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Some Biological and Biochemical Aspects of *Agrotis ipsilon* (Lepidoptera: Noctuidae) Larvae as Influenced by Methoprene (JHA)

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ABSTRACT

In this study, we aimed to demonstrate the effects of the juvenile hormone (methoprene) on biological, biochemical and histological changes of *Agrotis ipsilon* after treatment the fourth larval instar. fourth instar larvae of *A. ipsilon* was treated with the 12.5, 25 and 50ppm concentration of methoprene. The developmental duration had been slightly prolonged. At the higher concentration level caused larval mortalities (50%) while the other two concentrations (17.7%). Also, the pupation program was impaired since some pupal-adult intermediates had been produced. The larval growth was directly proportional with the methoprene (JH) concentration levels. Furthermore, different levels of significant changes in the total protein and total lipids contents of the total body were estimated. Our results appear that, the total proteins and total lipids contents in supernatant of the homogenate larvae post-treatment was generally slightly increased, as affected by the tested material at different concentrations as compared to control. Moreover, different abnormal histological structures of mid-gut were noticed. Such as destruction of the gut epithelium and its separation from the basement membrane.

Key words: Methoprene (JHA), total protein, lipids, histopathological studies. *Agrotis ipsilon*

Introduction

Synthetic IGRs and hormone analogues replicate natural hormones and the physiological processes of insects and are confidential as juvenile hormone analogues, ecdysteroid hormones or as ecdysis inhibitors (Mondal and Parween, 2000). These compounds are meant to shrink adult coming out (Pedigo, 2002; Arthur, 2003) so that the effect of juvenile hormone analogue may be more prevalent in the next generation and their sublethal effect may be more obvious (Rumpf *et al*., 1998). Some reports observed that many biochemical and physiological changes in metabolic pathway caused by IGRs while their target sites are endocrine system (Kim and Kim, 2002; Leonardi *et al*., 2001).

Methoprene (Altosid ZR-515), a juvenile hormone analogue, (JHA) is a highly effective sesquiterpenoid hormone that controlling growth and development in insects. So that, JHA coding is specific to insects and other arthropods, compounds disrupting JH circle in insects are a perfect target for pest management, due to their low toxicity to non target organisms (Arrese and Soulages, 2010).

Both of total protein and total free amino acid in haemolymph and fat body have been affected by Juvenile hormone analogues (Hiremath and Jones, 1992; Etebari *et al.,* 2007). Protein synthesis is very important for body growth and reproduction. (Cohen, 2010; Sugumaran, 2010). Proteins enter in various reactions such as the hormonal regulation as the same as the carbohydrates and the lipids, proteins also integrated in the cell as the structural element of all the groups of insects probably lepidopteran group inflicts maximum economic injury. Virtually all the staple crops of the world like rice, wheat, maize, cotton are infested by these insects. The adults as well as larvae can cause severe damage. And among all the lepidopteran. In Egypt and many other countries of the world, the black cutworm, *Agrotis ipsilon* (Lepidoptera: Noctuidae) is a serious pest of corn and several agricultural

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crops, Stenerson, (2004). The control of this pest has become a serious basic requirement facing applied entomologists at the present time with regarding to resistance of insects to almost all available conventional insecticides.

The objectives of this research were to evaluate the susceptibility of 4th instars of *A. ipsilon* to methoprene (JHA). Then, its effect was studied on development, mortality and larval biochemical composition (total lipids, total proteins content). The morphological changes during mid-gut remodeling were studied using histological techniques.

Materials and Methods

Experiment Insect:

The colony of the black cutworm, *A. ipsilon* were obtained from Plant Protection Research Institute Ministry of Agriculture Dokki, Giza, without any insecticidal contamination. The larvae were fed on castor bean leaves, *Ricinus communis,* L. maintained at 22 ± 2 °C and 65 ± 5% relative humidity. Rearing technique was the same adopted by (Abdin, 1979).

Juvenoid Treatments:

The juvenile hormone mimic ZR-515 (Altosid®) methoprene
Chemical Name: Isopropyl (2E-4E)-11-methoxy-3, 7, 11-trimethyl-2, 4-dodecadienoate (56)
Trade Name(S):Altosid, Apex, Diacon, Dianex, Kabat, Minex,

Bioassay technique:

From the maintained insect culture, the 4th instar larvae were obtained. Then, they feed on castor oil leaves (*Ricinus communis*) discs 2.5cm diameter were dipped in the concentrations (50, 25 and 12.5ppm) of the tested compounds for two minutes and left to dry at room temperature. Biological studies were recorded including, the fresh body weight was recorded every day using an electric digital balance.

The weight gain was calculated as follows: initial weight (before the beginning of experiment) – final weight (at the end of experiment).

Larval duration, larval mortality%, pupation%, pupal duration, pupal weight. Fifteen larvae were carried out for each treatment, other disc of castor leaves were dipped in distilled water only and used as control.

Biochemical studies

To determine the proteins and lipids content in the whole insect homogenate were centrifuged at 8000 rpm for 20 min at 4 °C and the supernatant was used directly for the determination of the following: Main contents.

(i)Total lipids according to Knight et al., (1972)

(ii)Total soluble protein as described by Bradford, (1976).

Histopathologicl studies of the larval mid-gut.

The treated and untreated larvae were dissected in saline solution (0.75% sodium choloride in distilled water), and the mid gut of the larvae were fixed in alcoholic Bouin`s fluid for 24 hrs and then processed using the routine technique for paraffin embedding sections of 5 µm thickness and stained with Haematoxylin and Eosin prepared for observation and photomicroscopy (Humason and Freeman, 1979).

Statistical analysis

The results obtained were analyzed using one way ANOVA, significant differences between treatments were determined using Duncan's test ($p<0.05$).

Results and Discussion

Effect of Methoprene (Altosid) on Growth and Development of *A.ipsylon.*

Table (1) contains data of affected on growth and development of *A. ipsylon* after treatment of 4th instar larvae with different concentration levels of methoprene (Altosid). As clearly shown in this table, the three concentrations were significantly prolonged the duration of larvae after treated by methopren when compared to the control. The larval duration at conc. 12.5ppm (15.9±2.33days), conc. 25ppm (15.57±3.34days) and at conc. 50 ppm (14.7±3.74days) were significantly longer than control (10.5±0.14days). Increasing the concentrations, resulted in increased pupal longevity, at the lowest conc. (7±1.15days), the middle concentration (9±8.16 days) and (11±1.41 days)in the last concentration significantly shorter than that of the control group (10.57±0.86days).There were no significant differences between the control 10.57±0.86 days and highest conc. (11±1.41 day).Larval weight recorded at conc. one (355.02±12.0), conc. two (286.50±65.26) and conc. three (409±199) were significantly higher than that of the control (325.64±10.4).The larval weight gain was directly proportional with the methoprene (JH) concentration levels. Pupal duration and weight were reduced in treated larvae of *A. ipsilon* with three concentration levels in comparison with untreated larvae (Table 1).

Table 1: Growth and development of *A. ipsilon* after treatment of the newly moulted 4th instar larvae with methopren (Altosid).

Conc. ppm	Larval duration (days) M±SD	Larval weight/mg M±SD	Larval weight Gain/mg M±SD	Larval Mortality (%)	Pupal Duration (days) M±SD	Pupal weight/mg M±SD	Pupation (%)
12.5	15.9±2.3^{HS}	355.02±12.0 ^{HS}	503.1±19.3 ^{HS}	17.7	7±1.15 ^{HS}	320.25±13.2^{NS}	33.3
25	15.57±3.34 ^{HS}	286.50±65.3^{HS}	523.1±15.3 ^{HS}	17.7	9±8.16 ^{NS}	404.53±75.4^{NS}	33.3
50	14.7±3.74 ^{HS}	409±199 ^{HS}	775.1±408.2 ^{HS}	50	11±1.41 ^{NS}	415±47 ^{NS}	20
Control	10.5±0.14	325.64±10.4	937.6±54.4	0	10.57±0.86	442.03±77.05	100

Each value in the treatment and control represents the mean ± SD.
Significant of differences from control at (P<0.05) according to Duncan.

Depending on these data, at two lower concentration levels of methopren, caused (17.7%) mortality of 4th larval instar, treated with 50ppm caused (50%) mortality vs. zero in controls.

Methoprene prohibited the pupation in a dose-dependent course. Methoprene at its two lower concentrations, succeeded to 33.3%of treated larvae to pupate but at the highest concentration level (20 %) pupation vs. 100% pupation of untreated larvae (control), finally the formation of pupal-adult intermediates. Considerably, these results match up many similar findings that reported some increasing effects on growth and development of other insects species by different IGRs and (JHA) such as Khatter, (2014), found that larval duration of *Spodoptera littoralis* was prolonged statistically at the higher concentration levels of methoprene, also the same result obtained with Tanani, (2015). Application of methoprene caused in prolongation of larval period. Therefore the larvae consumed allot of food and this resulted in the increased body weight. A correlation study relating different dosages of methoprene with larval weight and weight gain, causing a positive relationship, this results in line with Santhy, (2015) when treated *bombyx mori* by methoprene.

Our results showed that the mortality was clearly methoprene caused appreciable stomach toxic effect on larvae of *A. ipsilon*, this result agreements with the reported toxic effects on other insect species by various IGRs, such as *Choristoneura fumiferana* by tebufenozide and methoxyfenozide (Sundaram (2002); *Eurygaster integriceps* by pyriproxyfen (Mojaver 2010). *Dysder cuskoenigii* by flufenoxuron (Khan and Qamar, 2011) *Papilio demoleus* by Diofenolan (Singh and Kumar 2011). *Halyomo rphahalys* by diflubenzuron (Kamminga *et al*., 2012). *Spodoptera litura* by chlorfluazuron Perveen (2012). *Locusta migratoria* var. *manilensis* by flufenoxuron, RH-5849 and pyriproxyfen (Hu *et al*., 2012); *A. ipsilon* by flufenoxuron and methoprene Khatter, (2014).

In the present study, treatment of 4th instar larvae of *A. ipsilon* with methoprene concentration levels, the pupation program was impaired since some pupal-adult intermediates had been produced.

The foremost symptoms and features can be described as reduction of pupation, production of pupal-adult intermediates. However, all or some of these features were observed after treatment with several IGRs, as flufenoxuron and methoprene Khatter, (2014), chlorfluazuron on *S. littoralis* Shaaban, (1993), Sammour, (2008), triflumuron Bakr, (2010), El-Naggar, (2013) and lufenuron Adel, (2012).

Effect of methoprene (altosid) on the total protein and total lipid content of the 4th larval instar of *A.ipsilon* at different concentrations

After 72 h post treatment of the newly moulted (4th) instar larvae of *A. ipsilon* with (50, 25 and 12.5 ppm) of the JH mimic methoprene, data of the total protein content were arranged in Table (2). An increasing on the total protein content was generally exhibited by all concentrations with an exception the lowest concentration. At the lowest concentration level (12.5 ppm), the slightly decrease in protein content was recorded after treatment with methoprene (10.86±0.52 mg/g.b.wt) in comparison with (11.51±1.2mg/ mg/g.b.wt) of control larvae. While the highest percentage of change in total protein with regard to control was recorded in body weight content of the treated larvae at concentration 50 ppm (+29.83%).But the least inducing effect was exhibited in protein content (change %1.22) at the concentration level 25 ppm and (change % 5.57) at the lowest (see Table2).

Table 2: Total protein content (mg/g ± SD) of total body of *A. ipsilon* larvae as influenced by methoprene after treatment of 4th larval instar.

Different parameters	Altosid concentration (ppm)			
	50	25	12.5	Control
Mean total protein mg/g.b.wt± SD	14.93±1.07^{HS}	11.36±0.67^{NS}	10.86±0.52^{NS}	11.51±1.2
Change %	29.83	1.22	5.57	

Each value in the treatment and control represents the mean ± SD.
Significant of differences from control at (P<0.05) according to Duncan.
Chang % = $\frac{T-c}{c} \times 100$, *where T=treated larvae, c =control.*
Total proteins% $= \frac{wt.of\,total\,proteins}{wt.of\,sample}$

In insects, JH exhibit a regulatory effect on protein synthesis. JH and the 20-hydroxyecdysone (20E) either inhibition or stimulate protein synthesis independently or antagonistically (Dubrovsky, 2005; Fang*et al*., 2005). However, these hormones can also interact synergistically and it can be more effective to motivate protein synthesis (Flatt *et al*., 2006). Effects of methoprene (Altosid) on total protein content of the (4th) instar larvae of *A. ipsilon* were determined in our results. Increasing the concentrations, resulted in increased protein content when compared to the control. Relatively similar findings were given by Ghoneim *et al*., (2012) found that after treatment of (5th) instar nymphs of the desert locust *Schistocerca gregaria* with the insect growth regulators (IGRs): pyriproxyfen (juvenoid), tebofenozide (ecdysone agonist) and lufenuron (chitin synthesis inhibitor). An inhibitory effect on haemolymph proteins was generally exhibited by all IGRs along the nymphal stage with an exception of the day after treatment (1-day old nymphs) at which pyriproxyfen and lufenuron enhanced the nymphs to attain increasing proteins. The results corresponded to those reported for silkworm *Bombyx mori* when topical application with (JH) fenoxycarb on (day-0) induced high total protein content in the hemolymph (Ramazan *et al*., 2009).Also, increasing protein content was determined in other insect species, such as cotton leafworm *S. littoralis* by some chitin synthesis inhibitors (Basiouny et al., 2016). Gnanamani and Dhanasekaran, (2017) reported that fifth instar larvae of *Pericallia ricini* which treatment with *Azadirachta indica* leaf extract, total protein levels were decreased to 0.22mg/g from 0.72mg/g when compared with control larvae.

The overall, changes in protein level may be mirror the balance between synthesis, storage, transport and degradation of structural and functional nutrients during ontogeny as well as response to particular physiological conditions (Shoukry *et al*., 2003).

Data of total lipids level after72 h post treatments of newly moulted 4th instar larvae with concentration levels (50, 25 and 1.25 ppm) of JH mimic methoprene have been illustrated in Table

(3). Just a look at these data, it can be concluded that the levels of lipids content of both concentrations (50 and 25 ppm) were observed to be very similar to those of the control. The decreased in lipids content were recorded (4.87±0.15 mg/g.b.wt) at the high concentration level and (5.23±0.58 mg/g.b.wt) at the middle concentration. However, this level increased to reach a mean (7.22±0.77 mg/g.b.wt) at concentration (1.25 ppm), vs.(4.86±0.24 mg/g.b.wt) of control groups.

The lipids are necessary as a source of energy in live animals such as insects. Insects obtain lipids from food or synthesize them from inside the bodies (Gilbert, 1967). It has been reported that the lipid accumulation is more likely to be related to need of juvenile hormone (Hill and Ezzat, 1974).

The whole body of *Plodia interpunctella* larvae, Lipids content was reduced as a result to the action of 20- hydroxyecdysone and azadirachtin (Rharrabe *et al.,* 2008) or to pyriproxyfen (Ghasemi *et al.,* 2010). Pyriproxyfen treatments resulted in decreasing lipid content in hemolymph and fat body of the sunn pest *Eurygaster integriceps* nymphs (Zibaee *et al*., 2011). Hamadah *et al*. (2012) reported that last instar nymphs of the desert locust *S. gregaria* when treated with pyriproxyfen, tebufenozide and lufenuron resulted in slightly decreased lipids in the hemolymph with an exceptional case, the lipid content non-significantly increased in (4-day) old adults after treatment with the lowest concentration of lufenuron. Also, the adult females of Sunn pest, *Eurygaster intedriceps* (Hemiptera), produced from nymphs grown on wheat, had the highest total body lipid content (Ameri *et al*., 2010). (Ghoneim *et al*., 2013) found that black blister beetle *Meloeprosca rabaeus* (Coleoptera: Meloidae) when Feeding on different host plants, *Trifolium* alexdandrinum or *Lactuca* sativa led to gradually increasing lipid content during the oviposition period and the next period.

Table 3: Total lipid content (mg/g ± SD) of total body of *A. ipsilon* larvae as influenced by methoprene after treatment of 4th larval instar.

Different parameters	Altosid concentrations (ppm)			
	50	25	12.5	Control
Mean total lipids mg/g.b.wt± SD	4.87±0.15^{NS}	5.23±0.58^{NS}	7.22±0.77^{HS}	4.86±0.24
Change %	0.21	7.61	48.56	

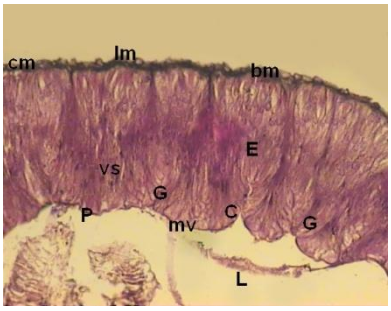
Histological Studies

Effect of methoprene (Altosid) on the mid-gut epithelial layer of the 4th larval instar of *A.ipsilon*

The mid-gut is the largest part of the digestive tract of Lepidopteran larvae. The mid-gut is lined with columnar cells. Preliminary studies indicated that change in mid-gut remodeling in *A.ipsilon.* coincided with the larval stages. JH methopren (Altosid) at concentration levels (50, 25 and 1.25 ppm) induced histological damage in the larval mid-gut, as some of the epithelial cells were vacuolated and destruction of nuclear content also occurred (Fig.1b, c & d) in contrast with untreated control (Fig.1 a).The treatment with (conc.12.5) of methoprene of *A ipsilon* caused vacuolation and detachment of the columnar epithelium cells Fig. (1 b).While at (conc.25) Fig. (1 c) the peritrophic membrane was slightly loose lying on the epithelial cells and the space in between the epithelium and peritrophic membrane was filled with few cytoplasmic vesicles. This became more apparent and severe after treated larvae at concentration (50 ppm).The severe degeneration of columnar epithelial cells and severe detachment of cells from their basement membrane, in addition degeneration of nuclear contents, granulation of cytoplasmic contents and vacuolization were clearly observed (Fig 1d).

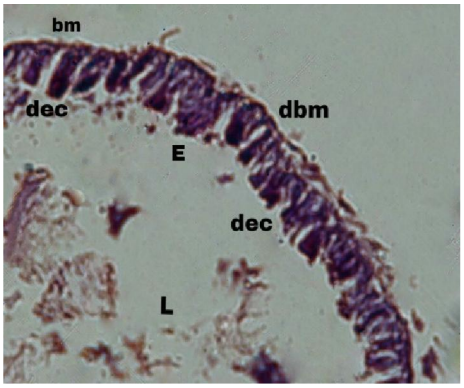
Similar observations were also obtained by Rawi *et al*., (2011) reported the histological changes occurred in the larval mid-gut of *S. littoralis* when treated with *Azadirachta indica* and *Citrulus colocynthis* extracts were vacuolated and necrosis of the epithelial cells and their boundaries and vacuoles occur as a result of cell elongation. Also Abedel-Hakim *et al*. (2016) observed the effects of triflumuron (Alsystin) on *A. ipsilon* larvae showed highly histopathological destruction of the mid-gut epithelium cells and its separation from the basement membrane with different degrees depending on the concentration level. Our results confirmed with Tappozada *et al*., (1968) investigated the histological and cytological changes in the mid gut of *S. littoralis* by some insecticides such as elongation of the epithelial cells and degeneration of some cells. Decamethrin, diflubenzuron and

methomyl caused vacuolation, elongation and breakdown of the epithelium, separation and detachment of peritrophic membrane of the pink bollworm *Pectinophora gossypiella* (Saad *et al*., 1985). Also Salah Eldin, (2016) found the histological changes in the mid-gut of 4th instar larvae of Khapra Beetle, *Trogoderma granarium* when treated with 0.05 mL pyriproxyfen for 5 days showed great destruction in mid-gut cells.

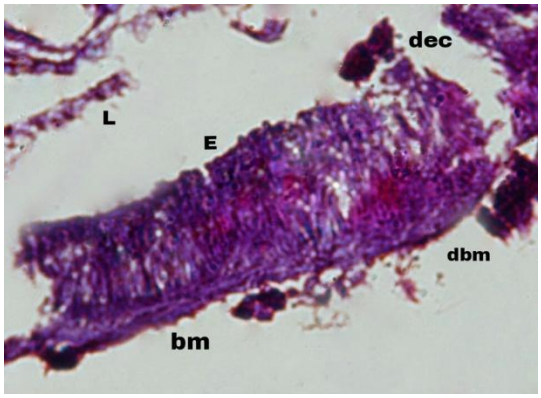


(a)

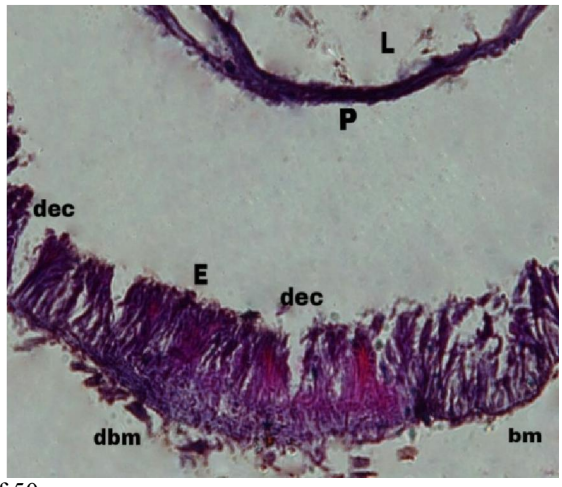
Fig. (1a): Photomicrograph of transverse section through the midgut of normal 4th larval instar of *Agrotis ipsilon.* bm: basement membrane, E:Epithelial layer, vs:vesicles, P:Peritrophic membrane, L:Lumen, cm:circular muscles, lm:longitudinal muscle, G:Goblet cells, C:Columnar cell, mv: microveilli. (X200).



(b) Methoprene at a concentration of 12.5 ppm



(c) Methoprene at a concentration of 25 ppm



(d) Methoprene at a concentration of 50 ppm

(Fig.1b, c & d) Cross sections in the mid-gut of *A.ipsilon* larva treated with JH methoprene (Altosid), dbm: detachment of basement membrane from epithelial cells
dec: destruction of epithelial cells
p: detachment of pretrophic membrane.

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