SNAIL CONTROL WITH DIFFERENT AND UNSPECIFIC PESTICIDES
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ABSTRACT

Brown snails (Eobania vermiculata), one of the most abundant gastropod of Egyptian farms, which causes many damages for economic crops and ornamentals plants. Methomyl, pirimicarb, and oxamyl as a symbol of carbamates pesticides group, malathion as a symbol of organophosphates pesticides group, thiamethoxam, imidacloprid, and acetamiprid as a neonicotinoid pesticides group symbol, lambda-cyhalothrin as a pyrethroid pesticides group symbol, pymetrozine as a symbol of Antifeedant pesticides group, and the symbols of fungicide pesticides group were carbandazim, and copper sulfate were used against snails, by baiting techniques. Bioassay results showed high activity of methomyl, oxamyl, acetamiprid and lambda-cyhalothrin against E. vermiculata, all over the different times of exposure, after 24 hrs the LC$_{50}$ values were = 0.259, 0.358, 1.049 and 5.976 % respectively, the LC$_{50}$ values after 48 hrs were = 0.058, 0.90, 0.37 and 0.305 % respectively, the LC$_{50}$ values after 72 hrs = 0.024, 0.023, 0.129 and 0.187 % respectively, and the LC$_{50}$ values after 96 hrs = 0.024, 0.004, 0.039 and 0.047 % respectively. Biochemical studies of the four mentioned pesticides showed significantly inhibition effect for snail enzymes: acetylcholinesterase (AChE), carboxyl esterase (CaE), catalase, glutathione-s-transferase (GST) and polyphenoloxidase (PPO). So, these pesticides is promising as a new identification as molluscicides, available in the markets with suitable prices for users, instead of classical molluscicides, which became rarely in the markets and very expensive.

Keywords: Brown snail; Eobania vermiculata; Pesticides; Molluscicides; Bioassay; Biochemical studies.

INTRODUCTION

Snail is a common name that is applied most often to land snails, terrestrial pulmonate gastropod molluscs. Snails are among the most bothersome pests in many gardens and landscapes (Dreistadt et al., 1994 and Flint 1998).

Snails are divided into two parts; Marin snails and Terrestrial snails, both of these divisions have many thousands species of snails (Valdés Á.and Warén A. 2005, Bouchet et al., 2005). Adult brown garden snails lay an average of 80 spherical, pearly white eggs at a time into a hole in the soil. They can lay eggs up to 6 times a year, and it spends about 2 years to get mature. Snails are mostly active at night and on cloudy or foggy days. On sunny days they seek hiding places out of the heat and bright light. The brown garden snail, Eobania vermiculata, is the most common snail causing problems in gardens. It was introduced from France during the 1850s for use as food (Valdés Á. and Warén A. 2005).

Our work aims to estimate different types of pesticides as molluscicides. Thirteen different pesticides were chosen, because of its
Materials and Methods

Materials

Tested pesticides
2- Pirimicarb: (2-(dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate), (Aphox).
3- Oxamyl: (methyl 2-(dimethylamino)-N-[[((methylamino) carbonyl] oxy]2-oxoethanimidothioate), (Vydate).
4- Malathion: (diethyl [(dimethoxyphosphinothioyl) thio] butanedioate), (Malathion).
5- Thiamethoxam: (3-[[2-chloro-5-thiazolyl)methyl]tetrahydro-5-methyl-N-nitro-4H-1,3,5-oxadiazin-4-imine), (Actara).
6- Acetamiprid: ([E]-4, 5-dihydro-6-methyl-4-(3-pyridylmethylenamino) -
1, 2, 4-triazin-3(2H)-one), (Pymetrozine).
7- Copper sulfate (copper(2+) sulfate (1:1)), (Blue stone).
8- Beauveria bassiana, (Bio – fly).

Tested snails

Specimens of the herbivorous land snail, brown garden snail Eobania vermiculata (Müller) (1.53± 0.076 mm in shell diameter and 0.93± 0.007 g in body weight), were collected during spring and autumn seasons from untreated area at El-Maamora gardens, Alexandria, Egypt. They were kept for three weeks in aerated cages (40 × 30 ×30 cm, with 100 individuals per cage) for acclimatization under laboratory conditions (26 - 30 °C and 63-64 RH), and fed on lettuce leaves ad-libitum. before the experiments.

Methods

Bioassay technique

The snails (E. vermiculata) were kept in a glass aquarium and were feed lettuce ad libitum. The aquarium was kept at laboratory conditions and the animals were kept moist by sprinkling with water every day. The aquarium was noticed daily, food was offered as it requires from the start of the experiment, and the glass aquarium was cleaned twice a week.

Number of dead snails of the tenth in petry dishes with filter paper (Whatman No.1) was recorded daily after 24, 48, 72, 96,120 and144 hours of adding 1 ml of different concentrations of tested compounds in three
replicates. Dead snails loss its response to a thin stainless-steel needle (WHO, 1965), and the cumulative mortality percentages were estimated and subjected to probit analysis and effectiveness were expressed as values.

**Biochemical studies**

**Homogenate preparation**

The remaining of digestive glands from each group was weighted and was homogenated with 10 volumes (w/v) of ice-cold saline solution using a polytron homogenizer (Tekmar tissumizer) for 30 seconds. The homogenates were centrifuged at 5000 rpm for 30 min at 4°C using IEC - CRU 5000 centrifuge, Model 2345. The supernatants were used as source of the following enzymes: Acetylcholine esterase (AChE), Catalase (CAT), Polyphenoloxidase (PPO), Carboxylesterase (CaE), and Glutathione - S - transferase (GST) activities as well as total protein content, (Radwan et al.,2008).

Total protein was determined according to the method described by (Lowry et al.,1951).

* **Glutathione- S-transferase (GST) activity assay:** was determined according to the method of (Vessey and Boyer 1984).
* **Catalase (CAT) activity assay:** was determined according to the method of (Beers and Sizer 1952).

\[
2\text{H}_2\text{O}_2 \rightarrow \text{CAT} \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\]

Catalase is an enzyme that scavenges hydrogen peroxide and converts it to water and molecular oxygen.

* **Acetyl cholinesterase (AChE) activity assay:** was described in details by (Ellman et al.,1961).
* **Polyphenol Oxidase (PPO) activity assay:** is a bifunctional, copper-containing oxidase having both catecholase and cresolase activity (Malmström and Rydén 1968).
* **Carboxyl esterase (CaE) activity assay:** was measured according to the method was described in detail by (Chanda et al.,1997).

**Statistical analysis**

The mortality percentages data were expressed as mean ± standard deviation; the data were analyzed by probit analysis to obtain LC<sub>50</sub> and its upper and lower limits for all treatments at 95% confidence limits, slope and intercept values of toxicity curves for all treatments were listed ± standard error Finney et al., (1971), while as the biochemical data were expressed as mean ± standard error; the data were analyzed using ANOVA test (Sedlak and Lindsey 1968).

**RESULTS AND DISCUSSION**

**Molluscicidal activity of tested pesticides**

The brown snail (Eobania vermiculata) was used as a target to evaluate the toxic effect of different pesticides, methomyl, pirimicarb, and oxamyl as carbamate pesticides, malathion as an organophosphate pesticides, thiamethoxam, imidachloprid, and acetamiprid as neonicotinoid
pesticides, lambda-cyhalothrin as a pyrethroid pesticides, pymetrozine as an antifeedant, a carbendazim, and copper sulfate as fungicides, however, *beauveria bassiana* as a bio-insecticides. The toxicity assays were presented in Table (1) after 24, 48, 72, and 96 hrs of treatment and the LC$_{50}$ values were calculated.

The mortality data of methomyl are proved that a significantly increased by increasing the concentration; also, the mortality was increased by increasing the time of the exposure. Methomyl was the most potent compound after 24, 48 and 72 hrs of application with LC$_{50}$ of 0.259, 0.058 and 0.024 as % concentration, followed by oxamyl and bio-insecticide (*Beauveria* sp.) LC$_{50}$ were 0.004 and 0.009 % concentration after 96 hrs of treatment. The data obtained is in agree with which found by Radwan *et al.*, (2008), that methomyl exhibited greater efficacy than other tested compounds. Also, data is agree with which found by Gadalah (2013), that carbamates (methomyl and oxamyl) was the most potent compounds followed by neonicotinoid (acetamiprid), also, bio-insecticide (*Beauveria* sp.) showed a good efficacy to control terrestrial snails. The mortality data of pirimicarb$_{DG}$ or pirimicarb$_{WG}$ evidenced that was significantly increased with increasing the concentration of compounds and time of the exposure.

Data in table (1) show clearly that there are different molluscicidal effects ranged from the most potent one are carbamates compounds followed by bio-insecticide, neonicotinoid, antifeedant, pyrethroid and organophosphates which agree with which found by Fouad *et al.*, (2004), who explained the differences in toxicity of tested pesticides against snails could be due to the basis of its chemical structure.

Our data was in agree with which found by Laguerre *et al.*, (2009), that toxicity of malathion is increased by time and concentration that LC$_{50}$ was 9.418 after 24 hrs of treatment, while it was 1.04, 0.485 and 0.069 after 48, 72 and 96 hrs of treatment.

Fouad *et al.*, (2004) tested the molluscicidal effect of sumithion as an organophosphate, they found that molluscicidal effects of tested pesticide ranged between death, hibernation, and normal state with different concentrations and different time of exposure for that compound.

Data in Fig (1) show the potency of LC$_{25}$ and LC$_{50}$ values of oxamyl, methomyl, acetamiprid and lambda-cyhalothrin as inhibited dose for different type of target enzymes activity of acetylcholinesterase (AChE) Fig (1.a), carboxylesterase (CaE) Fig (1.b), catalase Fig (1.c), glutathione-s-transferase (GST) Fig (1.d) and polyphenoloxidase (PPO) Fig (1.e) in brown snail *Eobania vermiculata*. The data show clearly that most of the used insecticides are not dose dependant to inhibit different type of tested enzymes and also, most of them can inhibit more than one target inside the effected body of the snail. This results is in agree with which found by Laguerre *et al.*, (2009). However, each compound is clearly to be has main target to effect by it can effect other targets which clear in the effect of methomyl to acetylcholinestrase and oxamyl, acetamiprid and lambda-cyhalothrin effect against catalase activity and methomyl effect against polyphenoloxidase. Our results are in agreement with the results were
obtained by Cacciatore et al., (2012), Ahamed et al., (2012) and Kristoff et al. (2012). Also, there are some targets clear to be a general target or non-specific target to inhibit as show with GST target effected by oxamyl, methomyl, acetamiprid and lambda-cyhalothrin compound no concentration dependant target. Khangarot and Das (2010),, Sawasdee et al., (2011) and Sangita & Khangarot, (2011).

Table (1); Toxicity of different pesticides against the brown snails (Eobania vermiculata) after 24, 48, 72, and 96 hr of the exposure.

<table>
<thead>
<tr>
<th>Pesticide class</th>
<th>Pesticide</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>LC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methomyl</td>
<td>0.259</td>
<td>0.058</td>
<td>0.024</td>
<td>0.024</td>
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<tr>
<td>Carbamate</td>
<td>Pirimicarb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.30</td>
<td>0.775</td>
<td>0.190</td>
<td>0.126</td>
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<tr>
<td>Neonicotinoid</td>
<td>Pirimicarb&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.537</td>
<td>1.298</td>
<td>0.498</td>
<td>0.092</td>
</tr>
<tr>
<td>Neonicotinoid</td>
<td>Oxamyl</td>
<td>0.358</td>
<td>0.090</td>
<td>0.023</td>
<td>0.004</td>
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<tr>
<td>Neonicotinoid</td>
<td>Thiamethoxam</td>
<td>3.354</td>
<td>2.697</td>
<td>1.776</td>
<td>0.664</td>
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<tr>
<td>Neonicotinoid</td>
<td>Imidaclopride</td>
<td>2.122</td>
<td>0.597</td>
<td>0.462</td>
<td>0.074</td>
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<tr>
<td>Neonicotinoid</td>
<td>Acetamiprid</td>
<td>1.049</td>
<td>0.378</td>
<td>0.129</td>
<td>0.039</td>
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<tr>
<td>Antifeedant</td>
<td>Pymetrozine</td>
<td>5.080</td>
<td>2.223</td>
<td>1.782</td>
<td>1.139</td>
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<tr>
<td>Fungicide</td>
<td>Carbendazim</td>
<td>23.39</td>
<td>4.617</td>
<td>4.330</td>
<td>1.374</td>
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<tr>
<td>Fungicide</td>
<td>Copper sulfate</td>
<td>1.621</td>
<td>1.139</td>
<td>0.734</td>
<td>0.672</td>
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<tr>
<td>Pyrethroid</td>
<td>Lambda-cyhalothrin</td>
<td>5.976</td>
<td>0.305</td>
<td>0.187</td>
<td>0.047</td>
</tr>
<tr>
<td>Organophosphate</td>
<td>Malathion</td>
<td>9.418</td>
<td>1.040</td>
<td>0.485</td>
<td>0.069</td>
</tr>
<tr>
<td>Bio-insecticide</td>
<td>Beauveria bassiana</td>
<td>0.479</td>
<td>0.216</td>
<td>0.042</td>
<td>0.009</td>
</tr>
</tbody>
</table>

*<sup>a</sup> = LC<sub>50</sub>: The lethal concentration causing 50% mortality.
Fig (1): Effect of LC$_{25}$ and LC$_{50}$ values of the most active pesticides on AChE (a), CaE (b), Catalase (c), GST (d), and PPO(e) activity in brown snails (E. vermiculata) after 96 hrs of exposure for pesticides.
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مكافحة القوارض الأرضية بمبيدات مختلفة وغير مخصصة.

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قام كيمياء وتقنية المبيدات بكلية الزراعة جامعة الإسكندرية

أجري هذا البحث خلال عام 2015-2014، بهدف إيجاد مبيدات قواصي بديلة للبيدات المخصصة المعروفة والتي أصبحت نادرة الوجود في الأسواق، لارتفاع سعرها، وإنخفاض كفاءتها، وتأثيرها السلبي على البيئة وعلى الكائنات الناشئة، لإستخدامها في مكافحة القوارض البيئية في قرى معمور وشاطئ، في الإسكندرية.

وقد تم اختيار ثلاثة عشر مبيد من المبيدات المتوفرة في السوق، والتي تستخدم في غراس الدات، Agrinate: Lambda, Pymetrozine, Vydate, Malatox, Bio-fly, Sunchlorbide, Setar. والمكافحة الأخرى غير القواص، لتعبر على مدى قدرتها على مكافحة القوارض وهي: Actara, Occidor, Copper sulfate, Pirimicarb, Aphox. وقد تم اختبار تأثير هذه المبيدات على القوارض البيئية التي تم تجميعها من مكان الإصابة (محمور الشاطئ)، بواسطة الاختبارات المعروفة وهي تنقسم إلى جزئين:

أولاً: التقييم الحيوي

والذلك تم قدر الفعل المثال للمبيدات التي تم اختيارها لمكافحة القوارض، وقد تم الاختبار تحت الظروف المعملية، وبدع قدرات مختلفة من التعوض للبيدات، حيث كانت القيم هي (24، 48، 72) ساعه، فقد أظهرت التجربة أن الLC 50 ساعه كانت أقل قيم Lambda, Setar, Vydate, Agrinate عن قيم المبيدات المختبر في كل القيم.

ثانيًا: التقييم الكميوجي

والذلك عن طريق تتب تشابه الإنزيمات الحيوية المستخلصة من جسم القوارض بعد تعرضها للبيدات المذكورة من cadre. في LC 100، لأمراض المبيدات السائدة اليوم. هذه الإنزيمات هي: Acetylcholinesterase, Carboxylesterase, Catalase, Glutathione-S-transferase, Polyphenoloxidase.

وأظهرت نتائج الدراسة الحيوية لتشابه الإنزيمات الحيوية التي تعرضت للمبيدات الأرضية المختارة، مما أظهرت تأثيرات التقييم الحيوي من فعاليتها في مكافحة القوارض. 

ومن الممكن أن نستخلص، أن هناك بعض المبيدات المحفزة بالفعل والتي لها قدرة على مكافحة القوارض البيئية.