EFFECT OF NON-STEROID COMPOUNDS ON COTTON LEAFWORM

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Abstract

The effects of the nonsteroidal ecdysteroids tebufenozide on some biochemical of the haemolymph *Spodoptera littoralis* larvae was studied. This include the effects of total protein, lactic acid dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G-6-PDH), malic acid dehydrogenase (MDH), alkaline phosphatase (Alkapase), acid phosphatase (Acpase), aspartate glutamate aminotransferase (GOT), alanine oxaloacetic aminotransferaase (GPT) and food consumption. The data show that tebufenozide have a significant effect on the above mentioned enzyme activities. However, MDH, Alkapase did not affect with tebufenozide. Also, tebufenozide has no effect on Acpase, but has a great effect on GOT and GPT. The relative consumed rate for *S. littoralis* was decreased as the concentration of tebufenozide increase.

Introduction

Tebufenozide is an analogue of the dibenzoylhydrazine- based nonsteroidal ecdysteroids, which represents a novel class of insect growth regulators. The morphogenic and developmental effects of some synthetic ecdysteroids were reported (Smagghe and Degheele, 1997; Retnakaran *et al*., 1997). However, no attention has almostly been paid to study the biochemical effect of such synthetic ecdysteroids. The purpose of this work was to study the effects of the nonsteroid tebufenozide on some of biochemical aspects of *Spodoptera littoralis* larvae.

Material and Methods

Haemolymph samples were collected from both 4*th* and 6*th* instar larvae which fed on castor oil leaves previously treated with LC_{50} of the tested compound tebufenozide [(1-N-t-butyl-1-(3,5-dimethyl) benzoyl-2-(4-ethyl benzoyl] hydrazine. Total protein in the haemolymph samples was determined by Lowry *et al*. (1951) method. Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities were carried out according to Reitman and Frankel (1957). Acid and alkaline phosphatase enzymes were determined according to Shinowara *et al*. (1942) method. Lactic acid dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) were determined by Kilgore and Painter method (1964) and malic acid dehydrogenase (MDH) were determined as Meizel and Market (1967) method. Relative consumed rate (RCR) was determined as Slansky (1993) method. The means and standard deviation were calculated for each test and the data were compared using the ANOVA test according to Snedecor (1971).

Results and Discussion

Effect of Tebufenozide on the Protein Contents of the 4*th* and 6*th* Instar Larvae of *S. Littoralis*

Feeding the 4*th* and 6*th* instar larvae of *S. littoralis* on castor oil leaves previously treated with LC_{50} of tebufenozide caused a significant decrease in the level of the total protein at all time intervals tested for the 4*th* and 6*th* instar larvae comparing with the untreated check as shown in Table (1).

The decrease of total protein may reflect the decrease in the activity of various enzymes. The results agree with Shebl (1979) who found the protein in haemolymph of *Heliothis zea* was decreased after treatment with Dimilin. Also, Abdel-Hafez *et al*. (1988) reported that some insect growth regulators caused a reduction in the level of protein of *S. littoralis*. Also, Gadallah *et al*. (1994) reported the same results.

Effect of Tebufenozide on the Ldh and G-6-pdh Activities of the 4*th* and 6*th* Instar Larvae of *S. Littoralis*

The activity of lactic acid dehydrogenase (LDH) and glucose 6-phosphatase dehydrogenase (G-6-PDH) in haemolymph of 4*th* and 6*th* instar larvae of *S. littoralis* was determined after treatment with LC_{50} of tebufenozide are shown in Table (2). Data revealed that, there were a significant reduction in the level of enzyme activity during different time intervals comparing with the check for the LDH but it was not the case for G-6-PDH since the data show there was no significant change in activities. The relative activities of LDH and G-6-PDH enzymes have been used as index to indicate the significance conversion pyruvate to lactate or to another reaction sequence (Chen and Awapara, 1969). Both LDH and G-6-PDH activation are considered as evidence for an alternative pathway of terminal anaerobic metabolism (Bianconcini *et al*., 1980).

Effect of Tebufenozide on Malic Acid Dehydrogenase of *S. Littoralis*

The results indicated that there are no significant differences between the treated and untreated larvae of *S. littoralis* at all time intervals for the 4*th* and 6*th* instar larvae for malic acid dehydrogenase as shown in Table (4). However, the activity of MDH during larval development period was almost constant in the haemolymph.

Effect of Tebufenozide on Acid and Alkaline Phosphatase of *S. Littoralis*

The effect of tebufenozide on acid and alkaline phosphatase are shown in Tables (5 and 6). The result indicated that no significant in the activity of acid phosphatase at all intervals in both tested larval instars. In contrast the tebufenozide have a significant in the activity of alkaline phosphatase in late development of 4*th* and 6*h* instar larvae of *S. littoralis*. It is known that phosphatase are defined as enzyme hydrolyzing and phosphorus esters (O'Brien, 1967). This increase or decrease in the activity of the enzyme during development is reflected in decrease or increase in acid-soluble phosphorus content. Same results were obtained when larvae of *S. littoralis* were treated with flufenoxuron and teflubenzuron (El-Kordy *et al*., 1995).

Effect of Tebufenozide on Got and Gpt of *S. Littoralis*

Data in Tables (7 & 8) showed the effect of tebufenozide on the activities of aspartate glutamate aminotransferase (GOT) and alanine oxaloacetic aminotransferase (GPT) of the 4*th* and 6*th* instar of *S. littoralis* larvae. The data revealed that, there was no significant increase in GPT activities during the different time intervals comparing with the check. GOT showed different trend in treated larvae with tebufenozide. In case of 4*th* and 6*th* instar larvae treated of *S. littoralis*, the enzyme activity was reduced comparing with the check.

It is generally accepted that, building blocks for protein synthesis comes from amino acid pool maintained mainly by transamination, and it known that GOT and GPT are the most active transaminase enzyme. Thus, tebufenozide could be considered as inhibitory agent for protein synthesis in treated *S. littoralis*. This results are in agreement with the finding of Abdel-Hafez *et al*. (1988) in larvae of *S. littoralis* with diflubenzuron and triflumuron.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:892-895 (2001)
National Cotton Council, Memphis TN

Effect of Tebufenozide on Food Consumption of *S. Littoralis*

The data in Table (9) show the effect of tebufenozide on food consumption on 4*th* and 6*h* instar larvae of *S. littoralis*.

The reduction on food consumption was reversely correlated to the concentration irrespective of the treated larvae. Relative consumed rate (RCR) was found to be associated with the amount of food consumed and showed the same trend, *i.e*. RCR was decreased as the concentration increased. This was in both 4*th* and 6*th* instar treated larvae.

The present results, show an inhibitory action of the tested ecdysteroid on the food consumption whether its determination was estimated as amounts in Mg of RCR. Such reductions in food consumption gave remarked reductions in relative weight gain (RWG). Same results in agreement with Sundaramurthy (1977 and Farag, 1991).

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Table 1. Changes in protein contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

	Protein contents (μg/g b. wt. $\pm$ S.D.)			
	0	**24 hr**	**48 hr**	**96 hr**
4th instar				
Tebufenozide	30.2±1.3 a	20.1±1.1 b	30.4±1.3 d	42.3±1.3 f
Check	33.8±1.1 a	24.2±1.2 c	36.3±1.4 e	50.3±1.4 g
6th instar				
Tebufenozide	31.1±1.9 a	32.0±1.4 a	40.1±1.6 b	36.5±1.9 a
Check	35.2±1.4 a	41.3±1.5 b	45.3±1.2 b	44.3±1.4 b

- Figures followed by the same letters are statistically insignificant (P > 0.05), those followed by the same letter are statistically significant (P > 0.5).

Table 2. Changes in lactic acid dehydrogenase contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

	LDH contents (μg/g b. wt. $\pm$ S.D.)			
	0	**24 hr**	**48 hr**	**96 hr**
4th instar				
Tebufenozide	10.14±2.0 a	15.32 ±1.0b	16.41±1.4b	21.43±1.3bc
Check	13.6 ±4.0 a	17.44±1.4b	18.23±1.5b	26.51±1.4c
6th instar				
Tebufenozide	4.1±1.3 a	6.90± a	7.22± b	24.54±1.5 c
Check	4.3±1.5 a	7.81± b	8.33±1.6 b	29.55±1.4 d

- Figures followed by the same letters are statistically insignificant (P > 0.05), those followed by the same letter are statistically significant (P > 0.5).

Table 3. Changes in Glucose-6-phosphate hydrogenase contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

G-6-PH contents (μg/g b. wt. $\pm$ S.D.)				
	0	**24 hr**	**48 hr**	**96 hr**
4th instar				
Tebufenozide	50.1±2.5 a	64.32±2.4 b	68.4±2.4 b	81.4±1.5 d
Check	51.31±3.9 a	66.44±2.8 b	71.54±1.9 b	85.32±1.8 d
6th instar				
Tebufenozide	60.25±1.4 a	68.5±1.5 b	74.31±2.3 c	80.11±1.6 d
Check	67.43±1.3 a	73.55±1.9 b	81.63±2.4 c	88.51±1.4 d

- Figures followed by the same letters are statistically insignificant (P > 0.05), those followed by the same letter are statistically significant (P > 0.5).

Table 4. Changes in Malic acid dehydrogenase contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

MADn contents (µg/g b.wt. ±S.D.)				
	0	**24 hr**	**48 hr**	**96 hr**
4*th* instar				
Tebufenozide	20.31±1.1 a	22.83±1.3 a	16.12±1.4ab	18.56±1.4 b
Check	21.44±1.4a	23.55±1.2 a	19.41±1.5ab	20.33±1.3 b
6*th* instar				
Tebufenozide	21.0±1.6 a	20.13±1.4 a	17.46±1.4 a	17.94±1.5 a
Check	22.44±1.2 a	21.52±1.5 a	18.90±1.9 a	19.43±1.6 a

Figures followed by the same letters are statistically insignificant (P > 0.05), those followed by the same letter are statistically significant (P > 0.5).

Table 5. Changes in acid phosphatase contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

Acid phosphatase contents (µg/g b.wt. ±S.D.)				
	0	**24 hr**	**48 hr**	**96 hr**
4*th* instar				
Tebufenozide	13.51±2.3 a	10.41±1.5 a	9.99±1.4 a	11.52±2.1 a
Check	14.31±1.5 a	11.59±1.4 a	11.45±1.5 a	12.3±1.6 a
6*th* instar				
Tebufenozide	15.63±1.4 a	12.40±1.5 a	12.13± a	10.55±1.4 a
Check	16.82±1.9 a	12.94±1.8 a	13.45± a	11.13±1.2 a

- Figures followed by the same letters are statistically insignificant (P > 0.05), those followed by the same letter are statistically significant (P > 0.5).

Table 6. Changes in alkaline phosphatase contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

Alkaline phosphatase contents (µg/g b.wt. ±S.D.)				
	0	**24 hr**	**48 hr**	**96 hr**
4*th* instar				
Tebufenozide	7.13±1.4 a	5.22±1.0 a	9.31±1.1 b	8.45±1.6 d
Check	8.45±1.1 a	8.92±0.9 a	5.14±1.3 c	15.52±1.7 e
6*th* instar				
Tebufenozide	8.49±1.2 a	6.43±1.2 b	7.55±1.2 c	11.32±1.4 e
Check	10.13±1.3 a	9.42±1.5 b	13.2±1.3 d	16.3±1.5 f

Table 7. Changes in GPT haemolymph contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

GPT haemolymph contents (µg/g b.wt. ±S.D.)				
	0	**24 hr**	**48 hr**	**96 hr**
4*th* instar				
Tebufenozide	31.61±2.4 a	14.42±1.3 c	31.52±1.4 a	38.4±2.3 e
Check	115.31±2.6b	46.50±1.5 d	28.3±2.1 a	32.6±2.6 a
6*th* instar				
Tebufenozide	21.51±1.3 a	11.54±1.5 c	41.32±2.4 d	50.4±3.4 f
Check	32.54±1.4 b	43.15±1.6 d	32.33±2.5 e	36.3±2.9 g

Table 8. Changes in GOT haemolymph contents in the 4*th* and 6*th* instar larvae of *S. littoralis* after treated with LC_{50} values of tebufenozide orally.

GOT haemolymph contents (μg/g b. wt. $\pm$ S.D.)				
	0	**24 hr**	**48 hr**	**96 hr**
4th instar				
Tebufenozide	10.4±1.2 a	48.63±1.4 b	34.12±1.9 c	29.55±1.4 c
Check	9.33±1.0 a	50.31±1.9 b	36.62±1.5 c	32.54±1.9 c
6th instar				
Tebufenozide	29.52±1.4 a	38.42±1.6 b	29.33±1.2 a	29.41±2.3 a
Check	31.55±1.5 a	41.34±1.4 b	33.22±2.1 a	33.66±2.9 a

Table 9. Effect of tebufenozide on food consumption (mg±S.D.) on the 4*th* amd 6*th* instar larvae of *Spodoptera littoralis* (Boisd.).

Conc. (ppm)	**Relative weight gain**	**Food consumed**	**Relative consumed rate**	**% of change**
4*th* larvae				
1.0	21.43±4.5 a	345.62±11.4 a	0.73±0.21 a	0.231
5.0	14.32±6.2 a	262.35±14.2 b	0.74±0.26 a	0.221
10.0	4.43±1.5 b	171.21±13.9 c	0.79±0.32 a	0.168
chek	63.46±4.2 c	614.21±12.5 d	0.95±0.12 b	-
6*th* larvae				
1.0	24.23±1.5 a	416.0±3.9 a	0.61±0.32 a	0.306
5.0	16.35±2.1 a	243.0±2.2 b	0.63±0.36 a	0.284
10.0	11.23±1.9 b	69.0±4.5 c	0.68±0.31 a	0.227
chek	46.45±3.2 c	745.0±3.2 d	0.88±0.50 c	-