BIORESIDUAL TOXICITY OF TWO CHITIN SYNTHESIS INHIBITORS AND THEIR LATENT EFFECTS ON NUTRITIONAL INDICES OF THE TORTOISE BEETLE, *CASSIDA VITTATA* (VILL.)

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Abstract

The initial toxicity of Lufenuron (Match 5% EC), Hexaflumuron (Consult 10% EC) and profenofos (Selecron 72% EC) was studied at their recommended rates, also its field and laboratory aged sugar beet leaf residues were tested against the adults of the tortoise beetle, *Cassida vittata* (Vill.). The effect of field-aged leaf residues of the anti-moulting agents compared with profenofos on the consumption and utilization of food by the *C. vittata* adults were also studied. Initial toxicity data revealed that profenofos exhibited 100 percent killing compared with 65% in case of Lufenuron, while Hexaflumuron was remarkably less toxic (60%). On the other hand, in field-aged leaf residue bioassays, indicated that the adult mortality of profenofos was significantly higher (88.75%) compared with Lufenuron (50%) and Hexaflumuron (50%). Comparison based on mean during the whole experimental periods, indicated significant reduction in growth rate (G.R.), consumption index (C.I.) and approximate digestibility (A.D.) for profenofos, Lufenuron and Hexaflumuron treatments compared with the control. Considering the bioresidual effect of the tested compounds on the efficiency of conversion of either ingested (E.C.I.) or digested (E.C.D.) food to body tissue, it was obvious that profenofos and Hexaflumuron resulted in significant increase in both parameters compared with Lufenuron treatments.

INTRODUCTION

The tortoise beetle, *Cassida vittata* (Vill.) is considered as one of the major and important economic pests of sugar beet in Egypt. Control of this insect has depended basically on insecticides. However, in general, the extensive and continued use of insecticides for pest control have resulted in emergence of several strategies for insecticides resistance management (IRM), which have been proposed and implemented in sugar beet pest control program. These strategies include using new compounds having new mode of action and rotation of different chemical classes of insecticides, such as using insect growth regulators and chitin synthesis inhibitors.

Chitin biosynthesis disruptor belonging to phenylthiourea group or/and juvenile hormone analogue (JHA) or mimics (JHM) have been considered early as promising alternative compounds for pest control, causing morphogenic abnormalities and physiological disorders (Radwan *et al.,* 1985) and known to affect the metabolic processes of ingested food (Radwan *et al.,* 1986).

The early studies of Wing *et al.* (1988) & Wing and Aller (1989) proved the insecticidal activity of some ecdysteroid agonists and noval insect growth regulators. Salnsky and Scriber (1985) indicated that the amount of consumed food by any adult influence its performance, growth rate, development time, final body weight and survival. Though an understanding of the nutritional indices in relation to food consumption, growth rate and food utilization in the feeding adults on Hexaflumuron-treated leaves will be of significant value.

Accordingly, the present study was undertaken to investigate the environmental toxicity either initial or/and bioresidual toxicity of the recommended rates of Lufenuron and Hexaflumuron compared with the organophosphorous Profenofos at various intervals after application. Also, the sublethal effects of residues at several intervals on the nutritional indices particularly on the rate of food ingestion, digestion, assimilation and conversion after feeding the adults of *Cassida vittata* (Vill.) on field-aged leaf residues of the tested compounds were tested.

MATERIALS AND METHODS

1. Experimentation:

The tested chemical compounds were the anti-moulting agents, Lufenuron (Match 5% EC) and Hexaflumuron (Consult 10% EC) and the Organophosphate compounds profenofos (Selecron 72% EC).

Field experiments were carried out at (Kafr El-Sheikh Governorate) in 2005 sugar beet season. The experimental area was divided into 16 plots (40 sq m/each) to serve 4 treatments including check treatment, each replicate was repeated 4 times in complete randomized block design. Each experimental plots was treated with the recommended rate of application for each insecticide, i.e. Lufenuron (Match) 200 m/200 L water (Fed.), Hexaflumuron (Consult) 200 ml/200 L water (fed.), and profenofos (Selecron) 750 ml/200 L water (fed.). Randomized samples of sugar beet leaves were collected from treated plots at specific days, through the experimental period (22 days) after spraying. Five samples of 10 leaves were collected after treatment with 0,7,14 and 21 days from each experiment, separately. Five replications of 10 adults/replicate were allowed to feed for 48h through different 4 time intervals (0-1 day, 7-8 days, 14-15 days and 20-21 days0 of treatments on the collected treated leaves with each compound, respectively. The adults that survived from each bioassay-testing time interval were transferred to new clean jars and feed on untreated sugar beet leaves for three successive days.

2. Biological evaluation:

To study the effect of the recommended rate of the tested compounds were studied at the previous testing intervals on food consumption, absorption and utilization parameters. The treated and control adults were weighed before and after feeding, also fresh leaves before offering to the adults and the remained leaves after consumption were weighed and recorded. Faeces discharged by adults were weighed daily. Feeding rate was expressed as the amount of food consumed during the feeding period.

Nutritional indices were calculated according to equations described by Waldbauer (1968) as follows:

Consumption index (C.I.) = C/(T.A.).

Growth rate (G. R.) = G/(T.A.).

Approximate digestibility (A. D.) = (C-F/C) X 100.

Efficiency of conversion of ingested food to body tissue (E.C.I.) = (G/C) X100.

Efficiency of conversion of digested food to body tissue (E.C.D.) = G/(C-F) X100.

Where :

C = Fresh weight of leaves consumed.

T = Duration of feeding period.

A = Mean fresh weight of the adult during the feeding period.

G = Fresh weight gain of the adult.

F= Faces weight during the feeding period.

During the experiment, the calculated mean weight (fresh) of the consumed treated leaves was corrected according to equation described by Ghanema (2002) as follows:

Corrected weight of consumed treated leaves= (Cb/Ca)X Ta

Where:

Cb = Initial fresh weight of leaves without adults.

Ca= Final weight (after exposure to natural dryness for 24 h) of leaves without adults.

Ta = Final weight of treated leaves after feeding the adults . for 24 h.

Daily weight (fresh basis) of consumed treated leaves/adult = (A-B)/C.

Where:

A= Initial weight (fresh) of treated leaves before feeding the adults.

B = Corrected weight (fresh) of treated leaves after feeding the adults.

C = Number of survived adults.

Statistical analysis:

Data were subjected to analysis of Variance (ANOVA) and means were separated by Duncan multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSION

Initial and bioresidual toxicity on *Cassida vittata* adults:

Results in Table (1) demonstrate the percentages of initial and bioresidual adult *C. vittata* mortalities occurring after 2 days of feeding on sugar beet leaves treated with Lufenuron, Hexaflumuron and profenofos at different time intervals after application under field conditions.

The percentages initial of mortalities after 48 h feeding on sugar beet treated leaves followed by 72 h feeding on untreated sugar beet leaves reached 65% for Lufenuron, 60% for Hexaflumuron and 100% for profenofos.

The bioresidual toxicity of the tested insecticides on the second group of adults fed for 48 h on of treated leaves sugar beet collected on day 7 and day 8 following by feeding 3 days on untreated leaves are also presented in Table (1). The adult's mortalities reached 50, 55 and 100% for Lufenuron, Hexaflumuron and profenofos, respectively.

Results of the 3rd group of fed adults for 48 h on of treated leaves of sugar beet collected from the field on day 14 and day 15 after application then fed for 3 days on treated leaves of sugar beet reached 45, 45, and 90% for Lufenuron, Hexaflumuron and profenofos, respectively.

Table (1) indicates that the bioresidual toxicity of the tested insecticides after 21 days of application reached 40, 40 and 65% for Lufenuron, Hexaflumuron and profenofos, respectively. The mean residual adults mortality percentages were 45, 46.66 and 85% for Lufenuron, Hexaflumuron and profenofos, respectively.

The statistical analysis of the overall mean adults mortality percentages revealed that profenofos (88.75%) was significantly higher compared with Lufenuron (50%) and Hexaflumuron (50%).

In this respect, El-Khouly (2001) indicated that the insect growth regulator (IGR) Lufenuron and the plant extract azadirachtin could be classified as Integrated Pest Management (IPM) compatible insecticides.

In general, it can be concluded that feeding on untreated leaves play an important role on the performance of the toxicity later on, particularly for the compounds with new mode of action such as insect growth regulators. However, Radwan and Rizk (1975) indicated that both new chitin biosynthesis inhibitors, PH-5040 and PH-6038 induced justified performance when the tested instar larvae of *Spodoptera littoralis* were fed continuously for 0-5 days after application.

The effect of field-aged leaf residues on feeding activity and nutritional indices:

1. Growth rate (G.R.):

Table (2) shows the effect of the three tested pesticides on growth rate (G.R.) after feeding of *C. vittata* adults on sugar beet treated leaves at specific intervals of feeding. Statistical analysis during the whole experimental periods indicated remarkable reduction in means of growth rates (G.R.) of the treated adults with Lufenuron, Hexaflumuron and profenofos than control.

The reduction percent in G.R. values for Lufenuron was low at the 1st testing intervals, (-9.65%) and it was high (-31.37%) at the 2nd testing intervals. In contrast, the reduction percentages in G.R. values for Hexaflumuron was relatively high at the 1st interval, -18.60% and extremely low at 2nd intervals, (-11.22%). The reduction percentages in G.R. values for profenofos were low at the 3rd and the 4th intervals, (-7.44 and -7.54%), while this reduction was high (-24.29%) at the 1st testing interval.

These findings coincide with the results of several insect species such as *Spodoptera littoralis* fed on diflubezuron-treated caster leaves (Radwan *et al.,* 1986); *Agrotis ipsilon* fed on pyriproxyfen treated castor been leaves (El-Dessouki and Omer, 2000).

2. Consumption index (C.I.):

One of the parameters associated with feeding activity is consumption index (C.I.). Table (3) shows that the means of consumption index (C.I.) of *C. vittata* were significantly decreased than control in all treatments. The changes percentages of C.I. for Lufenuron were almost similar after the four testing intervals, they were (29.92, -28.49, -23.74 and -21.22%) for each interval, respectively. Table (3) reveals slight increase in C.I. changes percentages of Hexaflumuron treated adults in the 1st and 2nd intervals but also they decreased at the 3rd and 4th intervals was detected these percentages were, (-16.12, 15.03, -33.79 and -32.49%) for the 4 intervals, respectively.

Table 1. Initial and bioresidual toxicity of different insecticides against the adults of *Cassida vittata* fed on treated sugar beet leaves for 48 h followed by 3 days feeding on untreated sugar beet leaves.

Treatment	Rate ml/Feddan	%Adult mortality after feeding on collected treated leaves during the indicated periods					*overall mean±SE
		Initial toxicity 1st interval (0-1 day)	Bioresidual toxicity				
			2nd interval (7-8 day)	3rd interval (14-15 day)	4th interval (21-22 day)	Mean	
Lufenuron	40	65	50	45	40	45.00	$50.00^{b}\pm10.80$
Hexaflumuron	200	60	55	45	40	46.66	$50.00^{b}\pm9.12$
Profenofos	750	100	100	90	65	85.00	$88.75^{a}\pm16.52$
Control	-	3	1	2	3	2.00	2.25 ± 0.95
F =							*** 22.57
LSD=							15.24

*Mean followed by the same letter is not significantly different according to Duncan's multiple range tests.

Table 2. Growth rate of *Cassida vittata* adults fed for 48 h on of treated leaves of sugar beet with Lufenuron, Hexaflumuron and profenofos.

Insecticides	Rate ml/Feddan	Mean of growth rate values (G.R.) at the indicated testing intervals								*Mean±SE
		1st interval (0-1 day)		2nd interval (7-8 day)		3rd interval (14-15 day)		4th interval (20-21 day)		
		G.R.	% Change	G.R.	% Change	G.R.	% Change	G.R.	% Change	
Lufenuron	40	0.290	-9.65	0.269	-31.37	0.283	-29.77	0.301	-29.00	0.285[a]±0.013
Hexaflumuron	200	0.261	-18.69	0.348	-11.22	0.336	-11.66	0.369	-12.97	0.333[a]±0.049
Profenofos	750	0.243	-24.29	0.285	-27.29	0.373	-7.44	0.392	-7.54	0.323[a]±0.070
Control	-	0.321	-	0.392	-	0.403	-	0.424	-	0.385±0.044
F =										Ns. 0.88
LSD=										0.08

*Mean followed by the same letter is not significantly different according to Duncan's multiple range tests.

Table 3. Consumption index (C.I.) of *Cassida vittata* fed for 48 h on of treated leaves of sugar beet with Lufenuron, Hexaflumuron and profenofos.

Insecticides	Rate ml/Feddan	Mean of C.I. at the indicated testing intervals								*Mean±SE
		1st interval (0-1 day)		2nd interval (7-8 day)		3rd interval (14-15 day)		4th interval (20-21 day)		
		C.I.	% Change	C.I.	% Change	C.I.	% Change	C.I.	% Change	
Lufenuron	40	3.98	-29.92	4.09	-28.49	2.73	-23.74	3.34	-21.22	3.53[a]±0.63
Hexaflumuron	200	4.73	-16.12	4.86	-15.03	2.37	-33.79	2.82	-33.49	3.69[a]±1.28
Profenofos	750	3.39	-40.31	4.05	-29.19	3.30	-7.82	3.81	-10.14	3.63[a]±0.35
Control	-	5.68	-	5.72	-	3.58	-	4.24	-	4.80±1.06
F =										Ns. 0.036
LSD=										1.36

*Mean followed by the same letter is not significantly different according to Duncan's multiple range tests.

Generally, Table, 3 shows that profenofos recorded significantly the least reduction in C.I. at all testing intervals compared with other treatments and control.

However, the moderate reduction in food consumption for both IGR's induced remarkable inhibition in foods consumption. Such observation is in agreement with earlier reports which indicated that insect growth disruptions interfere with feeding (Abid *et al.,* 1978). Furthermore, previous reports have also confirmed that the chitin biosynthesis disruptor Diflubenzuron acts on the peritrophic membrane by affecting its chitin-protein structure, hinding its role in protecting secreting cells from any damage (Clarke *et al.,* 1977). Likewise, it was found recently that feeding *S. littoralis* larvae on the ecdyson agonist chromfenozide Ghanema, 2000) and/or Tebufenozide (Hashem, 2002) resulted in reduction in food consumption (I.C.).

3. Food utilization (E.C.I. and E.C.D.):

The ability of insect to utilize food for growth is measured by the efficiency of conversion of ingested food (E.C.I.), together with the efficiency of conversion of digested food (E.C.D.) to body substance.

Considering the bioresidual effect of the tested insecticides on E.C.I. values it was obvious that Lufenuron treatment exhibited lower and significant E.C.I. values when compared with control (Table 4).

Based on mean of the efficiency of conversion of digested food (E.C.D.) for adults fed on treated leaves with Lufenuron and Hexaflumuron, they were in general, remarkably decreased than that of the control during the four intervals. Such decrease in case of Hexaflumuron in addition to that of profenofos were significantly lower than either Lufenuron or/and control treatment.

Our results in this respect are in agreement with those reported by Hashem (2002) who indicated that the decreases in E.C.D. values of IGR-treatments were more obvious in treatment of higher sublethal concentration than in the lower one.

Similarly, previous studies indicated that different IGR's, reduced E.C.I. and E.C.D. in *S. littoralis* (Radwan *et al.,* 1986, and Ghanema, 2002).

4. Approximate digestibility (A.D.):

Data shown in table (5) revealed that the treatment of adults food with Lufenuron and Hexaflumuron at recommended rates resulted in considerable increase in A.D. values within the 4 testing intervals. Based on the mean of A. D. values within all experimental period, revealed that both compounds induced in A.D. increase with 96.08% for Lufenuron and 95.77% for Hexflumuron compared with a relatively lower A.D. of 95.56% for control. Our results in this respect agree with those obtained by El-Dessouki and Omr (2002) using Tebufenozide against 4th instar larvae of *S. littoralis.*

In conclusion, the obtained results strengthen the notion that Lufenuron and related compounds may be useful in the control of leopteran insectspests such as *C. vittata* completely unrelated to that of other classes of conventional neurotoxic insecticides in addition to their long bioresidual activity.

Table 4. Efficiency of conversion of ingested food to body tissue (E.C.I.) and efficiency of conversion of digested food to body tissue (E.C.D.) after feeding adults of *Cassida vittata* for 48 h on of treated leaves of sugar beet with Lufenuron, Hexaflumuron and profenofos.

Insecticides	Rate ml/Feddan	Mean of E.C.I. at the indicated testing intervals								*Mean±SE
		1st interval (0-1 day)		2nd interval (7-8 day)		3rd interval (14-15 day)		4th interval (20-21 day)		
		E.C.I.	% Change	E.C.I.	% Change	E.C.I.	% Change	E.C.I.	% Change	
Lufenuron	40	49.60	-12.13	52.63	-17.91	44.74	+10.41	47.06	-15.67	48.50ᵃ±3.39
Hexaflumuron	200	55.10	-2.39	65.45	+2.07	58.03	+16.19	43.59	-21.89	55.89ᵃ±13.35
Profenofos	750	71.88	+27.33	70.27	+9.59	46.67	-6.54	43.59	-21.89	58.10ᵃ±15.04
Control	-	71.11	-	64.12	-	49.94	-	55.81	-	60.24±9.29
F =										* 0.92
LSD=										16.53

Insecticides	Rate ml/Feddan	Mean of E.C.D. at the indicated testing intervals								*Mean±SE
		1st interval (0-1 day)		2nd interval (7-8 day)		3rd interval (14-15 day)		4th interval (20-21 day)		
		E.C.D.	% Change	E.C.D.	% Change	E.C.D.	% Change	E.C.D.	% Change	
Lufenuron	40	52.35	-12.85	58.32	-13.71	46.20	-5.38	49.08	-15.10	51.48ᵃ±5.20
Hexaflumuron	200	57.69	-3.96	67.29	-0.44	61.69	+26.33	45.31	-22.41	54.08ᵃ±11.21
Profenofos	750	74.68	+24.32	71.23	+5.38	48.05	-1.59	45.09	-22.00	59.76ᵃ±15.43
Control	-	60.07	-	67.59	-	48.83	-	57.81	-	58.57±7.72
F =										* 0.65
LSD=										17.29

*Mean followed by the same letter is not significantly different according to Duncan's multiple range tests.

Table 5. Approximate digestibility (A.D.) for groups of *Cassida vittata* fed for 48 h on treated leaves of sugar beet with Lufenuron, Hexaflumuron and profenofos.

Insecticides	Rate ml/Feddan	Approximate digestibility (A.D.) at the indicated testing intervals								*Mean±SE
		1st interval (0-1 day)		2nd interval (7-8 day)		3rd interval (14-15 day)		4th interval (20-21 day)		
		A.D.	% Change	A.D.	% Change	A.D.	% Change	A.D.	% Change	
Lufenuron	40	95.91	-0.06	95.71	+0.46	96.84	+0.74	95.88	+1.05	$96.08^{a}\pm0.51$
Hexaflumuron	200	96.06	+0.09	95.44	+0.17	95.63	-0.50	96.92	+2.15	$95.77^{a}\pm0.89$
Profenofos	750	96.25	+0.29	98.65	+3.54	97.11	+1.02	96.67	+1.88	$97.17^{a}\pm1.04$
Control	-	95.97	-	95.27	-	96.12	-	94.88	-	95.56±0.58
F =										Ns. 2.82
LSD=										1.24

*Mean followed by the same letter is not significantly different according to Duncan's multiple range tests.

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الآثر الباقي لسمية إثنين من مثبطات تخليق الكيتين وأثرهما المتأخر علي الإستهلاك الغذائي لحشرة خنفساء البنجر السلحفائية

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١. *معهد بحوث وقاية النباتات – مركز البحوث الزراعه – جيزة*

٢. *المعمل المركزي للمبيدات– مركز البحوث الزراعية – الدقي – جيزة*

في تجربة حقلية معملية لدراسة الآثر الباقي لسمية إثنين من مثبطات تخليق الكيتين وهما ليوفينورون (ماش ٥% EC) وهكسافينورون (كونصلت ١٠% EC) بالإضافة للمبيد الفوسفوري بروفونفوس (سليكرون ٧٢% EC) على حشرة خنفساء البنجر السلحفائية وجد أن الآثر الفوري للبروفونفوس ١٠٠% و ليوفينورون ٦٥% وهكسافينورون ٦٠% أما الآثر الباقي فكان للبروفونفوس (٨٨,٦٥%) ذات معنوية عالية مقارنة باليوفينورون وهكسافينورون ٥٠%.

وبمقارنة متوسطات فترات التغذية الأربعة التي درست علي معدل النمو ودليل الإستهلاك ومعامل الهضم التقريبي لمبيدات بروفونفوس و ليوفينورون و هكسافينورون ظهرت ذات معنوية منخفضة عن المقارنة. وبمقارنة الآثر الباقي لتأثير كلا من كفاءة تحويل الغذاء المهضوم لمبيدات بروفونفوس و هكسافينورون وجد أنها ذات معنوية عالية عن ليوفينورون.