BIOCHEMICAL RESPONSES OF *SPODOPTERA LITTORALIS* (BOISD.) TO THE TREATMENT WITH SPINETORAM, TEFLUBENZURON AND TEBUFENOZIDE

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Abstract

The susceptibility of the laboratory 4th instar larvae of cotton leafworm *Spodoptera littoralis* to the treatments with the green chemical spinetoram and two IGR's (teflubenzuron and tebufenozide) was studied. Teflubenzuron exhibited high level of toxicity with low LC_{50} value (8.9 ppm) followed by tebufenozide (65.7 ppm) and finally spinetoram (121.1 ppm). The LC_{50} value of each compound was used to determine the biochemical responses of *Spodoptera littoralis* to the treatment with the tested compounds (Spinetoram, Teflubenzuron and Tebufenozide), the treatment with spinetoram caused significant increase in chitinase, phenol-oxidase, trehalase and acetylcholine esterase activities, while the activity of amylase was significantly reduced. The treatment with tebufenozide reflected in high α-esterase activity. In contrary teflubenzuron remarkably reduced chitinase, trehalase and α-esterase activities.

INTRODUCTION

Among more than 1300 insect species recorded from cotton, the most important pest to have spread is the cotton leafworm *Spodoptera littoralis* (Boisduval), which is found almost every where cotton is grown (Matthews and Tunstall, 1994). It is one of the most notorious and destractive phytophagous insect pests in Egypt, not only to cotton, but also to other field crops and vegetables (Kandil *et al.*, 2003). Although, chemical insecticides are very effective mean of preventing the major damage caused by *S. littoralis*, the extensive and continuous use of traditional insecticides to control cotton pests created many problems, mainly the incapability of toxic agents in controlling the target pest at the recommended doses. Therefore, insecticides with novel modes of action are required. Spinetoram is a new class of insect management tools developed by Dow, produced from fermentation of *Saccharopolyspora spinosa* as other spinosyns, but fermentation is followed by chemical modification to create the unique active ingredient in spinetoram which owned the 2008 presidential green chemistry challenge award (USEPA, 2008). Also insect growth regulators (IGR's) received great attention as a hope for the future of insect control. Among these IGR's, chitin synthesis inhibitors (CSI's) acylureas interfere with the chitin deposition. Occurrence of chitin is mainly restricted to arthropods, fungi and nematodes, ingestion of chitin synthesis inhibitors by insect larvae disturbed

endocuticular deposition during molting process because it blocks chitin synthesis (Mulder and Gijswijt, 1973). Another group of IGR's is the ecdysone receptor agonist (diacylhydrazines) that exert the same action as 20-hydroxy-ecdysone such as tebufenozide. These compounds mimic the biological activity of the natural insect molting hormone 20-hydroxyecdysone (20E) by binding to the ecdysteroid receptor complex in a competitive manner with ecdysteroids (Wing, 1988). The bisacylhydrazine derivatives are nonsteroidal ecdysteroid agonists that mimic the action of moulting hormones and induce a precocious and incomplete moult in several insect orders (Khebbeb *et al.,* 2008). Therefore, the aim of this work was to spot light the effect of the treatment with the tested compounds (Spinetoram, Teflubenzuron and Tebufenozide) on the enzymes related to the nervous system or to the cuticle synthesis of 4th instar larvae of *S. littoralis* as a way to understand the mode of action of each compound.

MATERIALS AND METHODS

Insects:

S. littoralis strain used in this study is a laboratory susceptible strain reared in the Plant Protection Research Institute, Pest Physiology Department. The culture was maintained under optimum conditions (25^{o}C ± 1 and 70 ± 5% R.H) and reared on castor-bean leaves until the 4th larval instar which used in the study.

Compounds:

Radiant, SC 12% (Spinetoram, a mixture of major component (3'ethoxy, 5,6-dihydro spinosyn J) and minor component (3'ethoxy spinosyn L). It is a trademark of (Dow AgroSciences).

Nomolt, SC 15% (Teflubenzuron, *N*-[[(3,5-dichloro-2,4 difluorophenyl) amino]carbonyl]-2,6-difluorobenzamide), a trademark of (BASF).

Mimic, WP 7% (Tebufenozide, 3,5-dimethylbenzoic acid 1-(1,1-dimethylethyl)-2-(4-ethylbenzoyl)hydrazide), a trademark of (Dow AgroSciences).

Bioassay:

Leaf-dipping bioassay method was used to determine the median lethal concentration (LC_{50}) values of the tested compounds (Spinetoram, teflubenzuron and tebufenozide). Series concentrations (in water) of the tested formulated compounds were prepared. Castor-bean leaves were dipped for 15 seconds in each concentration then left to dry. The treated leaves were offered to newly molted 4th instar larvae for 48 hr. then replaced by untreated leaves for 24 hr. Mortality percentages were recorded after 72 hr. and corrected according to natural mortality (Abbott, 1925). To

estimate the LC_{50} values, the corrected mortality percentages were subjected to probit analysis according to Finney (1952).

Preparation of samples for biochemical studies:

Castor-bean leaves were dipped for 30 seconds in an aqueous solution of each of the tested compounds at the LC_{50} level, and then left for drying in room temperature before being offered to the 4th instar larvae of *Spodoptera littoralis* . Larvae were fed for 48 hours on the treated leaves, and then transferred to fresh untreated leaves. Larval samples for biochemical assays were collected after 72 hr. during treatment periods, then placed away of food and starved.

The starved larvae were homogenized in distilled water using a teflon homogenizer-(MECHIANIKA PRECYZYJNA warszawa type MPN-309-Poland)-surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 6000 r.p.m. for 10 minutes at 5°C -(by BECKMAN GS-6R Centrifuge)-, and the supernatants were used directly for the biochemical analyses.

Enzymes measurements:

-non-specific esterases (α and β- esterase) activities were measured using the method of Van Asperen (1962), using α- and β- naphthyl acetate as substrates.

-Acetyl choline-esterase activity was measured according to the method described by Simpson *et al.,* (1964), using acetylcholine bromide (AChBr) as substrate

-Carbohydrates hydrolyzing enzymes (Amylase, trehalase and invertase) were determined using the method of Ishaaya and Swiriski (1976) using starch, trehalose and sucrose as substrates.

-Chitinase activity was determined using 3, 5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexoamines liberated on chitin digestion according to the method described by Ishaaya and Casida (1974)

-Phenol oxidase assay was based on the method described by Ishaaya and Casida (1974)

Statistical analysis:

The significance of the main effects was determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test ($p< 0.05$). All analyses were made using a software package "Costat", a product of cohort software, Berkley, California (Duncan, 1955).

RESULTS AND DISCUSSION

1-Toxicological studies:

The toxicological studies of spinetoram and two insect growth regulators, the CSI (teflubenzuron) and the ecdysone receptor agonist, tebufenozide, were

represented in Table (1) which showed the levels of sensitivity of *S. littoralis* to the tested compounds after 3 days from the treatment of the 4th instar larvae.

Treatment of *S. littoralis* larvae with each of the tested compounds revealed that, teflubenzuron was more toxic to the cotton leafworm than the other IGR (tebufenozide) and spinetoram, the LC_{50} values were 8.9, 65.7 and 121.1 ppm, respectively.

The need to find environmentally safe insecticides as well as to combat species resistant to conventional pesticides had spurred increase interest in alternative insecticides (Herbert and Herber, 1987). Insect growth regulators (IGR's) gained attention as a new type of insecticides because of their unique mode of action which is different from conventional insecticides. In this respect, attention has also been paid to the insect endocrinology and insect hormones. The acylureas such as teflubenzuron and diflubezuron had a remarkably chitin synthesis inhibition character Clarke and Jewess (1990), and were more toxic (up to 140-fold at the LC_{50}) than the most neuro-active compounds such as pyrethroids (Fisk and Wright 1992). In 2002, Raslan reported that IGR's can be considered as a possible alternative way for controlling the newly hatched *S. littoralis* larvae. Tebufenozide has great potential for use as chemical protection in the stored product industry, especially against Lepidoptera (Hami *et al.*, 2005). This compound has a similar hormonal activity with the natural hormones in the insects and particularly in Lepidoptera of which the principal effect is to cause incomplete and lethal molts (Dhadialla *et al.*, 2005).

Spinetoram was recorded as a potential bio-insecticide in controlling moveable stages of *Tetranychus utricae* (El Kady *et al.*, 2007), and the 2nd and 4th larval instars of *S. littoralis* (Elbarky *et al.*, 2008)

2-Biochemical studies:

The changes in enzymatic activities of different enzymes related to the nervous system or to the cuticle synthesis of 4th instar larvae of *S. littoralis* (laboratory strain) as responses of treatment with the LC_{50} of the tested compounds. The data were expressed as percentages in the activity relative to control.

2.1.- Acetyl choline-esterase (AChE) and Non-specific esterases:

Table (2) shows the changes in acetyl choline-esterase (AChE), alpha (α-E) and beta-esterase (β-E) enzymes activity. The data obtained showed that, in general, the treatment with tested compounds caused different levels of significant increase in the AChE activity, the highest level of the enzyme activity was recorded in the larvae treated with spinetoram (248.05%) relative to control followed by teflubenzuron and tebufenozide (116.07 and 113.7%), respectively.

Very similar trend was obtained in non-specific esterases activities, appeared in increase in the enzymes activity as a response of the treatment with spinetoram and tebufenozide (187.73 and 142.78%) in α-esterase activity, respectively and (114.68 and 146.20%) in β-esterase activity, respectively. In contrary, treatment with teflubenzuron caused a significant decrease in both α- and β- esterases (56.55 and 93.11%) relative to control, respectively.

2.2.- Carbohydrases:

Table (3) shows the changes in the carbohydrases (amylase, trehalase and invertase) enzymes activity. In general, the treatment with the LC_{50} values of the tested compounds caused significant reduction in the carbohydrases activities except the treatment with spinetoram, it caused a very significant increase in trehalase activity, as it recorded 135.36% relative to control. Amylase appeared as the most affected enzyme activity with high level of significant reduction, spinetoram recorded the highest level in reduction (4.79%) relative to control, followed by teflubenzuron and tebufenozide (32.45 and 67.69% relative to control), respectively. While spinetoram caused an increase in trehalase activity, teflubenzuron and tebufenozide reduced slightly the enzyme activity (91.53 and 98.52% relative to control), respectively. The three tested compounds reduced the invertase enzyme activity by different levels (77.81, 83.21 and 95.80%) caused by tebufenozide, teflubenzuron and spinetoram, respectively.

2.3.- Chitinase:

The response in larval chitinase activity to the treatment with the LC_{50} values of the tested compounds showed in table (4). The results obtained showed remarkable increase in the enzyme activity in the larvae treated with spinetoram compared to untreated larvae (164.75% relative to control). In contrary effect, teflubenzuron caused reduction in chitinase activity (81.44%), while tebufenozide had no effect on the enzymatic activity.

2.4.- Phenol oxidase:

Treated 4th instar larvae of *S. littoralis* with the three tested compounds recorded high levels of phenol oxidase activity compared to control (untreated larvae). Data shown in table (4) can be ordered dissentingly as follow: 289.84 > 227.06 > 207.41%, recorded for teflubenzuron > tebufenozide >Spinetoram treated larvae.

The increase in AChE activity plays an important role in resistance development. Elevated esterase activity in field population was also detected by Rodriguez *et al.* (2002) in *Aedes aegypti*. Srinivas *et al.* (2003) found that field strain of *Helicoverpa armigera* larvae was characterized by less sensitivity of AChE, high activities of esterases and phosphatases when compared with susceptible larvae. They

added that the high level of resistance detected in the field pests could be due to a combined effect of the decreased sensitivity to AChE and the increased levels of esterases. The same conclusion was found by Farag (2005) in his studies on *S. littoralis*.

In general, α- and βesterases activities in spinetoram and tebufenozide (20-hydroxyecdysone mimic) treated insects were higher than that found in teflubenzuron treated insects. As mentioned by Gilbert *et al.* (1977), High haemolymph titers of JH maintain the larval or nymphal state, while low JH titers initiate pupal and/or adult development. That makes it logically, the treatments of ecdysone mimic and spinetoram may be due to needs an increase in α- and β esterases activities to regulate the JH titer.

Carbohydrases play a significant role in the supply of energy to the insect, thus the activity of trehalase might serve as an indicator of energy reserves resulting from availability of carbohydrate nutrients (Wyatt, 1967). In this concern Ishaaya and Ascher (1977) concluded that carbohydrates might be affected due to the reduced levels of amylase, trehalase and invertase of the 4th larval instar of *T. castaneum*.

Lee *et al.*, (1994) recorded that, amylase and beta-fructofuranosidase activities were lower in *Hyphantria cunea* after treatment with tebufenozide and other IGR's than those in untreated larvae.

It is well known that in insects, trehalase degrades the disaccharide trehalose to glucose for internal energy supply and generates (during molting) glucose needed for chitin build-up. So the inhibition of the carbohydrases observed in the present work might affect this process.

The elevations of chitinase and phenoloxidase were indicated by Verloop (1977) as secondary effect of acylureas (diflubenzuron), and in 1994, Lee *et al.* found that, the treatment of *Hyphantria cunea* with the LC_{50} of tebufenozide and other insect growth regulators caused increases in chitinase activity compared to untreated larvae. Ecdysis is initiated by apolysis, the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysial membrane formation. The molting fluid contains proteases and chitinases, enzymes that digest the main constituents of the old endocuticle (Reynolds and Samuels, 1996).

The enzyme phenoloxidase (PO) is one of the first immune molecules that was identified in invertebrates. Recently, the immune function of PO has been challenged. Bidla *et al.*, (2009) tested how PO is activated following injury in 2 insects (the fruit fly *Drosophila melanogaster* and the wax moth *Galleria mellonella*). Rapid PO activation in *Drosophila* was limited to discrete areas of the hemolymph clot which forms after injury.

The data obtained in the present work could let us suggest the lethal effect of spinetoram and IGR's may be occurred through the alternation of enzyme balance of both chitinase and phenol oxidase, since these two enzymes played a known role in ecdysis and metamorphosis.

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الاستجابات الكيميائية الحيوية لحشرة سبودوبتيرا ليتوراليس (بويزد.) تجاه المعاملة بالسبينتورام، التيفلوبينزيورون و التيبيوفينوزايد

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تم دراسة حساسية العمر اليرقى الرابع للسلالة المعملية لدودة ورق القطن (*سبودوبتيرا ليتوراليس*) تجاه المعاملة بمركب سبينتورام ومركبين من منظمات النمو الحشرية (التيفلوبينزيورون والتيبيوفينوزايد). أظهر مركب التيفلوبينزيورون أعلى مستوى من السمية بقيمة اقل من الجرعة النصفية السامة (٨,٩ جزء فى المليون) تلاه التيبيوفينوزايد (٦٥,٧ جزء فى المليون) وأخيرا السبينتورام (١٢١,١ جزء فى المليون). تم استخدام قيم الجرعات النصفية السامة للمركبات المختبرة فى تقدير الإستجابات البيوكيميائية لدودة ورق القطن تجاهها، وقد سببت المعاملة بالسبينتورام زيادة معنوية فى نشاط إنزيمات الكيتينيز، الفينول أوكسيديز، التريهاليز والأسيتايل كولين إستيريز، بينما سببت المعاملة به إنخفاض معنوى فى نشاط إنزيم الأميليز.

إنعكست المعاملة بالتيبيوفينوزايد فى صورة نشاط عالى من إنزيم الألفا إستيريز. وعلى العكس سببت المعاملة بالتيفلوبينزيورون إنخفاض ملحوظ فى نشاط إنزيمات الكيتينيز، التريهاليز و الألفا إستيريز.