EFFECT OF THREE INSECT GROWTH REGULATORS ON SOME BIOLOGICAL AND PHYSIOLOGICAL ASPECTS OF *SPODOPTERA LITTORALIS* (BOISD.)

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INTRODUCTION

Cotton plants have been infested by many pests specially cotton leaf worm, *Spodoptera littoralis* (Boisd.) which led to great crop loss. The control of this pest is based mainly on foliage treatments with several traditional insecticides. The extensive and continuous use of such insecticides has created many problems, mainly the incapability of toxic agents in controlling the target insect pest at the recommended doses. Therefore, insecticides with novel modes of action with high selectivity towards vertebrates, such as insect growth regulators (IGRs) are required. This group of insecticides has developed and classified into (i) chitin synthesis inhibitors (CSI's) like Hexaflumuron (consult) and Teflubenzuron (No moult) and (ii) juvenile hormone mimics or ecdysone agonist (molting accelerating compounds) such as Tebufenozide (MAC). IGR's act by altering normal insect growth patterns, eventually and indirectly resulting in death of treated insects (Chandler *et al.*, 1992). Many IGR's have shown potentiality against lepidopterous insects including *S. littoralis* (Ahmed *et al.*, 1990; El-Deeb *et al.*, 1991; Abdalla and Sammour, 1992; Fisk and Wright, 1992; Rao *et al.*, 1994; Sokar, 1995; Auda and Salem, 1997; Farag, 2001 and Abdel-Al, 2003 and Seth *et al.*, 2004). The aim of present work is to evaluate three IGRs against *S. littoralis* by studying their toxicity, and their efficacy on certain biological aspects as well as on feeding, consumption, digestion and utilization of treated cotton leaves by *S. littoralis* larvae.

MATERIAL AND METHODS

S. littoralis egg masses were obtained from the Research Division of the Cotton Leafworm, Plant Protection Research Institute. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber bands and supplied

daily with clean and fresh castor oil bean leaves, *Ricinus communis*, as larval food. Jars were left under laboratory conditions of 27 ± 2 °C and 65 ± 5% RH and were examined daily. The 4th instar larvae were used in the present experiments.

Three insect growth regulators (IGRs) were used, two of which are chitin synthesis inhibitors *i.e.* hexaflumuron 10% E.C. (Consult) and teflubenzuron 15 % E.C. (No moult) and the third is nonesteroidal ecdysone agonist, Tebufenozide24 % F.L. (MAC) molting accelerating compound.

1. Toxicity test

Leaf –dipping technique was used to study the susceptibility of newly moulted 4th instar. *S. littoralis* larvae were exposed to the tested IGRs according to (Abo El-Ghar *et al.*, 1994). Five Different aqueous concentrations (10, 5, 3, 2, 0.5 ppm) of the IGRs (Hexaflumoron, Teflubenzuron and Tebufenozide) were prepared. Castor bean leaves were dipped in each concentration level,for 5 sec., left to dry at room temperature and offered to the newly moulted 4th instar larvae. Larvae were allowed to feed on treated leaves for 24 hrs, then, they were provided daily with untreated leaves until pupation. Four replicates of 50 larvae were used for each concentration. Control (untreated) larvae were fed on castor bean leaves dipped in distilled water only. The mortality percentages of treated larvae were corrected against those of the control by using Abbott's formula (Abbott, 1925). The data were then subjected to probit analysis (Finney, 1971) to obtain the LC_{50} values.

2. Biological studies

Newly moulted 4th instar larvae treated with the LC_{50} values of the three tested IGRs were examined daily. The larval and pupal durations, pupal weight, percentage of pupation, number of eggs deposited by mated female moth and the percentage of egg hatchability were recorded.

Mating processes occurred according to the following combinations:

- Treated female x Treated male
- Treated female x Untreated male
- Untreated female x Treated male
- Untreated female x Untreated male (Control)

3. Consumption and utilization of food

Under the laboratory conditions of 27 ± 2° C and 65 ± 5 % R.H., *S. littoralis* larvae reached the 4th instar on the 6th day after hatching. Castor bean leaves were dipped for 5 seconds in each tested IGR (LC_{50} values), and then left to

dry for 1h at room temperature. Newly moulted 4th instars were confined for 24 hrs with treated leaves in 400-ml glass jars covered with muslin cloth. Treated leaves were then removed and fresh untreated ones were offered regularly until death or pupation. Untreated and treated tests were replicated 4 times with 50 larvae each. Dead larvae were discarded while the fresh weight of survivors, faeces and castor bean leaves in each rearing jar were recorded daily and related to the number of survivors in either control or treatment. Fresh leaves were kept in a similar rearing jar under the same conditions to estimate the actual loss of moisture, which was used for calculating the corrected weight of consumed fresh leaves. Food consumption and utilization were calculated according to the equations given by Waldbauer (1968) and Slansky and Scriber (1982) as follows:

$$\textbf{-Consumption index (CI)} = \frac{I}{TA}$$

Where: A = Mean body weight of larvae during the feeding period (mg).

I = Weight of food ingested (mg).

$$\mathbf{I = [I - a / 2]\ [w - (L + bL)]}$$

a: the ratio of loss of water to the initial weight of leaf.

b: the ratio of loss of water to the final weight of leaf.

w: weight of food introduced.

L: weight of uneaten food.

T = Feeding period (days).

$$\textbf{- Relative growth rate (RGR)} = \frac{W}{TA}$$

Where: W = Weight gain of larvae (mg).

- Approximate weight of digested food (mg) (D) = I – F

Where: F = weight of faeces (mg).

- Approximate Digestibility (AD) =

$$\frac{\textbf{Approximate weight of food digested}}{\textbf{Amount of food ingested}} \textbf{ X 100}$$

- Efficiency of conversion of ingested food into body matter (ECI) =

$$\frac{\textbf{Weight gain of larva (mg.)}}{\textbf{Amount of food ingested (mg.)}} \textbf{ X 100}$$

- Efficiency of conversion of digested food into body matter (ECD) =

$$\frac{\textbf{Weight gain of larva (mg.)}}{\textbf{Approximate weight of food digested (mg.)}} \textbf{ X 100}$$

The data obtained were statistically analyzed by Student's-*t*- test between control and treatments.

RESULTS AND DISCUSSION

1-Toxicological effects of tested IGRs

Table (1) shows the susceptibility of the 4th instar *S. littoralis* larvae towards the tested IGRs. Based on LC_{50} values the toxicity of the IGRs can be arranged in a descending order as follows: Hexaflumuron > Teflubenzuron > Tebufenozide as the LC_{50} values were 2.264, 4.1 and6.61 ppm, respectively. However the respective values for LC_{90} were 15.8, 38.4 and 48.4 ppm. The bioefficiency of the IGRs Hexaflumuron and Teflubenzuron as chitin synthesis inhibitors causing a slow detoxification in the insect body, was reported by Rao *et al.*, (1994); Mansour (1997); Shaurub *et al.*, (1999); Retnakaran, *et al.*, (2001) and Abdel-Al (2003). According to Smagghe and Degheele (1995), tebofenozoid was found to induce a premature and lethal larval moult by direct interference with the ecdysteroid receptors (ECR).Comparative toxicity studies of RH-5992 (tebofenozoid) and RH -2458 (methoxyfenozid) agonists on the sixth instar larvae of *S. littoralis* showed that methoxyfenozid was 3 to 7 times more potent than tebofenozoid agonist susceptible and 7 to 17 times agonist a pyrethroid resistant strain (Ishaaya *et al.* 1995).

2- Biological effects of the tested IGRs

The data (Table 2) show that treatments with the tested IGRs significantly ($P < 0.05$) decreased both the larval and pupal durations of *S. littoralis*, where means of 11.80, 10.60 and 9.10 days for larval duration and 13.80, 12.40 and 12.90 days for pupal duration were recorded after treating the cotton leaves with the preceding IGRs, respectively. The larval and pupal durations after feeding on untreated leaves were 12.2 and 14.30 days, respectively. On the other hand, all treatments decreased the pupal weight significantly except in case of tebufenozide treatment, where the decrease was insignificant. In 2003, Butter *et al.*, (2003) evaluated the IGR Lufenuron against *Helicoverpa armigera* on cotton under laboratory conditions. They found that IGR treatment in the larval stage significantly affected both pupal period and pupal weight. Pupal duration of the test insect was significantly extended by IGR treatment. Williams *et al.*, (2002) show that the tebufenozide appear more potent ecdysteroid agonists in Lepidoptera.On the other hand, these compounds decreased the pupation percent which was arranged descendingly as follows: Tebufenozide (78%)>Teflubenzuron (67%)> Hexaflumuron (42 %) compared to control (95 %).

The efficacy on fecundity and fertility of *S. littoralis* given in Table (3) show that the lowest number of eggs laid per female was obtained for treated females mated with treated males, followed by treated females mated with normal males, as compared to control. On the other hand, the highest number of eggs laid per female occurred when the males were the treated sex only. This may indicate that females were more sensitive to IGRs than males. In all the mating combinations, hexaflumuron was the most effective IGR; whereas tebufenozide was the least effective one. The data also show that in all mating combinations the egg-hatch percent was decreased, as compared to control. This reduction was much obvious in case of hexaflumuron treatment, followed by Teflubenzuron and Tebufenozide treatments. The fertility followed the same pattern as that of fecundity, *i.e.*, the lowest egg hatch was obtained in the mating combination containing treated females. These females were more sensitive to IGRs than the males. The impaired reproductive potential of moths emerging from treated larvae could be explained by the short life span of adults and/or interference with oogenesis and spermatogenesis. Moursy and Bartlett (1992) found that the IGR, pyriproxyfen decreased the number of spermatophores transferred to *P.gossypiella* females. Ibrahim and Shebl (2002) suggested that reduced female fecundity could be a result of a low metabolic rate. Chandler et al (1992) indicate that lepidopterous larvae ease feeding within hours of exposure to ecdysteroid agonists and soon undergo an unsuccessful moult, which results in their death (usually in 2-4 d).

3- Food consumption and utilization of leaves treated with tested IGRs

To evaluate the effect of tested IGRs on consumption and utilization of cotton leaves in the body tissues of treated *S. littoralis* larvae, the nutritional indices and related parameters are presented in Table (4). The data obtained clarified that the fourth instar larvae consumed an average of 195.34 mg of untreated leaves during the whole instar. After feeding the larvae on treated cotton leaves for 24 h. then on untreated leaves, the ingested food significantly decreased, being 123.0, 89.70 and 83.56 mg/larvae after treating with Tebufenozide , Hexaflumuron and Teflubenzuron , respectively. These amounts are reduced by 37.03, 54.08 and 57.22 % as compared with the untreated check.

This indicated that the tested IGRs, especially chitin synthesis inhibitors (Hexaflumuron and Teflubenzuron) act as antifeedant. These agree with the results given by Sokar (1995), El -Sheikh (2002) and Abdel Al (2003). The same trend could be detected for the approximate weight of food digested by the fourth instar *S. littoralis* larvae, as being affected by the tested IGRs.

In this case, larva digested about 164.21 mg till full grown, this amount was reduced to 84.10, 47.10 and 47.16 mg whene larva supplied with cotton leaves treated with the LC_{50} of Tebufenozide, Hexaflumuron and Teflubenzuron, for one day, respectively, then fed on untreated leaves. These treatments reduced the amounts of digested leaves by 48.79, 71.32 and 71.28 respectively, as compared with the amount of digested untreated leaves. Reduction in approximate digestibility (AD) was also noticed to be 68.30, 56.44 and 52.50 % for Tebufinozide, Teflubenzuron and Hexaflumuron, respectively with regard to the control (84.06 %) (Table 4). This lead to delaying of larval development resulting a reduction in the pupal weight (Table 2). This delaying in development may be attributed to the amount of energy spent by larvae in order to detoxify the IGRs. As the tested IGRs displayed considerable toxic effects, therefore, the observed decrease in the measuring parameters of *S. littoralis* may be explained according to the assumption of Lu *et al.* (1978) that the accumulation of toxic xenobiotics which is a complicated balance of such factors as absorption, excretion, intoxication and detoxication, in any organism may be expected to affect the rate of development of insect. .The ability of treated larvae to convert the digested food (ECD) into body tissues was increased in case of treatment with both CSIs while decreased in case of tebufinozide, which acts as moulting accelerating compound and the all anabolism and metabolism was occurred rapidly, so the ECD in the control was less than in both CSIs and tebufinozide. On the other hand, all treatments specially benzophenylyreas increased the ability of treated larvae to converts the ingested food (ECI) into body tissues. Ibrahim and Shebl (2002) found that the effect of insect growth regulators against grasshopper, *Euprepocnemis plorans plorans* and *S. littoralis* on the metabolism and on the reproduction potential, indicated a potential use as a pesticide.

TABLE (I)

Susceptibility of *S. littoralis* 4th instar larvae to the tested IGRs

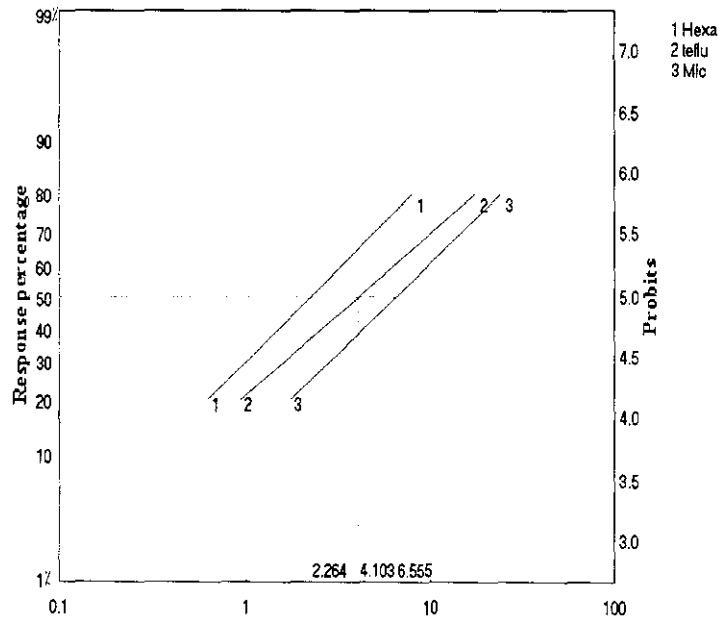
IGRs	LC_{50} (ppm)	LC_{90} (ppm)	95 % Fiducial limits		Slope
			Upper	Lower	
Hexaflumuron	2.264	15.8	2.8	1.86	1.0
Teflubenzuron	4.1	38.4	5.28	3.33	1.8
Tebufenozide	6.61	48.4	8.75	5.26	2.9

TABLE (II)

Some biological aspects of *S. littoralis* treated as 4th instar larvae with LC_{50} values of the tested IGRs (means ± S.E.)

IGRs	Larval duration (days)	Pupal duration (days)	Pupal weight (mg)	Pupation (%)
Hexaflumuron	11.80* ± 0.12	13.80* ± 0.09	389.00* ± 8.00	42
Teflubenzuron	10.60* ± 0.05	12.40* ± 0.05	352.00**± 3.00	67
Tebufenozide	9.10* ± 0.09	12.90*± 0.16	420.00 ns ± 0.32	78
Control (check)	12.2 ± 0.30	14.30 ± 0.10	425.00 ± 0.24	95

ns: not significant.
*: Significant at P< 0.05
**: Highly significant



1. Hexaflumuron. 2. Teflubenzuron. 3. Tebufenozide.

Fig (1): Toxicity regression lines 24 hours after feeding the fourth instar *S .littoralis* larvae on castor bean leaves treated with the tested IGRs.

TABLE (III)

Number of deposited eggs and egg hatchability for mated female moth of *S. littoralis* treated as 4th instar larvae with LC_{50} of the tested IGRs (Means ± S.E.)

IGRs	Mating combinations					
	Treated female x Treated male		Normal female x Treated male		Treated female x Normal male	
	No. of eggs/female	Egg Hatchability (%)	No. of eggs/female	Egg Hatchability (%)	No. of eggs/female	Egg Hatchability (%)
Hexaflumuron	185 ± 0.98 (85.4)**	12 (87.76)	236 ± 0.6 (81.42)	23 (76.53)	140 ± 2.3 (88.98)	18 (81.63)
Teflubenzuron	321 ± 2.4 (74.72)	34 (65.31)	368 ± 2.5 (71.02)	38 (61.22)	210 ± 1.5 (38.46)	31 (68.37)
Tebufenozide	695 ± 0.9 (45.28)	56 (42.86)	795 ± 1.2 (37.40)	62 (36.73)	750 ± 2.2 (40.94)	54 (44.90)

(*) Mean number of deposited eggs female and hatchability of eggs (%) laid by moths fed as larvae on untreated leaves (control) were 1270 ± 6.5 eggs/ female and 98.00 %, respectively.

(**) Values between brackets represent percentages of reduction as compared with control

TABLE (IV)

Some nutritional values of *S. littoralis* 4th larvae treated with LC_{50} of the tested IGRs (Means ± S.E.)

Physiological aspect	Control	Hexaflumuron	Teflubenzuron	Tebufenozide
Weight of food ingested/ larva (mg)	195.34 ± 2.30	89.70 ± 4.90 (54.08)*	83.56 ± 5.70 (57.22)	123.0 ± 6.90 (37.03)
Weight of food digested/ larva (mg)	164.21 ± 2.20	47.10 ±3.80 (71.32)	47.16 ± 4.4 (71.28)	84.10 ± 4.20 (48.79)
Approximate digestibility (%)	84.06 ± 2.10	52.50 ± 5.60	56.44 ± 6.70	68.30 ± 4.80
Relative growth rate (%)	30.00 ± 0.30	28.00 ± 0.40	35.00 ±0.20	60.00 ± 0.10
ECI (%)	17.56 ± 2.70	33.00 ± 3.60	34.20 ± 3.40	28.90 ± 3.90
ECD (%)	20.89 ± 3.10	63.00 ± 2.60	60.64 ± 5.40	43.30 ± 3.60

(*) Values between brackets represent of reduction as compared with control

SUMMARY

Hexaflumuron and Teflubenzuron, as chitin synthesis inhibitors and Tebufenozide as nonsteroidal ecdysone agonist (dibenzoyl hydrazine) were toxicologically, biologically and physiologically evaluated as insect development inhibitors (IDI's) against the fourth instar larvae of the cotton leaf worm, *Spodoptera littoralis* (Boisd.). IGRs significantly decreased both larval and pupal durations of S. *littoralis*, as compared to control. On the other hand, all treatments significantly decreased the pupal weight, except in case of Tebufenozide where an insignificant decrease in weight was obtained.

The fecundity of the treated females resulted from all mating combinations and egg-hatch percent remarkably decreased, as compared to control. Such reduction was much obvious in case of Hexaflumuron treatment, followed by Tebufenozide and Teflubenzuron treatments. The same trend was recorded for fertility of the resulted eggs as being affected by the tested IDI's s. The data also showed that the females were more sensitive to IDI's s than the males.

Consumption and utilization of food in treated larvae resulted in reduced approximate digestibility (AD), being 47.16 and 52.50 % for Teflubenzuron and Hexaflumuron, respectively compared to (84.06 %) for the control. The ability of treated larvae to convert the digested food (ECD) into body tissues increased in case of treatment with both chitin synthesis inhibitors, while decreased in case of ecdyson agonist.

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