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MOLLUSCICIDAL EFFICACY AND TOXICITY OF SOME PESTICIDES UNDER LABORATORY AND FIELD CONDITIONS

[56]

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ABSTRACT

Laboratory and field trials were carried out at Koom Hamada district and Etay El-baroud research station during 2004/ 2005 season to clarify the molluscicidal activities of the following: indoxacarb, lufenuron, *Bacillus thurringiensis*, *kurestaci* (*Bt*.) and methomyl against glassy clover snails *Monacha cartusiana* (Müler). Results indicated that all tested pesticides decreased snails population compared with control. Under the field conditions, the efficiency of the tested compounds were 98.0, 93.4, 93 and 71.58% for methomyl, lufenuron, indoxacarb and *Bt*. after 5, 6, 13, and 28 days of treatment, respectively. Moreover the effect of LC_{50} and 0.5 LC_{50} of these compounds were investigated on some biochemical parameters *in vivo*. The activities of acetylcholinestrase (AChE), alanine aminotransferases (ALT) , aspartate aminotransferases (AST) and protein contents at different time intervals were evaluated. The specific activity of AChE reached 0.009, 0.002, 0.006 and 0.001 μmole/ mg protein/ min for indoxacarb, lufenuron, *Bt.* and methomyl, respectively, after one day of treatment with (LC_{50} for each compound). In general, *Bt.* and indoxacarb caused slight inhibition on AChE enzyme. All treatments decreased ALT and AST enzyme activity in the tested animals. However, *Bt.* Showed slight effect on the activity of aminotransferases enzymes.

Keywords: Snails, Molluscs, Pesticides, Toxicity, Enzymes.

INTRODUCTION

Mollusks are a large and diverse group of animals distributed worldwide (**El-Okda, 1980; Godan, 1983 and Nakhla *et al* 1993).** Terrestrial gastropods (mollusks: snails and slugs) are being abundantly distributed in the North coast, new reclaimed lands and addition to delta region of Egypt **(Kasaab and Daoud, 1964); (El-Okda and Khalil, 1981) and (Abou-Baker, 1997)**. Also, snails are becoming serious agricultural animal pests in Egypt, especially in the Northern coastal areas. Their damage to ornamental plants resembles that done by caterpillars or

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wireworms. The brown garden snail (*Helix aspersa*), is the common snail causing problems in California gardens; it was introduced from France during the 1850s for use as food. Snails and slugs move by gliding along on a muscular "foot". Snails and slugs feed on a variety of living plants as well as on decaying plant matter. On plants, they chew irregular holes with smooth edges in leaves and can clip succulent plant parts (**Flint, 1998**). They can also chew fruit and young plant bark. A good snail and slug management program relies on a combination of methods. The first step is eliminate, all places where snails or slugs can hide during the day. Boards, stones, debris, weedy areas around tree trunks, leafy branches growing close to the ground, and dense ground covers such as ivy are ideal sheltering spots safe (**Salagado, 1990**). Synthetic molluscicides are still considered the most effective measures for the control of snails. Lufenuron is a new insecticide being effective against resistant pests to organophosphates and pyrthroides. It was soft on adult beneficial and predatory mites, safe on a wide range of crops and suitable for integrated pest management (**Harder *et al* 1996**). One of the most promising biological control approach that has received attention of many scientists is the development of *Bacillus thuringiensis* (*Bt.*) toxins as insecticides (**Belfore *et al* 1994**). Diple - 2X is one of the biological insecticides containing proteins that are highly toxic to insects. Indoxacarb is the common name proposed for the S-isomer of oxadiazine derivative, which is insecticidal active isomer. This compound represents a new class of compounds has broad-spectrum insecticidal activity and yet be environmentally safe (**Salagado, 1990**). The present study was aimed to evaluate the efficacy of some safe pesticides such as indoxacarb, *Bacillus thuringiengsis* (*Bt.*) and lufenuron as molluscicides compared with methomyl under field conditions, and examined their effects on some biochemical parameters in the laboratory.

MATERIAL AND METHODS

I- Field Studies

The field study was conducted in clover fields at Koom Hamada district, El-Behera governorate during May, 2005. The treatments were done with 0.5 LC_{50} and LC_{50} which cited from **Abou- El Khear *et al* (2005**). The LC_{50} values were 6.7, 6.4, 7.0, and 5.9 $x10^4$ ppm for indoxacarb (Avanet® 15% SC) methyl (s)-N- [7-chloro-2, 3, 4, a, 5-tetrahydro-4a (methoxy carbonyl) indeno [1,2-e] [1, 3, 4] oxadiazin-2-yl carbonyl]-4-(trifloromethoxy) carbonilate; lufenuron (Match® 5% EC): N-[[[2, 5-dichlro-4-(1, 1,2,3,3,3, -hexafluro-propoxy)-(phenyl] amino] carbonyl]-2, 6-difluorobe-zamide (CA); Dipel® -2X 10.3% WP: *Bacillus thuringeinsis, kurestaci.* and methomyl (Lannate® 90% SP): S-methyl-N- (methylcarbamoyl) oxyl- thioacetimidate, respectively. The experiments were carried out in plots of ¼ feddan each ($1000m^2$). Each treatment was replicated five times in random blocks. Bait were distributed before sunset using twelve bait stations in different areas of the plot. Wheat bran was kept in bait stations for three days, and then the poisoned baits were added in 200 gm each. The bait stations were inspected and replenished on each subsequent two days until the cessation of the snails' activity, nearly for one month. Daily bait consumption and dead animals were counted and removed from each plot. Daily observation and snails traps in this area showed that, *Monacha cartusiana* was the most common and important species. Mortality percentage was calculated and the molluscicidal potencyexpressed as population reduction was measured for all tested compounds according to Henderson and Tilton equation (1955):

Reduction %

$$= 1-\left\{\frac{\text{No. of control before baiting}}{\text{No. of control after baiting}} \times \frac{\text{No. of treatment after baiting}}{\text{No. of treatment before baiting}}\right\}$$

II- Biochemical Studies

The glassy clover snails *Monacha cartusiana* (Müller) were collected from clover fields in 2004/2005 season from Koom Hamada district and transferred to laboratories at Etay El-Broud Research Station, El-Behera governorate. The shell diameter of adult snails was measured at average 1.6 cm. They were acclimatized for a week under laboratory conditions and fed on lettuce (*Lactuca sativa*) *ad lib*. Toxic white bran baits were prepared containing each LC_{50} or 0.5 LC_{50} values of tested pesticides. The bait consisted of grain bran: molasses: blue dye with the ratio 8: 1: 1, respectively, (WHO, 1961). Baits were distributed in three replicates and 15 healthy animals were used in each replicate. In the case of control, pesticides were substituted with distilled water. Replicates were kept under laboratory conditions and suitable humidity required for snail activity. The dead animals were discarded, while the healthy adults were taken at different time intervals, i.e. 1, 3, 5, and 7 days of treatment. The soft tissues were weighed at average (0.86 gm), pooled, and homogenized as 1: 10 (w/v) in buffer phosphate (0.1 M) PH 7.0. The homogenate was centrifuged at 5000 rpm for 20 min under cooling. The supernatant was decanted and frozen at -20 °C until used as enzymes source. Activities of some enzymes were measured such as alanine aminotransferases (ALT), aspartate aminotransferases (AST) according to **Retman and Frankel** method (**1957**). Additionally, acetylcholiesterase (AChE) activity was measured according to **Ellman *et al* (1961**) and its specific activity was expressed as µmole substrate hydrolyzed/mg protein/min. Total protein was measured according to **Lowry *et al* (1951).**

Statistical Analysis

In filed treatments, analysis of variance was used to compare means among treatment. The least square means were compared for significant differences between treatments using student- New man- Keals test (**Sokel and Rohlf, 1969**). While, in biochemical studies all data were calculated as X ± S.E. and comparison between two groups was performed by student t-test (**Motulesky, 1987**). P-value of 0.05 was considered significant.

RESULTS AND DISCUSSION

1. Molluscicidal activities of tested insecticides

The Survey and observation of the studied area showed that, glassy clover snails Helix aspersa (Müller) is the common species snails on winter crops. The efficacy of tested pesticides under the field conditions was evaluated as snails' population reduction, bait consumption, days of pest control and the required poison baits (Kg/feddan). The results in **Table (1)** indicated that, methomyl and lufenuron were the most effective against brown snails causing (96.96 and 98.03%) and (85.44 and 94.66%) reduction in population for 0.5 LC50 and LC50, respectively. The population reduction by indoxacarb reached 79.02 and 93.33% with 0.5 LC50 and LC50, respectively. The lowest effective pesticide as molluscicide was Diple-2X, where the population reduction of snails reached 45.82 and 71.58% with the same concentrations, respectively.

Concerning the amounts of consumed baits, it was found that, Bt. bait exhibited the highest consumption by snails (7.6 Kg /feddan). While, the low consumptions were 2.5, 4.0, and 5.5 Kg/feddan for methomyl, lufenuron, and indoxacarb, respectively. On the other hand, the periods required to achieve successful control of the snails were 5, 12, 14, and 28 days for methomyl , lufenuron, indoxacarb and Diple-2X, respectively.

Generally, lufenuron and indoxacarb showed high molluscicidal effect against clover snails, which considered an important pest on most field crops, under field conditions compared with methomyl, in addition to other positive properties of the compounds low toxicity to non-target organisms and short persistence in the environment (**Salagado, 1990; Harder, *et al* 1996 and Pluschkell *et al* 1998). Abou-El Khear *et al* (2005)** found the same results under laboratory conditions. These results agree with the findings by **Hussien (2003)** who indicated that, methomyl was more effective as molluscicidal compound using topical application and poisoned food techniques than indoxacarb. **Abou-Baker (1997)** illustrated that, the chemical control is the most powerful tool available for controlling snails. **Zedan *et al* (1999**) found that, bacterial formulation was the most effective against land snails compared with methomyl.

2. Toxicolgical activities of tested insecticides

Data on the Aspartate aminotransferases (AST), alanine aminotransferases (ALT), acetylcholinesteras activities and protein concentration in animals treated with 0.5 LC_{50} and LC_{50} of the tested pesticides at different time intervals are shown in **Tables (2 and 3).** AST activity was reduced by 85.4 and 80.6% (Diple-2X); 69.6 and 53.3% (indoxacarb); 79.8 and 72.6% (lufenuron) and 63.6 and 67.7%(methomyl) for animals treated with 0.5 LC_{50} and LC_{50}, respectively, after three days of treatment. Alanine aminotransferase was also significantly reduced compared with control animals. The inhibitory effects by 0.5 LC_{50} and LC_{50} for methomyl, lufenuron, indoxacarb, and Diple-2X on the AChE activity of snails are shown in **Table (3).** The results indicated that, the highest inhibitory effect against AChE enzyme activity of treated animals was occurred with LC_{50} of methomyl. The specific activity of AChE was expressed as µmole/mg protein/min which accounted for 0.004, 0.004, 0.002, and 0.001 µmole/mg protein/min for Diple-2X, indoxacarb, lufenuron, and methomyl, respectively. Slight inhibition in AChE enzyme activity was done with lufenuron and Diple-2X treatments. In addition, the protein

Table 2. Effect of tested pesticides against alanine aminotransferases (ALT), aspartate aminotransferases (AST) activities of glassy clover snails *Monacha cartusiana* (Müler) at different time intervals

Treatment	Enzyme Activity					
	Dose	Time interval (day)	AST (U / 1)	% of Control	ALT (U / 1)	% of Control
Control	-	1	47.3±7.5	100	18.7±2.4	100
		3	41.3±3.2	100	28.8±4.3	100
		5	43.0±3.1	100	26.8±4.6	100
		7	42.3±4.2	100	22.5±2.1	100
Bt	$0.5LC_{50}$	1	25.7±4.6**	54.3	19.0±2.5	101.6
		3	35.0±4.8**	85.4	21.8±3.1**	75.6
		5	5.8±2.3**	13.5	12.0±3.2**	44.7
		7	7.7±1.3**	18.2	14.3±2.6**	63.5
	LC_{50}	1	26.7±3.6 **	56.4	13.9±3.4**	74.2
		3	33.3±2.5 **	80.6	23.8±4.1**	82.3
		5	13.0±1.3**	30.2	18.7±3.6**	70.0
		7	7.73±2.1**	33.0	12.1±3.2**	53.8
indoxacarb	$0.5LC_{50}$	1	22.0±3.5**	46.5	10.7±3.5**	57.2
		3	28.7±3.4**	96.6	12.1±1.3**	42.1
		5	5.7±1.2**	13.3	13.4±2.3**	50.0
		7	6.7±1.4**	28.7	22.2±1.8**	98.7
	LC_{50}	1	33.0±5.2 **	69.7	9.1±2.1**	48.7
		3	22.0 ±1.2**	53.3	15.3±2.9**	56.8
		5	5.47±1.1**	12.7	12.1±3.7**	42.5
		7	7.47±1.4**	32.1	14.0±3.8**	62.2
lufenuron	$0.5LC_{50}$	1	24.3±2.2*	51.3	13.2±2.4**	70.5
		3	34.3±3.2*	79.8	18.3±2.4**	63.6
		5	31.0±3.5	75.1	16.9±1.8**	63.3
		7	11.0±1.3**	47.2	19.6±3.7	87.5
	LC_{50}	1	16.0±0.0**	33.8	12.9±1.7**	68.9
		3	30.0±0.8*	72.6	23.1±1.9*	80.2
		5	24.3±0.7**	56.5	20.2±2.3*	75.3
		7	15.0±0.2**	64.3	22.1±2.6	98.2
methomyl	$0.5LC_{50}$	1	24.3±2.3**	51.4	19.3±3.1	103.2
		3	26.3±3.6**	63.6	14.2±1.6**	49.4
		5	4.5±1.1**	10.4	16.5±2.9**	61.2
		7	6.7±1.4**	28.8	14.9±2.9**	66.3
	LC_{50}	1	27.3±7.5**	57.6	14.4±2.4**	48.3
		3	31.6±2.2**	76.7	17.0±3.5	93.7
		5	11.4±1.3**	26.5	14.0±1.8**	52.4
		7	3.2±0.0**	13.7	22.4±2.3	100

Each value is the mean of three replicates ± S. E.

* Significant at 0.05 ** Significant at 0.01

Table 3. Effect of tested pesticides against acetyl cholinesterase (AChE) activity and total protein of glassy clover snails *Monacha cartusiana* (Müller) at different time intervals

Treatment	Enzyme Activity					
	Dose	Time interval (day)	AChE Activity (µmole/mg protein/min)	% of Control	Total protein (mg/ml)	% of Control
Control	-	1	0.002±0.0	100	120.6±2.5	100
		3	0.004±0.0	100	121.3±0.48	100
		5	0.003±0.001	100	128.6±3.8	100
		7	0.033±0.009	100	128.9±4.3	100
Bt	$0.5LC_{50}$	1	0.003±0.00	67.23**	95.77±3.8**	78.51
		3	0.004±0.001	102.3	105.2±3.7*	86.65
		5	0.007±0.00	64.69**	96.67±2.8**	75.00
		7	0.023±0.001	36.20**	10.44±100*	77.88
	LC_{50}	1	0.006±0.002	47.13**	28.00±5.6**	23.33
		3	0.005±0.001	95.83	77.00±4.6**	63.63
		5	0.004±0.00	57.24**	68.67±1.2**	53.42
		7	0.061±0.001	92.19	97.13±2.4	75.35
indoxacarb	$0.5LC_{50}$	1	0.002±0.00	62.77**	65.0±4.9**	54.16
		3	0.005±0.001	62.69**	65.78±6.2**	54.36
		5	0.012±0.002	95.14	39.78±3.4**	30.93
		7	0.012±0.003	31.48**	99.65±4.6**	77.30
	LC_{50}	1	0.009±003	68.98**	66.45±3.8**	55.09
		3	0.004±0.00	57.71**	59.78±5.7**	49.27
		5	0.023±0.012	58.06**	44.22±3.7**	34.46
		7	0.026±0.00	30.63**	76.09±1.3**	59.03
lufenuron	$0.5LC_{50}$	1	0.003±0.00	69.82**	64.33±3.9**	53.34
		3	0.002±0.00	28.01**	56.33±4.6**	46.42
		5	0.003±0.00	39.64**	55.22±4.4**	42.9
		7	0.040±0.01	34.25**	82.00±5.3*	63.6
	LC_{50}	1	0.002±0.00	58.87**	73.78±5.6**	61.17
		3	0.002±0.00	33.84**	87.78±2.5*	72.36
		5	0.003±0.00	63.85**	79.67±3.5**	61.95
		7	0.013±0.00	14.49**	73.00±7.5**	56.63
methomyl	$0.5LC_{50}$	1	0.002±0.00	51.74**	81.78±3.2**	67.17
		3	0.004±0.00	63.30**	76.67±2.7**	61.25
		5	0.006±0.00	61.43**	53.33±2.5**	41.46
		7	0.028±0.01	23.53**	53.89±1.3**	41.46
	LC_{50}	1	0.001±0.00	31.98**	74.33±4.6**	61.63
		3	0.003±0.00	60.75**	57.67±5.7	55.88
		5	0.004±0.00	47.00**	31.22±3.1**	47.60
		7	0.042±0.01	31.55**	46.56±1.1**	36.20

Each value is the mean of three replicates ± S.E.
* Significant at 0.05   ** Significant at 0.01

content was significantly decreased after methomyl, lufenuron, indoxacarb, and Diple-2X treatment. The highest reduction was recorded in the animals treated with the high concentration (LC_{50}) of methomyl. AChE was more vulnerable proteins than other enzyme system especially against methomyl that attributed to its toxic effect. Elevation of AST and ALT had been reported in pesticide treatments (**El-Wakil *et al* 1992; Radwan *et al* 1992; Radwan *et al* 1993; and Mohamed, 1995)**. The possible mechanisms involved in the elevation of AST and ALT activities observed in the present study may be based on tissue damage, increased synthesis or decreased catabolism of both enzymes (**Todior and Van-Heemastra, 1980).** On the other hand, the decreased activity of AST and ALT may be due to rather leakage of the enzyme into extra cellular compartments or to actual enzyme inhibition by tested pesticides **(Abou-Baker, 1997; Abdalla *et al* 1998 and Hussein, 2003).**

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مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ،14(2) ، 861-870 ، 2006

الكفاءة الابادية وسمية بعض المبيدات ضد قوقع البرسيم الزجاجي تحت الظروف الحقلية والمعملية

[56]

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1– **المعمل المركزي للمبيدات – محطة البحوث الزراعية – وزارة الزراعة – ايتاى البارود – مصر**

2– معهد بحوث وقاية النباتات–قسم الحيوانات الضارة والطيور–المركز الدولي للبطاطس–كفر الزيات– مصر

أجريت هذه الدراسة تحت الظروف الحقلية بمركز كوم حمادة محافظة البحيرة ومحطة البحوث الزراعية بايتاى البارود للموسم الزراعي 2005/2004 لدراسة كفاءة ثلاث مركبات حديثة وآمنة بيئيا من مجا ميع مختلفة هى مركب الماتش (ليفينيرون 15 %) (اندوكساكارب 5%)والمبيد البكتيري- الدايبل (*Bt* **10.3**.) ومقارنتها بمركب اللانيت (ميثوميل90%) الموصى به في مكافحة القواقع الأرضية.

تم استخدام تركزين من هذه المركبات تمثل التركيز النصفى قاتل LC_{50} و نصف هدا التركيز $0.5\ LC_{50}$ كطعوم سامة أظهرت نتائج الدراسات الحقلية أن:-

* مركب اللانيت أعطى كفاءة ابادية عالية حتى 98 %خلال خمسة أيام من المعاملة يليه مركب الماتش (93,33%) ثم الافانت (94,66%) وأخيرا مركب الدايبل (71,58%) وكانت المدة اللازمة للوصول لأعلى إبادة هي (5 و12 و14 و28 يوما على التوالي).

*كانت كمية المبيد اللازمة لتحقيق المكافحة الناجحة للقواقع 7.6 - 5.5 - 4 - 5.2 كجم /فدان للدايبل ، الافانت ، الماتش ثم اللانيت على التوالي.

* أظهرت دراسة تأثير هذه المركبات على نشاط بعض الإنزيمات الحيوية داخل جسم القواقع المسممة أن التأثيرات المثبطة للكل من اللانيت والافانت على نشاط إنزيم الاستيل كولين استريز كانت أعلى من الماتش يليها الدايبل.

* حدث انخفاض واضح في نشاط إنزيمات ALT, AST مما أدى ألي تدهور النشاط الجسمي للقواقع وقد حدث أعلى تثبيط بعد ثلاثة أيام من المعاملة خاصة في حالة استخدام التركيز النصفى القاتل LC_{50}.

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