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Toxicity and biochemical studies of some pesticides against small sand snail *Helicella vestalis* (Pfiffer)

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Abstract

The present research aimed to study the sensitivity of snails, adults small sand snail *Helicella vestalis* (Pfiffer) as non-target organisms towards, Kuik (insecticide); Brominal (herbicide); Sencor (herbicide) and Goal (herbicide) under laboratory conditions.

The tested pesticides exerted lethal effect against land snail *H. vestalis* after seven days post-treatments. The mortality percentages increased with increasing the concentration values and the duration of exposure towards all the tested pesticides at laboratory experiments. The 7-days/LC50 values were, 0.38%, 1.15%, 1.83% and 2.30% for Kuik, Brominal, Sencor and Goal against *H. vestalis* respectively. Moreover, the impacts of sub lethal concentration (1/8 LC50) of Kiuk on certain biochemical parameters were advised for dissimilar intervals thereupon request. Results expressed that the total protein level heightened thereupon 1st day then diminished peicemeally thereupon 3 rd and 7th day post - treatment comparatively with control. The level of total lipid was hesitated whereas it minimized thereupon 1st day but it raised post 3rd day then, minimized thereupon 7th day. The vitality of glutamic pyruvic transaminase (GPT) piecemeally lessend with interval thereupon treatment while various findinas were recorded in state of glutamic oxaloacetic transaminase (GOT), as the identical treatment augment the enzyme vitality symmetry post 1st and 3rd, then it tessened thereupon 7th day. Regarding lactic acid dehydrogenase (LDH), results showed strict diminished in its level thereupon 1st, 3rd and 7th day pot-treatment. Moreover, the vitality of acetylcholinestrase (AchE) was significantly diminished traverse the identical intervals.

Keywords: Land Snails, Toxicity, biochemical, pesticides.

Introduction

Land snails are important pests for a wide range of fruit orchards ,ornamental plants ,vegetables and field crops all over the world ,they tend to feed on the softer parts of plants causing feeding damage to plant seedlings ,irregular holes in leaves ,roots tubers and fruits ,decrease yield or cause loos of quality due to presence of snails or their feces in the harvested product (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 pesticides e.g. Kuik, Brominal , Sencor and Goal. Proved promising efficiency for control of land snail species (Gabr, et al., 2006 and Lokma, 2013).

The present work aims to study the biochemical effects of the certain pesticides i.e., Kuik, Brominal, Sencor and Goal against the small sand snail species, *H. vestalis* one of the most common and harmful species in Egypt.

Materials and Methods

1- The tested chemicals:

1- Goal 24% EC (herbicide):

Trade name: Goal 24% EC.

Common name: fenthion or oxyfluorfen (Goal or Kotlax).

Chemical name: 2-chloro-a, a,a trifluoro-p-tolyl3-ethoxy-4-nitrophenyl

2- Sencor 70% WP (herbicide):

Trade name : Sencor 70% WP.

Common name: metribuzin.

Chemical name: methyl 2 (4-methoxyl-1,3,5-triazin) ymethylamino carbonyl

3- Brominal 24% EC (herbicide):

Trade name : Brominal 24% EC.

Common name: propineb.

Chemical name : 2.6-dibromo-4-cyanophenyl-actanoate

4- Kuik 90% SP (insecticide):

Trade name : Kuik 90% SP.

Common name: rotam .

Chemical name : 5-methy-N-((methyl carbamoyl) oxy) thioacetimidate

2- Experimental animals:

Land snails, *H. vestalis* were collected from the Faculty of Agriculture Research Station at Abbis area, Alexandria University, during spring and autumn 2012. The snails were kept under laboratory conditions in Agricultural. Zoology and Nematology Department. Faculty of Agriculture, Al- Azhar University for 14 days and fed on fresh lettuce leaves (El-Deeb, et al., 2003). For each treatment, 40 healthy animals were divided into four replicates (10 for each).

3- Contact treatment:
Evaluated pesticides were prepared as poisonous baits by mixing calculated weight or volume of the pesticide with wheat bran to give the concentrations 1, 2, 4, and 8% for *H. vestalis*. Ten grams of the poisonous baits were spread into each plastic box (1/2 kg capacity). Ten healthy adult snails were introduced for each box, closing boxes with musline cloth and secured with rubber band to prevent snails from escaping. Each concentration was replicated four times. Control treatment was prepared using bran bait only without any pesticides. Mortality percentages were recorded after 1, 3, 5, 7, 14 and 21 days post-treatment. Observation of mortality entailed using stainless steel needle according to El-Okda (1991). Dead snails were removed after testing and mortaility percentages were calculated until the end of the experiment (21days). The data were analyzed using the probit analysis (Finney, 1971) and LCP lines (toxicity lines), the LC50 values were estimated for the tested pesticides. Analysis of variance was conducted to test significance between treatments using L.S.D. values according to Snedecor (1957).

4- Biochemical studies:
The biochemical response of *H. vestalis* to sub lethal concentration of Kuik (which proved to be the most toxic compound) was studied. Animals were treated with 1/8 LC50 of the tested compound using the same contact technique mentioned before.
4-1- Samples preparation for biochemical tests:
Shells of snails were removed and tissues were homogenized in 10 folds (w/v) of distilled water by using glass homogenizer. Homogenates were centrifuged at 5000 rpm for 30 minutes using a cooling centrifuge at 4ºC and the supernatant was used as a source of protein and enzyme assay.
4-2- Determination of total proteins and lipids
Total proteins were colorimetrically determined according to Gornall, et al., (1968) while total lipids were assayed by the method of Zollner and Kirsch (1962).

4-3- Determination of GOT and GPT:
The supernatants were sued for assaying glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) activities (Reitman and Frankle, 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 Ach E:
Acetyl cholinesterase (AchE) was determined according to Ellman, et al., (1961).

Results and Discussions

1- Molluscicidal Effect:
Data in Table (1) showed that the toxic efficiency of the tested chemicals against *H. vestalis* individuals along the duration of the experiment (21days) under laboratory conditions. A kuik gave the highest mortality percentages i.e. the mean value of mortality percentage ranged between 28.33 and 63.33% after 21 days post-treatment with 7 days/LC50 (0.38%). On the other hand, Goal had the lowest toxic effect against *H. vestalis* i.e, the mean value of mortality percentage ranged between (6.67 and 40.00%) with 7-days/LC50 (2.30%). According to the men value of the mortality percentage of *H. vestalis* adults and 7- days/LC50 , the tested pesticides could be arranged descending as follows, Kuik, Brominal, Sencor and Goal. i.e. the highest and the lowest mean values of mortality percentage after 21 days post-treatment were (63.33 and 28.33%); (56.67 and 11.64%); (51.61 and 10%) and (40.00 and 6.67%) for Kuik, Brominal, Sencor and Goal respectively. On the other hand, the values of 7-days/LC50 were (0.38, 1.15, 1.83 and 2.30%) for Kuik, Brominel, Sencor and Goal, respectively. Generally, the efficiency of the tested pesticides against *H. vestalis* was arranged in descending order according to their general mean of mortality percentages as follows (48.75, 35.84, 30.84 and 20.42%) for Kuik, Brominal, Sencor and Goal, respectively. On the other hand statistical analysis of data indicated that Kuit was the most toxic compound, while Goal was the least toxic one against *H. vestalis* snails.

2- Biochemical response:
The biochemical impacts of Kuik 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 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 an intimate role in the structure of cell membranes and the organelles found in the cell (Wilson, 1986).

Table 1. Mortality percentages and the values of 7-days/LC50 with confidence limits of *H. vestalis* treated with different pesticides under laboratory conditions.

Toxicant	Conc. %	Mortality % days						Mean	General mean	LC50	95 % confidence limit	
		1	3	5	7	14	21				Lower	Upper
Kuik	1	0	0	10	40	50	70	28.33	48.75	0.38	0.051	0.70
	2	0	0	30	70	80	90	45				
	4	10	20	50	80	90	100	58.33				
	8	10	30	50	90	100	100	63.33				
Brominal	1	0	0	0	20	20	30	11.67	35.84	1.15	0.23	3.05
	2	0	10	20	30	50	60	28.33				
	4	10	20	30	50	80	90	46.67				
	8	10	30	40	70	90	100	56.67				
Sencor	1	0	0	0	10	20	30	10	30.84	1.83	0.83	11.20
	2	0	0	20	30	40	70	26.67				
	4	0	10	20	30	70	80	35				
	8	20	30	30	60	80	90	51.67				
Goal	1	0	0	10	10	10	10	6.67	20.42	2.30	1.35	7.48
	2	0	10	10	10	20	30	13.33				
	4	0	10	10	30	30	50	21.67				
	8	0	30	30	50	60	70	40				
L.S.D at 0.05								38.8 30.03 32.27 27.00	24.58			

L.S.D= Least significant differences at 5% level of probability.

Effect of sub-lethal dose (1/8 LC50) of Kuik compound on total protein and total lipid in *H. vestalis* is shown in Table (2). Data obviously revealed that kuik business addition total protein up to 12.34 after 1st day from request, while it decrease this price to be -5.55 and -14.81 post 3rd and 7th day, successively in comparison to control. concerming, total lipid, vice-versa occurred as it diminish with 48.70% after 1st day but it raised up to 46.12% after 3rd day then lower to 71.20% after 7th day comparatively to control. The anterior data establish that the hesitation in the level of both total protein and total lipid might be resulted from disequilibrium between the grade of synthesis and grade of devolution.

El-Wakil and Radwan (1991) recorded that either methomyl or thiodicard caused significant decreased in total lipid and total protein after 1-10 days of treatment for *Eobania vermiculata*. Our results are agreed with Godan (1983) who observed that there are different susceptibility levels at different times in the life cycle of snails. Also, she mentioned that the period of snails sensitivity, which is important for control program of snails.

Table 2. Impact of 1/8 LC50 of Kuik of the total protein and total lipid in *H. vestalis* at different periods post-treatment.

Parameter	Control	Different periods post - treatment				
		1		3		7	
	Mean ± S.E	Mean ± S.E	% difference	Mean ± S.E	% difference	Mean ± S.E	% difference
Total protein (gm/100ml)	1.62 ± 0.03	1.82 ± 0.18	12.34	1.53 ± 0.03	- 5.55	1.38 ± 0.92	- 14.81
Total lipid (gm/100ml)	2.71 ± 0.2	1.39 ± 0.3	48.70 **	1.46 ± 0.14	-46.12 *	0.78 ± 0.35	71.20**

* Significant ** Highly significant.

2-2- Enzymes activity:

The accedence of dissimilar enzymes i.e. GPT, GOT, LDH and AchE to request with 1/8 LC50 of Kuik was presented in Table (3). Regarding the energy of enzymes GPT and GOT, results compliance that GPT vivacity diminished piecemeal with expatiation the period after request as it diminish than control with -36.64, -22.72 and -16.64% after 1, 3 and 7 post-treatment, successively. The adversary surfaced in state of GOT as the vera treatment improved the enzyme vitality symmetry to 42.94 and 70.17% post 1 and 3days then it diminished to 64.50% after 7 post-treatment than control.

Our results are in agreement with those reported by Lebsack et al., (1980) who mentioned that the possible mechanism involved in the elevation of GPT and GOT levels may be due to tissue damage or due to increase of it.

Table 3. Impact of 1/8 LC50 of Kuik of the activity of some enzymes in *H. vestalis* at different periods post-treatment.

Enzyme	Control	Different periods post – treatment					
		1		3		7	
	Mean ± S.E	Mean ± S.E	% difference	Mean ± S.E	% difference	Mean ± S.E	% difference
GPT (µ/L)	17.6 ± 0.38	11.15 ± 0.65	- 36.64 **	13.6 ± 2.1	-22.72 *	14.67 ± 0.5	- 16.64 *
GOT (µ/L)	6.17 ± 0.68	8.82 ± 0.25	42.94*	10.5 ± 0.8	70.17**	10.15 ± 0.55	64.50**
LDH (µ/L)	106.5 ± 0.44	77.4 ± 3.11	-26.98*	39.9 ± 1.7	- 62.23**	71.0 ± 8.5	-33.33*
AchE (nol/min/mg)	1031.7 ± 0.04	382.4 ± 1.93	-62.90**	368.6 ± 0.01	-64.20**	169.3 ± 7.5	- 83.50 **

* Significant ** Highly significant.

El-Wakil (1990) found that GPT and GOT activities were not significantly affected by the Bayluscide, Dursban and Gramaxon however a significant damages in activities of these enzymes were noticed among the different periods after treatment. Radwan et al., (1993) reported that chlorfluazuron and cascade increased the activities of GPT and GOT in *Helix aspersa* snail tissues.

Concerning the lactic acid dehydrogenase (LDH), sequences showed that its level diminished with -26.98, -62.23 and -33.33% after 1, 3 and 7days from request successively than regulator. LDH is an enzyme regarded with the lessention in the attendance lowered diphosphonucleotide (DP/VH) of alpha-kito and alpha gamma-dikito acids. LDH vitality of plasma, serous effusions and cerebrospinal liquid may be measured by the reduction in the appearance of DPNH of pyrovic acid to lactic acid .Variation in the lactic dehydrogenase (LD) vitality and serous effusions have been processed in diverse aliment. The measurement of LD vitality may be profitable in the diagnosis and prognosis of myocardial infarctin, incisive hepatitis, leukemia, meningitis and other sickness (Cabaud and Wroblewski, 1958), Abd El-Aal ,(2004) mentioned that the reproduce in the vitality of LDH might due to the effect of the insecticide on the membranes of the intracellular organoids and on the membrane of the cell it self, reproducing its permeability to the LDH enzyme which appeared in the liver at first and in the plasma after wards, also, they processed that LDH vitality minimized in the liver tissue, while it reproduced in the plasma. This quite expected since the insecticide may state damage to the liver cells leading to the appearance of the enzyme in the plasma.

Acytylcholinesterase (AchE) is an enzyme that acts directly on the acetylcholine. Acetylcholine function as chemical transmitter at all cholinergic sites in the body. Organophosphorous and carbamate compounds act by inhibiting acetyl cholinesterase enzyme thus preventing the hydrolysis of acetylcholine leading to its accumulation.

Data tabulated in Table (3) indicate the effect of LC50 of Kiuk on the activity of cholinesterase in land snail, *H. vestalis* after different periods. Data obviously indicated that high significant symmetry diminish was perceived to the enzyme level with -62.90, -64.20 and -83.50% comparatively with divergent after 1, 3 and 7days post divergent successively.

Westalke et al., (1981) mentioned that the administration of carbamate and organophosphorous pesticides produce a dramatic neuromuscular effect. The various response of AchE which is apparent in different species may be common to both compounds. Also, El-Deeb et al., (1999) found that osbac insecticide elevated the AchE activity in land snail *Monacha contiana* after one day from treatment while it reduced after three days post-treatment.

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دراسات سمية وبيوكيميائية لبعض مبيدات الآفات ضد قوقع الرمال الصغير

Helicella vestalis

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الملخص العربى

تم دراسة مدى حساسية الافراد البالغة لقوقع الرمال الصغير *Helicella vestalis* عند معاملتها ببعض المبيدات الكيميائية وهى السنكور (مبيد حشائش) – بروماينال (مبيد حشائش) – كويك (مبيد حشرى) – جول (مبيد حشائش) والتى استخدمت عن طريق الملامسة لكل مركب تحت الظروف المعملية .

وقد أوضحت النتائج ما يلى:

أظهرت المبيدات الكيميائية المختبرة تأثير أبادى ضد قوقع الرمال الصغير وذلك بعد سبعة أيام من المعاملة كنسب مئوية للموت والتى ازدادت بزيادة قيمة التركيز وفترة التعرض. وقد أظهرت النتائج أن مركب الكويك كان الأكثر سمية ضد قوقع الرمال الصغير يليه مركب البروماينال ثم مركب السنكور بينما احتل مركب الجول المرتبة الأخيرة حيث كان أقلها تأثيرا ، حيث بلغ التركيز القاتل لنصف تعداد الأفراد معمليا بعد سبعة أيام من المعاملة هى كالتالى (0.38 ، 1.15 ، 1.83 ، 2.3%). وكذلك تم دراسة التأثيرات البيوكيميائية لمركب الكويك على قوقع الرمال الصغير *Helicella vestalis* حيث تمت معاملة القواقع بتركيز تحت مميت (1/8 LC50) لمركب الكويك عن طريق الملامسة باستخدام الطبقة الرقيقة . أشارت النتائج أن مستوى البروتين الكلى زاد بعد يوم واحد ثم انخفض تدريجيا بعد 3 أيام و7 أيام مقارنة بالكنترول. بينما حدث تذبذب لمستوى الليبيدات الكلى حيث انخفض بعد يوم واحد بينما ارتفع بعد 3 أيام ثم انخفض مرة أخرى بعد 7أيام. وبالنسبة لنشاط إنزيمى GOT, GPT فقد أظهرت النتائج حدوث انخفاض تدريجى لإنزيم GPT مع زيادة الفترة بعد المعاملة بينما حدث العكس مع إنزيم GOT حيث ارتفع نشاط الإنزيم طرديا بعد يوم واحد ، 3 أيام ثم انخفض بعد 7 أيام. أما بالنسبة لإنزيم LDH أكدت النتائج حدوث انخفاض شديد فى مستوى الإنزيم بعد يوم ، 3 ، 7 أيام من المعاملة. كذلك لوحظ حدوث انخفاض تدريجى معنوى فى نشاط إنزيم AchE خلال نفس الفترات الثلاثة.