Effect of Insect growth regulator, Flufenoxuron on pheromone production and perception by *Agrotis ipsilon* (Lepidoptera: Noctuidae)

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**Abstract** The present study aimed to evaluate the biological effect of insect growth regulator (flufenoxuron) against 3rd larval instar of *Agrotis ipsilon* as a chitin synthesis inhibitor. The effect of sublethal dose LC$_{50}$ was used to investigate its effect on sex pheromone production and perception by *Agrotis ipsilon*. Results indicated that the response of the cutworm larvae increased with increasing in pheromone concentration. The calculated 50% response threshold (RD50) after 1 minute exposure to the sex pheromone extract was equal to 0.014 female equivalents. found that ethylether and methylene chloride were excellent and potent solvents for extracting sex pheromone from females in comparison with acetone, benzene, chloroform, methanol and 95% ethanol.

**Keywords:** Flufenoxuron, *Agrotis ipsilon*, Insect growth regulator, sex pheromone, pest control

**1 - Introduction**

Treated and untreated cutworm *Agrotis ipsilon* secreted two kinds of pheromones. The first pheromone called an aggregation pheromone was secreted by males which was stimulated and attracted both sexes while, second pheromone called sex pheromone was secreted by females which was excited and attracted males more than females.

IGR was introduced to describe a new class of bio-rational compounds. 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 organisms and, usually, are rapidly degraded in the environment (Zurleuf, 1974, Carter, 1975; Staal, 1975; Oberlander, 1978; Ishaya et al., 1987; Oberlander, 1997; Ishaya & Horowitz, 1998; Kostyukovsky et al., 2000). 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; ecdysteroids, which affect molting and chitin synthesis inhibitors (CSIs), which interfere with cuticle formation (Ishaya & Casida, 1974; Post et al., 1974; Smet et al., 1990; Binnington & Retnakaran, 1991; Cohen, 1993; Oberlander, 1997; Ishaya & Horowitz, 1998 and Oberlander & Silhacek, 1998). Among the diverse in vivo actions of CSIs on the life cycle of insects of various orders are ovicidal and larvicidal effects (Ascher et al., 1987). Impairment of cuticle secretion in affect edembryos may be the cause of hatchability reduction due to treatment with CSIs (Grosscurt, 1978; Grosscurt & Anderson, 1980 and Elek, 1998 a & b). The larvicidal effects of CSIs are most likely achieved through interference with the formation of a new cuticle (Oberlander and Silhacek, 1998); Bakr et al., 2005 and Mojaver & Bandani, 2010.

Pheromone must be considered as a major mode of intraspecific communication in insects that acts to elicit a specific behavioral or developmental response from other organisms of the same species (Karlson & Luscher, 1959 and Nordlund, 1981). They offer several possibilities for the manipulation of populations and behavior of such destructive insects.

Burkholder and Dicke (1966 Le Vinson and Bar lion (1970), investigated the presence of *sex* pheromone in virgin female adults of the black carpet beetle, *Attagenus piceus* (Olivier), *Trogoderma inclusum* (Leconte) and *Trogoderma glabrum* (Herbst) that influenced behavior of the respective males. Also, many investigators showed the presence of aggregation pheromone in male red flour beetle, *Tribolium castaneum* (Herbst) and confused flour beetle, *Tribolium confusum* (Duv), (Suzuki, 1981; Faustini et al., 1982 Suzuki et al., 1984; Schlyter et al., 1992); Narayanan and Nadarajan 2005; Mazomenos 2006; Ruther et al., 2007and Ali 2010, found that males of many insects produce a sex pheromones.

Ethylether and methylene chloride were excellent and potent solvents for extracting sex pheromone from females. In comparison with acetone, benzene, chloroform, methanol and 95% ethanol, ether was potent solvent for extracting pheromones as a repellent substance from male and females, Roelofs and Feng (1967) Yinon and Shulov (1968) Kanaujia and Sidhu (1980b Faustini et al., 1982 Abd El-kader et al. (1986b), Lima et al. (2008) and Xiang et al. (2010).

Barak and Burkholder (1977), El-Kader and Barak (1979), noticed that the response of black carpet beetle males, *Attagenus elongatus* (Casey),
increased with increasing in pheromone concentration.

The aim of the present study act mainly with the sex pheromone secreted by female butterflies and the perception of males to sex pheromone extracts. the response of treated and untreated (males to their own sex, the response of female butterflies to the other sex, the response of males to females and the response of females to their own sex. According to the potency of solvents tried in the extraction of pheromone, the tested solvents could be arranged descendingly.

2- Material 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+1°C 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.

3- Larval treatment (Dipping technique)

Larvae of uniform age were obtained and dipped for 10 seconds in IGR, flufenoxuron diluted at the concentrations of 0.1, 0.5, 1.5 and 10 ppm, then transferred into suitable media. Four replicates (25 larvae for each) were run for each concentration. The larvae were examined after eight days. The dipping technique used was according to Oberlander (1997). Evidence of pheromone production was carried out by bioassay treated males against treated females in compare with untreated one. Also bioassay treated females against treated males and treated females against treated males in compare with untreated one. Also bioassay treated females against treated males and treated females against treated females in compare with untreated one. The tested males and females were placed separately in individual olfactometer. The olfactometer used in the present study was a vial type similar to that used by Burkholder (1970). It consisted of a glass vial (15x1.5 cm). Which had a rubber plug with a movable glass rod (Fig.1). The latter had a broad inner end at which a small piece of masking tape was fixed. The insect tested for pheromone production was held by the masking tape, while that tested individual for response was placed on the bottom of the vial. The distance between the two insects was 4 cm. Ten replicates each one contains 10 vials and in each vial two individuals (male and female) were placed separately. The tested males and females were 8-10 days old. Assays were conducted at 1p.m. under conditions of 30°C and 70% R.H.

![Fig: 1 Olfactometer](image)

4- Extraction and Bioassay procedures

Several solvents were tried for extracting pheromones in the present study. These included hexane, acetone, diethylether and chloroform. Extraction was prepared by placing 30 treated beetles of the same sex (females) and of known age (8-10 days) in one ml of the solvent. The latter was confined in a screw-top glass vial of 5 ml in capacity. The tops of the vial caps were foil-lined to avoid solvent loss or contamination. The butterflies were held in solvents for 24 hours in a refrigerator, then the insects were removed. Extracts were stored in a deep freezer at -20°C until used. A vial containing 30 untreated butterflies was held under the same conditions and served as a control. The test extract was pipetted directly into a bioassay disc (filter paper) which was fixed by a masking tape at the inner end of the glass rod of the olfactometer. The bioassay disc was placed 4 cm above the insect. The concentration of the female extracts was (0.3) female equivalents (FE) per solvent according to Hussien (1982). For each test, ten replicates (each one contains 10 treated males that placed individually in 10 vials) were used. Untreated males and females used according to the previous manner for comparison. One day before a bioassay was to be conducted, the tested butterflies were placed separately in individual olfactometer vials which had been washed by acetone and oven dried to prevent contamination. Bioassay was conducted using a technique similar to that applied by (Burkholder, 1970).

Insects were exposed to cutworm extracts for 1 minute. A positive response was recorded if the insect become aroused and showed locomotory
movements. Only restive insects were used in the bioassay. In the present study all bioassays were conducted at constant conditions of 30 °C, 70% R.H. and at 1 p.m.

To investigate the effect of pheromone concentration on treated male response, extracts were prepared by placing 10, 20, 30, 50, 80, and 90 treated virgin female butterflies of 8-10 days old in one ml of hexane only. The female extracts were then tested against 8-10 days old males. For each test, ten replicates each one contains 10 treated males placed individually in 10 vials were used. Untreated males and females were used according to the previous manner for check.

3. Results and discussion:

1- Effect of LC50(1.2ppm) of flufenoxuron on responsiveness and production of pheromones in male and female adult which resulted from treated 4th larval instar of *Agrotis ipsilon*:

Results on the response of treated virgin females and males of the cutworm, *A. ipsilon* to pheromone produced by either treated sex , under constant conditions of 30°C and 70% R.H., are given in Table (1).

The following is an explanation of the response behavior of each sex to either male or female. 

- Male response behavior to female:-

  The level of response 29.79 % (corrected experiment) was reached when treated males were tested against treated females. While in untreated one and used solvent only the response reach 80.85 % and 6.00 %, respectively. The response behavior of treated male insects to treated female consisted of a sequence of increasing levels of excitation. The treated males exhibited a sequence of events from the resting state. The first level of response included the raising of antennae, head and thorax. The second level of response included moderate activity of circular running, on the surface of the olfactometer vial. During the excitation, antennal and legs vibrations occurred. The duration of any level of excitation was variable. The treated males occasionally progressed from the resting state to active in a fast and continuous motion; but most commonly paused for variable lengths of time at lower levels of excitation.

- Male response behavior to male:-

  Treated males also responded at a level 21.28 to treated male insect but response of untreated one and used solvent only were 61.70 and 6.00 %, respectively. The response of treated male insect to their own sex also consisted of a sequence of events the first level of response included the raising of antennae, head and thorax. The second level of response included low activity of circular running, on the other hand the activity was moderate at untreated butterflies. No vibration or bobbing appeared.

- Female response to female:-

  Treated females tested against their own sex showed a level 12.50 % of response. While response of untreated one and used solvent only 54.17 and 4.00 %, respectively as well as treated males.

- Female response behavior to male:-

  The level of response 08.16 % was reached when female tested against males but response of untreated one and used solvent only were 38.78 and 2.00 %, respectively. In this case, females also exhibited a sequence of events similar to those mentioned in male response behavior to male. Statistical analysis of the data of the response of treated males to their own treated sex and the response of treated females to the other treated sex showed significant difference.

While showed significance difference and in used solvent only no significance appear among previous groups.


<table>
<thead>
<tr>
<th>Types of Experiment</th>
<th>Percentage of response</th>
<th>P- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
<td>Corrected</td>
</tr>
<tr>
<td>Male tested against female</td>
<td>43</td>
<td>29.79 ±0.51</td>
</tr>
<tr>
<td>Male tested against male</td>
<td>29</td>
<td>21.28 ±0.24</td>
</tr>
<tr>
<td>Female tested against female</td>
<td>36</td>
<td>12.50±0.24</td>
</tr>
<tr>
<td>Female tested against male</td>
<td>20</td>
<td>8.16 ±0.32</td>
</tr>
</tbody>
</table>

ANOVA P-Value: Student's (t) test: **= Significantly different at P<0.01. N.S= non Significantly different
2)-Pheromone extraction by different solvents:-
In order to find out the efficiency of different solvents in sex pheromone extraction, the following solvents were tested: hexane, diethylether, acetone and chloroform. These solvents were provided in a pure condition. Results on the response of males of *A. ipsilon* to extracts (by different solvents) of virgin females are given in Table (2).

According to the percentage of treated male response to extracts of treated virgin females, the tested solvents could be arranged descendingly in the following manner: hexane 23.91 %, diethylether 22.92 %, acetone 17.02 % and chloroform 16.33 %. While in untreated one the response reach 78.26, 75.00, 63.83 and 61.22 %, respectively.

Statistical analysis of the data indicated that the difference in response between extracts by either hexane and diethylether, or between acetone and chloroform was not significant and the difference between the two groups of solvents was not significant at both treated and untreated case, according to Grant (1975), the potency of the extract depends on the solvent used.

Table (2): Efficiency of different solvents in sex pheromone extraction of (8-10 days old) virgin *A. ipsilon* females produced by treated 4th larval instar by flufenoxuron.

<table>
<thead>
<tr>
<th>Types of Experiment</th>
<th>Treated</th>
<th>Corrected experiment</th>
<th>Untreated</th>
<th>Corrected experiment</th>
<th>With only solvent</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>30</td>
<td>23.90±0.32</td>
<td>80</td>
<td>78.26±0.11</td>
<td>8</td>
<td>**</td>
</tr>
<tr>
<td>Diethylether</td>
<td>26</td>
<td>22.92±0.24</td>
<td>76</td>
<td>75.00±0.84</td>
<td>4</td>
<td>**</td>
</tr>
<tr>
<td>Acetone</td>
<td>22</td>
<td>17.02±0.20</td>
<td>66</td>
<td>63.83±0.24</td>
<td>6</td>
<td>**</td>
</tr>
<tr>
<td>Chloroform</td>
<td>18</td>
<td>16.33±0.20</td>
<td>62</td>
<td>61.22±0.35</td>
<td>2</td>
<td>**</td>
</tr>
<tr>
<td>P-value</td>
<td>N.S</td>
<td>-</td>
<td>N.S</td>
<td>-</td>
<td>N.S</td>
<td>-</td>
</tr>
</tbody>
</table>

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

3)-Effect of pheromone concentrations on male response:-
The response of males to different pheromone concentrations or titers of virgin females is given in Table (3). The treated male response started with low level 4.26 % at 0.1 female equivalent and increased with the increase of female equivalent to reach the maximum level of response 48.89 % at 0.9 female equivalent. While in untreated one the lowest response 44.68 % and the highest response 91.11%. According to the previous results, more untreated male had more excitation at higher extract concentrations. This coincides with the findings of Bartell et al. (1969) and Brady & Delay (1972). The percentage of male response, therefore was taken as a criterion for amount of sex pheromone titers produced by females.

Table (3): Response of male *A. ipsilon* to pheromone concentrations of virgin females, produced by treated 4th larval instar by flufenoxuron.

<table>
<thead>
<tr>
<th>Different pheromone Concentrations (titers)</th>
<th>Treated</th>
<th>Corrected experiment</th>
<th>Untreated</th>
<th>Corrected experiment</th>
<th>With only solvent</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>10</td>
<td>04.26±0.32</td>
<td>48</td>
<td>44.68±0.15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>22</td>
<td>15.22±0.17</td>
<td>62</td>
<td>58.70±0.44</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>0.3</td>
<td>36</td>
<td>33.33±0.40</td>
<td>80</td>
<td>79.17±0.24</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>44</td>
<td>39.13±0.24</td>
<td>86</td>
<td>84.78±0.65</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>50</td>
<td>46.81±0.32</td>
<td>88</td>
<td>87.23±0.45</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>54</td>
<td>48.89±0.24</td>
<td>92</td>
<td>91.11±0.35</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>**</td>
<td>-</td>
<td>**</td>
<td>-</td>
<td>N.S</td>
<td></td>
</tr>
</tbody>
</table>

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

The results concluded that both treated and untreated sexes of the cutworm moth could secrete a pheromone that was able to stimulate the other sex as well as its own sex. Although responsiveness and production of pheromone in untreated groups were significantly higher than treated one. The degree of response varied according to the source of pheromone. Thus, Females secreted a pheromone that stimulated and highly excited males more than females. Thus the female pheromone appeared to be a sex pheromone, while treated male beetles of *A. ipsilon*, produced a pheromone, apparently an aggregating pheromone, that was able to excite a high percentage of treated males.

Production and perception of pheromone by untreated butterflies were significantly higher
than production and perception of pheromone by treated one. The solvents were arranged in the following manner: Hexane, diethylether, acetone and chloroform. Consequently, hexane according to their effect on the pheromone production.

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