Sublethal effect of certain insecticides on biological and physiological aspects of *Spodoptera littoralis* (Boisd.)

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Abstract: The toxicity and sublethal effects of three bioinsecticides; (spinosad; spinetoram and emamectin benzoate) and two insect growth regulator; (flufenoxuron and triflumuron) on some biological and physiological aspects were evaluated using a laboratory strain of ***Spodoptera littoralis*** **(Boisd.)** 4th larval instar. On bases of LC_{50} values**,** emamectin benzoate was the most toxic insecticides followed by flufenoxuron while spinosad was the least toxic one.Triflumuron and spinetoram had a moderate effect. There was a negative relationship between the time elapsed post treatment and the LC_{50} values for all the tested insecticides.The treatment of the 4th instar larvae with LC_{25} values of the tested insecticides increased the larval, pupal duration and malformed pupae and decreased the percentage of pupation,adult emergence and egg hatchability compared to the control. Also, the tested insecticides at LC_{50} concentration reduced food consumption, larval growth rate, efficiency of converting ingested and digested food into body tissue. In contrast, all the tested insecticides did not significantly affect on approximate digestibility except emamectin benzoate as its effect increased. Thus, the tested boinsecticides and IGRs could be used into integrated pest management programs of any crop.
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1. Introduction:

The cotton leafworm, *Spodoptera littoralis* (Boisd.) considered as one of the most series pest for many different crops in Asia, Africa and Europe (Horowitz *et al.*, 1994 and Smagghe and Degheele, 1997). The intensive use of conventional pesticides led to several important problems, i.e. environmental pollution, destruction of the natural enemies and insect resistance to different insecticides Therefore, there is a great need to develop alternative or additional techniques, which would allow a rational use of pesticides and provides adequate crop protection for sustainable food, feed and fiber protection. Among the most promising alternative to the conventional insecticides, is the discovered bacterial insecticides spinosad.Sspinosad is a metabolite of *Saccharopolyspora spinosa*. It is the active ingredient in Tracer. The extensive worldwide testing that spinosad provides an effective control on key pests in numerous crops, including vegetables and cotton (Bret *et al*., 1997; Notling *et al.,* 1997). Also spinosad offers approaches to integrated pest management (Peterson *et al.,*1997) and insecticide resistance management (Salgado,1997) as it provides excellent crop protection with a relatively low toxicity to non- target organisms (Thompson *et al*.,2000) 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 avermactins (Crouse and Sparks, 1998). Also, emamectin benzoate (Radical) is a novel semi-synthetic derivative of natural product abarmectin in Avermactin family. Abamectins (Avermectin B1) are a fermentation product from the soil microorganisms, *Streptomyces avermactitis* (Burg *et al.*, 1979) Avermactins have been shown to be effective against broad spectrum of arthropod pests (Putter *et al.*, 1981). This materials act by interfering with the action of gamma aminobutyric acid (GABA) (Fritz *et al.*, 1979). It blocks post-synaptic potentials of neuromuscular junctures, leading to paralysis. Avermectin B1 has been shown to inhibit pheromones production (Writght,1984) and inhibit feeding (Pienkowski and Mehring,1983) Radical shows high potential effect against Lepidopterous larvae (Mrozik, 1994 and White *et al.*, 1997). Abamectin also is more environmentally acceptable because it binds to soil, does not bioaccumulation, and degrades rapidly (Lasota and Dybas, 1991). On the other hand, the insect growth regulators (IGRs) are biorotional 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 and Nicholas *et al*., 1999).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 adults stage. However, many IGRs have shown potentiality against Lepidopterous

insects (Farag, 2001,Abdel-Aal, 2003 and Seth *et al*., 2004). Therefore, this study aimed to evaluate the toxicity of certain insecticides belonged to bioinsecticides (spinosad, spinetoram and emamectin benzowate) and IGRs (flufenoxuron and triflumuron) against the 4th larval instar of *Spodoptera littoralis* The study 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).

Tested insecticides

A. Bioinsecticides:

1- Spinosad (Tracer 24% SC) was produced by Dow Agro. Sceinces, Co.

2- Spinetoram (Radiant 12% SC) is the second generation of the spinosyn group, and was produced by Dow Agro. Sciences Co.

3- Emamectin benzowate (Radical 0.5% EC) is a new semi-synthetic derative of avermectin B1 was produced by El-Aserah Company.

B. Insect growth regulators (IGRs):

1- Flufenoxuron (Cascade 10% EC) was produced by American Cyanamid Co.

2- Triflumuron (Alsystin 48% SC) was produced by Bayer Crop Science.

Toxicological studies

The tested compounds were belonged to two classes, the bioinsecticides (spinosad, spinetoram and emamectin benzowate) and IGRs (flufenoxuron and triflumuron). To assess the insecticidal activity of the tested compounds, a serious of aqueous concentrations for each compound was prepared using the commercial formulations. The leaf dipping technique was adopted according to Abo El-Ghar *et al.* (1994), where freshly castor bean leaves were dipped for 10 seconds in one of the prepared concentrations. The treated leaves allowed to dry under laboratory conditions before being offer to *S. littoralis* larvae. Hundred larvae distributed in five replicates (20 larvae/replicate) were used for each concentration. Also, larvae were fed on leaves immersed in only water as a control. Newly moulted 4th larval instars were fed on the treated leaves in a glass jar covered with muslin for 24 hrs. for spinosad, spinetoram and emamectin benzowate and for 48 hrs. for flufenoxuron and triflumuron. 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). The $LC_{25,}$ LC50 and slope values of the tested compounds were calculated using Finney's equation (1971), through software Computer program.

2-Biological studies:

Castor bean leaves were soaked in determined LC_{25} for each insecticide which calculated after 48 hrs and used for feeding the newly 4th instar larvae. Four hundred 4th instar larvae in four replicates, one hundred each were used for each insecticide. The larvae were placed in a glass jar and provided with the treated leaves. After 24 hrs for the bioinsecticides and 48hrs for the IGRs, survived larvae were transferred to jars containing fresh untreated leaves and observed daily to determine larval duration, the pupation, malformed pupae and emerged. One female and two male of resulted adults were placed together in a glass jar to maximize successful mating, then provided with a 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 oviposition. Also, mating of adult male and female which resulted from feeding the larvae on untreated leaves was used as a control. 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 hatchability was recorded.

3-Physiological studies:

Newly ecdysed 4th instar larvae were starved for three hours before used in the tests to insure on empty intestine (El-Malla and Radwan, 2008). Five replicates of (20 larvae each) were allowed to feed on castor bean leaves treated with LC_{50} values of the tested bioinsecticides for 24 hrs and the IGRs for 48 hrs. After that, the survived larvae were transferred to untreated leaves in clean jar and left to feed until pupation or death. The fresh weight of larvae, faeces and castor bean 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 h 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) and Senthil-Nathan and Kalaivani (2005) as follows:

Consumption index (CI) = E/TA

Relative growth rate (RGR) = P/TA

Approximate digestibility (AD) = 100 x (E-F)/E

Efficiency of conversion of ingested food (ECI) = 100

x P/E

Efficiency of conversion of digested food (ECD) = 100 x P/(E-F)

Where:

A = Means dry weight of larvae during the experimental period.
E = Dry weight of food eaten
F = Dry weight of faeces produced
P = Dry weight gain of larvae
T = Duration of experimental period.

All data were subjected to analysis of variance (ANOVA) through SPSS Computer program (2004) and the means values were compared using Duncan's ultiple Range Test (1955)

3. Results and Discussion

Toxicity of some insecticides against the 4th instar larvae of *Spodoptera littoralis* (Boisd.):

The results presented in Table (1) show the toxicity of three bioinsecticides (Radical, spinosad and spinetoram) and two insect growth regulators (flufenoxuro and triflumuron) against the 4th larvae instar of *S littoralis* at different exposure times. Among the bioinsecticides, emamectin benzoate proved to be the most effective compound followed by spinetoram, while spinosad the least effective after 24 and 48 hrs of exposure. Its important to note that there was a negative relationship between the time elapsed post treatment and the LC_{50} values of all the tested insecticides. The LC_{50} values were 0.464, 7.900 and 32.500 ppm for emamectin benzoate, spinetoram and spinosad after 24 hrs of exposure. Increasing the period of exposure till 48 and 72 hours decreased the LC_{50} values to reach 0.019, 4.250 and 14.870 ppm after 48 hours and 0.011, 1.92 and 10.14 pmm after 72 hours for emamectin benzoate, spinetoram and spinosad respectively. The present findings confirm the results of with Abdel-Rahim *et al.* (2009); Dahi *et al.*, (2009); Ezz El-Din *et al.* (2009) and Abdu-Allah (2010), who reported that emamectin benzoate was the most effective compound against the 4th instars larvae of *S. littoralis*. Also, Abdel-Raman and Abou-Taleb (2007) showed that spinetoram was more toxic than spinosad against the larval instar of *S. littoralis* after 24, 48 and 72 hours of exposure.

The obtained results also, indicated that the tested IGRs; flufenoxuron and triflumuron were more toxic than the tested bioinsecticides against the 4th instars larvae of *S. littoralis*. This may be due to slow metabolism of IGRs used in the insect body (Haga *et al.,* 1984, Shaurub *et al.*1999 and Abdel-Aal,2003. The results revealed that flufenoxuron was more effective than triflumuron after 48 and 72 hrs of exposure. In contrast El-Sheikh and Abdel-Aal (2007) reported that triflumuron was more effective compound against the 4th instar larvae of the laboratory strain of *S. littoralis* followed by flufenoxuron.

Table (1): Toxicity of some tested insecticides against 4th larval instar of *Spodoptera littoralis* (Boisd.) by dipping technique at different exposure times.

Insecticides	Time (hrs)	LC_{25} (ppm)	LC_{50} (ppm)	LC_{50}		Slope values
				Upper	Lower	
Emamectin benzoate	24	0.220	0.464	0.567	0.372	2.053
	48	0.007	0.0187	0.024	0.015	1.570
	72	0.005	0.011	0.014	0.008	2.003
Spinosad	24	11.62	32.50	41.19	22.510	1.530
	48	5.40	14.87	20.51	11.110	1.200
	72	2.51	10.14	13.89	7.256	1.113
Spinetoram	24	2.91	7.90	11.44	6.13	1.54
	48	1.37	4.25	5.67	3.32	1.37
	72	0.63	1.92	2.44	1.49	1.39
Flufenoxuron	48	0.021	0.085	0.128	0.062	1.11
	72	0.012	0.036	0.047	0.028	1.37
Triflumuron	48	0.055	0.258	0.360	0.189	1.00
	72	0.028	0.135	0.185	0.097	0.98

2-Sublethal effect of certain insecticides on some biological aspects of *Spodoptera littoralis*:

The main biological aspects of *S. littoralis* after feeding the 4th instar larval for 24 hours for tested bioinsecticides and 48 hours for IGRs on castor bean leaves treated at their LC_{25} values were shown in Table (2). All the tested insecticides resulted a significant increase in both larval and pupal durations as well as malformed pupae in compared to control. Also, these effects were more pronounced for the IGR (flufenoxuron and triflumuron) than for the bioinsecticides (emamectin benzoate, spinosad and spinetoram). The larval duration was 14.7, 14.5, 14.3, 12.2 and 13.6 days for triflumuron, flufenoxuron, spinetoram, spinosad andemamectin benzoate, respectively, in compared to the control (10.5days),

while pupal duration was 11.5; 10.7; 11.2; 10.5 and 10.3days for the previous insecticides, respectively in compared to the control (8.5 days). The percentage of malformed pupae ranged from 10.8% (spinosad) to 23.6 % (flufenoxuron) in compared to 4.5% 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 effect of the tested insecticides with exception of spinosad effect on pupation as it induced the highest percentage (75.0%). However, the pupation varied from 59% for triflumurun to 75% for spinosad in compared to 97% for the control. The adult emergence ranged from 67% (flufenoxuron) to 68.4% for emamectin benzoate in compared to 96.5% for the control. However, the decrease in adult emergence could be due to the fact that the toxic blocks the maturation of imaginal discs which are primordial for many adult integument structure in endopetrygote insect (Schneiderman, 1972). The results were accordance with the findings of Abdel-Rahim *et al.* (2009), who reported that Radical and spinosad at LC_{50} increased the larval and pupal duration of *S. littoralis* and decreased pupation and adult emergence percentages as compared to the control. Also, Reda *et.al.*, (2010) 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 contrast, Abdel-Aal and Abdel-Khalek (2006), El-Sheikh and Abdel-Aal (2007) reported that IGRs as chitin synthesis inhibitors (Hexaflumuron, Teflubenzuron, Triflumuron and Flufenoxuron) decreased both larval and pupal duration of *S. littoralis* as compared to the control.

Table (2): Effects of certain insecticides at their LC_{25} values on some biological aspects of laboratory strain of *Spodoptera littoralis* (Boisd.) when fed at the 4th instars larval on treated castor bean *leaves*

Insecticides	Larval duration days ± SE*	Pupal duration days ± SE*	% Pupation ± SE*	% Malformed pupae ± SE*	% Adult emergence ±SE*	% Hatchability ±SE*
Emamectin benzoate	13.6±0.404 b	10.3±0.35 b	66±2.83 bc	15.7±1.1 c	71.0±3.30 b	68.4+ 1.8 b
Spinosad	12.2±0.61 c	10.5±0.40 b	75±3.20 b	10.8±0.72 e	75.0±2.40 b	63.5±1.74 b
Spinetoram	14.3±0.24 a	11.2±0.19 ab	68±2.40 bc	13.33±0.17 d	70±0.90 b	61± 1.40 b.
Flufenoxuron	14.5±0.50 a	10.7±0.52 ab	63±3.3 c	23.6±0.9 a	67.0±1.4 b	58± 1.42 b
Triflumuron	14.7±0.30 a	11.5±0.32 a	59±1..9 c	20.4±0.40 b	69.0± 1.90 b	60± 1.73 b
Control	10.5±0.80 d	8.5±0.35 c	97±1.40 a	4.5±0.12 f	96.0±2.02 a	96.5± 1.40 a

SE* = Standard error

Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test (1955).

3 - Sublethal effect of certain insecticides on the food utilization and nutritional indices of *Spodoptera littoralis:*

The nutritional indices and related parameters of three bioinsecticides (emamectin benzoate, spinosad and spinetoram) and two IGRs (flufenoxuron and triflumuron) against *S. littoralis* 4th larval instar were 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 check larvae. Also, it was clear that all the tested insecticides showed a significant decrease in food consumption (CI), the 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 emamectin benzoate which had a significant high value compared to the check larvae. 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). Abo-El-Ghar *et al.* (1993) found that Abamectin had remarkable antifeeding 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 were 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 (AD%) when 4th larval instar of *S. littoralis* fed on treated plants compared to the control. El-Malla and Radwan (2008) 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 decreased compared to check except 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.

Table (3): Effect of certain insecticides at their LC_{50} values on the food utilization and nutritional indices of *Spodoptera littoralis* of 4th larval instars. (means ±SE)

Insecticides	Mean of larval weight (gm) ±SE*	Consumption index (CI) ± SE*	Relative growth rate (RGR)% ±SE*	Approximate digestibility% (AD) ±SE*	Converting ingested food % (ECI) ± SE*	Converting digested food % (ECD) ± SE*
Emamectin benzoate	0.024±0.01 c	2.9±0.21 d	7.12±0.25 e	96.2±0.33 a	2.20±0.2 e	2.39±0.21 d
Spinosad	0.038±0.01 b	3.8±0.31 c	15.17±0.39 b	88.6±0.38 b	4.39±0.21 b	4.83±0.27 b
Spinetoram	0.036±0.006 b	3.5±0.12 c	11.61±0.24 d	88.00±0.32 b	2.98±0.19 c d	3.3±0.18 c
Flufenoxuron	0.037±0.006 b	4.0±0.16 b c	13.4±0.18 c	86.5±0.37 b	3.5±0.14 c	3.79±0.16 c
Triflumuron	0.034±0.01 b	4.5±0.16 b	12.58±0.14 c	87.3±0.29 b	2.82±0.21 d	3.08±0.07 c
Control	0.047±0.02 a	5.5±0.32 a	20.6±0.43 a	89.2±0.43 b	7.6±0.18 a	8.7±0.26 a

SE* =Standard error

Means in the same column followed by the same letter are not significantly different according to Duncan Multiple Range Test (1955)

Finally, it can be concluded that the tested bioinsecticides and IGRs can be used in the integrated pest management program of any crop to minimize bad effects of the chemical insecticides on the environment as well as beneficial insects.

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