Field study to evaluate the joint action of certain insecticides, IGR's and baculoviruses against *Spodoptera littoralis* (Bosid.)

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Abstract

As a selective biological insecticide, spinosad has been widely used for the control of pests including *Spodoptera littoralis*. It was studied very well in the laboratory last decade but there is a lack in knowledge in both field evaluations, lethal and sublethal effects to obtain a complete analysis of spinosad impact. This study attempts to evaluate the lethal and sublethal effects of spinosad on this pest by recording and analyzing various toxicological and physiological parameters. The toxicity of spinosad against *S. littoralis* was determined under Egyptian field conditions on Tomato plants in Fayoum Governorate in the period extended from June to July of 2014 conditions by oral exposure of late second-instar larvae to the compounds. The LC50 values of spinosad to *S. littoralis* tested at 24, 48, 72, 96, 120, 144 and 168 h after treatment were 37.580, 19.050, 9.028, 7.019, 5.0182, 4.0181 and 2.0109 mg x kg(-1), respectively. Spinosad at sublethal concentrations significantly extended the developmental period of survivor larvae, and reduced larval wet weight. These doses were significantly reduced when Spinosad tested in combination with *SpLi*NPV trend at 1x 103 PIB's/ml to reach 17.3583, 8.5878, 4.1247, 2.1474, 1.2204, 0.1241 and 0.0147 mg x kg(-1) the same trend was obtained when Flufenoxuron was determined. However these data were compared with the recommended pesticide Diazinon. Some biological aspects were investigated and it was found that the effect of combination of Flufenoxuron with the NPV was the best in the field application followed by Spinosad in combination with the NPV virus. Also, the tested insecticides at LC50 concentration reduced food consumption, larval growth rate, efficiency of converting ingested and digested food into body tissue. This work concluded that combination of both bio-rational pesticides Flufenoxuron and Spinosad with virus is recommended in field.

Keywords: NPV–Spinosad interaction, Baculovirus, Flufenoxuron, Diazinon.

1. Introduction

The cotton leafworm, *Spodoptera littoralis* is considered as one of the major and economic pests in Egypt, infesting over 112 plant species. The larval stage is known as a leaf eater accepting almost all herbaceous plants Abdel- Wahab (2002) \[4\] and in Asia and Europe too Horowitz et al., (1994) \[23\] and Smagghe and Degheele (1997) \[33\]. The use of insecticides for control of such pest proved to be the most accepted during the recent years. However, the practical application of different insecticides extensively has resulted in several problems such as development of resistance in field population of insects Frank et al., (1990) \[21\]. The current application of chemical insecticides on other crops is considered as one of the main factors affecting the agro ecosystem (plant, soil, water and other organisms). From this point of view, it is necessary to minimize the application of insecticides that considered as a main source of environmental pollution and use other compounds may proof as good alternative of insecticides, among these compounds is spinosad. Spinosad offers approaches to integrated pest management Peterson et al., (1997) \[25\] and insecticide resistance management Salgado, (1997) \[26\] as it provides excellent crop protection with a relatively low toxicity to non-target organisms Thompson et al., (2000) \[34\]. 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 neonicotinoids and fibroses avermectins Crouse and Sparks, (1998) \[10\]. On the other hand, insect growth regulators (IGRs) are bio rational insecticides with novel modes of action that disrupt the physiology or development of target pest. Such compounds tend to be selective and generally less toxic to no-target organisms than conventional insecticides Biddinger and Hull, (1995) \[9\] and Nicholas et al., (1999) \[24\]. The use of IGRs compounds in insect control is known as insect development...
Inhibition, which inhibits or prevents normal metamorphosis of immature stages to the adult's stage. However, many IGRs have shown potentiality against Lepidopterous insects Farag, (2001) [19], Abdel-Aal, (2003) [2] and Seth et al., (2004) [13]. Therefore, this study aimed to evaluate the toxicity of certain insecticides belonging to bioinsecticides (spinosad, spinetoram) and IGRs (flufenoxuron) against the 2nd larval instars of *Spodoptera littoralis* alone and in combination with *Spli*NPV at sublethal concentrations. The investigation also, involved the sublethal effects of the previous compounds on some biological and physiological aspects of the insect.

2. Material and Methods

Test insect

A laboratory strain of cotton leafworm *Spodoptera littoralis*, was reared in the laboratory on castor bean leaves under constant laboratory conditions of 25±2°C and 65±5% R.H. El-Defrawi et al., (1964) [17].

Tested insecticides

1. Spinosad: One of the new classes of insect control products, derived from the metabolites of the naturally occurring bacteria, Saccharopolyspora pinosa.

   Active ingredient: Spinosad
   
   Common name: Spinosad
   
   Trade name: Tracer ®
   
   Chemical Structure: Spinosad is a mixture of spinosyn A and spinosyn D. Spinosad (Tracer 24% SC) was produced by Dow Agro. Sciences, Co.

2. Baculovirus

   *Spodoptera littoralis* nucleopolyhedrovirus (*Spli*NPV) was the baculovirus used in present work.

3. Insect growth regulators: Flufenoxuron (Cascade 10% EC) was produced by American Cyanamid Co.

4. Diazinon: is the common name for a synthetic organophosphate, is a non-systemic insecticide used in agriculture to control soil and foliage insects and pests on a variety of fruit, vegetable, and field crops.

Field experiments

Field experiments were carried out during 2014, the period extended from June to July in a tomato field located at Fayoum Governorate. The experiments were done under local ethological conditions of 32°C average temperature 61% average R.H. and the wind velocity was 1.8 m/sec., we used 0.5 hectare which split into 20 plots and control plot. The experimental field was sprayed with recommended rate. The tested compounds were belonged to two classes, the bioinsecticides (spinosad) and IGRs (flufenoxuron). To assess the insecticidal activity of the tested insecticides and their combinations with *Spli*NPV at 1x10^3 PIB'S/ml, each treatment was represented in five replicates. The tomato plants 20 days post planting were covered with insecticides and one leaf was removed daily and put in jars with 10 second instar larvae of the tested insect. After that, the treated leaves were replaced by another untreated ones and allowed to feed till the pupation. The jars were daily examined to determine the larval mortality. The corrected mortality of larvae was carried out using Abbott’s formula Abbott, (1925) [1]. The LC25, LC50 and slope values of the tested compounds were calculated using Finney’s equation (1971) [20].

Biological studies

Tomato leaves were soaked in LC25 concentration of each insecticide which calculated after 48 hrs and used for feeding the newly 2nd instar larvae. Fifty 2nd instar larvae in five replicates, ten each were used for each insecticide. The larvae were placed in a glass jar and provided with the treated leaves. After 24 hrs exposure period, survived larvae were transferred to jars containing fresh untreated leaves and observed daily to determine larval duration, pupation %, malformed pupae and adult emergence. One female and two male of resulted adults were placed together in a glass jar to maximize successful mating, then provided with piece of cotton soaked in 10% sugar solution as a source of food for moth and was internally covered with soft sheet of paper for opposition. Also, mating of adult male and female which resulted from feeding the larvae on untreated leaves was used as a control. In order to determine the fertility (hatchability percentage of eggs), two or three patches having not less than 100 eggs were collected during the first 3 days of oviposition and incubated under the laboratory conditions until hatching, then hatch ability was recorded.

Physiological studies

Newly 2nd instar larvae were starved for three hours before used in the tests to insure on empty intestine El-Malla and Radwan, (2008) [15]. Five replicates of (10 larvae each) were allowed to feed on tomato leaves treated with LC50 values of the tested bio-insecticides for 24 hrs exposure period. After that, the survived larvae were transferred to untreated leaves in clean jars and left to feed until pupation or death. The fresh weight of larval faces and tomato leaves in each rearing jar were daily recorded. Fresh leaves were kept in a similar rearing jar without larvae under the same conditions to estimate the actual loss of moisture, which was used, to calculate the corrected weight of consumed fresh leaves. The quantity of ingested food was estimated by subtracting dry weight of the larvae remaining at the end of each experiment from the total weight of the diet provided. Food consumption and utilization were calculated according to the equation given by Waldbauer (1968) [35] and Senthil-Nathan and Kalaivani (2005) [36]. Data were subjected to analysis of variance (ANOVA) through SPSS Computer program (2004) and the means values were compared using Duncan’s multiple Range Test (1955) [12].

3. Results and Discussion

The toxicity of tested insecticides and their combination with baculovirus against the 2nd instar larvae of *Spodoptera littoralis*

The results presented in Table (1) showed the toxicity of spinosad and flufenoxuron and their combination with NPV virus against the 2nd instar larvae of *S littoralis* at different exposure times. Treatment with flufenoxuron+ virus proved to be more effective than spinosad+virus or Spinosad alone, while Diazinon treatment showed the least effective after 24 and 48 hrs post exposure. It is important to note that there was a negative relationship between the times elapsed post treatment and the LC50 values of all tested insecticides.
Sublethal effect of tested insecticides on some biological aspects of *Spodoptera littoralis*

The results obtained from feeding the 2nd instar larval for 24 hours on LC_{50} treated tomato leaves of tested insecticide are shown in Table (2). All the tested insecticides illustrated a significant increase in larval mortality. The LC_{50} values of spinosad tested against *S. littoralis* at 24, 48, 72, 96, 120, 144 and 168 hrs after treatment were 37.58, 19.05, 11.7, 11.7, 11.8, 11.7 and 11.7 days for the tested previous insecticides, respectively compared to the control (8.9 days). The percentage of malformed pupae ranged from 4.02 and 2.01 mg x kg (-1), respectively. Spinosad at sublethal concentrations significantly extended the developmental period of survivor larvae, and reduced larval wet weight. These doses were significantly reduced when Spinosad tested in combination with *Sp/LNPV* at 1x 10^{5} PIB/ml to be 17.34, 8.59, 4.15, 2.15, 1.25 and 0.01 mg x kg (-1). For Flufenoxuron the LC_{50} values give 7.02, 5.02, 2.37, 1.37, 1.98, 0.98 and 0.89 mg x kg (-1) in combination with virus these values decreased to 0.06, 0.03, 0.02, 0.014, 0.0132, 0.011and 0.010. However, these data are comparable with the recommended pesticide Diazion. The present findings confirm the results of Abdel-Rahim et al. (2009) [6]; Dahi et al., (2009) [11]; Ezz El-Din et al. (2009) [18] and Abu-Allah (2010) [7], who reported that spinosad was the most effective compound against the 4th instar larvae of *S. littoralis*. Also, Abdel-Rahman and Abou-Taleb (2007) [3] showed that spinetoram was more toxic than spinosad against the larval instars of *S. littoralis* after 24, 48 and 72 hours of exposure. Obtained results also, indicated that the tested IGR "flufenoxuron" was more toxic than the tested bioinsecticides against the 2nd instar larvae of *S. littoralis*. This may be due to slow metabolism of IGRs used in the insect body Haga et al., (1984) [22] Shaurub et al., (1999) [22] and Abdel-Aal (2003) [2]. In contrast, El-Sheikh and Abdel-Aal (2007) [16] reported that triflumuron was more effective compound against the 4th instar larvae of the laboratory strain of *S. littoralis* followed by flufenoxuron, as well as malformed pupae in compared to control. Also, these effects were more pronounced for the IGR (flufenoxuron) than for the bioinsecticides (spinosad). The larval duration was 12.5, 15.3, 14.2 and 13.7 days for spinosad and Spinosad+virus, Flufenoxuron+virus, flufenoxuron, and diazinone respectively, in compare with the control (11-2 days), while pupal duration was 10.2, 11.1, 11.8, 11.7 and 11.7 days for the tested previous insecticides, respectively compared to the control (8.9 days). The percentage of malformed pupae ranged from 4.6% (virus alone) to 29.6% (flufenoxuron alone and in combination with virus) compared to 5.1% for the control. On the other hand, the tested insecticides induced a significant suppression in pupation, adult emergence and egg hatchability when compared with a control. Also, there was insignificant differences between the effects of the tested insecticides with exception of spinosad effect on pupation as it induced the highest percentage (76.0%). However, the pupation varied from 55% for spinosad+virus to 76% for spinosad compared to 95% for the control. The adult emergence ranged from 63.8% (flufenoxuron) to 77.8% for Diazion compared to 97% for the control.

### Table 2: Results of nutritional indices and related parameters of *Spodoptera littoralis* 2nd larval instars are presented in Table (3). The results revealed that the larvae fed on the leaves treated with LC_{50} value of the tested insecticides induced a pronounced reduction in its weight compared to the check larvae. Also it was clear that all the tested insecticides showed a significant decrease in food consumption (CI), relative growth rate (RGR), efficiency of converting ingested (ECI) and digested food (ECD) into body tissue compared to the control. On the other hand, the ability of larvae to utilize food for growth was measured by approximate digestibility (AD)
which measures the digestion of food ingested by larvae. This value was not significantly affected for the tested insecticides except for spinosad which had a significant high value compared to the check larvae alone or in combination with virus. Blocks the maturation of imaginal discs which are primordial for many adult integument structure in endopertgoyta insect Schneiderman (1972) [27]. The results are in accordance with the findings of Abdel-Rahim et al. (2009) [6] and Reda et al., (2010) who showed that flufenoxuron increased the larval and pupal duration and decreased the pupation, adult emergence and fertility of the eggs produced by adult progeny. In general, it was observed that emamectin benzoate was more effective in all the mentioned measured parameters. However, the reduction in the efficiency of converting ingested (ECI) and digested (ECD) food and approximate digestibility (AD) were drastically reduced as affected by treatments. Abdel-Aal and Abdel-Khalek (2006) mentioned that IGRs reduced approximate digestibility (AD) when the 2nd larval instar of S. littoralis fed on treated plants compared to the control. El-Malla and Radwan (2008) [15] found that growth rate (GR), consumption index (CI), approximate digestibility (AD), efficiency of conversions of either ingested (ECI) or digested (ECD) food to body tissue of S. littoralis larvae fed on Abamectin and Sumialfa were decreased compared to the check except for spinosad treatment which gave slight increase in the same parameters. Bedia and Gesara (2012) indicated that spinosad and Pyriban reduced food consumption larval growth rate (GR), efficiency of converting ingested (ECI) and digested (ECD) food into body tissue. On the other hand, the approximate digestibility (AD) was considerably not affected in all treatments except in trancer with higher concentration treatments. Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test (1955) [12], finally, it can be concluded that the tested bioinsecticides and IGRs can be used in the integrated pest management program.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Mean of larval weight (gm)</th>
<th>Consumption index (CI)</th>
<th>Relative growth rate (RGR)%</th>
<th>Approximate digestibility%(AD)</th>
<th>Converting ingested food % (ECI)</th>
<th>Converting digested food % (ECD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.047±0.02a</td>
<td>6.6±0.14a</td>
<td>19.9±0.24b</td>
<td>93.5±0.42b</td>
<td>6.7±0.33c</td>
<td>7.82±0.214c</td>
</tr>
<tr>
<td>Spinosad</td>
<td>0.033±0.01b</td>
<td>4.2±0.16b</td>
<td>11.2±0.21c</td>
<td>69.3±0.25c</td>
<td>1.6±0.34c</td>
<td>2.11±0.27c</td>
</tr>
<tr>
<td>Flufenoxuron</td>
<td>0.034±0.01b</td>
<td>4.5±0.12b</td>
<td>12.9±0.24c</td>
<td>89.3±0.17c</td>
<td>2.1±0.35c</td>
<td>3.45±0.25c</td>
</tr>
<tr>
<td>SpinoNPV</td>
<td>0.045±0.01b</td>
<td>4.1±0.13c</td>
<td>13.7±0.16c</td>
<td>97.3±0.17c</td>
<td>1.2±0.33c</td>
<td>2.26±0.26c</td>
</tr>
<tr>
<td>SpinoNPV + Spinosad</td>
<td>0.045±0.01b</td>
<td>6.1±0.14c</td>
<td>14.9±0.27c</td>
<td>94.3±0.19b</td>
<td>1.3±0.36c</td>
<td>2.42±0.25c</td>
</tr>
<tr>
<td>SpinoNPV + Flufenoxuron</td>
<td>0.044±0.01b</td>
<td>6.7±0.12b</td>
<td>16.9±0.22c</td>
<td>95.3±0.39b</td>
<td>2.3±0.35c</td>
<td>3.36±0.26c</td>
</tr>
<tr>
<td>Diazinon</td>
<td>0.024±0.01c</td>
<td>2.3±0.12c</td>
<td>8.9±0.24c</td>
<td>101.3±0.39b</td>
<td>2.1±0.33c</td>
<td>2.46±0.26d</td>
</tr>
</tbody>
</table>

Table 3: Effects of tested insecticides alone and in combination with SpliNPV virus at their LC50 values on the food utilization and nutritional indices of the 2nd larval instar of Spodoptera littoralis laboratory strain.

SE*: standard error

4. References
12. Duncan DB. Multiple range and multiple F test.