Efficacy of Spinetoram on some bio-chemical activities of *Spodoptera littoralis* larvae

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Abstract

The efficacy of Radiant (Spinetoram 12% Sc) on newly ecdysed 2nd and 4th instar larvae of *Spodoptera littoralis* (Boisd.) was evaluated. Results of the conducted bioassay showed that 2nd instars were more susceptible than 4thinstars as the LC_{50} and LC_{90} values were 0.5 and 3.9 ppm for 2nd instar larvae and 1.2 and 9.2 ppm for 4th instar larvae, respectively. The biochemical study carried out on 2nd and 4th instar larvae, 24 h following their feeding on castor oil bean leaves treated by their determined LC_{50} of spinetoram, showed that treatment of 2nd instar larvae caused a significant decrease in the content of protein, Meanwhile, it caused a significant elevation in total carbohydrates in larvae than their value in the untreated. These two respective mentioned components were also reduced in treated 4th instar larvae by 38.3 and 53.5% than their untreated.. The disturbance in carbohydrate level was expressed by impairment in activity of carbohydrate enzymes in treated 2nd and 4th instar larvae. In both treated instar larvae, there was a significant increase and decrease in the enzyme activity of trehalase and invertase as well as in protease. Meanwhile, an unsignificant decrease in the enzyme activities of both acetyl choline esterase and alpha esterase was recorded in treated larvae.

Keywords: *Spodoptera littoralis*, Spinetoram, Radiant, Toxicity, Enzyme activities.

INTRODUCTION

The Egyptian cotton leafworm, *S. littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a polyphagous insect of economic importance with a wide range of host plants (**Hosny *et al.*, 1986**). Chemical insecticides are an effective mean for the control and preventing of major damage caused by this pest, however, the extensive and continuous use of traditional insecticides create environmental contamination and could lead to development of resistance. Reduced pesticides risk are considered to be safer from human health and the environment and therefore are in constant demand for the control of insect pests. The relatively novel Spinosine insecticide Radiant is (Spinetoram 12% SC.), Spinetoram is a new member of the spinosyn class of insect management tools developed by Dow AgroScience. It is derived from fermentation of *Saccharopolyspora spinosa*, as are other spinosyns; it was a mixture of spinosyn J (major component) and spinosyn L (minor component), which have a reactive hydroxyl group at the 3′ position. Spinosyns J and L are both modified through the addition of the 3′-*O*-ethyl group and the reduction of the 5,6 double bond on spinosyn J. Spinetoram will provide long-lasting control of a broad spectrum of insect pests in a variety of crops. It is applied at low rates and has low impact on most beneficial insects. The new insecticide is more effective than spinosad because it is more stable in sunlight and provides longer residual control. It also has improved potency at the target site and improved penetration through the larval cuticle, **(James *et al.*, 2008 and Kirst, 2010).** The objective of the present work was to test Radiant (Spinetoram 12% SC.) as a untreated agent the Egyptian cotton

leafworm *S. littoralis* as well as its effect on some biochemical aspects of treated larvae.

MATERIALS AND METHOD

1. Maintenance of *Spodoptera littoralis* laboratory culture:-

The original colony of the cotton leafworm, *S. littoralis* was obtained from a well-established culture at the Department of cotton leafworm; Plant Protection Research Institute. The insects were maintained under laboratory conditions at 27+2^{0}C and 70+5% R.H. Larvae were reared on the fresh leaves of castor oil *Ricinus communis* supplied daily in sufficient amounts, maintenance of the insects different development stages of the insect were conducted according to method described by(**Gamil, 2004).**

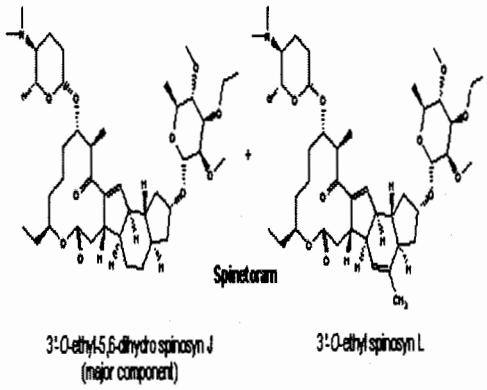
2. Tested compound:-

Common name: (Spinetoram 12% Sc.).

Trade name: Radiant

- **IUPAC of Chemical name**:

Chemical Structure:



3. Toxicological Studies

To assess the activity of Spinetoram a serial concentrations were prepared in distilled water which were 7.5, 3.75, 1.88, 0.94, 0.47, 0.23, 0.12 and 0.059 ppm. The dipping technique was adopted, where fresh clean castor oil leaves were immersed in tested concentrations, the leaves were allowed to dry at room temperature before being offered to newly ecdysed 2nd and 4th instar larvae *S. littoralis*. Larvae

were fed on treated leaves for 24h, subsequently they were reared on untreated castor oil leaves for the duration of the following larval instars. Each considered concentrations comprised 10 larvae and was replicated five times (i.e. 50 larvae / treatment). A similar number of larvae were considered as a untreated in which larvae were offered castor oil leaves immersed in distilled water. fter 24h mortality % was recorded. and corrected according to Abbott's formula, (**Abbott 1925**). Results were presented graphically as log/probit regression lines and LC_{50} values calculated by the computer program Sigma Plots for Windows (version 11).

4. Biochemical studies:-

The following biochemical studies were carried out on the 2nd and 4th instar larvae of *S. littoralis* treated with the LC_{50} of Spinetoram 12% SC. and the untreated one

4.1. Preparation of samples for biochemical analyses:

On the second day following treatment of the 2nd and 4th instar larvae of *S. littoralis* treated with the LC_{50} of Spinetoram 12% SC (i.e. 0.5 and 1.2 ppm respectively), surviving larvae exhibiting toxic symptoms were used to quantify enzymes activities. The larvae of each instar were anaesthetized and rinsed with 5 ml acetone to remove surface residues, the larvae were weighed then homogenized in phosphate buffer (pH 7) using a Teflon tissue homogenizer surrounded by crushed ice. The homogenates were centrifuged at 8000 rpm for 20 min at 4^0C and the supernatant was used directly for the determination of the following:

4.2. Main contents

i - Total carbohydrates were determined according to **Singh** and**Sinha (1977).**

ii- Total lipids were determined according to **Knight *et al.*, (1972).**

iii-Total soluble were determined protein as described by **Bradford(1976).**

4.3. Enzymes assay

The following enzymes activity was determined:

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

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

Non-specific α and β esterase activity was measured as described by **Van Asperen,(1962)** using α naphthyl acetate and β naphthyl acetate, respectively, as substrates.

5. Statistical analysis

Mortality percentages of all treatments were rated at one day after treatment and corrected according to Abbott's formula,(**Abbott ,1925).** Results were illustrated graphically as log/probit regression lines using Sigma Plots software for Windows (version 11)depending on **Finney,(1972).** Mortality data were subjected to probit analysis using the Statistical Analysis System Version 9.1 program PROC PROBIT , **(SAS Institute, 2003)** and statistical value of LC_{50} determined to reflect the efficiency of tested insecticides.

RESULTS AND DISCUSSION

1. Toxicological studies:

A bioassay was conducted to determine the toxicity of Spinetoram 12% Sc on Egyptian cotton leafworm, *S. littoralis* .A range of concentrations was prepared from Spinetoram. These preparations were tested on 2nd and 4th instar larvae of *S. littoralis* for 24h. As shown in **Tables (1)**;the percentage

of corrected larval mortality after 24h. of treatment for the 2nd instar larvae were 6.7, 22.3, 33.3, 49.3, 65.0, 73.3 and 93.3 while they were 0.0 , 4.0, 20.7, 35.0, 48.3, 61.7 and 70.0 for the 4th instar larvae at 0.0586, 0.1172, 0.2344, 0.4688, 0.9375, 1.8750 and 3.7500 ppm of spinetoram respectively. The results showed that the different applied concentrations of the present insecticide clearly affected the percentage of larval mortality, increasing gradually with an increase with the tested concentrations.

Table (1): Susceptibility of the 2nd and 4th instar larvae of *S.littoralis* to different concentrations of Spinetoram after 24h. of treatment.

(ppm)	Corrected mortality % after 24 h.	
	2nd instar	4th instar
0.0586	6.7	0.0
0.1172	22.3	4.0
0.2344	33.3	20.7
0.4688	49.3	35.0
0.9375	65.0	48.3
1.8750	73.3	61.7
3.7500	93.3	70.0

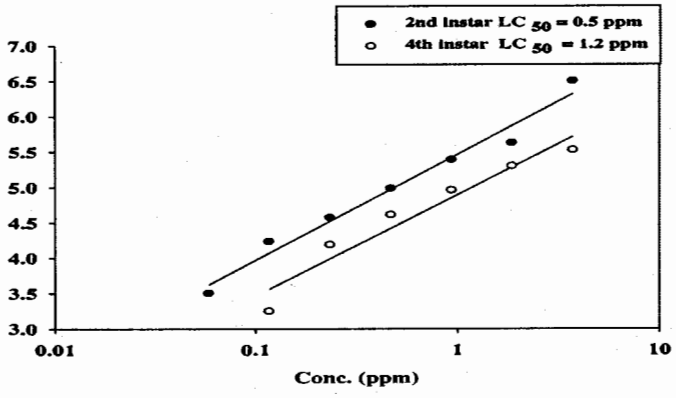


Fig (1): Toxicity regression lines of Spinetoram against 2nd and 4th instar larvaeof *S. littoralis*.

Table (2): Toxicity data for Spinetoram against 2nd and 4th instar larvae of *S. littoralis*.

Calculated Values	2nd instar	4th instar
LC_{50} (ppm)	0.5	1.2
LC_{90} (ppm)	3.9	9.2
Slope	1.5	1.4

As shown in Table (1)and(2) the 2nd instar larvae were more susceptible than older 4th instar this is depicted by the calculated LC_{50} and LC_{90} values which were 0.5 and 1.2 ppm for 2nd instar larvae and 3.9 and 9.2 ppm for 4th instar larvae, respectively. The slope values were 1.5 and 1.4 for the respective mentioned instars larvae proving the homogeneity of the treated insects.**(Table 2 and Fig 1).**

Treated insects exhibited symptoms of toxicity starting by sluggish slow movement, cessation of feeding followed by vomiting, subsequently, tremor followed by insect paralysis then death. The toxic sings were dose dependent, as they were quite rapid with the higher concentrations and slower with the lower concentrations.

2. Bio-chemical studies:-

2.1. Effect on main contents

As seen in **Table (3)**, treatment of 2nd instar *S. littoralis* larvae with LC_{50} of spinetoram caused an elevation in the total carbohydrates from 5.98 to 8.4 mg/ ml, giving a 40.5 % rise than their value in the untreated. Whereas, a marked reduction of 33.3% in protein content, as its value was reduced

from 23.7 in the untreated to 15.8 mg/ml in treated 2nd instar larvae. These respective mentioned two components were also reduced respectively by 38.3 and 53.5 % than their control, in 4th instar larvae treated by LC_{50} of spinetoram. Meanwhile, total lipid content was slightly decreased from 20.6 to 18.2 mg/ ml (i.e. 11.7% decrease) following treatment of 2nd instars, and a significantly lowered by 11.4 % (i.e. from 13.2 mg/ ml in the untreated to 11.7 mg/ ml in treated larvae) following the treatment of 4th instar larvae.

Table (3): Changes in main components of 2nd and 4th instar larvae of *S. littoralis*after treated with LC_{50} concentrations of Spinetoram.

Main Components	2nd instar			4th instar		
	untreated	Spinetoram	% Of untreated	untreated	Spinetoram	% Of untreated
Total Carbohydrates (mg/ml)	5.98± 0.3	8.4±0.4**	40.5	5.4±0.3	3.33±0.2**	-38.3
Total Proteins (mg/ml)	23.7± 0.9	15.8±0.7**	-33.3	32.7± 1.2	15.2±0.5**	-53.5
Total Lipids (mg/ml)	20.6± 0.8	18.2±0.6^{ns}	-11.7	13.2± 0.4	11.7±0.2*	-11.4

Of untreated = (Spinetoram – untreated) / untreated X 100

The disturbance in carbohydrates was expressed by impairments in activity of carbohydrate enzymes in treated larvae. A similar observation was reported in *S. littoralis* larvae exposed to the bio-insecticide Methylamine Avermactin , **(Dahi *et al.*, 2009**. And **Haga *et al.*, (1984)**showed that proteins help to synthesize microsomal detoxifying enzymes which assist in the detoxification of toxicants that inter the insect body. Decrease in total protein content in larvae following treatment with insecticides could signify suppression of protein synthesis as suggested by **Nath *et al.*,(1997).**However it, could be due to the breakdown of protein into amino acids to supply energy for the insect as interpreted by **Baker *et al.*, 1991.**

2.2. Enzyme assay

2.2.1. Effect on carbohydrate and protein enzymes activity

As seen in **Table (4)**, treatment of 2nd instar larvae with LC_{50} of spinetoram caused a slight reduction4.3% in amylase activity than that in untreated larvae. However, activities of trehalase, invertase and protease were significantly increased by 41.6, 94.7 and 57.9 % than the untreated, respectively. Meanwhile; treatment of 4th instar larvae caused an increase of 5.5 and 37.3 % in amylase and protease activity, respectively, and Ahighly significant was decreased of 54.2 and 40.5 % in trehalase and invertase activity than that in untreated larvae.

Table (4): Changes in carbohydrases enzymes activity in 2nd and 4th instar larvae *S. littoralis*after treated with LC_{50} concentrations of Spinetoram.

Enzyme Activities (μg Glucose/ml /min.)	2nd instar			4th instar		
	untreated	Spinetoram	% Of untreated	untreated	Spinetoram	% Of untreated
Amylase	172.7 ±6.4	165.3±3.2 ns	-4.3	251.0± 9.5	264.7±6.9 ns	5.5
Trehalase	360.7 ±6.0	510.7±7.4***	41.6	981.3± 26.1	449.3±4.7***	-54.2
Invertase	385±8.7	749.7±10.2***	94.7	1233.3 ±49.1	734.3±25.0***	-40.5
Protease and protein	15.9± 1.4	25.1±1.6*	57.9	29.2±2.1	40.1±2.2*	37.3

Of untreated = (Spinetoram – untreated) / untreated X 100

Trehalase is the only enzyme capable of hydrolyzing trehalose to its glucose monomeric units, **(Temesvari and Cotter, 1997).**Trehalase might be an interesting target in the development of new techniques controlling insects, **(Silva *et al.,* 2004).** In many organisms, changes in trehalase activity are closely linked to alteration in physiological conditions or development, indicating that this enzyme plays an important role in such biological functions as homeostasis and developmental events, **(Temesvari and Cotter, 1997).** Since metabolic utilization of trehalose is dependent upon trehalase, the increase in it activity may be due to higher metabolic utilization of trehalose reserves under induced insecticidal stress conditions, (**Friedman , 1978). In addition Nath, 2000** revealed a

significant decrease in fat body glycogen on exposure to organophosphorus insecticides which supports our findings.

2.2. Effect on acetyl choline esterase, alpha and beta esterase activity

The activity of alpha and beta esterase in *S. littoralis* 2nd and 4th instar larvae 24 hours following treatment with the calculated LC_{50} of spinetoram are shown in **Table (4)**. The activity of beta esterase in treated 4th instar larvae was 885.3 µg β -naphthol /ml /min/ g larval weight as compared to 1433.3 µg β -naphthol /ml /min/g larval weight in the untreated, being an decreased by 38.2 %. While it was a minor decrease by 0.4% following the treatment in 2nd instar larvae. Treatment of 2nd instar larvae with LC_{50} of spinetoram caused insignificant reduction in acetyl choline esterase and alpha esterase activities than that recorded in untreated larvae by 5.31 and 2.8 %., Acetyl choline esterase activityalso was reduced in treated 4th instars, i.e. 3.5%, meanwhile; there was a slightly increase in alpha esterase activity by 2.3 than that recorded in untreated larvae.

Esterase-based resistance to organophosphorus and carbamate insecticides is common in a range of different insect pests, **(Field *et al.,* 1988 and Hemingway and Karunaratne, 1998).** The esterases either produce broad spectrum insecticide resistance through rapid-binding and slow turnover of insecticide, i.e. sequestration, or narrow spectrum resistance through metabolism of a very restricted range of insecticides containing a common ester bond, **(Herath*et al.,* 1987).** The majority of esterases which function by sequestration are elevated through gene amplification,**(Vaughan and Hemingway, 1995).** Since enhanced metabolism is an important insecticide mechanism, thus oxidative, hydrolytic and conjugative detoxication enzyme activities toward universal substrates were measured in insecticide, **(Abo Elghar *et al.,*2005).** No fixed trend was observed in

the activity of general estresases in the present work suggesting that they are not involved in the contribution of the detoxification mechanism.

Table (5): Changes in acetyl choline-esterase and non-specific esterases enzymes activity in 2nd and 4th instar larvae of *S. littoralis* after treated with LC_{50} concentrations of Spinetoram.

Enzyme Activities	2nd instar			4th instar		
	untreated	Spinetoram	% of untreated	untreated	Spinetoram	% Of untreated
Acetyl Choline-esterase (μg AchBr/ml/ min)	97.8± 3.5	92.6±1.6 ns	-5.31	51.3±3.2	49.5±1.9 ns	-3.5
α - Esterase (μg α-naphthol released/ml. /min.)	614± 4.9	597±4.6 ns	-2.8	766.7± 8.8	784.7 ns	2.3
β - Esterase (μg β-naphthol released/ml. /min.)	732± 6.7 ns	729±2.6	-0.4	1433.3 ±54.6	885.3±10.1***	-38.2

Of untreated = (Spinetoram – untreated) / untreated X 100

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المخلص عربى

تم تقييم فاعلية مبيد الرادينت ١٢% Sc (اسبينوتورام) على العمرين اليرقين الثانى والرابع لدودة ورق القطن واوضحت نتائج التقييم الحيوي ان العمر اليرقى الثانى كان اكثر حساسية من العمر اليرقى الرابع حيث بلغت قيمتى LC90 Lc 50- ٠,٥ و ٣,٩ جزء فى المليون للعمر اليرقى الثانى بينما كان ١,٢ و٩,٢ جزء فى المليون للعمر اليرقى الرابع.

كما تم دراسة التغيرات البيوكيميائية الناتجه فى كل من العمر اليرقى الثانى والرابع نتيجة المعاملة بالتركيز النصفى القاتل لكل منهما واوضحت النتائج ما يلى وجود انخفاض معنوى فى المحتوى الكلى للبروتينات فى يرقات العمر الثانى المعاملة بالمقارنة بالغير معاملة فى حين زاد المحتوى الكلى للكربوهيدرات فى يرقات العمر الثانى نتيجة للمعاملة بينما اثرت المعاملة بالتركيز النصفى القاتل للعمر اليرقى الرابع سلباً على كل من المحتوى للبروتين والكربوهيدرات حيث انخفض بنسبة ٣٨،٣ و٥٣،٥ % لكل منهما على التوالى وهذا التغيير فى المحتوى الكلى للكربوهيدرات فى كل من العمرين الى حدوث تغيرات واضحة فى الانزيمات المحللة للكربوهيدرات حيث اوضحت النتائج وجود زيادة وانخفاض معنوى فى نشاط كل من التريهاليز والانفرتيز والبروتيز فى حين ان انخفاض نشاط كل من انزيمات الاستيل كولين استيريز والالفا ستيزيز نتيجة المعاملة فى كل من العمرين لم يكن معنويا .