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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, imidachloprid, 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, o.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: acetylcholinestrase (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 availability in the market with suitable prices; agrinate, aphox, pirimicarb^{WG}, pirimicarb^{DG}, copper sulfate, occidor, actara, setar, sunchlorobide, bio-fly, malatox, vydate, pymetrozine, and lambda., to make a contribution that each compound can act on different targets inside animal body.

MATERIALS AND METHODS

Materials

Tested pesticides

1- Methomyl: (methylN-[[(methylamino) carbonyl] oxy] ethanimidothioate)., (Agrinate).
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-4*H*-1,3,5-oxadiazin-4-imine).,(Actara).
6-Acetamiprid((*E*)-*N*-[(6-chloro-3-pyridinyl)methyl]-N'-cyano-N-methylethanimidamide). ,(Setar).
7-Imidachloprid(1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine)., (Sunchlorobide).
8- Lambda-cyhalothrin([1 (*S**),3 (*Z*)]-(±)-cyano(3-phenoxyphenyl)methyl3-(2-chloro3,3,3trifluoro1propenyl)2,2dimethylcyclopropanecarboxylate),(Lambda).
9- Pymetrozine: ((*E*) - 4, 5-dihydro-6-methyl-4-(3-pyridylmethyleneamino) -1, 2, 4-triazin-3(2*H*)-one)., (Pymetrozine).
10- Copper sulfate(copper(2+) sulfate (1:1)).,(Blue stone).
11- Carbandazim: (methyl 1*H*-benzimidazol-2-ylcarbamate)., (Occidor).
12- *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 ℃ 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 (Whattman 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℃ 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 (G S T) 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).

$2\ H_2O_2 \rightarrow CAT \rightarrow 2H_2O+O_2$

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 percentags data were expressed as mean ± standard deviation; the data were analyzed by probit analysis to obtain LC_{50} 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 biochmical 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 carbandazim, 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 acetylcholinestrase (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.

Pesticide class	Pesticide	LC_{50}[a] (%) 24 hr	LC_{50}[a] (%) 48 hr	LC_{50}[a] (%) 72 hr	LC_{50}[a] (%) 96 hr
Carbamate	Methomyl	0.259	0.058	0.024	0.024
	Pirimicarb[DG]	23.30	0.775	0.190	0.126
	Pirimicarb[WG]	9.537	1.298	0.498	0.092
	Oxamyl	0.358	0.090	0.023	0.004
Neonicotinoid	Thiamethoxam	3.354	2.697	1.776	0.664
	Imidaclpride	2.122	0.597	0.462	0.074
	Acetamiprid	1.049	0.378	0.129	0.039
Antifeedant	Pymetrozine	5.080	2.223	1.782	1.139
Fungicide	Carbandazim	23.39	4.617	4.330	1.374
	Copper sulfate	1.621	1.139	0.734	0.672
Pyrethroid	Lambda-cyhalothrin	5.976	0.305	0.187	0.047
Organophosphate	Malathion	9.418	1.040	0.485	0.069
Bio-insecticide	*Beauveria bassiana* (after120hr)	0.479	0.216	0.042	0.009

***a = LC50 : The lethal concentration causing 50% mortality.**

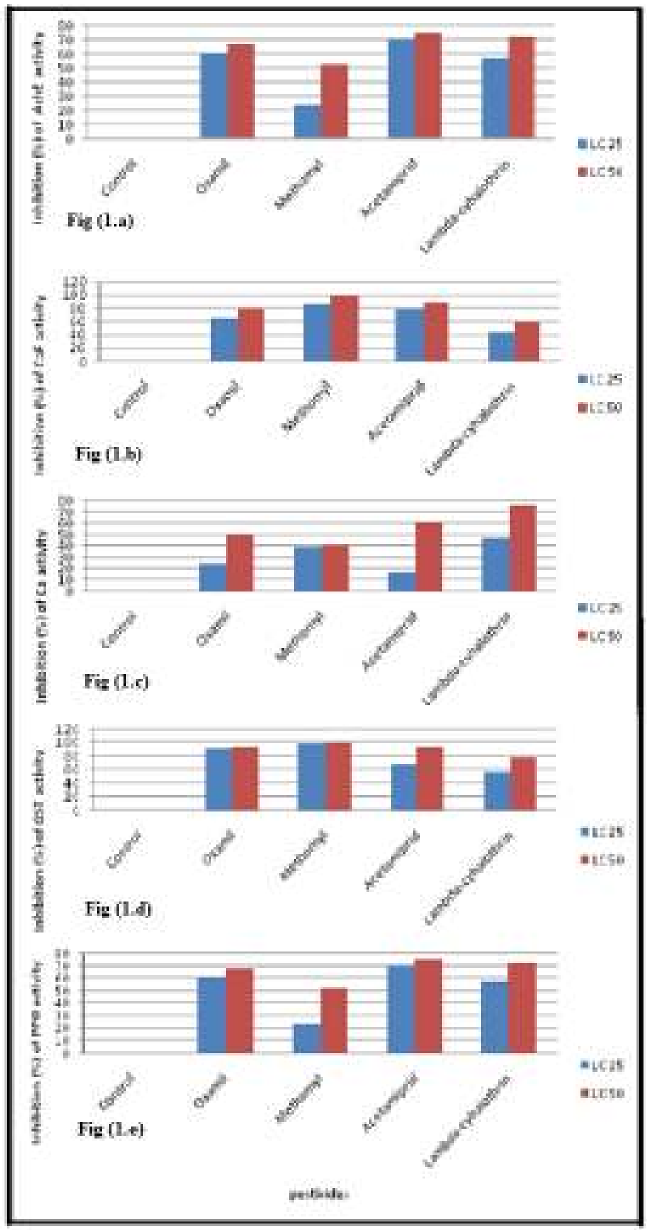

Fig (1); Effect of LC_{25} and LC_{50} values of the most active pesticides on AChE (a), CaE (b), Catalase (c), GST (d), and PPO(e) activity in brown snails (*E. vermiculata*) after 96 hrs of exposure for pesticides.

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مكافحه القواقع الأرضيه بمبيدات مختلفه وغيرمتخصصه.

نادرشاكر , محمدالطاهرإبراهيم بدوى وأشرف محمد عبد الحميد حسين
قسم كيمياء وتقنيه المبيدات بكليه الزراعه جامعه الإسكندريه

أجرى هذا البحث خلال عامي ٢٠١٤، ٢٠١٥ بهدف إيجاد مبيدات قواقع بديله للمبيدات المتخصصه المعروفه والتي أصبحت نادرة الوجود في الأسواق , لأرتفاع سعرها , وإنخفاض كفائتها , وتأثيرها السلبي علي البيئه وعلي الكائنات النافعه Non target organisms , لإستخدامها في مكافحه القواقع البنيه في قريه معموره الشاطيء السياحيه بالإسكندريه .

ولقد تم إختيار ثلاثه عشرمبيدا من المبيدات المتاحه في السوق - والتي تستخدم في أغراض المكافحه الأخري غير القواقع- للتعرف علي مدي قدرتها علي مكافحه القواقع وهي :Agrinate , Aphox , Pirimicarb , Copper sulfate , Occidor , Actara , Setar, Sunchlorobide , Bio-fly , Malatox , Vydate , Pymetrozine, Lambda .

وقد تم إختبارتأثيرهذه المبيدات علي القواقع البنيه التي تم تجميعها من مكان الإصابه (معموره الشاطيء) , بواسطه الإختبارات المعروفه وهي تنقسم إلي جزئين :

أولا : التقييم الحيوي

وذلك لتقدير الفعل السام للمبيدات التي تم إختيارها لمكافحه القواقع ، وقد تم الإختبار تحت الظروف المعمليه وبعد فترات مختلفه من التعرض للمبيدات , حيث كانت الفترات هي (٢٤, ٤٨ , ٧٢ ,٩٦) ساعه ،لقد أظهرت التجارب أن ال Lambda , Setar , Vydate , Agrinate لهم أقل قيم $LC_{٥٠}$ عن بقيه المبيدات المختبره في كل الفترات .

ثانيا : التقييم الكيموحيوي

وذلك عن طريق تتبع نشاط الإنزيمات الحيويه المستخلصه من جسم القواقع بعد تعرضها لل$LC_{٥٠}$, $LC_{٢٥}$ لأنواع المبيدات السابقه الذكرلمده ٩٦ساعه للمبيدات , وهذه الإنزيمات هي:

Acetylcholinestrase , Carboxylesterase , Catalase , Glutathione-S-transferase , Polyphenoloxidase.

وأظهرت نتائج الدراسه الحيويه لنشاط الإنزيمات الختبره و التي تعرضت للمبيدات الأربعه المختاره (لما أظهرته نتائج إختبارات التقييم الحيوي من فعاليتها في مكافحه القواقع) , إنخفاض عام في نشاط هذه الإنزيمات, مما يدل علي التأثيرالمثبط لهذه المبيدات علي فسيولوجي القواقع.

ومما سبق نستخلص أن هناك بعض المبيدات المجهزه بالفعل والتي لها القدره علي مكافحه القواقع البنيه .