 days from treatment while the increase was declined to 50% after 3 days compared with 16.7% post 4 days. From the previous results, it is clear that land snail *E. vermiculata* was more susceptible to Neemix compound than Vertemic as AST activity reduced to -64.3% for Neemix treatment and decreased to -60.2% for Vertemic after 4 days. Also, Neemix treatment enhanced the ALT activity to 33.3% while it increased to 16.7 only in case of Vertemic after the same period. El-Deeb *et al.*, (2003) recorded that methomyl compound decreased the AST activity to -26.7, -26.7 and - 13.3% for *Monacha obstructa* after 24, 48 and 72h post-treatment, respectively. Tilkian *et al.*, (1983) stated that the amount of AST was directly proportional to the number of cell damaged and the intervals after administration. Also, Amer *et al.*, (1994) reported that the increase of AST and ALT activity may be referred to the diffusion of this enzyme from its intracellular sites due to the damage caused by the insecticide on the sub cellular level. In contrast, the decrease of the enzyme level may be due to either: the diffusion of these enzymes from the liver to the blood and then through the kidney to outside with the urea or / and due to the decrease in its synthesis due to liver tissue disorders.

**2- Effect on Total Protein and Total Lipid:**

Plasma protein serves as source for rapid replacement of tissue proteins during tissue depletions, as buffers in acid base balance and as transporters for the constituents of the blood such as lipid, vitamins, hormones and certain enzymes. Also, lipids play extremely important roles in the normal function of cell. Not only do lipid serve as highly reduced storage forms of energy, but they also play an intimate role in the structure of cell membranes and the organelles found in the cell (Wilson, 1986).

The effect of sub-lethal concentration ($^1/_4$ $LC_{50}$) of the two tested compounds on the total protein and total lipid in *E. vermiculata* was shown in Table 2. Data showed that Neemix treatment increased total protein with 9.5 and 30.5% after 1 and 2 days but it reduced this percentage to be -6.3 and -20.0% post 3 and 4 days in comparison with control. On the other side Vertemic biocide increased total protein by 2.1, 5.3, 8.4 and 10.5% than control after 1, 2, 3 and 4 days from the treatment. Concerning total lipid, the two tested compounds took one way as total lipid percentage raised to 70.1, 66.7, 47.2 and 33.3% for Neemix after 1, 2, 3 and 4 days of treatment, consecutively while it enhanced to 98.9, 83.9, 78.2 and 52.9% with Vertemic after the same periods.

Discussing the results mentioned above, it is clear that both compounds showed the same effects on total lipid, but their effect differed on total protein specially at the third and fourth days after treatment as Neemix reduced total protein to -6.3 and -20.0% comparing with enhance effect by 8.4 and 10.5% for Vertemic, respectively. The increase in total protein could be attributed to the increased biosynthesis process occurred by high enzyme stress (Khater *et al.*, 1990). Also, El-Essely (2002) reported that the fluctuation in total protein level might be resulted from imbalance between the rate of synthesis and the rate of degradation. The depression in total lipid may be due to decline in lipid synthesizing capacity and / or due to an increase in the hydrolysis of hepatic lipid to combat the stress conditions as reported by Saxena *et al.* (1989).

**3- Effect on Peroxidase and Catalase Activities :**

The oxidoreductase enzymes peroxidase and catalase play an important role in the conversion of hydrogen peroxide $H_2O_2$ to $H_2O$. The importance of this reaction is attributes to the toxic effect of $H_2O_2$ on the life cells. $H_2O_2$ attack the unsaturated fatty acids of the cell membrane causing its oxidation and injury. Data in Table 3 revealed the effect of sub-lethal concentration ($^1/_4$ $LC_{50}$) of both tested compounds on the activity of respiratory enzymes (peroxidase and catalase). Results indicated that peroxidase activity decreased gradually with increase the period after treatment with the two tested compounds. Peroxidase activity recorded 75.7 & 98.3, 65.7 & 80.1, 46.4 & 74.0 and 32.6 & 60.8% increase than normal levels for Neemix and Vertemic after 1, 2, 3 and 4 days post-treatment, consecutively. Neemix proved more effective on peroxidase than Vertemic as it reduced the enzyme activity with high ratios at the all tested periods. Concerning catalase enzyme it showed adverse pattern as it's activity increased gradually with 111.9 & 153.1, 139.2 & 156.6 and 167.8, 183.9% for Neemix and Vertemic compounds after 1, 2, 3 days post-treatment, respectively. Thereafter, it decreased to 105.6 and 115.4 at fourth day for both compounds, consecutively to be approximately near the control range (100%). Abd El-Aal (2004) found that Niclosamide molluscicide increased the activity of peroxidase and catalase after 24h with 183.3% and 137.5%, respectively, then, peroxidase activity decreased gradually to reach 64.1% after 96h while catalase activity increased to reached its maximum 150% after 72h, thereafter decreased to 103.1% post 96h. Also our results are in agreement with the finding obtained by El-Deeb *et al.*, (2002). From the previous results it could be concluded that Neemix was more effective than Vertemic against *E. vermiculata* in all tested biochemical parameters. This variation in the toxic effect may be due to type of compound and its chemical structure.

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Table 1. Effect of $^1/_4$ $LC_{50}$ of Neemix and Vertemic on the activity of AST and ALT enzymes in *Eobania vermiculata* at different periods post-treatment.

| Tested compound | Enzyme | Control | Days after treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | | 2 | | 3 | | 4 | |
| | | Mean ± S.E | Mean ± S.E | % diff. | Mean ± S.E | % diff. | Mean ± S.E | % diff. | Mean ± S.E | % diff. |
| Neemix 4.5% | AST (U/L) | 23.8 ± 5.0 | 21.0 ± 1.2 | - 11.8 | 14.5 ± 1.9 | - 39.1 | 12.3 ± 1.4 | - 48.3** | 8.5 ± 0.9 | - 64.3** |
| | ALT (U/L) | 6.0 ± 1.2 | 7.0 ± 1.9 | 16.7 | 10.0 ± 1.2 | 66.7** | 9.0 ± 1.6 | 50.0** | 8.0 ± 1.6 | 33.3 |
| Vertemic 1.8% | AST (U/L) | 23.8 ± 5.0 | 19.3 ± 1.4 | - 18.9 | 10.8 ± 1.9 | - 54.6** | 10.0 ± 1.2 | - 58.0* | 8.5 ± 0.9 | - 60.2** |
| | ALT (U/L) | 6.0 ± 1.2 | 8.0 ± 1.6 | 33.3 | 10.0 ± 1.2 | 66.7** | 9.0 ± 1.0 | 50.0** | 7.0 ± 1.9 | 16.7 |

* Significant ** highly significant

Table 2. Effect of $^1/_4$ $LC_{50}$ of Neemix and Vertemic on theTotal protein and Total lipid in *Eobania vermiculata* at different periods post-treatment.

| Tested compound | Biochemical parameter | Control | Days after treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | | 2 | | 3 | | 4 | |
| | | Mean ± S.E | Mean ± S.E | % diff. | Mean ± S.E | % diff. | Mean ± S.E | % diff. | Mean ± S.E | % diff. |
| Neemix 4.5% | Total protein (g/100ml) | 9.5 ± 0.6 | 10.4 ± 1.0 | 9.5 | 12.4 ± 1.1 | 30.5* | 8.9 ± 1.1 | - 6.3 | 7.6 ± 0.7 | - 20.2* |
| | Total lipid (g/100ml) | 0.9 ± 0.1 | 1.5 ± 0.03 | 70.1** | 1.5 ± 0.2 | 66.7** | 1.3 ± 0.1 | 47.2* | 1.2 ± 0.1 | 33.3* |
| Vertemic 1.8% | Total protein (g/100ml) | 9.5 ± 0.6 | 9.7 ± 1.7 | 2.1 | 10.0 ± 0.2 | 5.3 | 10.3 ± 0.7 | 8.4 | 10.5 ± 0.8 | 10.5 |
| | Total lipid (g/100ml) | 0.9 ± 0.1 | 1.7 ± 0.3 | 98.9** | 1.6 ± 0.1 | 83.9** | 1.6 ± 0.2 | 78.2* | 1.3 ± 0.2 | 52.9* |

* Significant ** highly significant.

Table 3. Effect of $^1/_4$ $LC_{50}$ of Neemix and Vertemic on peroxidase and catalase activities in *Eobania vermiculata* at different periods post-treatment.

| Tested compound | Enzyme | Control | | Days after treatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | | 2 | | 3 | | 4 | |
| | | O.D | % activity | O.D | % activity | O.D | % activity | O.D | % activity | O.D | % activity |
| Neemix 4.5% | Peroxidase | 0.181 | 100 | 0.137 | 75.7* | 0.119 | 65.7** | 0.84 | 46.4** | 0.059 | 32.6** |
| | Catalase | 0.143 | 100 | 0.160 | 111.9* | 0.199 | 139.2* | 0.240 | 167.8* | 0.151 | 105.6 |
| Vertemic 1.8% | Peroxidase | 0.181 | 100 | 0.178 | 98.3 | 0.145 | 80.1 | 0.134 | 74.0* | 0.11 | 60.8** |
| | Catalase | 0.143 | 100 | 0.219 | 153.1* | 0.224 | 156.6** | 0.263 | 183.9** | 0.165 | 115.4 |

O.D = Optical densit

* Significant. ** highly significant.

# التأثيرات البيوكيميائية لإثنين من المبيدات الطبيعية علي قوقع الحدائق البني

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*معهد بحوث وقاية النباتات – مركز البحوث الزراعية– الدقي – جيزة*

تم دراسة التأثيرات البيوكيميائية لأثنين من المركبات الطبيعية هما نيميكس ٤,٥% (مستخلص نباتي) ، فيرتيميك ١,٨% (مركب حيوي ابامكتين) علي قوقع الحـــــدائق البني *Eobania vermiculata* . تم معاملة القواقع بتركيز تحت مميت ($^1/_4$ $LC_{50}$) لكل مركب عن طريق الملامسة بإستخدام طبقة رقيقة من كل مركب . تم تقدير نشاط أنزيمات الكبد AST , ALT وكذلك البروتين الكلي والليبيدات الكلية، كذلك تم قياس نشاط أنزيمات التنفس البيروكسيديز والكتاليز بعد يوم ويومين وثلاثة وأربعة أيام من المعاملة. أظهرت النتائج أن كلا من المركبين المختبرين أحدثا إنخفاضا تدريجيا في نشاط أنزيم AST كلما زادت الفترة بعد المعاملة بينما حدث العكس مع إنزيم ALT حيث أرتفع نشاط الإنزيم تدريجيا بالنسبة للمركبين مقارنة بالكنترول. وبالنسبة لليبيدات الكلية فإن كلا المركبين كان لهما نفس التأثير حيث أرتفعت نسبة الليبيدات بينما كان للمركبين تأثير مختلف علي نسبة البروتين الكلي خاصة فيْ اليوم الثالث والرابع حيث أحدث مركب النيميكس إنخفاضا ملحوظا في نسبة البروتين مقارنة بزيادة ملموسة في حالة مركب الفيرتيميك . أما بالنسبة لأنزيم البروكسيديز فقد حدث إنخفاض تدريجي مع زيادة الفترة بعد المعاملة بالنسبة للمركبين علي السواء. أما أنزيم الكتاليز فقد أخذ إتجاه معاكس حيث زاد نشاطه بعد اليوم الأول والثاني والثالث ، بينما إنخفض نشاطه في اليوم الرابع لكلا المركبين وبصفة عامة كان مركب النيميكس أكثر تأثيرا من الفيرتيميك علي جميع العوامل البيوكيميائية المختبرة في القوقع .