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Original Research Article

EFFECT OF TWO INSECT GROWTH REGULATORS ON THE DEVELOPMENT OF *AGROTIS IPSILON* HUFN. (LEPIDOPTERA: NOCTUIDAE)

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Abstract: The present study aimed to evaluate the biological effect of two insect growth regulators flufenoxuron and juvenile hormone analog methoprene against 3 rd larval instar of *Agrotis ipsilon* to determine their toxicity. Both tested compounds significantly induced larval, pupal and adult motalites which were concentration dependant the tested IGRs induced some morphological abnormalities in larval stages, larval- pupal intermediates were also recorded pupae with C-shaped and adults with incomplete wings were also recorded. Both tested IGRs significantly increased the larval and pupal duration. On the other hand decrease the percentage of adult emergence, fecundity, fertility of the eggs produced by the adults.

Key words: Insect growth regulators, IGRs, juvenoids, *Agrotis ipsilon*, pest control, flufenoxuron, methoprene), metamorphpsis, life span.

1- Introduction

IGRs was introduced to describe a new class of bio-rtional compound through greater selectivity of action, these compounds appear to fit the requirements for third generation pesticides. Generally, IGRs have very low toxicity to mammals and other non – target organism and, usually are rapidly degarded in the environment (Carter, 1975, Staal 1975, Zurfleuh, 1976; Oberlander, 1987; Ishaaya et al 1987; Oberlander, 1997; Ishaaya and Horowitz, 1998; Kostyukovsky et al, 2000). (Ishaaya and Casida, 1974; Post et al, 1974; Smet et al, 1990; Binnington and Retnakaran, 1991, Cohen, 1993; Oberander, 1997; Ishaaya and Horowitz, 1998 and Oberlander and Silhacek,1998). Among the diverse in vivo action of CSTs on the life cycle of insect of various orders are ovicidal and larvcidal effect, (Arthur et al, 2009). Impairment of cuticle secretion was affect embryos may be the cause of hatchabitity reduction due to treatment with CSIs (Grosscurt, 1978; Grosscurt and Aderson, 1980 and Elek, 1998 a and b). The lavicidal effects of CSIs are most likely achieved through

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interference with the formation of a new cuticle (Oberlander and Silhacel 1998).

The present work was planned to achieve the following proposes: study the susceptitity of the 3rd instar larvae of the cutworm *Agrotis ipsilon* to deferent concentrations of the two insect growth regulators (flufenoxuron and methoprene) as well as to evaluate the effects of these concentrations on some biological parameters.

2- Materials and Methods

A-Maintenance of Culture

1- Origin of *Agrotis ipsilon* colony

Individuals of cut worm, *Agrotis ipsilon* (Herbst.) was obtained originally as adult and immature stages from cabbage fields of the Hadaelsham region.

2- Rearing in laboratory

Stock culture of the insect were reared in the laboratory in an incubator adjusted 30+1C° and 70% relative humidity. Certain numbers of butterflies were reared in glass jars containing the rearing larval food of alfalfa plant leaves). The jars were covered by muslin secured in its place by rubber bands. The rearing medium was changed from time to time to keep the culture in a good condition for a long time. All precaution were taken to prevent any contamination by any bacterial or viral infection or any parasites or predators.The eggs hatch at 80 F (26.6 °C). It molted 5 times until convert into pupae. Moths were sexed as pupae, according to the structure of the genital lobes.

1- Larval treatment

A laboratory colony of *A. ipsilon* was utilized with this experiment. Stock solutions were diluted as necessary to prepare IGR's- water solutions containing the quantity of IGRs (flufenoxuron or methoprene) needed to give different concentrations (0.1, 0.5, 1, 5, 10 ppm). These concentrations applied to food substrates 2525cm×of The treated leaves in petridishes (25cm),. Twenty five individuals of the 3rd larval instars of *A.ipsilon* were fed on the appropriate treated diet for 48 hrs which they allowed to complete their larval periods on untreated plant leaves. Also, one hundred individuals in four replicates were fed on untreated leaves for check. The feeding technique used was according to Oberlander (1997). The dead larvae, pupae, adults and exuvia were removed during examination of the treated larvae. Percentage mortality of larvae, pupae, adults, adult emergence was estimated on the base of the number of adult emerged in relation to the number of larvae per petridish, uncompleted emerging adults were counted as dead, and also the duration sof both larvae and pupae were estimated. The healthy and active adults produced from males and females were counted and inhibition of adult emergence was estimated according to Khazanie (1979).Ten replicates in a plastic vials (25 ml) for each concentration were used. One male and female were put each vial and left for mating for 10 days and oviposition on a suitable media. The eggs laid were counted after 10 days. Both males and females were removed to record the number of eggs laid, hatchability percentage. Also, sterility according to Toppozada *et al.* (1966) and oviposition deterrent index (O.D.I) according to Lundergren (1975) were estimated. The larvae obtained from hatched eggs were reared in untreated media to adults. All tests were performed at constant conditions (30 °C and 70% R.H.). All chemical stocks and prepared solutions were stored at 1°C when they weren't used.

D- Calculations and Data Analysis

a- Data were subjected to probit analysis (Finney, 1971) to evaluate the values of LC50.

b- The percent of adult emergence inhibition was calculated according to Khazanie (1979) .

c- The ovipositor deterrent index (O.D.I) was calculated according to Lundergren (1975). Percentage of sterility was estimated according to the formula of Toppozada *et al.* (1966). The toxicity index of the tested IGRs was calculated according to Sun (1950).

d- Statistical analysis

The values of each measured and calculated parameters were expressed as four replicates ± SE at toxicological work were expressed as ten replicates ± SE. The results obtained were evaluated using one way analysis of variance

"ANOVA" (Snedecor, 1971) and t-test ($P < 0.01$).

3-Results and disscution

To study the effect of the present IGRs on some biological aspects of treated larval stage of *Agrotis ipsilon*, the experiments were carried out on the 3 rd larval instars. In the present study Flufenoxuron and methoprene caused appreciable stomach toxic effect on larvae of *A. ipsilom* and they interfere with formation of new cuticle and destroy it. Data obtained in Table (1) showed that the lowest percentages of larval mortality of 15 and 1% occurred at 0.1 ppm.

Table (1) : Effect of flufenoxuron on *Agrotis ipsilon* treated as 3rd larval instars

Conc. (ppm)	% Larval hatch. ±SE	% Larval malformation ±SE	% Pupal mortality ±SE	% Pupal malformation ±SE	% Adult mortality ±SE	% Adult malformation ±SE	% Emerged adult ±SE	% Inhibition of adult emergence
0.1	15.00±0.25	2.00±0.28	00.00	00.00	00.00	00.00	95.00.25	15.00
0.5	18.00±0.74	18.00±0.41	6.00±0.47	5.00±0.47	4.00±0.47	4.00±0.28	79.00±1.22	24.00
1	31.00±0.57	26.00±0.71	8.00±0.28	8.00±0.25	14.00^0.41	14.00±0.41	67.00±0.57	34.00
5	37.00±0.90	33.00±0.47	11.00±0.47	11.00±0.47	19.00±0.47	17.00±0.47	58.00±1.37	45.00
10	67.00±0.87	45.00±1.37	12.00±0.47	10.00±0.85	23.00±0.64	19.00±0.25	24.00±0.47	69.00
Control	00.00	00.00	00.00	00.00	00.00	00.00	100	00.00
p-value	**	**	**	**	**	**	**	-

**= significantly different at $P < 0.0$, N.S=non significantly different

Table (2) : Biological activity of flufenoxuron on Agrotis ipsilon treated as 3rd larval instars.

Conc. (ppm)	Larval duration(days) ±SE	Pupal duration (days)	No. of eggs\female (fecundity) ±SE	% Fertility ±SE	% Sterility	% Oviposition deterrent index (O.D.I)
0.1	18.87±0.41	9.75±0.06	162.00±1.71	89.69±1.37	44.89	10.07
0.5	19.10±0.41	10.80±0.14	87.00±1.51	67.73±0.92	66.17	41.57
1	17.27±1.19	12.31±0.05	77.00±0.81	25.56±0.47	84.04	60.60
5	19.79±1.05	13.50±0.06	44.00±1.33	00.00	77.87	100.0
10	8.05±0.46	6.63±0.09	00.00	00.00	98.57	100.0
Control	19.07±0.22	8.10±0.048	205.00±1.74	100±0.0	00.00	00.00
p-value	**	N.S	**	**	-	-

**= significantly different at $P < 0.01$, N.S=non significantly different

In the present study, flufenoxuron and methoprene caused appreciable stomach toxic effect on larvae of *A.ipsilon*. They interfere with formation of new cuticle and destroy it. Data obtained in Table (1, and 2) showed that the lowest percentages of larval mortality of 15.00% occurred at 0.1 ppm, and significantly (P< 0.01) increased with the increase of flufenoxuron concentration, while it was 15.00 for methoprene, reach 67.00 % at 10 ppm. The present results is similar to that obtained by (Neumann & Guyer, 1983 and Ishaaya et al, 1984 Abdu et al., '1985, Gazit et al., 1989, Malinowski & Pawinsk, 1992, Elek, 1998a and El- Metwelly et al., 2003).The larval mortality could be due to chitin deposited in the endocuticle and the characteristic lamellae are replaced with amorphous material (Mulder and Gijswijt, 1973). Also, mortality may be due to preponderance of oval vesicles filled with amorphous material in epidermal cells, consequently, block of secretion outside the cell; the microvilli of the plasma membrane were swollen (Percy-Cunningham *et* al., 1987). In addition, higher toxicity of IGR against larvae may be explained by its stability, lack of metabolism, weaker detoxification, long retention time in the larval body and slow elimination (Neumann and Guyer, 1987).

Table (3): Effect of methoprene against Agrotis ipsilon, treated as as 3rd larval instars

Cone. (ppm)	% Larval mortality ±SE	% Larval malformation ±SE	% Pupal mortality ±SE	% | Pupal malformation ±SE	% Adult mortality ±SE	% Adult malformation ±SE	%Emerged adult ±SE	% Inhibition of adult emergence
0.1	1.00 ±0.00	00.00	9.00±0.63	8.00±0.71	00.00	00.00	90.00±0.75	10.00
0.5	3.00±0.49	00.00	11.00±0.25	11.00±0.25	1.00±0.25	1.00±0.25	86.00±0.48	14.00
1	7.00±0.47	3.00±0.48	28.00±0.29	20.00±0.71	11.00±0.43	8.00±0.00	65.00±0.57	35.00
5	13.00±0.50	8.00±0.82	35.00±0.85	27.00±1.11	13.00±0.72	7.00±0.85	52.00±0.48	48.00
10	15.00±0.49	1 5.00±0.49	39.00±0.48	39.00±0.48	19.00±0.48	19.00±0.48	46.00±1.31	54.00
Control	00.00	00.00	00.00	00.00	00.00	00.00	100	00.00
p-value	**	**	**	**	**	**	**	-

**= significantly different at P < 0.01,N.S=non significantly different

The percentages of pupal mortality were 0.0, 4.00, 6.00, 9.00 and 12.00 and it was 8.00, 11.00, 20.00, 27.00, and 39.00, at the concentrations of 0.1, 0.5, 1, 5 and 10 ppm, of the two IGRs respectively. This result is in harmony with the results obtained by (Ishaaya *et aL*, 1987; Ishaaya and Yablonski, 1987) on *T. castaneum* and (Haseeb *et* al., 2005) on *Diadegma semiclausum* and *Oomyzus sokolowskii*. The explanation of this result could be due to Chlorfluazuron at higher concentrations have antifeeding effect (Riddiford and Truman, 1978).

Also, percentages of adult mortality increased significantly (P< 0.01) with the increasing of concentration and ranged between 0.00- 18.00 %. This result is in conformity with those obtained by (Perveen, 2000a, Ross & Brown, 1982 Ishaaya, 1990).These abnormal adults were due to the resistance of insect to metabolic detoxification during the larval and pupal stages (Ishaaya et al., 1987).

The percentage of adult emergence was concentration dependent, decreased significantly with increasing in concentrations as compared to

control. Higher inhibitions of adult emergence were, 79.00 and46.00 % at the concentrations of 10 ppm for the two IGRs, (flufenoxuron and methoprene) respectively, compared to 0.0 % in case of the control. These results are in agreement with those obtained by (Ishaaya & Yablonski, 1987; Degheele, 1990; Mostafa, 2002; Abdel Fattah & Khaled, 2008 and Khaled, 2009).

As shown in Table (3 and 4), the duration of larvae was significantly affected after feeding 3rd instar on flufenoxuron and methoprene - treated media at previous concentrations. The larval duration gradually increased with the increasing of concentrations, but, in case of the highest concentration of 10 ppm, the larval duration was sharply decreased to 7.05 days comparing to 14.07 days for untreated individuals. These observed results are in agreement with those obtained by (Kandil et al., 2005 and El-Barkey, 2009).

Percentages of morphogenic aberriations and abnormalities in larval, pupal and adult stages, and the intermediates of larval-pupal forms were increased significantly (P< 0.01) with the increasing in concentration as compared to control. These findings are in agreement with those results obtained by (Williams & Amos, 1974, Omatsu *et al.* 1991 and Salokha *et* al., 2008).These abnormalities could be due to changes in cuticle build up as a result of the decreasing level of chitin caused by an increased level of phenoloxidases (Ishaaya and Casida, 1974) or failure to transport UDP-N-acetylglucosamine (uridine diphosphate-N-acetylglucosamine) into the cuticular region, (Cohen, 1985 and Miyamoto *et* al., 1993 and Mayer *et aL,* 1990).

Both fecundity and fertility decreased significantly (P< 0.01) as a result of treatment with flufenoxuron and methoprene (Table 4). This decrease was negatively correlated with concentration. On the other hand, the oviposition deterrent index (O.D.I) and percentage of sterility were positively correlated with the tested concentrations. These obtained results are in agreement with (Yoshida, 1994; Perveen, 2006; 2008 and Sammour *et* al., 2008). In contrast to previous findings Kostryukousky and Trostanetsky 2006, Shaurub *et al*, 1998, Perveen and Miyata, 2000), found that some IGRs cause reduction in total number of eggs per female, this could be due to interference of the tested IGRs with oogenesis; it induced decrease in the concentration of yolk proteins, carbohydrates, lipids or disrupted ovarian development.

In conclusion, the two used IGRs have very low toxicity to mammals and other non-target organisms and, usually, are rapidly degraded in the environment. In the present study, flufenoxuron and methoprene caused appreciable stomach toxic effect on larvae of *A.ipsilon*. they interfere with formation of new cuticle and destroy it. These characteristics make IGRs as potential alternatives to conventional insecticides According to their mode of action, IGRs are divided into three main groups: juvenoids, which mainly affect larval metamorphosis by mimicking juvenile hormone; ecdystroids, which affect molting and chitin synthesis inhibitors (CSIs), which interfere with cuticle formation.

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