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# Field evaluation and biochemical studies of novel insecticide on the cotton leafworm, *Spodoptera littoralis* (Boisd).

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## ABSTRACT

Field evaluation for the efficacy of indoxacarb, spinetoram and methoxyfenozide against larval instars of S. *littoralis*, infested clover plants *Trifoliuma lexandrium* was studied at two different Governorates (Al- Qalyubia and El- Fayoum) during cultivation season 2012 - 2013.All three insecticides, of indoxacarb, spinetoram and methoxyfenozide had no great difference among themselves in reducing the larval population of *S. littoralis* with difference in the time needed to induce this reduction according to the mode of action of these insecticides.The initial reductionwas recorded after 1 day from spraying for indoxacarb and spinetoram while after 3 days from spraying for methoxyfenozide. Indoxacarb was the most effective insecticide followed by methoxyfenozide and spinetoram.Under semi-field conditions, results showed that, at Al- Qalyubia Governorate, persistence of indoxacarb is higher than other tested insecticides while, the half life of all tested compounds is short at El-Fayoum Governorate.Tolerance to the toxicity of the three tested insecticides increased as the larvae grew older from 2$^{nd}$ to 4$^{th}$instar.Biochemical clarification was carried out in an attempt to disclose the effect of sub-lethal concentration ($LC_{50}$) of the three tested insecticides; on haemolymph contents of 6$^{th}$ instar larvae of *S. littoralis* which was treated as 4$^{th}$larval instar with $LC_{50}$ of the tested insecticides and their effects on main components of insect metabolites and changes in protein patterns.

**Keywords:** Indoxacarb; spinetoram; methoxyfenozide; *Spodoptera littoralis*; field experiment; biochemical analysis; SDS (PAGE).

## INTRODUCTION

Egyptian clover, *Trifoliuma lexanderium* L. is considered the most important agricultural crop for Egyptian farmers because it is the principal food for their animals. On the other hand, it is a good reservoir for natural enemies and pests (Boraei, *et al*. 2005). The cotton leaf worm, *Spodoptera littoralis* is the most serious and destructive pest that attacks all parts of Egyptian clover plant from seedling to harvest. In addition, it is a very harmful potential pest of many field crops and vegetables, causing highly economic losses in both greenhouses and open field on a broad range of ornamental, industrial and vegetable crops. To overcome the losses and to increase the yield, application of chemical insecticide is the most important (Aslam *et al*., 2004). Currently new groups of chemical compounds are being tested against lepidopteran pests such as, indoxacarb (Steward 15% EC), spinetoram (Radiant 12% SC) and methoxyfenozide (Runner 24% SC).

Indoxacarb, Steward™, is a new oxadiazine insecticide discovered by the E. I. DuPont Co that has shown outstanding field insecticidal activity, environmental compatibility, and safety to non-target organisms (Wing *et al*., 2000). Indoxacarb is especially active on foliar-feeding lepidopteran larvae. When lepidopteran larvae ingest sprayed foliage or are sprayed directly, they stop feeding and either go into mild convulsions or a passive paralysis from which there is no recovery (Wing *et al*., 1998).

Spinetoram is a new generation of spinosyn group. It causes excitation of the insect nervous system by altering the function of nicotine and GABA-gated ion channels. It does not interact with the known binding sites of other classes of insecticides such as of neonicitinoids, fiproles or avermectins (Crouse and Sparks, 1998).

Methoxyfenozide, an insect growth regulator (IGR), is the newest and most potent member of the molt-accelerating compounds (MACs) against Lepidoptera (Smagghe *et al*. 2003). This compound mimicsthe natural insect molting hormone by binding competitively to ecdysteroid receptors in insect cells, thus inducing a premature larval molt (Dhadialla *et al*. 1998). Due to a high specificity of its action against Lepidoptera, it is considered as an environment-friendly compound (Palli and Retnakaran 2001).

Present studies were designed to evaluate the efficiency of these compounds against *S. littoralis* which infest Egyptian clover under field condition and residual efficacy of them. Also to study their effect on the main insect metabolites such as total protein and protein fraction, total carbohydrate and total lipid in the haemolymph of the 6$^{th}$ instar larvae of *S. littoralis*.

## MATERIALS AND METHODS

**Experimental Insecticides**

Three new chemical insecticides, indoxacarb (Steward 15% EC, DuPont), spinetoram (Radiant 12% SC, Dow Agro sciences) and methoxyfenozide( Runner 24% SC, Dow Agro sciences) were obtained from their respective manufacturers and used in the present studies.

**Field Experiments:**

Field experiments were conducted at Kaha Research Station, Toukh district, Al-Qalyubia Governorate and at Abshiway district, El-Fayoum Governorate, Egypt during cultivation season 2012 - 2013.The experiment area of two third feddan (2800 m$^2$) was divided into 4 equal randomized blocks (one for either treatment plus one for control). Each block was divided 4 experimental plots as replicates (175 m$^2$ for each)A motor sprayer was used. The volume of spray solution was 200 liters/feddan. The number of larvae were recorded on one meter lengthwise for five times (four at corners and the last one on plot center), before the spray and after 1, 3, 5, 7 and 10 day from experiment. Reduction percent in *S. littoralis* population was estimated using equation of Henderson and Tilton (1955).

**Residual Efficacy in Field-Lab. Experiment:**

From the same experiment area of the treated clover, plant samples were collected after zero time, 3,5and 7 days and transfer directly to the laboratory for feeding 2nd and 4th larval instars of *S. littoralis*. Four experiments were applied to estimate the mortality percent at different time intervals Exp.(1) at zero time, Exp.(2) at 3rd day from application, Exp.(3) at 5th day from application and Exp.(4) at 7th day from application.

**Statistical Analysis:**

The percentage of mortality was corrected according to the Abbott formula(Abbott, 1925) for correction wherever required. Probit analysis was determined to calculate $LC_{50}$(Finney, 1971), through software computer program. Statistical significant differences between individual means were determined by one way analysis of variance (ANOVA).

**Biochemical assays of larval main metabolites:**

Haemolymph was collected from normal and treated 6th instar larvae which were fed as 4th instar for 48 hrs. on castor-oil leaves treated with $LC_{50}$ values of each tested insecticide. The haemolymph was placed in 1.5ml ice-cold micro centrifuge tubes that contained few crystals of phenylthiourea (PTU) to prevent melanization. The samples were centrifuged at 2500 rpm for 5 minutes under cooling (4°C) to remove the blood cells. After centrifugation, the supernatant fluid was stored at –20 °C until analysis. Total protein was determined by the method of Bradford (1976), using coommasie brilliant blue G-250 reagent (CBB).Total carbohydrate were determined by the method described by Singh and Sinha (1977).While total lipid were estimated according to Knight *et al*. (1972), using phosphovanillin reagent. Fraction protein (SDS-PAGE) of haemolymph samples of untreated and treated 6th instar larvae of *S. littoralis* was performed in 12% acrylamide slab gel according to method of Laemmli, (1970) and modified by Studier, (1973).

## RESULTS

**A- Field efficacy of indoxacarb, spinetoram and methoxyfenozide on larvae of *S. littoralis* at Al-Qalyubia and El-Fayoum Governorates.**

Data presented in Table (1) showed the potency of those three commercial insecticides, indoxacarb, spinetoram and methoxyfenozideat Al-Qalyubia and Fayoum Governorates. At Al-Qalyubia Governorate indoxacarb achieved the highest reduction in number of different larval instars of *S. littoralis* infested clover plants by 97.98% followed by Spinetoram gave 89.59% whereas, Methoxyfenozide gave 66.78% reduction at zero time from application. While percentages reductions were 98.45, 91.43 and 89.82 at 3rd day from application. While at 5th and 7th days from application, reductions were 98.91, 91.38 and 94.58, and were 98.81, 94.03 and 97.36, respectively.

Methoxyfenozide showed relatively low percentage reduction in *S. littoralis* larvae after 1 day from spraying, but reduction percent began to increase after 3 days from spraying until reached 99.34% after 10 days. At 10th day from application both indoxacarb and methoxyfenozide recorded the highest percentage reduction in *S. littoralis* larvae followed by Spinetoram as it reached 99.26%, 99.34% and 93.91%, respectively. At EL-Fayoum Governorate data presented in Table(1) showed that initial reductions of different larval instars treated with indoxacarb and spinetoram after 1 day of spraying were (91.79and 89.51), respectively, while for methoxyfenozide initial reduction calculated after three days from spraying with percentage reduction was 80.97.Percentages reductions in *S. littoralis* larvae reached highest activity after applying indoxacarb 90.12, 92, 94.58 and 97.56 followed by spinetoram 88.49, 87.77, 88.79, and 92.83 at 3rd day, 5th day, 7th day and 10th day from insecticidal application, respectively. While methoxyfenozide gave 87.69, 90.07, 95.67 at 5th day, 7th day and 10th day from insecticidal application, respectively. At 10th day from application both indoxacarb and methoxyfenozide recorded the highest reduction percentage followed by spinetoram, as it reached 97.56, 95.67 and 92.83 reductions in number of different instars of larvae, respectively.

Table 1: Reduction percentage of *S. littoralis* larvae after treatment of *Ttrifoliuma lexandrium*in the field by recommended rate of tested compounds at two Governorates.

| compounds | Recommended rate/feddan | Qualyobia Governorate | | | | | Fayoum Governorate | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Reduction percentage | | | | | Reduction percentage | | | | |
| | | Zero time | 3rd day from application | 5th day from application | 7th day from application | 10th day from application | Zero time | 3rd day from application | 5th day from application | 7th day from application | 10th day from application |
| Indoxacarb (Steward 15% EC) | 105ml | 97.98 | 98.45 | 98.91 | 98.81 | 99.26 | 91.79 | 90.12 | 92.00 | 94.58 | 97.56 |
| Spinetoram (Radiant 12% SC) | 35ml | 89.59 | 91.43 | 91.38 | 94.03 | 93.91 | 89.51 | 88.49 | 87.77 | 88.79 | 92.83 |
| Methoxyfenozide (Runner 24% SC) | 37.5ml/100 Liter water | 66.78 | 89.82 | 94.58 | 97.36 | 99.34 | 70.04 | 80.97 | 87.69 | 90.07 | 95.67 |

colored cells indicate to initial effect

## B- Residual efficacy of indoxacarb, spinetoram and methoxyfenozide on *S. littoralis* larvaeat Al-Qalyubia and El-Fayoum Governorates.

At Al-Qalyubia Governorate data in Table (2) showed the potency of the recommended doses for the tested insecticides on2nd and 4th larval instars of *S. littoralis*. Experiment (1) declared the efficiency of tested compounds at zero time from spraying and percentage mortality was recorded after 24 hrs.72 hrs. and 120 hrs. to determine the initial effect of tested compounds. While experiments 2, 3, 4recorded the residual effects of tested compounds as shown in Table (2). Indoxacarb recorded the highest mortality at allexperiments which recorded 100% mortality for both tested larval instars after 120 hrs. these results mean that persistence of Indoxacarb was high. While, Spinetoram caused high mortality to 2nd and 4th larval instars reached 100 and 83.33% after 120hrs., respectively in Exp.(1) and decreased gradually to reach 66.67 and 53.33%, respectively in Exp.(4).These results indicated that, the persistence of Spinetoram is short. Methoxyfenozide showed the same effect as that of Spinetoram. It caused 96.67% and 100% mortality after 120hrs.in Exp. (1) and decreased gradually to reach 63.33 and 60.0% mortality in Exp.(4)for 2nd and 4th larval instars, respectively. Results showed that 2nd larval instars of *S. littoralis* were significantly more susceptible to insecticidal application than the 4thones.

Table 2: Corrected mortality % for the 2nd and 4th instar larvae of *S. littoralis* after treatment of *Ttrifoliuma lexandrium* by recommended rate of tested compounds at two Governorates.

| Qualyobia Governorate | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| compounds | Recommended rate/feddan | Corrected larval mortality % at different time intervals | | | | | | | | | | | | | | | | | | | | | | | |
| | | Initial effect | | | | | | Residual effect | | | | | | | | | | | | | | | | | |
| | | Exp. (1) Zero time | | | | | | Exp (2) 3rd day from application | | | | | | Exp (3) 5th day from application | | | | | | Exp (4) 7th day from application | | | | | |
| | | 24h | | 72h | | 120h | | 24h | | 72h | | 120h | | 24h | | 72h | | 120h | | 24h | | 72h | | 120h | |
| | | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th | 2nd | 4th |
| Indoxacarb 15% EC | 105ml | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 96.67 | 100 | 100 | 100 | 100 | 100 | 96.67 | 100 | 96.67 | 100 | 100 |
| Spinetoram 12% SC | 35ml | 80.0 | 46.67 | 100 | 63.33 | 100 | 83.33 | 56.67 | 30.0 | 70.0 | 60.0 | 73.33 | 66.67 | 43.33 | 23.33 | 63.33 | 53.33 | 66.67 | 60.0 | 16.67 | 10.0 | 43.33 | 36.67 | 66.67 | 53.33 |
| Methoxyfenozide 24% SC | 37.5ml/100 Liter water | 23.33 | 16.67 | 90.0 | 83.33 | 96.67 | 100 | 16.67 | 13.33 | 83.33 | 66.67 | 96.67 | 96.67 | 10.0 | 3.33 | 46.67 | 23.33 | 73.33 | 86.66 | 3.33 | 0.0 | 43.33 | 26.67 | 63.33 | 60.0 |
| Fayoum Governorate | | | | | | | | | | | | | | | | | | | | | | | | | |
| Indoxacarb 15% EC | 105ml | 76.67 | 73.33 | 100 | 83.33 | 100 | 100 | 83.33 | 63.33 | 93.33 | 73.33 | 100 | 90.0 | 73.33 | 46.67 | 83.33 | 56.66 | 93.33 | 73.33 | 36.67 | 26.67 | 63.33 | 50.0 | 73.33 | 56.67 |
| Spinetoram 12% SC | 35ml | 73.33 | 53.33 | 90.0 | 83.33 | 100 | 100 | 73.33 | 56.67 | 83.33 | 70.0 | 100 | 70.0 | 53.33 | 46.67 | 83.33 | 73.33 | 96.67 | 73.33 | 53.33 | 30.0 | 76.67 | 46.67 | 80.0 | 50.0 |
| Methoxyfenozide 24% SC | 37.5ml/100 Liter water | 23.33 | 13.33 | 90.0 | 73.33 | 96.67 | 83.33 | 23.33 | 13.33 | 86.67 | 60.0 | 90.0 | 73.33 | 13.33 | 6.67 | 60.0 | 50.0 | 66.62 | 73.33 | 13.33 | 6.67 | 46.67 | 36.67 | 56.67 | 53.33 |

At El-Fayoum Governorate Data in Table (2) showed that indoxacarb recorded the highest mortality in Exp. (2), 3rd day from application, which recorded 100% and 90 % for 2nd and 4th larval instars and decreased gradually to reach 73.33% and

56.67% in Exp.(4) for both instars, respectively. Spinetoram, also caused high mortality reached 100% and 70% after 120 hrs. in Exp.(2), 3rd day from spraying, and decreased gradually to reach 80% and 50% after 120hrs. In Exp.(4),7th day from spraying for 2nd and 4th larval instars, respectively. Methoxyfenozide showed the same effect as that of Spinetoram. It had 96.67% and 83.33% mortalities for 2nd and 4th larval instars after 120 hrs. in Exp. (1) and decreased gradually to reach 56.67% and 53.33% in Exp. (4) for both instars, respectively. Results showed that 2ndlarval instars of *S. littoralis* were significantly more susceptible than the 4th larval instars. In addition, the percentage mortality decreased gradually from Exp. (1) to Exp. (4) in all tested insecticides.

**C. Biochemical studies**

**Determination of main components of insect metabolites of haemolymph sample of untreated and treated 6th instar larvae of *S. littoralis*.**

The effect of sub-lethal concentration ($LC_{50}$) on main insect metabolites such as total protein, total carbohydrate and total lipid in the haemolymph of the 6th instar larvae of *S. littoralis* after treatment of 4thinstar larvae with $LC_{50}$ values of the three tested insecticides, Indoxacarb, Spinetoram and Methoxyfenozide reported in Table (3) Results indicated that the three tested insecticides induced highly significant reduction in the total protein, carbohydrate and lipid contents in treated larvae compared with control at $p \leq 0.05$.Total protein in the haemolymph of treated 6th instar larvae of *S. littoralis* were 15.3, 17.8 and 21.0 mg/ml as comparing with control (29.5 mg/ml) with percentage change-48.1, -39.7 and – 28.8 respectively.

Table 3: Determination of the treated main metabolites in the haemolymph of 6th instar larvae of *S. littoralis* resulting from the4th instar treated with $LC_{50}$ of tested insecticides.

| Insecticides used | Total protein (mg/ml) Mean ±SE | % Change | Total carbohydrate (mg/ml) Mean ±SE | % Change | Total lipid (mg/ml) Mean ±SE | % Change |
|---|---|---|---|---|---|---|
| **Indoxacarb** | 15.3±0.03**d** | -48.1 | 9.2±0.03**d** | -61.0 | 21.1±0.05**c** | -31.7 |
| **Spinetoram** | 17.8±0.03 **c** | -39.7 | 11.03±0.20 **c** | -53.3 | 14.3±0.09**d** | -53.7 |
| **Methoxyfenozide** | 21.0±0.57 **b** | -28.8 | 14.2±0.23**b** | -39.8 | 19.2±0.06**b** | -37.9 |
| **Control** | 29.5±0.28**a** | _ | 23.6±0.33**a** | _ | 30.9±0.31**a** | _ |
| **F. value** | 632.46*** | _ | 615.98*** | _ | 2344.83*** | _ |
| **LSD** | 0.9958 | | 0.9956 | | 0.998864 | |

Haemolymph carbohydrate of 6th instar larvae of *S. littoralis* showed highly significant reduction due to the treatment with the three tested insecticides. Mean values of this reduction were 9.2 mg/ml for indoxacarb, followed by 11.03 mg/ml for spinetoram and 14.2 mg/ml for methoxyfenozide as compared with control (23.6 mg/ml)with percentage changes -61.0, -53.3 and – 39.8 respectively.

The three tested compounds caused highly significant reduction in lipid contents as compared with control. Obviously treatment with spinetoram recorded 21.1 mg/ml while indoxacarb and methoxyfenozide were 14.3 and 19.2 mg/ml, respectively compared with control(30.9mg/ml) with percentage changes -31.7, -53.7 and - 31.9 respectively.

**E. Electrophoretic fraction protein patterns (SDS-PAGE) of haemolymph samples of untreated and treated 6th instar larvae of *S. littoralis*.**

Changes in the protein fractionation profile of larval haemolymph of untreated and treated 6th instar larvae of *S. littoralis* which fed as 4th instar with $LC_{50}$ of Indoxacarb, Spinetoram and Methoxyfenozide were detected. The fraction electrophoretic protein patterns (SDS-PAGE) were presented in Table (4), and illustrated in Fig. (1). The achieved results were compared by electrophoretic runs of proteins extracted from both control and treated samples.

Table 4: Molecular weights and percentage amount of larval haemolymph SDS-electrophoretic protein patterns for both untreated and treated samples of 6th larval instar of *S. littoralis*.

| Rows number | Molecular weights | Percentage amount of bands | | | |
|---|---|---|---|---|---|
| | | Control | Indoxacarb | Spinetoram | Methoxyfenozide |
| 1 r | 186.61 | — | — | 0.19 | 0.12 |
| 2 r | 144.57 | 0.42 | 7.11 | — | 0.08 |
| 3r | 117.33 | 0.67 | — | 1.76 | — |
| r4 | 100.60 | 0.78 | 1.13 | 0.99 | 0.13 |
| r5 | 84.86 | 0.97 | 1.10 | 1.69 | 0.48 |
| r6 | 73.48 | 1.11 | — | — | — |
| r7 | 70.86 | 1.22 | 12.03 | 1.71 | 0.74 |
| r8 | 60.49 | 2.23 | — | 1.69 | 0.72 |
| r9 | 55.16 | 2.89 | 8.50 | 1.56 | 0.95 |
| r10 | 52.89 | 2.25 | — | — | 0.81 |
| r11 | 50.46 | 1.59 | — | 1.99 | 0.97 |
| r12 | 47.11 | 1.52 | 5.15 | 2.81 | 1.02 |
| r13 | 44.17 | 2.21 | 2.95 | 3.12 | — |
| r14 | 40.32 | 2.83 | 3.60 | 1.41 | 2.61 |
| r15 | 38.09 | 3.60 | 3.39 | 1.71 | 1.07 |
| r16 | 35.56 | 3.87 | 4.06 | 3.40 | — |
| r17 | 33.32 | 5.04 | 3.36 | — | 2.95 |
| r18 | 31.07 | — | 4.72 | 5.37 | 4.44 |
| r19 | 28.88 | 5.37 | 1.82 | 5.28 | — |
| r20 | 26.87 | 5.12 | 6.67 | 5.95 | 5.25 |
| r21 | 24.94 | 5.24 | 4.96 | 6.82 | 5.02 |
| r22 | 23.87 | 4.71 | 5.38 | — | 5.83 |
| r23 | 22.10 | 3.69 | 1.82 | 6.12 | 8.28 |
| r24 | 21.05 | 3.65 | 2.60 | — | — |
| r25 | 20.07 | 2.97 | 2.01 | 4.36 | 6.62 |
| r26 | 18.31 | 4.52 | 2.02 | 6.09 | 8.68 |
| r27 | 16.62 | 7.49 | 5.03 | 6.07 | 9.84 |
| r28 | 16.03 | — | 1.64 | — | — |
| r29 | 15.11 | 6.38 | 1.92 | 5.66 | — |
| r30 | 14.17 | — | 2.13 | 6.40 | — |
| r31 | 13.18 | 4.80 | 2.26 | 5.02 | 11.26 |
| r32 | 11.98 | 4.97 | 1.53 | 5.30 | 9.81 |
| r33 | 10.76 | 4.39 | 1.12 | 7.51 | 8.80 |
| r34 | 10.13 | 2.00 | — | — | 3.52 |
| r35 | 9.63 | 1.52 | — | — | — |

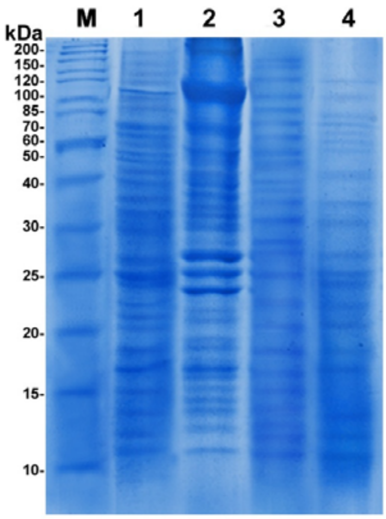


Fig. 1:SDS-electrophoretic protein patterns of larval haemolymph of *S. littoralis* as control and treated samples with tested insecticides.

M: Protein marker. 1: Haemolymph samples of Control.
2: Haemolymph samples of Indoxacarb. 3: Haemolymph samples of Spinetoram.
4:Haemolymph samples of Methoxyfenozide.

The SDS protein patterns of larval haemolymph revealed different numbers of protein bands according to their molecular weights; they were separated into 35 bands. The total number of bands in untreated samples were 31 bands with molecular weights ranged between (9.63 – 144.57) kDa. In the control samples, there were two characteristic bands appeared at $r_6$ and $r_{35}$ with percentage amounts (1.11 and 2.00) and molecular weights (73.48 and 10.13) kDa, respectively. While the total number of bands in treated samples were distributed as 27, 26 and 25 bands in Indoxacarb, Spinetoram and Methoxyfenozide with molecular weights ranged between (10.76–144.57), (10.76-186.61) and (10.13-186.61) kDa for the fore mentioned insecticides, respectively.

There were 16 common bands appeared in the rows ($r_4$, $r_5$, $r_7$, $r_9$, $r_{12}$, $r_{14}$, $r_{15}$, $r_{20}$, $r_{21}$, $r_{23}$, $r_{25}$, $r_{26}$, $r_{27}$, $r_{31}$, $r_{32}$ and $r_{33}$) represented protein extracted from *S. littoralis* larval stage either treated with Indoxacarb, Spinetoram and Methoxyfenozide or untreated one. Treatment with Indoxacarb caused appearance of unique band at $r_{28}$ with percentage amount 1.64 and molecular weight 16.03 kDa. This was in association with appearance of 3 abnormal bands as compared to control samples with percentage amount values 4.72, 1.64 and 2.13 and molecular weights 31.07, 16.03 and 14.17 kDa, respectively. Indoxacarb treatment caused disappearance of 7 normal bands at the rows $r_3$, $r_6$, $r_8$, $r_{10}$, $r_{11}$, $r_{34}$ and $r_{35}$.

Treatment with Spinetoram and Methoxyfenozide caused disappearance of normal bands and /or appearance of abnormal bands as compared to the control samples.

The deleterious effect occurred as a result of the treatment with Spinetoram was represented by alterations in the SDS electrophoretic protein pattern. It caused disappearance of 8 normal bands from the rows $r_2$, $r_6$, $r_{10}$, $r_{17}$, $r_{22}$, $r_{24}$, $r_{34}$ and $r_{35}$. This was in association with appearance of 3 abnormal bands at $r_1$, $r_{18}$ and $r_{30}$ with percentage amounts 0.19, 5.37 and 6.40 and molecular weights 186.61, 31.07 and 14.17 kDa, respectively.

The effect occurred as a result of treatment with Methoxyfenozide was represented by disappearance of 8 normal bands from the rows $r_3$, $r_6$, $r_{13}$, $r_{16}$, $r_{19}$, $r_{23}$, $r_{29}$ and $r_{35}$. This was in addition to appearance of 2 abnormal bands at $r_1$ and $r_{18}$ with percentage amounts 0.12 and 4.44 and molecular weights 186.61 and 31.07 kDa.

## DISCUSSION

### 1. Field and semi-field studies.

In field trials, the obtained results show that all three insecticides, of indoxacarb, spinetoram and methoxyfenozide had no great difference among themselves in reducing the larval population of *S. littoralis* with difference in the time needed to induce this reduction according to the mode of action of these insecticides. Residual efficacy at the recommended doses of tested compounds against 2nd and 4th larval instars of *S. littoralis* under field-lab. Condition showed that, at Al-Qalyubia Governorate, Indoxacarb recorded the highest mortality at all time intervals of the experiment which recorded 100 % mortality for both tested larval instars. In addition, the accumulative mortality percentage after 120 hrs. remained constant during all experiments (Exp. (2) at 3rd day from application, Exp.(3) at 5th day from application and Exp.(4) at 7th day from application.).These results mean persistence of indoxacarb is high. These findings are in full agreement with Liu *et al*. (2003) who revealed that, the toxicity of field-aged leaf residues of indoxacarb 0, 3, 5, 7, 10, 14, 17 and 21 day-old residues) declined slowly and gradually under the field conditions in South Texas. Almost all larvae of *Plutellaxy lostella* died on day 5 after feeding on the leaves with 0 – 14 day residue, and the mortalities were as high as 94 and 78% for the 14 and 17 day-old leaf residues. While, results declared that, the persistence of spinetoram is short. The short residual effect of spinetoram in this study is compatible with the results reported by (Elbarky *et al.* 2008) who found that spinetoram has short residual time in cotton under semi-field condition. Methoxyfenozide showed the same trend of spinetoram. On the other hand, the half life of all tested compounds is short at El-Fayoum Governorate. Indoxacarb, at Al-Qalyubia Governorate, had long half life while, at El-Fayoum Governorate showed short half life this observation may be due to geographical distribution of soil salinity in El-Fayoum District soil. Soil pH of more than 8.00 was found in about 73% of Fayoum District soils, whereas the soils with pH >8.5 are 3.96% in Tamia and 9.53% in Fayoum District (Abd-Elgawad *et al.*, 2013). Indoxacarb degrades quickly with a half-life of about 1 day at pH 9, but degrades slower at lower pHs (e.g. half-life ~500 days at pH 5). As a result, residues of this chemical in water resources can be expected (U.S. EPA 2000).

So, the pH factor had great effect on insecticides degradation, but the influence of pH differed with different pesticides and soils. 14C-labeled spinetoram-J and -L were stable in buffer solutions at pH 5 and 7, while they gradually degraded *via N*-demethylation at pH 9. The degradation half life of spinetoram-J could not be accurately calculated due to its slow degradation, but that of spinetoram-L (25°C) was estimated to be 154 days at pH 9 (Shimokawatoko, *et al*. 2012).

**2- Biochemical studies.**

**a. Determination of total protein, lipid and carbohydrate in haemolymph samples of untreated and treated 6$^{th}$ instar larvae of *S. littoralis*.**

Main metabolites; carbohydrate, protein, and lipid were very efficiently utilized by insects and most species derived the main part of their nourishments from these nutrients. Feeding the 4$^{th}$ instar larvae of *S. littoralis* for 2 days on castor oil leaves previously treated with $LC_{50}$ of Indoxacarb, Spinetoram and Methoxyfenozide until reached the 6$^{th}$ larval instar caused in general an obvious significant reduction in protein levels. The significant decrease of total protein contents was also reported in studies on *Musca domestica* treated with tebufenozide as ecdysone agonist (Assar *et al*., 2010); on the 6$^{th}$ instar larvae of *Spodoptera littoralis* when treated with spinosad compounds(El-Sheikh, 2012) and on *Sesamia cretica* larvae treated with the same compound (Osman *et al*., 2014). The decrease in protein content might be due to a mechanical lipoprotein formation which will be used to repair damaged cells, tissues, and organs (Saravana Bhavan & Geraldine, 2001; Ribeiro *et al*., 2001; Mosleh *et al*., 2003) or might be referred to mobilization of amino acids during insecticide stress to meet the energy. Also, the reduction of protein level might be due to the destructive effect on some of the cerebral neurosecretory cells of the brain responsible for secretion of the protein of the treated larval instars of *S. littoralis* (Hamouda and Dahi, 2008)who proved that, spintoram has a neurotoxic effect manifested as defined in histopathologiacal changes in nerve and neurosecretory cells of *S. littoralis*. Also, the treated larvae might produce hormones that utilize protein. Methoxyfenozoid compound bind to the ecdysteroid receptors, accelerating the molting process and thereby disrupting the insect hormonal balance (Wing, 1988 and Palli, 1996).

Carbohydrates are of vital importance since they can be utilized by the insect body for production of energy or conversion to lipid. Our results induced highly significant inhibition in carbohydrate levels with all tested insecticides and the most inhibited one was after treatment with Indoxacarb then Spinetoram and finally

Methoxyfenozide. These results showed that there were significant differences between the effect of the three tested insecticides and control. The present results agreed with that recorded by Abdel-Aal, 2006 and Dahi *et al*., 2009 who recorded inhibition effect of chlorfluzuron and methylamine avermactinon total carbohydrate of *S. littoralis*. Also, El-Sheikh, 2012 state that the total carbohydrates content in 6$^{th}$ larval instar treated with spinosad significantly decreased.

Lipids are essential structural component of cell membrane and cuticle. They provided a rich source of metabolic energy. The obtained results declare that the three tested insecticides caused a highly significant decrease in lipid contents as compared with untreated insects. The great reduction in total lipids might be due to breaking down lipids to simpler moieties that could be utilized as a carbon source for growth. Bennett and Shot well,(1972) suggested that the infected larvae might produce enzyme that utilizes lipids for energy requirement.

**b- Electrophoretic patterns of haemolymph protein of untreated and treated 6$^{th}$ instar larvae of S. *littoralis*.**

Polyacrylamide gel electrophoresis (PAGE) had been extensively used as an excellent tool for the separation of protein, lipoprotein and glycoprotein from both plant and animal sources (Zacharius *et al*., 1969). The electrophoretic analysis of SDS-PAGE protein was carried out for untreated and treated 6$^{th}$ larval instar of *S. littoralis*. The present findings showed differences in the protein patterns between treated and untreated larvae. The treatment with Spinetoram and Methoxyfenozide caused disappearance of normal bands and /or appearance of abnormal bands as compared to the control samples. While the appearance of new protein bands might be due to increase of protein synthesis. These results were in harmony with that found by (Smagghe and Degheele, 1992) who investigated differences in the protein pattern of the cuticle between treated and untreated 6$^{th}$ instar larvae indicated that in the newly induced larval cuticle, some proteins were missed or expressed with less density. Also, (Hassan and Osman, 2012) showed that Emamectin benzoate significantly decreased the concentration of protein of haemolymph of 6$^{th}$ instar larvae of *S. littoralis* and affected both the number and prominence of the major protein bands in the electrophoretic protein profiles of haemolymph. These finding coincided with those of (Anita *et al.,* 1998; Bakr, 2005 and El-Bermawy, 2005).

Results of protein patterns were in agreement with those of (Hassan and Abdel-hafez 2009) who studied the effect of two acetylcholine receptor modulator (Spinosad and Radiant) on protein pattern from the haemolymph of 6$^{th}$ instar larvae of *S. littoralis* treated as 2$^{nd}$ larval instar. Thus, the separation and characterization of the individual protein facilitated the study of the chemical nature and physiological function of each protein (Mohamed, 1990). The reduction in developed protein bands of haemolymph of treated 6$^{th}$ instar larvae of *S. littoralis* might be attributed to the toxic action of tested insecticides which inhibited the synthesis and expression process of these deleted protein.

In the light of the present findings, the three tested insecticides, Indoxacarb, Spinetoram and Methoxyfenozideare effective in reducing *S. littoralis* population especially, indoxacarb can play a useful role in resistance management programs. It has no cross-resistance with the current insecticides, such as pyrethroids, organophospates, and carbamates (Lapied *et al*. 2001). It has no appreciable effect on some important natural enemies (Bostanian and Akalach 2006). Also, spintoram has broad-spectrum insect control and low environmental effect on non-target species (Crouse, 2007). In addition, the diacylhydrazines (as Methoxyfenozoid) are considered highly selective with no harm to natural enemies (Dhadialla *et al*. 1998;

Smagghe and Degheele 1998). Hence, they can be considered as important addition to insect pest management programs. With attention for pH of soil and irrigation water because of at El-Fayoum Governorate, Soil pH of more than 8.00 was found in about 73% of El- Fayoum District soils (Abd-Elgawad, *et al*. 2013), persistence of indoxacarb was lower than that at Al-Qalyubia Governorate. So, we recommend that pH of soil and irrigation water should be considered before applying these insecticides.

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## ARABIC SUMMERY

**تقييم حقلي ودراسات كيمو حياتية لمبيدات حديثة على دودة ورق القطن الكبرى (اسبودبتر اليتورالز).**

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تم تقييم كفاءة ثلاثة مبيدات (الاندوكساكارب والسبينوتورام و الميثوكسى فينوزيد) ضد الأعمار اليرقية لدودة ورق القطن التي تصيب نبات البرسيم في محافظتي القليوبية والفيوم و ذلك اثناء الموسم الزراعى ٢٠١٢-٢٠١٣.وقد اثبتت النتائج عدم وجود فروق كبيره بين المبيدات الثلاثة (الاندوكساكارب والسبينوتورام و الميثوكسى فينوزيد) في احداث خفض لاعداد يرقات دودة ورق القطن مع الاخذ فى الاعتبار الوقت اللازم لاحداث هذا الخفض وذلك لاختلاف طريقة عمل كل مبيد . وقد سجلت نسبة الخفض الاولية بعد اليوم الأول من الرش لمركب الاندوكساكارب والسبينوتورام وبعد اليوم الثالث من الرش بمبيد الميثوكسيفينوزيد . وقد كان مبيد الاندوكساكارب الاكثر فعالية يليه الميثوكسى فينوزيد و السبينوتورام. كما أظهرت النتائج تحت الظروف شبه الحقلية أن بقاء الاندوكساكارب في محافظة القليوبية أعلى من المبيدين الاخرين المختبرين بينما كانت فترةبقاء المبيدات المختبرة جميعها بمحافظه الفيوم قصيره وقد اعزيت هذه النتائج إلى ملوحة التربة في محافظة الفيوم. كما لوحظ ان قدرة تحمل سمية المبيدات الثلاثة المختبرة ازداد مع زيادة عمر اليرقات من العمر اليرقى الثاني إلى العمر اليرقى الرابع . وقد تم استخدام الكيمياء الحيوية فى محاولة للكشف عن تأثير الجرعة نصف القاتلة للمبيدات الثلاثة المختبرة (الاندوكساكارب والسبينوتورام و الميثوكسى فينوزيد) على هيموليمف الطور اليرقى السادس ليرقات دودة ورق القطن التي قد تمت معاملتها في الطور اليرقى الرابع بالجرعة النصف قاتلة بالمبيدات المختبرة وذلك على المكونات الرئيسية لنواتج الأيض للحشرة والتغير في انماط البروتين .