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

Najat A. Khatter

King Abdulaziz University, Faculty of Science for Girls- Biology Department, Jeddah, Saudi Arabia
najat.khatter4@gmail.com

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 (RD5o) 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.
[Najat A. Khatter. **Effect of Insect growth regulator, Flufenoxuron on pheromone production and perception by *Agrotis ipsilon*.** *Life Sci J* 2014;11(12):745-751]. (ISSN:1097-8135). http://www.lifesciencesite.com. 138

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 (Zurfleuh, 1974, Carter, 1975; Staal, 1975; Oberlander, 1978; Ishaaya *et al.,* 1987; Oberlander, 1997; Ishaaya & 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 (Ishaaya & Casida, 1974; Post *et al.,* 1974; Smet *et al.,* 1990; Binnington & Retnakaran, 1991; Cohen, 1993; Oberlander, 1997; Ishaaya & 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).

Pheromones 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 elongatulus* (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^{0}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 l0 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 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.

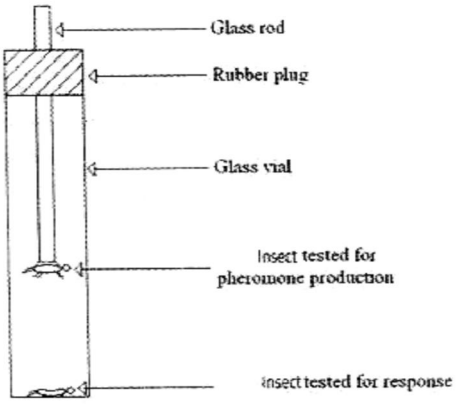

Fig: 1 Olfactometer

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 LC_{50}(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.

a- 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 in contrast the activity high in untreated cutworms . In third level of excitation the treated male was bobbing up and down 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.

b- 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.

c- 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.

d- 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.

Results obtained in the present study about untreated sexes are in agreement with those results obtained by August (1971) on *Tenebrio molitor;* Suzuki *et al.* (1984) on the rust red flour beetle, *T. castaneum*; Narayanan and Nadarajan (2005) on *Antigastra catalaunalis* and Ruther *et al.* (2007) on jewel wasps, *Nasonia vitripennis.*

Table (1): Response of virgin *Agrotis ipsilon* males and females (8-10 days old) to adults of both sexes produced by treated 4th larval instar by flufenoxuron

Types of Experiment	Percentage of response					*P*- Value
	Treated	Corrected experiment	Untreated	Corrected experiment	With only solvent	
Male tested against female	43	29.79 ±0.51	82	80.85±0.43	6	**
Male tested against male	29	21.28 ± 0.24 b	64	61.70±0.81	6	**
Female tested against female	36	12.50 ±0.24	56	54.17±0.23	4	**
Female tested against male	20	8.16 ±0.32 a	40	38.78±0.22	2	**
P- Value	**	-	**	-	N.S	-

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.. In contrast, Rotundo and Tremblay, 1975 on citras mealybug, *Planococcus citri*.

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.

Types of Experiment	Percentage of response					*P*- Value
	Treated	Corrected experiment	Untreated	Corrected experiment	With only solvent	
Hexane	30	23.90±0.32	80	78.26±0.11	8	**
Diethylether	26	22.92±0.24	76	75.00±0.84	4	**
Acetone	22	17.02±0.20	66	63.83±0.24	6	**
Chloroform	18	16.33±0.20	62	61.22±0.35	2	**
P- Value	N.S	-	N.S	-	N.S	-

**= 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.

Different pheromone Concentrations (titers)	Percentage of response				
	Treated	Corrected experiment	Untreated	Corrected experiment	With only solvent
0.1	10	04.26±0.32	48	44.68±0.15	6
0.2	22	1S.22±OJ7	62	58.70±0.44	8
0.3	36	33.33±0.40	80	79.17±0.24	4
0.5	44	39.13±0.24	86	84.78±0.65	8
0.8	50	46.81±0.32	88	87.23±0.45	6
0.9	54	48.89±0.24	92	91.11±0.35	10
P- Value	**	-	**	-	N.S

** = 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.

References

1. Abd El-kader, M.M.; Abd El-Rahman, H.A. and Hussien, M.A. (1986): Changes hi sex pheromone release and perception by the rust-red flour beetle, Tribolium castaneum (Herbst). Ain Shams Sci. Bull., 25: 40-54.
2. Abd El-Kader, M.M. and Barak, A.V. (1979): Evidence for a sex pheromone, in the hide beetle. Dermestes maculates (Degeer) (Coleoptera: Dermestidae). J. Chem. Ecol., 5(5): 805- 813.
3. Abdel Fattah, H.M. and Khaled, A.S. (2008): Morphological and biochemical disruption in development of Tribolium castaneum (coleopteran:Tenebrionidae). J. Egypt. Acad. Soc. Environ. Dvelop., 8 (3): 1-9.
4. August, S. J. (1971): The role of male and female pheromones in the mating behavior of Tenebrio molitor. J. Insect Physiol., 17: 739-751.
5. Bakr, R.F.A.; Soliman, F. EI.; El-Sayed, M.F.; Hassan, H.Abd-W. and Zohry, N.M.H. (2007): Effect of sublethal dosage of Flufenoxuron and Chlorfiuazuron on haemocytic, inorganic ions and total protein changes in haemolymph of 6th larval instar of Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae). The second international conference of economicentbmology, Cairo, Egypt, 2: 211- 238.
6. Barak, A.V. and Burkholder, W.E. (1977): Behavior and pheromone studies with Attagenus elongatulus (Casey) (Coleoptera: Dermestidae). J. Chem. Ecol., 3(2): 219- 237.
7. Rarratt, B.I.P. (1974): Timing of production of a sex pheromone by females of Stegobium paniceum (L.) (Coleoptera: Anobiidae) and factors affecting male response. Bull. Ent. Res., 64: 621- 628.
8. Bartell, R.J., Shorey, H.H and Browne, L.B. (1969): Pheromonal stimulation of the sexual activity of males of the sheep blowfly Lucilia cuprina (Calliphoridae) by the female. Animal Behaviour, 17: 576-585.
9. Binnington K. and Retnakaran, A. (1991): Epidermis-a biologically active target for metabolic inhibitors. In: K. Binnington and A. Retnakaran, Editors, Physiology of Insect Epidermis, CSIRO, Melbourne, PP. 307-326.
10. Brady, U.E. and Daley, R.C. (1972): Identification of a sex pheromone from the female raisen moth, Cadra figuliella. Ann. Entomol. Soc. Amer., 65: 1356-1357.
11. Burkholder, W.E. (1970): Pheromone research with stored- product coleopteran, pp. 1-20, in D. wood, R. silver stein and M. Nakajima (eds.). Control of insect behavior by natural products. Academic press, New York: pp. 345.
12. Burkholder, W.E. and Dicke, R.J. (1966): Evidence of sex pheromones in females of several species of Dermestidae. J. Econ. Entomol., 59(3): 540-573.
13. Burkholder, W.E.; Ma, M.; Kuwahara, Y. and Matsumura, F. (1974): Sex pheromone of the furniture carpet beetle, Anthrenus flavipes (Coleoptera: Dermestidae). Can. Entomol., 106(4): 835- 839.
14. Carter, S.W. (1975): Laboratory evaluation of three noval insecticides inhibiting cuticle formation against some susceptible and resistant stored product beetles, J. Stored Products Res., 11: 187-193.
15. Coffelt, J.A.; Vick, K.W.; Sower, L.L. and Moclellan, W.T. (1978): Sex pheromone of the sweet potato weevil, Cylas formicarius elegantulus: Laboratory bioassay and evidence for a multiple component system. Environ. Entomol., 7(5): 756- 758.
16. Cohen, E. (1993): Chitin synthesis and degredation as targets for pesticide action, Archives of Insect Biochemistry and Physiology, 22: 245-261.
17. Faustini, D.L., Rowe, J.R., and Burkhoder, W.E., (1981): A male produced aggregation pheromone in Tribolium brevicornis (LeConte) (Coleoptera: Tenebrionidae) and interspecific responses of several Tribolium species. J. Stored Products Res., 18 (4): 153-158.
18. Finney, D.G., (1971): Probit analysis. A statistical treatment of the sigmoid response curve. 7th Ed., Cambridge Univ. Press, England.
19. Ginnis, A.J., (1968): Quantitative responses of males of limonius californicus (Coleoptera: Elateridae) to female sex pheromone. Can. Entomol. 100 (10): 1071-1078.
20. Grant, G.G., (1975): Extraction and bioassay of female sex pheromone of white marked tussock moth, Orgyia leucostigma (Lepidoptera: lymantriidae). Can. Entomol., 107 (3): 303-309.
21. Grosscurt, A.C., (1978): Effect of difiubenzuron on mechanical penetrability, chitin formation, and structure of the elytra of leptinotarsa decemlineate, Journal of Insect Physiology, 24: 827-831.
22. Grosscurt, A.C., and Anderson, S.O., (1980): Effect of diflubenzuron on some chemical and mechanical properties of the elytra of leptinotarsa

decemlineata, proceedings of the koninklijke Nederlandse Akademie Van Wetenschappen Series C: Biological and Medical Sciences 83: 143-150.
23. Gullan P.J., and Cranston, P.S., (1994): The insects an outline of entomology. Chapman & Hall, London.
24. Hitoshi K., Ichiro K., and Goro, S., (1989): Laboratory evaluation of a new insect growth regulator pyriproxyfen, as a cockroach control agent. Jpn. J. Sanit. Zool., 40 (3): 195-201.
25. Hussien, M.A., (1982): A study on factors influencing sex pheromone production and perception in the rust-red flour bettle, Tribolium castaneum (Herbst) M.Sc. thesis, Department of Entomology, Faculty of Science, Ain Shams University, Egypt.
26. Ikan, R., Bergmann, E.D., Yinon, U. and Shulov, A., (1976): Production of aggregation pheromone in khapra beetle, Trogoderma granarium. Nature. PP. 223-317.
27. Ishaaya, I., and Casida, J.E., (1974): Dietary TH 6040 altera composition and enzyme activity of house fly larvae cuticle, pesticides Biochemistry and Physiology, 4: 484-490.
28. Ishaaya, I., and Horowitz, A.R., (1998): Insecticides with noval modes of action: an overview. In: I. Ishaaya and D. Degheele, Editors: Mechanisms and application springer, Berlin, PP. 1-24.
29. Ishaaya I, Yablonske, S and Ascher, K.R.S., (1987): Toxicological and biochemical aspects of noval acylureas on resistant and susceptible strains of Triboluium castaneum. In Editors, Proceedings of 4th International working conference on stored-product protection E., Donahye and S. Navarro, PP. 613-622.
30. Kanaujia, K.R. and Sidhu, H.S. (1980b): Extraction of sex pheromone and behavioral response of angoumois grain moth, Sitotroga cerealella (Olivier). Z. Angew. Entomol., 88(2): 188- 193.
31. Karlson, P. and Luscher, M. (1959): 'Pheromones': a new term for a class of biologically active substances. Nature (London) 183: 55-56.
32. Kawada, H.; Kojima, I. and Shingo, G. (1989): Laboratory evaluation of a new insect growth regulator, pyriproxyfen, as a cockroach control agent. J Sanit Zool, 40:195-201.
33. Keil, T. A. (1999): Morphology and development of the peripheral olfactory organs. In insect olfaction, pp. 5- 47 (ed. B. S. Hansson). Springer, New York.
34. Khazanie, R. (1979): Elementary statistics (Good year Publishing. Co., California, U.S.A., 488P).
35. Kostyukovsky, M.; Chen, B.; Atsmi, S. and Shaaya, E. (2000): Biological activity of two juvenoids and two ecdysteroids against three stored product insects, Insect Biochemistry and Molecular biology, 30: 891-897.
36. Le Vinson, H.Z. and Bar lion, A.R. (1970): A sex pheromone and an aggregation pheromone of the khapra beetle, Trogoderma granarium. J. Insect Physiol., 16(1): 561.
37. Lima, E.R.; Vilela, E.F.; Lucia, T.M.C.D. and Ataide, L.M.S. (2008): Age and time related pheromone production in coffee leafminer Leucoptera coffeella Gue>in-Meneville (Lepidoptera: Lyonetiidae). J. Braz. Chem. Soc., 19(8): 40- 50.
38. Lundergren, L. (1975): Natural plant chemicals acting as oviposition deterrent on cabbage butter flies, Pieris rapae and P. napi. Zool. Seri., 4: 250-258.
39. Mazomenos, B.E. (2006): Effect of age on pheromone production in the female olive fruit fly, Dacus oleae (Gmel.). J. Insect Physiol., 30(10): 765-769.
40. Mojaver, M. and Bandani, A.R. (2010): Effects of the insect growth regulator pyriproxyfen on immature stages of Sunn pest, Eurygaster integriceps Puton (Heteroptera: Scutelleridae). Mun. Ent. Zool, 5(1): 187-197.
41. Narayanan, U.S. and Nadarajan, L. (2005): Evidence for a male-produced sex pheromone in sesame leaf webber, Antigastra catalaunalis Duponchel (Lepidoptera, Pyraustidae). Current Science, 88(4): 631-634.
42. Neumann, R. and Guyer, W. (1983): A new chitin synthesis inhibitor CGA-112D 913: its biochemical mode of action as compared to diflubenzuron. In: Proc. The 10th Int. Congr. Plant Protection, Brighton, vol. 1, Oxford, Pergamon Press: 445- 451.
43. Neumann, R. and Guyer, W. (1987): Biochemical and lexicological differences in the modes of action of the benzoyl ureas, Pestic. Sci., 20: 147.
44. Nordlund, D.A. (1981): Semiochemicals: a review of terminology, pp. 13-28. In D. A. Nordlund, R. L. Jones, and W. J. Lewis, [eds.], Semiochemicals: Their Role in Pest Control. John Wiley and Sons. New York, N.Y.
45. Oberlander, H. (1978): Advances in insect growth regulators research with grain insects, symposium on the prevention and control of insects in stored food products, Manhattan, Kansas, PP. 247-263.
46. Oberlander, H. (1997): Current status and future perspectives of the use of insect growth

regulators for the control of stored product insects, J. Stored Product Res., 33: 1-6.
47. Oberlander, H. and Silhacek, D.L. (1998): New perspectives on the mode of action of benzoyl phehyl urea insecticides. In: I. Ishaaya and D. Degheele, Editor, Insecticides with noval modes of action: Mechanisms and application Springer, Berlin, PP. 92-105.
48. Post, L.C.; De Jong, B.J. and Vincent, W.R. (1974): l-(2, 6-disubstituted benzoyl)-3-phenyl-urea inseticides: inhibitors of chitin synthesis, Pesticides Biochemistry and Physiology, 4, PP. 473-483.
49. Roelofs, W.L. and Feng, K. (1967): Isolation and bioassay of the red-banded leafroller, Argyrotaenia velutinana. Ann. Entomol. Soc. Amer., 60(6): 1199-1202.
50. Rotundo, G. and Tremblay, E. (1975): Simple extraction and bioassay of the female sex pheromone of the citrus mealybug, Planococcus citri. Ann. App. Biol., 82(1): 165-167.
51. Ruther, J.; Stahl, L.M.; Steiner, S.; Garbe, L.A. and Tolasch, T. (2007): A male sex pheromone in a parasitic wasp and control of the behavioral response by female's mating status. J. Exp. Biol., 12: 210-216.
52. Ryan, M.F. and O'ceallachain, D.P. (1976): Aggregation and sex pheromones in the beetle, Tribolium confusum (Col., Tenebrionidae). J. Insect Physiol., 22(11): 1501-1503.
53. SsSchlyter F., Birgersson G., Byers J.A. and Bakke A. (1992): The aggregation pheromone of Ips duplicatus and its role in competitive interactions with/, typographus. Chemoecology, 3: 103-1 12.
54. Schneider, D. (1964): Insect antennae. Annual Review of Entomol., 9: 103- 22.
55. Selander, J. (1978): Evidence of pheromone-mediated behavior in the large pine weevil, Hylobins abietis (Coleoptera, Curculionidae). Ann. Entomol. Fenn., 44(4): 105- 112.
56. Smet, H.; Rans, M. and De Loof, A. (1990): Comparative effectiveness of insect growth regulators with juvenile hormone, anti-juvenile hormone and chitin synthesis inhibiting activity against several stored food insect pests. In: F. Fleurat-lessard and P. Ducom. Editors, Proceedings of the fifth International Working Conference on stored-product protection, Bordeaux, France, Imprimerie Medocaine, Blanquefort Cedex, PP. 639-657.
57. Snedecor, G. W. (1971): Statistical methods, 14th. Ed. The Iowa College Press, Am., U.S.A.
58. Staal, G.B. (1975): Insect growth regulators with juvenile hormone activity, Ann. Rev. Entomol., 20: 417-460.
59. Sun, Y.P. (1950): Toxicity index. An improved method of comparing the relative toxicity of insecticides. J. Econ. Ent., 43: 45- 53.
60. Suzuki, T. (1981): Identification of the aggregation pheromone of flour beetles, Tribolium castaneum and Tribolium confusum. Agric. Biol. Chem., 45(6): 1357-1363.
61. Suzuki, T.; Kozaki, J.; Sugawara, R. and Mori, K. (1984): Biological activities of the analogs of the aggregation pheromone of Tribolium castaneum (Coleoptera: Tenebrionidae). Appl. Entomol. and Zool., 19: 15-20.
62. Toppozada A., Abd-Allah, S and El-Defrawi M.E., (1966): Chemosterilization of larvae and adult of the Egyptian cotton leafworm, prodenia litura by apholate J. Econ. Entomol., 59, 1125-1128.
63. Xiang, Y.Y., Yang, M.F., and Li, Z.Z., (2010): Calling behavior and rhythms of sex pheromone production in the black cutworm moth in china. J. Insect Behavior, 23 (1): 20-31.
64. Yinon, U. and Shuloy, A., (1968): Bioassay of the response of tribolium castaneum to repellent substance extracted by Trogoderma. Entomol. Exper et Appl., 12 (2): 191-205.
65. Zurfleuh, R.C. (1976): Phenyl ethers as insect growth regulators: Laboratory and field experiments. In: L. I. Gilbert. Editor, the juvenile hormones, Plenum Press, New York, PP. 61-74.

12/1/2014