EFFECT OF JUVENILE HORMONE ANALOGOUS ON COTTON LEAFWORM

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Abstract

The effects of the juvenile hormone analogues pyriproxyfen [2-(1-methyl 1-2-(phenoxyphenoxy) ethoxy) pyridine] on total protein, amino acid tranferases, acid and alkaline phosphatases of Spodoptera littoralis were studied after feeding the 4th and 6th instar larvae on the LC_{50} (225 ppm) of the tested compounds. The results showed that pyriproxyfen caused a significant reduction in the level of the total protein at all time intervals tested (48, 72, 96 and 144 hours). The data revealed that there was a significant increase in activity of glutamatic Oxaloacetic transaminase (GOT) during the different time intervals comparing with the untreated check. While there was a significant reduction in the level of glutamatic pyruvic transaminase (GPT) after 48 and 72 hours in both 4th and 6th instar larvae. In contrast, at 96 and 144 hours, there was a significant increase. Pyriproxyfen also cause a significant decrease in activity of alkaline phosphatase (alkapase) and acid phosphatase at different time intervals.

Introduction

The juvenile hormone analogues as insect growth regulators have gained attention for insect control, because of their unique mode of action, that differ from the conventional insecticides. Recently, several compounds of this group have been develped which are still much more active such as pyriproxyfen. In addition to their effect on the reproductive system, these compounds disrupt the insect development from egg, through instar stages, to pupa and imago by controlling the timing for molting of the insect exoskeleton (Abdallah, et. al., 1974 and 1975). On the other hand, understanding of resistance mechanism of insects toward insecticides is an important factor in the IPM programmes. It is necessary to determine the vital role of the enzyme system of insect in destroying such compounds. This phenomena have been studied by (Abdel-Fattah et. al., 1986; Abdel-Hafez et. al., 1988; Gadallah et. al., 1990 and 1994.

The purpose of this work was carried out to study the effects of the juvenile hormone mimic pyriproxyfen on the activities of some biochemical aspects of Spodoptera littoralis that main problem of cotton and other crops in Egypt.

Materials and Methods

Test Insect

Laboratory strain of the cotton leafworm, Spodoptera littoralis (Boisd.) which has been reared in the laboratory for ten generation away from any insecticide contamination was used in these studies. The larval instars were fed on castor bean leaves, Ricinus communis (L.) After pupation, the pupae were placed in a wide glass jars until the adults emergence. Then, the emerged adults were supplied with a piece of cotton wetted with 10% sugar solution and branches of tafla (Nerium oleander) as suitable site for oviposition (El-Defrawi et. al., 1964). All stages were reared and treated under constant conditions of 25±2 °C and 65± R.H.

Chemicals Treatment Procedure

The 4th and 6th instar laravae were fed on castor oil leaves treated with different concentrations of the tested compound during the preliminary experiments. Then LC_{50} values were determined from the mortality regression lines (Finney, 1952).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:890-892 (2001)
National Cotton Council, Memphis TN

Collection of Haemolymph Samples

Haemolymph samples were collected from both 4th and 6th instar larvae which fed on castor oil leaves previously treated with LC_{50} (225 ppm) of the tested compound. The haemolymph samples were collected at 48, 72, 96 and 144 hour after transferring to untreated leaves. The procedure described by Chen and Levenbook, 1960 was followed. Collected haemolymph was kept inconvenient small well stoppered glass (7 x 0.5 cm). After being centrifuged at 2500 r.p.m. for 10 minutes, the samples were stored in refrigerator at - 20°C.

Protein Determination

Total protein in the haemolymph samples was determined according to Lowry et. al. (1951).

Determination of Transaminase Activities

Determination of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities were carried out according to Reitman and Frankel (1957).

Determination of Acid and Alkaline Phosphatase

The activity of acid and alkaline phosphatase enzymes were determined according to the method of Shinowara et. al. (1942).

Statistical Analysis

The means and standard deviation were calculated for each experiment and the data were compared using the ANOVA test according to Snedecor (1971).

Results and Discussion

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

The decrease of total protein might reflect the decrease in the activity of various enzymes. The pyriproxyfen could be considered as inhibitory agents for protein synthesis in S. littoralis. These results are in agreement with Abdel-Hafez et. al. (1988) and Gadallah et. al. (1994).

Data in Table (2) indicated that pyriproxyfen treatment showed a significant increase in the level of glutamic oxaloacetic transaminase (GOT) actvity after 48, 96 and 144 hours of feeding the 4th instar larvae on treated castor bean leaves with LC_{50}, while highly significant decrease was found after 72 hours of treatment in comparison with the untreated larvae.

As for the 6th instar larvae, application with the LC_{50} revealed significant decrease in GOT activity after 48 and 72 hours, in contrast the enzyme activity was increased after 96 and 144 hours post-treatment comparing with untreated check.

Considering alanine amino tranferase activity (GPT), Table (3) showed that there was a significant reduction in the level of glutamatic pyruvic transaminase (GPT) after 48 and 72 hours in both 4th and 6th instar larvae. In contrast, at 96 and 144 hours, there was a significant increase.

It is clear from our results that pyriproxyfen revealed an pronounced increase in transaminases (GOT & GPT) at most time intervals after treatment. Since transferases are group of enzyme which responsible for maintenance of the balance amino acid pool in insects. Consequentely, the degradation in hemolymph protein increased resulted in reduction in protein content. It has been reported by many workers that the level of amino acid transferases varies with the amount of protein synthesis (Chen, 1966; Gilbert, 1967; Gadallah et. al., 1994). Zidan et. al. (1996) found that

immediately after treatment, B. thuringiensis had the most significant effect on reduction of protein content of the larvae, but KZ oil showed the highest overall reduction 72 h after treatment. Pyriproxyfen resulted in the best immediate inhibitory effect on AchE [acetylcholinesterase] whereas B. thuringiensis and KZ oil caused better latent effects. Pyriproxfen and B. thuringiensis caused considerable reduction in ACP (acid phosphatase) activity (72 h) after treatment. The inhibition of GPT [alanine aminotransferase] was greatest within 24 h for all 3 insecticides used.

The effect of pyriproxyfen on the activities of haemolymph acid phosphatase (Acpase) and alkaline phosphatase (Alkpase) in 4th and 6th instar larvae were determined at different time intervals, as shown in Tables (4 and 5).

The data revealed that pyriproxyfen caused a significant reduction in the activities of Acpase enzyme at all time intervals treated for the two instar larvae. Alkpase activities showed a different trend. There was a significant difference at all time interval except at 24 hours after treatment.

The present results agree with the finding of Abdel-Hafez et. al. (1988) and El-Kordy et. al. (1995).

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Table 1. Changes in protein contents in the 4th and 6th instar larvae of S. littoralis after treated with LC_{50} values of pyriproxyfen with leaf dipping technique.

Protein contents (μg/g b. wt. ± S.D.)				
***h.p.t.**	**48 hr**	**72 hr**	**96 hr**	**144 hr**
4th instar				
Pyriproxyfen	26.5±1.5a	28.43±1.4a	29.43±1.3a	30.11±1.4a
Check	33.8±1.9b	24.28±1.8a	36.25±1.7b	50.32±2.1b
6th instar				
Pyriproxyfen	27.44±1.7a	36.55±1.3a	34.14±1.7a	35.24±2.1a
Check	35.22±1.5b	41.32±1.3b	45.32±1.2b	44.33±1.1b

- Figures followed by the same letters are statistically insignificant (P>0.05), those followed by the different letter are significantly different (P>0.5).
*h.p.t.: hours post treatment.

Table 2. Changes in GOT haemolymph contents in the 4th and 6th instar larvae of S. littoralis after treeated with LC_{50} values of pyriproxyfen with leaf dipping technique.

GOT haemolymph contents (μg/g b. wt. ± S.D.)				
***h.p.t.**	**48 hr**	**72 hr**	**96 hr**	**144 hr**
4th instar				
Pyriproxyfen	26.6±0.8a	19.4±1.0a	48.3±2.3a	41.4±2.2a
Check	9.3±0.7b	50.3±2.3b	36.3±1.7b	32.5±2.1b
6th instar				
Pyriproxyfen	29.4±1.1a	11.9±0.8a	41.61±1.3a	42.3±1.4a
Check	31.5±1.1b	41.3±2.1b	33.2±1.3b	33.6±1.1b

- Figures followed by the same letters are statistically insignificant (P>0.05), those followed by the different letter are significantly different (P>0.5).
*h.p.t.: hours post treatment.

Table 3. Changes in GPT haemolymph contents in the 4th and 6th instar larvae of S. littoralis after treated with LC_{50} values of pyriproxyfen with leaf dipping technique.

GPT haemolymph contents (μg/g b. wt. $\pm$ S.D.)				
***h.p.t.**	**48 hr**	**72 hr**	**96 hr**	**144 hr**
4th Instar				
Pyriproxyfen	35.5±1.9a	14.5±1.4a	34.4±1.3a	45.3±1.2a
Check	115.3±2.3b	46.5±2.9b	28.3±0.9b	32.6±1.0b
6th Instar				
Pyriproxyfen	26.4±0.8a	12.4±1.2a	49.5±1.1a	55.4±1.4a
Check	32.5±1.4b	43.1±2.4b	38.3±0.7b	36.3±1.5b

- Figures followed by the same letters are statistically insignificant (P>0.05), those followed by the different letter are significantly different (P>0.5).
*h.p.t.: hours post treatment.

Table 4. Changes in acid phosphatase contents in the 4th and 6th instar larvae of S. littoralis after treated with LC_{50} values of pyriproxfen with leaf dipping technique.

Acid phosphate contents (μg/g b. wt. $\pm$ S.D.)				
***h.p.t.**	**48 hr**	**72 hr**	**96 hr**	**144 hr**
4th instar				
Pyriproxyfen	8.3±0.6a	6.9±0.7a	3.4±0.5a	2.3±0.6a
Check	14.3±0.8b	11.5±0.8b	11.4±0.7b	12.32±0.7b
6th instar				
Pyriproxyfen	11.4±0.7a	7.3±0.7a	5.8±0.7a	3.3±0.8a
Check	16.8±0.9b	12.9±1.0b	13.4±0.9b	11.1±0.9b

- Figures followed by the same letters are statistically insignificant (P>0.05), those followed by the different letter are significantly different (P>0.5).
*h.p.t.: hours post treatment.

Table 5. Changes in alkaline phosphatase contents in the 4th and 6th instar larvae of S. littoralis after treated with LC_{50} values of pyriproxyfen with leaf dipping technique.

Alkaline phosphate contents (μg/g b. wt. + S.D.)				
***h.p.t.**	**48 hr**	**72 hr**	**96 hr**	**144 hr**
4th instar				
Pyriproxyfen	6.32±0.4a	4.21±0.3a	8.91±0.6a	9.5.3±0.6a
Check	8.45±0.6a	8.92±0.5b	12.14±0.8b	15.52±1.1b
6th instar				
Pyriproxyfen	7.92±0.7a	5.2±0.4a	8.4±0.7a	10.1±0.9a
Check	10.13±1.1a	9.4±0.8b	13.2±0.9b	14.3±1.2b

- Figures followed by the same letters are statistically insignificant (P>0.05), those followed by the different letter are significantly different (P>0.5).
*h.p.t.: hours post treatment.