MOLLUSCICIDAL ACTIVITY OF SOME PESTICIDES AGAINST GLASSY CLOVER SNAIL *MONACHA OBSTRUCTA* MULLER

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Abstract

The molluscicidal activity of 5 different pesticides was tested against land snail, *Monacha obstructa* Muller in comparison with methomyl (The recommended compound). The study cleared that carbofuran compound (nematicide) was the most toxic one followed by methomyl (insecticide), pyriproxyfen (insect growth regulator), benthiocarb (herbicide) and fenthion (insecticide) while dicofol (acaricide) had the lowest effect. Also, the effects of sub-lethal concentration ($^1/_4$ LC_{50}) of carbofuran on some biochemical parameters were studied for different periods after treatment. Results indicated that the total protein level increased after 24h, then reduced gradually after 48 and 72h post-treatment comparatively with control. The level of total lipid was fluctuated whereas it decreased after 24h but it raised post 48h then, reduced after 72h. The activity of aspartate aminotransferase (AST) gradually reduced with time after treatment while contrary findings were recorded in case of alanine aminotransferase (ALT), as the same treatment enhanced the enzyme activity proportionally post 24 and 48h, then it decreased after 72h. Concerning lactic acid dehydrogenase (LDH), results showed severe reduce in its level after 24, 48 and 72h post-treatment. Also, the activity of acetylcholinestrase (AchE) was significantly reduced through the same periods.

INTRODUCTION

Land snails are injurious pests to a wide variety of vegetables, fruits, ornamental flowers and shrubs (Miller *et al.*, 1988). These animals attack plants at their different growth stages and reduce their yields. Recently, the terrestrial snails became an economic serious pest in several governorates in Egypt. Therefore, the present study aims to test and evaluate the molluscicidal activity and biochemical effects of certain pesticides against the glassy clover snail, *Monacha obstructa*, which is considered one of the most common species in Egypt, to introduce some effective compounds to control their numbers under the injury level.

MATERIALS AND METHODS

1- Pesticides Used:

a - Admeral 10% EC.

Common name: Pyriproxyfen.

Chemical name: 4- phenoxyphenyl (Rs) -2- (2- pyridyloxy) propyl ether.

Action : Insect growth regulator.

b- Lebaycide 50% EC.

Common name: Fenthion, mercaptophos.

Chemical name: 0,0-dimethyl -0-[3- methyl-4-(methyl thio) phenyl] phosphorothioate.

Action : Insecticide.

c- Kelthane 18.5% EC.

Common name: Dicofol.

Chemical name: 2, 2, 2-bichloro- 1, 1- bis (4- chlorophenyl) ethanol.

Action : Acaricide.

d- Saturn 50% EC.

Common name: Benthiocarb.

Chemical name: S - 4 chlorobenzyl diethyl(thiocarbamate).

Action: Herbicide.

e - **Furadan 10% G.**

Common name: Carbofuran.

Chemical name: 2, 3 -dihydro -2, 2-dimethyl benzofuran -7- yl methyl carbamate.

Action : Nematicide.

f - **Newmyl 20% SL.**

Common name: Methomyl.

Chemical name: S- methyl N- (methyl carbamoyloxy) thioacetimidate.

Action : Insecticide.

2- Tested Animals:

Adult individuals of glassy clover snail, *Monacha obstructa*, were collected from the infested Egyptian clover fields at Giza Governorate. Animals were transferred to laboratory, kept in glass boxes and fed on fresh lettuce leaves. For each treatment, 40 healthy animals were allocated and divided into four replicates, (10 for each).

3- Toxicity Tests

The toxic effects of the tested compounds were evaluated using thin layer film technique according to Ascher and Mirian (1981). Serial concentrations for each tested compound (1500 - 7500 ppm for pyriproxyfen, 3000 - 15000 ppm for benthiocarb, 7400 - 74000 ppm for dicofol, 5000 - 50000 ppm for fenthion, 500 - 6000 ppm for carbofuran and 400 - 1600 ppm for methomyl) were applied in Petri- dishes using water. Two ml of each concentration were spreaded on the inner surface of the 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 concentrations of each tested compound for 72h. A parallel control test was conducted using pesticide-free water. The killed animals were daily counted. The corrected mortality rates as well as LC_{50} & LC_{99} values were estimated for each tested compound according to Finney (1971).

4- Biochemical Tests

The biochemical response of *M. obstructa* to sub-lethal concentration of carbofuran (which proved to be the most toxic compound) was studied. Animals were treated with $^1/_4$ LC_{50} of the tested compound using the same contact technique mentioned before.

4-1- Samples Preparation

Samples were prepared according to Bergmeyer (1963). Ten animals were homogenized for 3 minutes with 10 ml of phosphate buffer, pH7 at 1- 4°C and centrifuged at 3500 r. p. m for 10 minutes.

4-2- Determination of Total Proteins and Lipids

Total proteins were colorimetrically determined according to Gornall *el al.* (1968) while total lipids were assayed by the method of Zollner and Kirsch (1962).

4-3-Determination of AST and ALT

The activity of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) was determined according to the method of Reitman and Frankel (1957) using commercial reagents.

4-4- Determination of LDH

Lactic acid dehydrogenase (LDH) was assayed using the colorimetric method described by Cabaud and Wroblewski (1958).

4-5-Determination of AchE

Acetyl cholinesterase (AchE) was determined according to Ellman *et al.* (1961).

RESULTS AND DISCUSSION

1- Molluscicidal Effect

Data in Table (1) represented the response of glassy clover snail, *Monacha obstructa*, to different pesticides in comparison with methomyl (The recommended compound). Results show that carbofuran compound was the most toxic one whereas its LC_{50} value was 0.18% followed by methomyl 0.39%, pyriproxyfen 0.49%, benthiocarb 0.90% and fenthion 1.54% while dicofol was the lowest effective one as its LC_{50} value was 4.05%. It means that *M. obstructa* was more susceptible to carbofuran than the other tested compounds. On the contrary it was more tolerant to dicofol. The differentiation of the susceptibility level of *M. obstructa* to the tested

pesticides may be due to the type of compound (chemical structure) and the physiological state of snail e.g., enzymatic system. El-Deeb *et al.* (2003) found that methomyl was more toxic than diazinon compound against both land snail species *M. obstructa* and *E. vermiculata.*

2- Biochemical Response

The biochemical impacts of carbofuran compound (which proved to be the most effective one) were studied on some biochemical parameters of the tested animals..

2-1- Total Protein and Total Lipid

Plasma protein serves as a 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 lipids, vitamins, hormones and certain enzymes. Also, lipids play extremely important roles in the normal function of cell. Not only do lipids 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 (Warnick and Carter, 1972). Effect of sub-lethal dose ($^1/_4$ LC_{50}) of carbofuran compound on total protein and total lipid in *M. obstructa* is shown in Table (2). Data cleared that carbofuran treatment increased total protein up to 18.3% after 24h from treatment, while it reduced this value to be -10 and -23.3 post 48 and 72h, respectively in comparison to control. Regarding total lipid, vice-versa occurred as it decreased with -53.0% after 24h but it raised up to 39.2% after 48h then, reduced to -76.1% after 72h comparatively to control. The previous data proved that the fluctuation in the level of both total protein and total lipid might be resulted from imbalance between the rate of synthesis and rate of degradation. Khater *et al.* (1990) mentioned that the increase in total protein could be attributed to the increased biosynthesis process occurred by high enzyme stress. Also, Saxena *et al.* (1989) reported that 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.

2-2- Enzymes Activity

The response of different enzymes i.e. AST, ALT, LDH and AchE to treatment with $^1/_4$ LC_{50} of carbofuran was presented in Table (3). Concerning the activity of liver enzymes AST and ALT, results proved that AST activity reduced gradually with prolongation the period after treatment as it decreased than control with -37.5, -18.8 and 12.5% after 24, 48 and 72h post-treatment, respectively. The contrary occurred in case of ALT as the same treatment enhanced the enzyme activity proportionally to 36.5 and 78.8% post 24 and 48h, then it decreased to 73.0% after 72h than control. Similar results were observed by Khidr *et al.* (2005). Alteration in the activity of AST

and ALT are known to be helpful in the diagnosis of hepatic disease and infarcts of the heart. 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 enzymes from its intracellular sites due to 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.

Regarding the lactic acid dehydrogenase (LDH), results showed that its level reduced with -16.7, -57.8 and -26.5% after 24, 48 and 72h from treatment, consecutively than control. LDH is an enzyme concerned with the reduction in the presence of reduced diphosphonucleotide (DPNH) of alpha-kito and alpha gamma-dikito acids. LDH activity of serum, serous effusions and cerebrospinal fluid may be measured by the reduction in the presence of DPNH of pyrovic acid to lactic acid. Alteration in the lactic dehydrogenase (LD) activity and serous effusions have been reported in various disease. The measurement of LD activity may be helpful in the diagnosis and prognosis of myocardial infarction, acute hepatitis, leukemia, meningitis and other diseases (Cabaud and Wroblewski, 1958). Amer *et al.* (1994) mentioned that the increase in the activity of HDL might be due to the effect of the insecticide on the membranes of the intracellular organoids and on the membrane of the cell it self, increasing its permeability to the LDH enzyme which appeared in the liver at first and in the serum after wards. Also, they reported that LDH activity decreased in the liver tissue, while it increased in the serum. This quite expected since the insecticide may cause damage to the liver cells leading to the appearance of the enzyme in the serum.

Concerning the response of acetyl cholinesterase (AchE) to carbofuran treatment, data cleared that high significant gradually decrease was observed to the enzyme level with - 63.7, - 63.9 and -85.1% comparatively with control after 24, 48 and 72h post treatment, respectively. AchE is an enzyme acts directly on the acetylcholine. Acetylcholine function as a chemical transmitter at all cholinergic sites in the body. Organophosphorous and carbamate compounds acts by inhibiting AchE thus preventing the hydrolysis of acetylcholine leading to its accumulation. El-Deeb *et al.* (1999) found that osbac insecticide elevated the AchE activity in land snail, *Monacha contiana*, after 24h from treatment while it reduced after 72h.

Table 1. LC_{50} and LC_{99} values of the tested pesticides against *Monacha obstructa*

Compound	Action	LC_{50}%	LC_{99}%	Slope value
Pyriproxyfen	Insect growth regulator	0.49	1.93	3.88
Benthiocarb	Herbicide	0.90	6.09	2.81
Dicofol	Acaricide	4.05	44.23	2.24
Fenthion	Insecticide	1.54	38.21	1.67
Carbofuran	Nematicide	0.18	4.60	1.65
Methomyl	Insecticide	0.39	1.11	5.11

Table 2. Effect of $^1/_4$ LC_{50} of carbofuran on total protein and total lipid in *Monacha obstructa* after different periods.

Parameter	Control	Period after treatment					
		24h		48h		72h	
	Mean ± S.E	Mean ± S.E	% difference	Mean ± S.E	% difference	Mean ± S.E	% difference
Total protein (gm / 100 ml)	0.6 ± 0.06	0.71 ± 0.05	18.3	0.54 ± 0.05	- 10.0	0.46 ± 0.04	- 23.3*
Total lipid (gm / 100ml)	1.3 ± 0.04	0.61 ± 0.01	- 53.0**	0.79 ± 0.01	39.2*	0.31 ± 0.004	- 76.1**

* Significant ** High significant

Table 3. Effect of $^{1}/_{4}$ LC_{50} of carbofuran on the activity of some enzymes in *Monacha obstructa* after different eriods.

Enzyme	Control	Period after treatment					
		24h		48h		72h	
	Mean ± S.E	Mean ± S.E	% difference	Mean ± S.E	% difference	Mean ± S.E	% difference
AST (U / L)	16.0 ± 0.44	10.0 ± 0.82	- 37.5**	13.0 ± 1.2	- 18.8*	14.0 ± 0.7	- 12.5*
ALT (U /L)	5.2 ± 0.42	7.1 ± 0.41	36.5*	9.3 ± 0.25	78.8**	9.0 ± 0.08	73.0**
LDH (U / L)	107.1 ± 0.43	89.2 ± 4.7	- 16.7*	45.2 ± 1.9	- 57.8**	78.7 ± 4.8	- 26.5*
AchE (n mol / min / mg)	924.9 ± 1.36	335.4 ± 3.0	- 63.7**	333.9 ± 1.86	- 63.9**	137.2 ± 2.4	- 85.1**

* Significant ** highly significant

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النشاط الإبادي للقواقع بإستخدام بعض مبيدات الآفات ضد قوقع البرسيم الزجاجي *Monacha obstructa*

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تم إختبار النشاط الابادي لخمسة مبيدات آفات مختلفة ضد القوقع الأرضي (قوقع البرسيم الزجاجي) بالمقارنة بمبيد الميثومايل الموصي به لمكافحة القواقع. أظهرت النتائج أن مركب كاربوفيوران (مبيد نيماتودي) كان الأكثر سمية ضد القوقع يليه مركب ميثومايل (مبيد حشري) ثم مركب بايريبروكسيفين (منظم نمو حشري) يليه بنثيوكارب (مبيد حشائش) ثم فينثيون (مبيد حشري) بينما أحتل مركب دايفيكول (مبيد أكاروسي) المرتبة الأخيرة حيث كان أقلهم تأثيراً.

كذلك تم دراسة التأثيرات البيوكيميائية لمركب الكاربوفيوران بعد المعاملة بتركيز تحت مميت ($^1/_4$ LC_{50}) علي فترات مختلفة من المعاملة. أشارت النتائج أن مستوي البروتين الكلي زاد بعد ٢٤ ساعة ثم أنخفض تدريجياً بعد ٤٨ ، ٧٢ ساعة مقارنة بالكنترول ، بينما حدث تذبذب لمستوي الليبيدات الكلي حيث أنخفض بعد ٢٤ ساعة بينما أرتفع بعد ٤٨ ساعة ثم أنخفض مرة أخري بعد ٧٢ ساعة. بالنسبة لنشاط أنزيمي الكبد ALT, AST فقد أظهرت النتائج حدوث إنخفاض تدريجي لأنزيم AST مع زيادة الفترة بعد المعاملة بينما حدث العكس مع أنزيم ALT حيث أرتفع نشاط الأنزيم طردياً بعد ٢٤، ٤٨ ساعة ثم أنخفض بعد ٧٢ ساعة. بالنسبة لأنزيم LDH أكدت النتائج حدوث إنخفاض شديد في مستوي الأنزيم بعد ٢٤، ٤٨، ٧٢ ساعة من المعاملة. كذلك لوحظ حدوث إنخفاض تدريجي معنوي في نشاط أنزيم AchE خلال نفس الفترات الثلاثة.