Biological and physiological effects of the bioinsecticide Spinosad on the cutworm, *Agrotis ipsilon* (Hufnagel)

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ABSTRACT

Fourth and fifth instar larvae of the cutworm, *Agrotis ipsilon* (Hufn.) were fed on castor-bean leaves treated with the bioinsecticide, Spinosad. Marked differences in susceptibility for Spinosad based on accumulative mortality between 4^{th} and 5^{th} instar larvae of *A. ipsilon* were observed. There was a reduction in the consumption of food caused by Spinosad treatment. Considerable decrease in relative weight gained (RWG) (especially after treatment of 4^{th} instar larvae) was also recorded. Feeding 4^{th} instar larvae on treated leaves by Spinosad increased the efficiency of converting ingested food (ECI) and efficiency of converting digested food (ECD) into body substances at 4^{th} and 5^{th} instars at higher concentrations (100 and 200 ppm) but decreased at 6^{th} instar. On the other hand, the approximate digestibility (AD) was negatively affected by Spinosad at 5^{th} instar. The growth rate (GR), the assimilation rate (AR) and the relative metabolic rate (RMR) exhibited inconsistent values after feeding of 4^{th} instar larvae by Spinosad. Interestingly, feeding the 4^{th} instar larvae on 100 and 200 ppm treated leaves, significantly increased the larval and pupal durations. However, this observation was detected only for larval duration after feeding the 5^{th} instar larvae. Spinosad-treated leaves consumed by 4^{th} and 5^{th} instar larvae resulted in decrement of deposited eggs/female and hatchability percentage.

Key Words: Bioinsecticide (Spinosad), *Agrotis ipsilon*, Food utilization.

INTRODUCTION

The cutworm, *Agrotis ipsilon* (Hufn.) is a major destructive lepidopterous pest of cotton, corn and vegetable plants. High population of *A. ipsilon* can also cause a reduction in the number of seedlings at the beginning of the season. Lepidopterous pest control on vegetable plants is vital because larval presence, feeding damage and presence of faeces which can seriously reduce the marketability of such vegetable plants. Despite, potential resistance problems, environmental hazards and general public disapproval are associated with extensive use of insecticides for controlling lepidopterous pests. However, the use of chemicals still remains the most practical way for reducing lepidopterous pest populations on cotton plants. One of the solutions of this dilemma is to shift the emphasis from using synthetic to using safer natural insecticides for control of *A. ipsilon* and other lepidopterous pests. Natural insecticides have been shown to be effective against lepidopterous pests.

Spinosad is a bioinsecticide derived from fermentation of *Saccharopolyspora spinosa,* a recently described species of actinomycete (Mertz and Yao, 1990). Spinosad has become a standard bioinsecticide in pest management programs for the production of vegetables and cotton. It has both contact and stomach activity against lepidopteran larvae, leaf miners and thrips with relatively long residual activity (Palumbo and Reyes, 1998; Palumbo, 1999 and Palumbo *et al.*, 2000). Previous researches have provided a strong basis for making wise decision on insecticide use, but there is still a need to study the effects of this compound on preventing plant damage. One assessment of plant damage is the leaf consumption by *A. ipsilon*, but this variable is impractical to study it in the field.

Therefore, the present study was conducted to indicate the effect of Spinosad on mortality percentage, food consumption and utilization by *A. ipsilon* larvae in the laboratory. Reproduction per female and hatchability percentage of eggs were also investigated.

MATERIALS AND METHODS

Cutworm rearing

A. ipsilon used in this study originated from a susceptible colony from Plant Protection Institute, Ministry of Agriculture, Dokki, Giza and reared on castor oil leaves (*Ricinus communis* L.) at Dept. of Plant Protection, Faculty of Agriculture, Al-Azhar University, Cairo. Larvae were reared on castor leaves under laboratory conditions at 25±2C° and 60-70% R.H.

The bioinsecticide (Spinosad)

Spinosad is a new insecticide belongs to Actinomycetales. It was originally isolated from an organism found in soil samples taken in 1982 and identified in 1988 as a new bacterium species, *Saccharopolyspora spinosa* (Mertz and Yao, 1990). This product is an identical formulation and contains 480 g/L of the new active ingredient Spinosad technical insecticide. The following aqueous concentrations of the tested compound: 5, 10, 25, 50, 100 and 200 ppm. were prepared.

Treatment technique:

Larvae of 4^{th} and 5^{th} instars were reared individually to avoid cannibalism and starved 4 hours, then fed for 48h. on a castor bean leaves treated with different concentrations of Spinosad (48%) (5, 10, 25, 50, 100 and 200ppm.) whereas, leaves were dipped in different concentrations then air dried and used as a contaminated food. The treated larvae were examined daily and mortality percentages were corrected according to natural mortality (Abbott, 1925). Food consumption and utilization, larval and pupal durations, egg production/female and hatchability percentage of eggs were recorded.

Food consumption, absorption and utilization parameter were estimated overall the 4^{th}, 5^{th} and 6^{th} instars after treatment of 4^{th} instar and the same parameter estimated overall the 5^{th} and 6^{th} instars after treatment of 5^{th} instar. Treated and control larvae were weighed before and after feeding, fresh food was weighed before offering to the larvae, and its fresh weight was recorded after feeding every day. The larvae of each replicate were starved for 3 h. before weighing to insure an empty intestine. Fresh leaves were kept in a rearing jar under the same conditions to estimate the natural loss of moisture, which was used for calculating the corrected weight of the consumed leaves. Faeces discharged by larvae were also weighed. Feeding rate was the amount of food consumed during the feeding period of the instar, generally expressed on a "per day" basis (consumption rate, CR) or on a "per day per unit body mass" basis (relative consumption rate, RCR) (Slansky, 1993). RCR = mg consumed food/ g mean fresh body weight/day (Slansky and Scriber, 1985). Approximate digestibility (AD) = [weight of ingested food- weight of faeces / weight of ingested food] x 100. The efficiency of conversion of ingested food to body substance (ECI) = [weight gain/weight of ingested food] x 100. The efficiency of conversion of digested food to body substance (ECD) = [weight gain/weight of ingested food – weight of faeces] x 100. Growth rate (GR) = fresh weight gain during feeding period/feeding period x mean fresh body weight of larvae during the feeding period (Waldbauer, 1968). Relative weight gain (RWG) = mg weight gain during the instar/days with correction for a single instar (Johnson and Mundel, 1987). Assimilation rate (AR) = RCR x AD (Scriber and Slansky, 1981). Relative metabolic rate (RMR) was calculated according to Slansky (1993) but corrected for fresh weight as follows: RMR = (mg weight ingested food – weight of faeces) / g mean fresh body weight / day.

Data obtained were statistically analyzed using the student t-distribution refined by Bessel correction (Morony, 1957) for testing the significance of difference between means.

RESULTS AND DISCUSSION

Effect of Spinosad on percentage of mortality

Data presented in Table (1) show the effect of different concentrations of Spinosad on the 4th and 5th instar larvae. It is noticed that 100 ppm caused 92.58 and 53.18 % as accumulative mortality percentage after treatment of the 4th and 5th instar larvae. The low concentrations 5 and 10 ppm induced only 28.43 and 43.11 % after treatment of the 4th instar larvae and 7.14 and 7.14 %, respectively with 5th instar larvae. From these data, it could be concluded that the 4th instar larvae were more susceptible than 5th instar larvae to Spinosad. Similarly, Temerak and Sayed (2001) stated that, all rates of Spinosad (10, 20, and 40 ml/100 liter) showed significant control of *Deudorix livia* on the date palm *Phoenix dactylifera* (100 % reduction) before harvest, in 1999 and 2000. Also, McPherson *et al.* (2003) represented that hornworm larvae (*Manduca sexta* L.) were highly susceptible to tobacco foliage dipped in Spinosad, with LC_{50} of 4.3 x 10^{-4} after 24h, 1.0 x 10^{-6} after 48h, and 5.7 x 10^{-7} after 72h (ml/ml). Also, data were coincided with other researches which indicated that, the active component of Spinosad, apparently had a mode of action different from all other insecticides or insect control agents, with high levels of both contact and oral activity against *Heliothis virescens* (Sparks *et al.*, 1995).

Effect of Spinosad on food consumption rates

Most of the total consumption and growth usually occur during the 5th and 6th instar larvae, therefore performance values calculated for these instars tend to be representative of those calculated for the entire larval stage (Mackey, 1978). There was a direct proportion relationship between the eaten food amounts and the relative weight gained by the 5th and 6th instar larvae after treatment of 4th instar larvae. The RWG decreased by decreasing the food consumption and also by increasing the concentration levels (39.35, 33.46 and 15.75 mg and 71.9, 37.6 and 35.62 mg at 5, 50 and 200 ppm in the 5th and 6th instars, respectively (Table, 2). As reported by Sundaramurthy, (1977) the amount of growth reduction was proportional to reduced food consumption. There were no significant differences in relative consumption rate by the 4th, 5th, and 6th instar larvae after treatment of the 4th instar larvae.

Data of Table (3) showed the food consumption by the 5th and the 6th instar, larvae after feeding of 5th instar. Food consumption decreased by increasing the concentrations and averaged 661.73, 666.98 and 621.59 mg and 1021.21, 788.31 and 744.21 mg at 5, 25 and 100 ppm, in the 5th and 6th instars respectively, compared with 731.59 and 1064.03 at control but the reverse was observed when 200 ppm was used. After treating the 5th instar larvae, last instar larvae gained larger body weights than those of 5th instar larvae relative to control treatment. Also, after treatment of the 5th instar larvae by Spinosad, the RCR did not significantly decreased compared to untreated

Table (1): Effect of different concentrations of Spinosad on corrected mortality percentages of larval, pupal and adult stages of *Agrotis ipsilon*

Conc. ppm	The treated larval instar							
	4th instar				5th instar			
	L.S	P.S	A.S	Acc. M%	L.S	P.S	A.S	Acc. M. %
5.0	28.43±8.5**	0.0	0.0	28.43±8.4**	7.14±1.2***	0.0	0.0	7.14±1.7**
10.0	43.11±6.6**	0.0	0.0	43.11±6.6**	7.14±1.5***	0.0	0.0	7.14±1.5**
25.0	67.84±9.6**	0.0	3.33±0.7	74.08±10.9**	14.29±2.0**	0.0	3.30±0.06	17.82±1.5**
50.0	78.56±10.2**	3.44±2.5	0.0	85.18±14.8**	21.36±1.6**	6.60±0.7	0.0	28.43±3.2**
100.0	89.27±12.9**	0.0	3.33±0.8	92.58±6.0**	50.03±3.6**	3.30±0.6	0.0	53.18±5.3**
200.0	92.82±6.1**	0.0	0.0	92.59±6.1**	78.46±3.2**	3.30±1.2	0.0	82.0±3.6**

L.S. = Larval stage, P.S. = Pupal stage, A.S. = Adult stage,
Acc. M. % = Accumulative mortality percent

Table (2): Food consumption rates (mg±SD) by the 4th, 5th and 6th instar larvae of *A. ipsilon* when the 4th instar was fed on leaves treated with Spinosad.

Conc. ppm	R.W.G.	F.C.	R.C.R.	Change %
		4th instar larvae		
5.0	28.55±3.6*	240.0±23.6**	0.64±0.1	0.0
10.0	26.62±4.4	230.0±32.4*	0.67±0.1	+3.1
25.0	13.46±2.2**	250.0±32.3**	1.13±0.2*	+73.8
50.0	11.79±1.4**	170.0±22.6**	0.79±0.3	+21.5
100.0	31.22±3.4	216.0±54.2**	0.56±0.0	-13.8
200.0	31.77±4.2	240.06±40.0**	0.62±0.0	-4.6
Contro l	33.11±4.1	283.73±3.6	0.65±0.1	---
		5th instar larvae		
5.0	39.35±6.1*	600.0±15.5**	0.53±0.1	-5.4
10.0	34.53±7.1*	600.0±20.5**	0.53±0.2	-5.3
25.0	40.94±14.5*	345.0±25.3**	0.36±0.1	-35.7
50.0	33.46±8.3**	205.0±15.6**	0.17±0.1*	-69.6
100.0	18.44±9.2**	267.7±30.5**	0.21±0.1	-62.5
200.0	15.75±10.1**	243.0±40.1**	0.20±0.1	-64.3
Control	45.79±5.0	641.5±13.2	0.56±0.1	---
		6th instar larvae		
5.0	71.9±14.2**	845.0±28.4**	0.17±0.1	+6.3
10.0	78.57±14.6**	769.0±50.0**	0.13±0.1	-18.8
25.0	72.64±13.1**	363.3±60.0**	0.08±0.0	-50.0
50.0	37.6±15.5**	378.11±50.5**	0.09±0.1	-43.8
100.0	38.98±16.1**	536.60106.0**	0.12±0.0	-25.0
200.0	35.62±16.0**	470.0±109.0**	0.11±0.1	-31.3
Control	93.9±16.5	949.5±36.3	0.16±0.5	---

RWG: relative weight gain,
FC: food consumption,
Conc.: concentration,
RCR: relative consumption rate of food,
*: significantly different (P>0.05) and
**: highly significant (P<0.01),

Table (3): Food consumption rates (mg±SD) by the 5th and 6th instar larvae of *A. ipsilon* when the 5th instar was fed on leaves treated with Spinosad

Conc. ppm	R.W.G.	F.C.	R.C.R.	Change %
		5th instar larvae		
5.0	44.25±4.7	661.73±46.3**	0.75±0.4	-9.6
10.0	42.21±6.8	669.20±211.4**	0.64±0.2	-22.9
25.0	30.26±5.9*	666.98±69.4**	0.57±0.1	-31.3
50.0	37.20±4.3*	608.30±71.5**	0.55±0.1	-33.7
100.0	39.27±8.3	621.59±160.4**	0.50±0.1	-39.8
200.0	43.62±5.6	925.63±204.7**	0.64±0.3	-22.9
Control	45.0±3.0	731.59±55. 6	0.83±0.3	---
		6th instar larvae		
5.0	86.65±9.6	1021.21±301.8*	0.20±0.1	-51.2
10.0	81.25±8.8	1008.40±311.4*	0.19±0.0	-53.7
25.0	107.30±8.8 *	788.31±205.8**	0.15±0.1	-63.4
50.0	76.60±6.9	1160.82±236.8**	0.33±0.0	-19.5
100.0	64.20±9.4*	744.21±47.9**	0.22±0.0	-46.3
200.0	59.10±0.8*	1041.13±234.6	0.30±0.1	-26.8
Control	78.30±4.6	1064.03±194.1	0.41±0.0	---

RWG, FC, RCR, Conc, *, **: see the footnote of Table (2).

larvae at the 5th and 6th instars. The values were 0.75, 0.55 and 0.64 mg compared with 0.83 in control at the 5th instar and 0.20, 0.33 and 0.30 mg compared with 0.41 mg in control at the 6th instar for 5, 50 and 200 ppm, respectively. These results are supported by Yee and Toscano, (1998) who stated that, the total leaf consumption of lettuce leaf by the 5th instar of *Spodoptera exigua* treated with 50 ppm of success was 0.58±0.5 cm^2 compared with 6.16±1.00 cm^2 at untreated control leaf disks. Also, they reported that this was the most effect bioinsecticide tested against *S. exigua* based on leaf consumption, survival, duration, and percentage of survival on lettuce. The lowest food-consumption level in *A. ipsilon* affected by Spinosad treatments was similar to that caused by IGR's (Diflubenzuron and Trifluron) on *S. littoralis* (Radwan *et al.*, 1986). Larvae fed on Spinosad-treated leaves exhibited a proportional relationship between food consumed and values of RWG throughout this study.

Effect of Spinosad on food utilization

The efficiency of conversion of ingested food to body substance (E.C.I.) is a measure of the insect's ability to utilize food for growth. Likewise, the metabolic efficiency expressed as efficiency of conversion of digested food (E.C.D.) estimates the percentage of assimilated food to biomass (Slansky and Scriber, 1985). After exposure of 4th instar larvae to Spinosad treated leaves, the approximate digestibility (AD) values for the 4th and 6th instar larvae were remarkably higher than the corresponding values for the untreated larvae (84.8, 87.5, and 89.68 for control, 5, and 50 ppm at the 4th instar, respectively; whereas, at the 6th instar, they were 80.86, 82.24 and 85.32 for control, 5, and 50 ppm, respectively. At the 5th instar larvae, the AD was lower especially at higher concentrations (62.60, 77.05 and 75.99 compared with untreated control (87.39)). These results indicate an increased ability to digest food at the 4th and the 6th instar larvae (Table, 4).

The ECI and ECD values increased during the 4th instar, the change percentages of ECI and ECD were +23.5 & +13.2; and +25.6 & +4.1 for 100 and 200 ppm, respectively. At the 5th instar larvae the change percentages of ECI and ECD were increased (+185.9, +32.7 and +24.9 for ECI while ECD values were +298.0, +52.6 and +196.7, respectively) especially when the 4th instar was fed on leaves treated with 50, 100 and 200 ppm of Spinosad, but at low concentrations there were no marked differences (Table 4). The prolongation of larval duration at higher concentrations (100 and 200 ppm) may be explained by the observation shown later on (Table 8). Interestingly, these treatments hindered the ability of last instar larvae to convert the ingested or the digested food to biomass at higher concentrations (50, 100 and 200 ppm). The ECI values were 55.25, 58.12 and 60.60 compared with 64.28 of untreated control. The ECD values were 70.0, 68.40 and 70.0 compared with 79.40 for untreated control. At the 4th and 5th instars, no clear differences for ECI or ECD were found at lower concentrations but increased at higher concentrations compared with untreated control.

Table (4): Food absorption and utilization by the 4th, 5th and 6th instar larvae of *A. ipsilon* when the 4th instar was fed on Spinosad – treated leaves.

Conc ppm	AD	Change%	ECI	Change%	ECD	Change %
			4th instar larvae			
5.0	87.50±3.8	+3.18	34.72±3.3	-1.1	40.80±2.4	-1.4
10.0	89.46±0.7*	+5.49	34.73±4.4	-1.1	30.80±5.5**	-25.6
25.0	90.50±2.2*	+6.72	16.15±2.8**	-53.9	17.80±4.3**	-56.9
50.0	89.68±1.2	+5.75	20.81±3.1**	-40.7	23.20±2.3**	-43.9
100.0	83.41±3.5	-1.63	43.36±0.5**	+23.5	51.98±5.1**	+25.6
200.0	92.17±2.2*	+8.69	39.72±3.2*	+13.2	43.09±2.9	+4.1
Control	84.80±3.5	-	35.10±4.2	-	-	-
			5th instar larvae			
5.0	85.00±1.8	-2.7	26.23±5.0	-8.1	30.80±3.5	-5.7
10.0	84.39±0.5	-3.4	21.23±1.9**	-25.7	25.0±2.9**	-23.5
25.0	78.91±2.5*	-9.7	47.47±5.2**	+66.3	60.0±5.4**	+83.7
50.0	62.60±5.0**	-28.3	81.64±4.1***	+185.9	130.0±2.2**	298.0
100.0	77.05±1.9**	-11.8	37.90±3.2***	+32.7	47.80±3.1**	52.6
200.0	75.99±3.3**	-13.0	35.66±5.2**	+24.9	96.90±4.9**	196.7
Control	87.39±4.2	-	28.55±4.3	-	32.66±4.0	-
			6th instar larvae			
5.0	82.24±2.5	+1.7	74.58±3.2*	+16.0	81.10±4.1	+2.1
10.0	84.82±3.2*	+4. 9	71.52±3.2*	+11.3	84.30±2.7*	+6.2
25.0	86.52±5.2*	+6.9	129.97±2.8**	+102.2	87.0±7.1**	+9.6
50.0	85.32±4.2*	+5.5	55.25±4.1**	-14.0	70.0±3.2**	-11.8
100.0	84.94±3.9*	+5.0	58.12±4.3**	-9.6	68.40±5.6**	-13.9
200.0	76.19±2.9*	-5.7	60.60±2.9*	-5.7	70.0±4.6**	-11.8
Control	80.86±1.2	-	64.28±1.9	-	79.40±3.2	-

Conc., *, **: see the footnote of Table (2).
AD: Approximate digestibility, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food.

Feeding of the 5th instar larvae on Spinosad-treated leaves cleared that the AD, ECI and ECD values showed no significant differences. But with the 6th instar, the change percentages at higher concentrations (100 and 200 ppm) were +34.5 & +45.7 for AD and +32.0 & +1.4 for ECI and -36.5 & -30.4 for ECD, respectively. This means that, Spinosad had positive effect on AD and ECI but negative effect on ECD compared with untreated control (Table, 5). Generally, it can be concluded that Spinosad-treated leaves increased the approximate digestibility than control indicating that, the quality of the food and the ability to digest material from the treated leaves to assimilate nutrients were unaffected. In this respect, Radwan *et al.,* (1986) observed that, the approximate digestibility coefficient increased considerably in larvae fed on leaves treated with Diflubenzuron and Trifluron.

The assimilation rate (AR) indicates the ability of larvae to assimilate the digested and observed food overall the instars. The data provided the correlation of growth rate to assimilation rate and relative metabolic rate. Highly significant declined rate of food assimilation occurred throughout the 5th larval instar as an effect of Spinosad treatment on the 4th or 5th instar larvae. The AR after treatment of the 4th instar larvae was 10.64, 16.18 and 15.19 at higher concentrations (50,100 and 200 ppm). Also, after treatment of the 5th instar larvae, the AR was 45.90, 40.77 and 53.92 for the previous concentrations, respectively (Table 6 &7). The rates of AR of last instar larvae were decreased significantly (7.67, 10.19 and 8.38 compared with 12.93 at control) after feeding the 4th instar larvae on Spinosad treated leaves with high concentrations (50, 100 and 200 ppm). Meanwhile, it insignificantly increased after treating the 5th instar larvae with all concentration levels. The higher concentrations (50, 100 and 200 ppm) of tested compound against the 4th instar larvae inhibited the RMR of the 5th instar, for instance the RMR was 2.68, 4.90 and 4.72 compared with 7.93 at untreated control. While no significant effect on the 6th instar larvae was found. Growth rate (GR) was increased with the 4th instar larvae while it decreased with the 5th and 6th instar larvae at higher concentrations (24.64, 8.08 & 7.21 for 100 ppm and 24.76, 7.32 & 7.12 for 200ppm at the 4th, 5th and 6th instars, respectively), after treatment of the 4th instar (Table 6). After treatment of the 5th instar larvae, the effect of Spinosad on the GR, AR and RMR were not significantly affected at the 5th and 6th instar larvae. In general, GR, AR, and RMR were increased at highest concentration (200ppm) in the 6th instar after treating the 5th instar and the values were 16.42, 24.71 and 3.03, respectively, compared with 11.09, 23.17 and 1.88 for untreated control (Table, 7). This may be due to the prolongation of the larval period (Table 8).

The potentially of insects for consumption and utilization of their food is known to be influenced by various factors such as: food derivation and host plant (Abdelfattah *et al.,* 1991- 1992, and Mohamed, 2003), larval instar (Banerjee and Haque, 1984), and rearing temperature (Reynold and Nottingham, 1975). These factors modify the ability of lepidopterous insects to consume or utilize their plant food. Other factors, such as the control measures taken against these insects were

Table (5): Food absorption and utilization by the 5th and 6th instar larvae of *A. ipsilon* when the 5th instar was fed on Spinosad – treated leaves.

Conc. ppm	AD	Change%	ECI	Change%	ECD	Change %
	5th instar larvae					
5.0	88.91±5.4*	+6.6	8.92±2.9	+8.8	10.02±0.9	+1.9
10.0	86.92±8.3	+4.2	9.46±0.8	+15.4	11.50±1.3	+16.9
25.0	85.40±1.6	+2.4	8.69±0.1	+5.9	10.01±2.9	+1.8
50.0	83.46±4.7	+0.7	10.19±2.5	+24.3	12.35±1.5	+25.6
100.0	81.55±7.4	+0.2	11.58±1.9*	+41.2	14.20±1.5**	+44.5
200.0	84.26±2.4	+0.4	8.64±1.5	+5.4	9.94±1.8	+1.1
Control	83.39±8.2	-	8.20±2.1	-	9.83±1.9	-
	6th instar larvae					
5.0	77.88±4.8**	+37.8	10.74±1.4	+9.59	13.79±3.0*	-20.5
10.0	79.84±2.2**	+41.2	11.0±1.7	+12.2	13.78±1.3*	-20.5
25.0	78.05±4.3**	+38.1	11.05±0.9	+12.8	14.41±1.0*	-16.9
50.0	83.34±4.7**	+47.4	9.24±2.0	-5.7	10.09±0.9**	-36.0
100.0	76.06±3.6**	+34.5	12.94±2.7*	+32.0	11.01±1.7**	-36.5
200.0	82.39±4.5**	+45.7	9.94±1.1	+1.4	12.07±1. 7**	-30.4
Control	56.53±3.1	-	9.80±1.9	-	17.34±1.5	-

Conc., *, **,: see the footnote of Table (2).
AD , ECI, ECD: see the footnote of Table (4)

Table (6). The correlation among GR, AR and RMR of the 4th, 5th and 6th instar larvae of *A. ipsilon* after treatment 4th instar by Spinosad.

Conc. ppm	GR (x100)	AR	RMR
	4th instar		
5.0	22.85±0.3	56.0±1.9	5.03±0.2
10.0	23.38±1.0	59.93±3.3*	5.42±0.4
25.0	18.26±0.8**	102.26±5.5*	9.20±0.3**
50.0	16.52±0.6**	70.84±3.5**	6.40±0.1**
100.0	24.64±0.2*	46.70±1.6**	4.26±0.2
200.0	24.76±0.8	57.14±2.2	5.17±0.4
control	22.83±0..5	55.12±2.9	4.96±0.1
	5th instar		
5.0	13.93±0.5*	45.05±1.9*	7.22±0.6
10.0	13.61±0.7*	44.72±3.3*	8.65±0.7
25.0	15.27±1.2*	28.40±2.9**	4.58±0.4**
50.0	14.02±0.8*	10.64±3.0**	2.68±0.3**
100.0	8.08±1.0**	16.18±3.0**	4.90±0.7**
200.0	7.32±1.2**	15.19±3.2**	4.72±0.8**
control	16.20±0.9	48.93±2.9	7.93±0.5
	6th instar		
5.0	9.58±1.1	13.98±0.9	6.02±1.1
10.0	9.82±0.8	11.02±1.0	5.70±0.8
25.0	10.02±0.9	6.92±1.1**	2.70±1.0*
50.0	7.23±0.7*	7.67±1.5**	4.65±0.8
100.0	7.21±0.9*	10.19±0.3**	6.75±0.7
200.0	7.12±1.3*	8.38±1.3**	6.50±0.8
control	10.51±1.0	12.93±0.8	5.58±0.9

Conc., * , ** : see the footnote of Table (2).
AR: Assimilation rate,
RMR: Relative metabolic rate,
GR: Growth rate

Table (7). The correlation between GR, AR and RMR of the 5th and 6th instar larvae of *A. ipsilon* after treatment 5th instar by Spinosad.

Conc. ppm	GR (x100)	AR	RMR
	5th instar		
5.0	6.73±1.1	66.68±2.9	10.74±3.0
10.0	6.09±0.9	55.62±1.6**	10.72±2.1
25.0	4.41±1.4	48.67±1.5**	12.24±0.7
50.0	5.68±1.5	45.90±2.1**	11.50±2.6
100.0	5.80±0.3	40.77±2.9**	12.37±0.5
200.0	5.40±1.6	53.92±1.6**	16.42±1.9**
control	6.81±1.9	69.21±3.3	11.09±1.7
	6th instar		
5.0	10.74±3.0	15.57±2.2**	2.23±0.7
10.0	10.72±2.1	15.16±1.0**	2.13±0.8
25.0	12.24±0.7	11.70±2.5**	3.01±1.0
50.0	11.50±2.6	27.50±3.9*	3.12±0.8
100.0	12.37±0.5	16.73±2.8**	2.85±1.0
200.0	16.42±1.9**	24.71±1. 5	3.03±0.9
control	11.09±1.7	23.17±2.1	1.88±0.8

Conc., *, **,: see the footnote of Table (2).
AR , RMR, GR: see the footnote of Table (6).

Table (8). The effect of Spinosad on the larval and pupal durations of *A. ipsilon* when the 4th and 5th instar larvae were fed on Spinosad - treated leaves.

Conc. ppm	Treated at 4th instar		Treated at 5th instar	
	Larval duration	Pupal duration	Larval duration	Pupal duration
5.0	13.50±0.8	11.50±0.7	10.33±0.6	11.33±0.8
10.0	14.0±0.6	11.0±0.8	11.33±0.8	11.33±0.9
25.0	13.50±0.9	12.66±0.9	12.0±0.4	12.0±0.6
50.0	15.50±1.1*	13.0±1.0	12.0±0.7	12.33±0.8
100.0	16.50±1.2*	13.20±0.8*	12.66±0.4	12.0±0.6
200.0	16.50±1.1*	13.20±0.8*	13.0±0.9	12.33±0.9
control	13.50±0.7	11.50±0.5	[illegible]-0.5	11.66±0.9

antifeedant activity (Vinson and Barras, 1970).

The effect of Spinosad on larval and pupal durations

The effect of Spinosad on larval and pupal durations of *A. ipsilon* treated as the 4th and 5th instar larvae were recorded in Table (8). No significant differences in the larval period were observed after treating the 4th or 5th instar larvae at lower concentrations (5-25 ppm). There were significant differences in larval duration (16.50 & 16.50 and 12.66 & 13.0 days compared with 13.50 and 10.66 days for untreated control, respectively at the 4th and 5th instars) at higher concentrations (100 and 200 ppm). No significant differences were noticed in pupal durations after treatment the 4th and 5th instar larvae with exception of the 100 and 200 ppm which averaged 13.20 & 13.20 days, respectively, after treating the 4th instar larvae . These results agree with Youssef *et al.* 1991, who stated that, the larval duration of *A. ipsilon* was prolonged when treated by 1 µl of juvenal hormone analogue.

The effect of Spinosad on fecundity eggs and hatchability percentage

Data in table (9) showed that the highest mean number of deposited eggs at the 4th instar was 230 eggs/female (5 ppm) and the lowest was 89.40 eggs/female (200 ppm). The hatchability percentages of eggs were 56.60 and 43.40%, respectively, for the two previous concentrations. The 5th instar treatments showed that, 10 & 200 ppm resulted 230.70 & 100.50 eggs/female and the hatchability percentages were 52.80 & 36.10%, respectively, while the deposited eggs/female by untreated control were 242.70 and 250.50 and the hatchability percentages of eggs were 69.80 and 65.70, respectively at the 4th and 5th instars. There were significant differences between different concentrations and untreated larvae for fecundity and hatchability percentages of eggs. Additionally, Temerak and Sayed (2001) cleared that, unhatched eggs of *Deudorix livia* ranged from 20 to 29 % in 1999 and from 17 to 23 % in 2000 for Spinosad at 10 to 40 ml/100 liters. Also, Fang *et al.* (2002) stated that, Spinosad killed all exposed *Rhyzopertha dominica* adults and significantly suppressed progeny production (84-100%) and kernel damage (66-100) at both rates (0.1 and 1 mg (AI)/kg) compared with untreated wheat. Spinosad was extremely effective against *Plodia interpunctella* on wheat at 1 mg/kg, based on larval mortality (97.6-99.6%), suppression of egg to adult emergence (93-100), and kernel damage (95-100), relative to similar effects on untreated wheat. *Corcyra cephalonica* was highly susceptible to Spinosad at 0.5 and 1 mg/kg. At both Spinosad rates, reduction in larval survival, egg-to-adult emergence, and seed damage relative to the control treatment was more than or equal to 93% on both corn and sunflower seeds (Huang and Subramanyam, 2004).

Table (9). The effect of Spinosad fecundity and hatchability % of *A. ipsilon* treated at the 4th or 5th instar larvae.

Conc. ppm	4th instar		5th instar	
	Laying eggs/female	Hatchability %	Laying eggs/female	Hatchability %
5.0	230.0±15.0**	56.60±6.7**	200.40±35.4**	57.86±10.6*
10.0	155.0±10.0**	57.0±2.0**	230.70±20.4**	52.80±7.5**
25.0	140.0±10.5**	51.60±9.1**	220.10±14.6**	46.20±11.6**
50.0	130.0±8.6**	45.38±4.9**	180.50±16.8**	36.40±7.7**
100.0	160.0±14.5**	40.0±6.8**	176.0±7.5**	38.80±10.6**
200.0	89.40±5.6**	43.40±10.5**	100.50±9.5**	36.10±11.5**
Control	242.70±11.9	69.80±7.1	250.50±15.2	65.70±9.8

The results assured that, the 4th instar larvae were more susceptible than the 5th instar to Spinosad. On the other hand, the growth reduction was proportional to the reduction of food consumption after feeding the 4th instar larvae on Spinosad–treated leaves, while this observation was not detected after feeding the 5th instar larvae on the same food. Spinosad has inconsistent effect on the treated 4th instar. AD, ECI and ECD values were positively correlated to high concentrations and negatively affected with the low concentrations in case of the 4th and 5th instar larvae, but the reverse effect was detected in case of the 6th instar. GR and AR values were decreased with the 5th and 6th instar after feeding 4th instar on Spinosad-treated leaves. Treated 5th instar, generally, had a positive effect on the 5th and 6th instars, except ECD in the 6th instar. Finally, the larval and pupal durations were affected only at higher concentrations. Fecundity and hatchability percentages were significantly affected by Spinosad treatment.

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