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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 lake 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 LC_{50} 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 10^3 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 LC_{50} 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) [31]. 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±2ºC 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 LC_{25}, LC_{50} and slope values of the tested compounds were calculated using Finney's equation (1971) [20].

Biological studies

Tomato leaves were soaked in LC_{25} 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 LC_{50} 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 larvae 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) [28]. 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 LC_{50} values of all tested insecticides.

Table 1: The Toxicity of Spinosad, Flufenoxuron, *SpLi*NPV and their combinations with *Spli*NPV in comparison with Diazinon tested against *Spodoptera littoralis.*

Insecticides	Time (hrs)	LC$_{25}$(ppm)	LC$_{50}$(ppm)	Slope values
Spinosad	24	11.951	37.580	1.242
	48	9.5025	19.050	1.369
	72	7.2628	09.0281	1.321
	96	5.4572	07.0190	1.952
	120	3.1231	05.0182	1.321
	144	2.0942	04.0181	1.981
	168	1.0512	02.0109	1.987
Flufenoxuron	24	2.0553	07.0251	1.987
	48	1.0568	05.0252	1.325
	72	1.0238	02.3695	1.321
	96	0.5187	01.3699	1.214
	120	0.4128	01.9841	1.301
	144	0.3079	00.9875	1.021
	168	0.1047	00.8974	1.016
Spinosad in combination with *Spli*NPV1x 10^3 PIB's per ml	24	6.5519	17.3583	1.124
	48	2.9026	08.5878	1.254
	72	1.2628	04.1247	1.231
	96	1.2225	02.1474	1.100
	120	1.1232	01.2204	1.321
	144	0.0748	00.1241	1.254
	168	0.0314	00.0147	1.231
Flufenoxuron in combination with *SpLi*NPV1x 10^3 PIB's per ml	24	0.0600	00.2492	1.254
	48	0.0305	00.2111	1.127
	72	0.0201	00.1589	1.214
	96	0.0140	00.1029	1.211
	120	0.0132	00.9214	1.212
	144	0.0111	00.8211	1.201
	168	0.0100	00.4213	1.191
Diazinon	24	5.4471	11.2571	1.547
	48	2.7112	05.8791	1.324
	72	1.3221	03.4781	1.214
	96	1.2042	01.6552	1.235
	120	0.0759	00.2584	1.321
	144	0.0238	00،1110	1.111
	168	0.0114	00.0943	1.0321

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$_{25}$- 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, 9،03, 7.02, 5.02, 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 *Spli*NPV at 1x 10^3 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 Diazinon. 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 Abdu-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) [5] 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) [32] 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, Flufexoron+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 Diazinon compared to 97% for the control.

Table 2: Effects of tested insecticides alone and their combination with *Spli*NPV on some biological aspects of *Spodoptera littoralis*.

Insecticides	Larval duration days	Pupal duration days	% Pupation	% Malformed pupae	% Adult emergence	% Hatchability
Spinosad	12.5±0.41^a	10.2±0.45^b	76± 4.85^c	13.6±0.39^e	71.8±2.3^b	68.5±1.7^b
Flufenoxuron	14.2±0.82^a	11.7±0.33^{ab}	68± 3.35^{bc}	29.6±0.39^d	67.8±1.3^b	61.2±1.2^b
*Spli*NPV	14.2±0.31^a	10.3±0.31^{ab}	59± 9.12^c	4.6±0.39^a	66.8±3.5^b	60.3±1.7^b
*SpLi*NPV+Spinosad	15.3±0.54^a	11.1±0.37^b	55± 3.20^c	14.6±0.39^b	65.8±2.6^b	59.4±1.3^b
*SpLi*NPV+Flufenoxuron	14.1±0.21^a	11.8±0.39^a	68± 8.21^c	29.6±0.39^b	63.8±2.6^b	55.5±1.5^b
Diazinon	13.7±0.58^b	11.7±0.35^b	65± 2.31^c	14.6±0.39^c	77.8±3.3^b	67.5±1.8^b

SE*= standard error

Sublethal effect of tested insecticides on the food utilization and nutritional indices of *Spodoptera littoralis*

Results of nutritional indices and related parameters of spinosad and flufenoxuron and their combination with virus against *S. 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 endopterygota 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 food Senthil-Nathan *et al.*, (2005a, b) [29, 30]. Abo-El-Ghar (1993) [8] found that Abamectin had remarkable anti-feeding activity on *S. littoralis* larvae, accompanied with reduced relative consumption index (C.I) and relative growth rate (RGR). In addition, the same authors indicated also that, the higher concentration of Abamectin caused a significant decrease in both the efficiency of conversion of either ingested (ECI) or digested (ECD) food to body tissue. El-Basyouni and Sharaf (2002) showed that food consumption of 4th larval instar of *S. littoralis* fed on treated plants with LC_{50} of some IGRs was significantly less than untreated one, relative growth rate (RGR) of larval instar was significantly reduced. Efficiency of conversion of 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. Ebeid and Gesraha (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 tracer 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 3: Effects of tested insecticides alone and in combination with *Spli*NPV virus at their LC_{50} values on the food utilization and nutritional indices of the 2nd instar larvae of *Spodoptera littoralis* laboratory strain.

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

SE*= standard error

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