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EFFECT OF GAMMA IRRADIATION ON PRODUCTION AND PERCEPTION OF SEX PHERMON IN THE BLACK CUTWORM, *Agrotis ipsilon* (HUFN)

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ABSTRACT

The females of the black cutworm, *Agrotis ipsilon* (Hufn), produce their sex pheromone early at the beginning of their emergence. Males also can percept this pheromone at the same age. After emergence, the pheromone production and perception increase rapidly, through the first day and continue till the 5*th* day after emergence.

The pheromone production and perception increases to reach its maximum on the second day and continues at a high titer except during the last few days of female life. As the black cutworm has a short life span, it could be concluded that the sex pheromone production by females and perception by males continues at a high level during most of their life.

Pheromone production and perception by the black cutworm moth was considerably affected by gamma irradiation applied during the full grown male and female pupae with 50, 100 and 150 Gy. Pheromone production in female decreased with increasing doses more than pheromone perception in P1 male.

INTRODUCTION

The black cutworm, *Agrotis ipsilon* (Hufn.) is a serious pest of several important field and vegetable crops in Egypt, such as cotton, soybean, corn, potatoes, and tomatoes, and control of this pest has usually depended exclusively on insecticides. Several methods have been tried for its control among these, the sterile insect technique (SIT) appeared to be promising in an integrated programme for the control of this and other serious insect pests. The high doses of gamma radiation required in such technique may affect some physiological and biological aspects, specially its reproductive potential. This effect may disturb the intraspecific communication between males and females by adversely affecting pheromone gland and hence pheromone production (Stimmann *et al.,* 1972; El-Shall *et al.,* 2005 and El-Naggar *et al.,* 2006). Trials to seek some of the factors that can prevent such communication in the black cut worm may be of important in its integrated control programme (Hussein *et al.* 2001).

The present study investigate the effect of gamma radiation doses on sex pheromone production by female and perception by male.

MATERIALS AND METHODS

1.Insect rearing Technique:

The culture of the tested insect, the black cutworm, *Agrotis ipsilon* (Hufn.) started by collecting healthy full grown larvae from an infested filed at Qualubia Governorate. These larvae were kept individually inside a glass tube with fresh castor oil plant leaves, *Ricinus communis.* Tubes were kept under laboratory conditions of 25°C and 60-70 % R.H. Just before pupation, tubes were provided with saw dust as pupating media and daily checked for collecting new formed pupae. Pupae were surface sterilized with formalin 10%, sexed and kept on moistened saw dust. Full grown pupa of each sex were transferred to adult emergence cages provided with small glass vials, with piece of cotton wool soaked with 10% sugar solution serving as adult's food. Chimney glass cages (used as mating cage) were lined and covered with toweling paper as oviposition site. The eggs were daily collected to start new culture. Hatching occurred within 3-5 days inside glass jars provided with fresh castor oil plant leaves. Green leaves were renewed daily in the early three larval instars. Fourth and fifth instars larvae were kept in low numbers to avoid cannibalism with fresh castor oil plant leaves which always occurred under the state of crowding (Ibrahim, 1981).

2. Irradiation Technique:

Full grown pupae of *A. ipsilon* (24 - 48 hours before emergence) were irradiated using a Gamma Cell (Cobalt 60 source) installed at the laboratory of Middle Eastern Regional Radioisotopes Centre for the Arab Countries, Cairo (Egypt). The irradiation dosimeter applied was 6.25 rad/second through the experimental work. Correction for decay was routinely run whenever needed at a half life of 5.3 years.

Gray (Gy) = 100 rad, krad = 1000 rad.

3.Experimental Procedures:

3.1. Sex pheromone extraction:

Sex pheromone was extracted from newly emerged females (1-2 days old) held under 25°C ± 2, 60-70 % R. H. The extraction procedures were similar to that described by Sower *et al.* (1973). The last three abdominal segments were cut and soaked for 24 hours in 10 ml. of the tested solvents. One hundred females' abdominal tips were used, to get a concentration of 0.0 1 female equivalent (FE/pJ). The extract was kept inside a small glass vial in complete darkness at (-10 DC). A vial containing the tested solvent only was used as a control.

3.2. Bioassay of male responses:

Each extract was bioassaily tested with live 1-2 days old males by using an olfactometer (Fig.1) similar to that used by Karlson and Butenandt (1959). It consists of a glass jar (20 x 10 cm) covered by muslin cloth, through which a plastic rod can be introduced. For each extract, a small filter paper strips impregnated with the tested extract was used. The bioassay strips were fixed in place by a masking tape at the inner end of the plastic rod, placed at 12 cm above the tested males. Five males (1-2 days-old) were tested in ten replicates (total of 50 males for each test). Males were exposed to females'

extract for 30 seconds. A position was recorded if the males moved their antennae, fluttered their wings or showed the usual courtship behavior. A bioassay strip impregnated with the solvent only was used as control. The bioassay was conducted under the aforementioned laboratory condition.

3.3 . Effect of the solvent on the potency pheromone extract:

Extraction of the sex pheromone gland of *A. ipsilon* was carried out by soaking the abdominal tips of virgin females in four solvents, including ethanol, acetone, diethyl ether and petroleum ether, to determent the effect of solvent on the potency of female extract.

3.4 . Effect of female age on pheromone production:

To determine the effect of age on sex pheromone production, extracts were prepared from virgin females of various ages (1, 2, 3, 4, 5, 6, and 7 days old). Each fifty female age's group extract was tested against 2-3 days old males in ten replicates.

3.5. Effect of male age on male response:

To investigate the effect of male age on male response, males of different ages (1-7 days) were tested against female extract of 1-2 days old virgin female. Fifty males in ten replicates were used in each test.

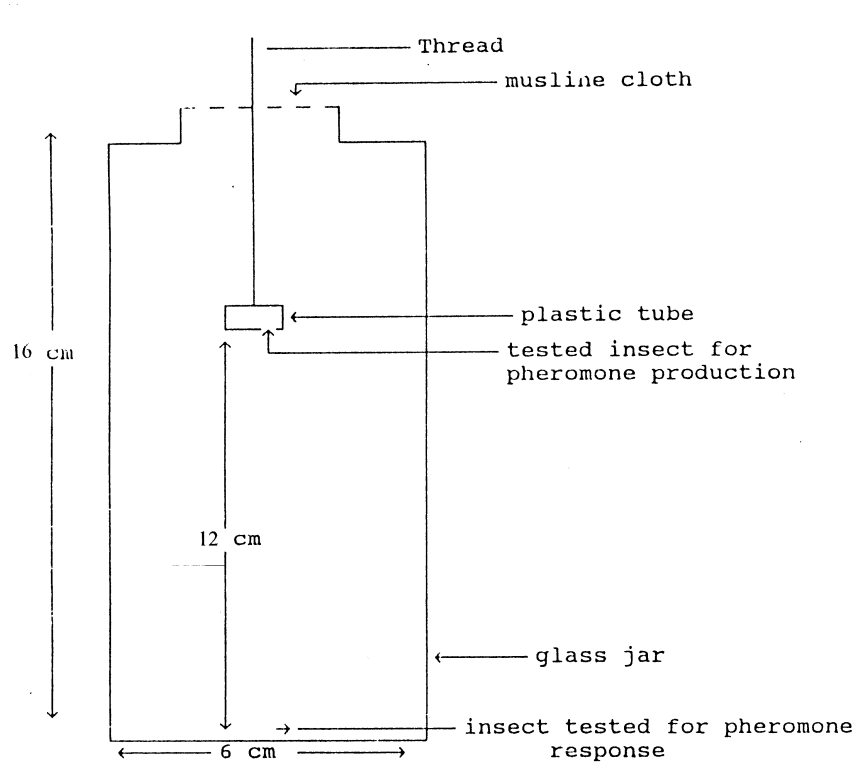

Fig. 1: Olfactometer used for testing pheromone produced by female moths.

3.6. Effect of gamma irradiation on female pheromone production:

To determine the effect of irradiation doses on pheromone production in females and receptors in males, full grown male and female pupae were irradiated with sub sterilizing doses 50, 100 and 150 Gy. Fifty males in ten replicates of 1-2 day- old were used in each test against untreated females of 1-2 days. Females of 1-2 days old were extracted by diethyl ether at a concentration 0.01 FE / *m*l . The response of untreated males to extract of untreated virgin females at the same concentration was used as control.

3.7. Effect of gamma irradiation on male response:

To determine the effect of irradiation doses on male response, full grown male pupae were irradiated with sup sterilizing doses of 50, 100 and 150Gy. Fifty males in ten replicates of 1-2 days old were used in each tested against the extract untreated female of 1-2 days. Females of 1-2 days old were extracted by diethyl ether at a concentration 0.01 FE/ *m*l. The response of untreated males to the extract of untreated virgin females at the same concentration was used as control.

RESULTS AND DISCUSSION

1. Sex Pheromone Production and Perception:

In order to evaluate the efficiency of different solvents on sex pheromone extraction, the following solvents were tested: diethyl ether, petroleum ether, acetone and ethyl alcohol. Results on the response of 1-2 days old male moth of *A. ipsilon* to the extracts of virgin females (1-2 days old) by the different solvents are given in Table (1).

Males' response to female extraction was carried for 6-7 hours after sunset. Bioassays were conducted under laboratory conditions. According to the percentage of male response to arranged in a descending manner the efficiency of solvents was: diethyl ether (71.66%), ethyl alcohol (68.13%), acetone (61.7%*)* and petroleum ether (50%).

Table (1): Response of *Agrotis ipsilon* males to female sex pheromone extracted by different solvents.

Solvent	Percentage of male response %		
	Experiment	Control	Corrected *Exp.
Acetone	64	6	61.7
Diethyl ether	73	9	71.66
Ethyl alcohol	71	9	68.13
Petroleum ether	52	4	50.0

*** Corrected Experiments = Experiment – Control / 100 - Control**

The obtained results revealed that diethyl ether and ethanol were the most potent solvents for extracting sex pheromone. Other solvents used (acetone & petroleum ether) were less efficient. The first two solvents probably have a more dissolving power, thus, were more extractable to pheromone titer than the other tested solvents. This agrees with the findings of Roe!ofs and Feng (1967), who found that diethyl ether was the excellent solvent for extracting the female sex pheromone of red banded leaf roller moth, *Arayrotaenia velutinana* (Walker) comparable with other solvents. EI-

Sayed (1990) proved that diethyl ether was the most potent solvent for pheromone extraction of the Indian meal moth *Plodia interpunctella* (Hubner).

In contrast, Grant 1975) proved that hexane is the most effective solvent for extracting *Orgyia leucostigmll* sex pheromone. This was found true by Kanaujia and Sidhu (1980) working on *Sitotroga cerealella* (Oliver) who reported that hexane is more effective solvent than the other tested solvents. According to these authors, the considerable difference in the efficiency of solvents could be attributed to their pheromone extraction ability, i.e. their dissolving power.

1.2. Effect of female age on pheromone production:

Results obtained on the response of 1-2 day- old males of *A. ipsilon* to sex pheromone of virgin female's extraction at different female age (1-7 day- old) are given in Table (2). Data indicate that, virgin females of *A. ipsilon* could secrete sex pheromone early at the beginning of their life (1 days old). This continued with varying titers as they became older up to 7 days old. The highest level of pheromone production by females however, was reached when the females were 2 days old and then slowly decreased until it reached its lowest titre when the females became older than six days old.

Table (2): Response of *A. ipsilon* males to sex pheromone extracted from virgin females at various ages.

Female age (days)	Percentage of male response %		
	Pheromone extract	Pure solvent (Control)	Corrected *Exp.
1	41	7	36.55
2	69	8	66.30
3	67	6	64.89
4	67	10	63.33
5	65	8	61.90
6	59	9	54.94
7	45	9	40.86

*** Corrected Experiments = Experiment - Control / 100 - Control**

Data obtained on the response of *A*. *ipsilon* males at different ages to female sex pheromone extract are summarized in Table (3). Males of different ages (1-7days old) clearly responded to sex pheromone extract of virgin females, the average male response, started at (44.9%) for (1 days old), and reached the maximum male response
(73.1) for males (2 days old). Older males (7 days old) showed decreased levels of response than (2days old). The lowest levels were reached at the oldest ages (7 days old). These results indicated that the optimum age of male response to sex pheromone extract was about (2 days old).

The obtained results indicated that females produce their sex pheromone early at the beginning of their emergence. Males also can perceptive this pheromone at the same age. This results agree with the findings of El-Sayed (1990) on the Indian meal moth, *Plodia interpunctella,* and Hazaa (1995) on cotton leaf worm, *Spodoptera littoralis.* After emergence, the pheromone production and perception increase rapidly, through the first day and continue till the 5 day after emergence.

Table (3): Response of *A. ipsilon* males at different ages to female sex pheromone extract.

Male age (days)	Percentage of male response		
	Pheromone extract	Pure solvent (Control)	Corrected *Exp.
1	51	11	44.9
2	75	7	73.1
3	69	9	65.9
4	68	12	63.6
5	55	10	50.0
6	51	4	48.9
7	48	8	43.4

*** Corrected Experiments = Experiment – Control / 100 - Control**

The pheromone production and perception increases to reach its maximum on the second day and continues at a high titer except during the last few days of female's life. As the black cut worm has a short life span, it can be concluded that the sex pheromone production by females and perception by males continues at a high level during most of their life. This appears to help the insect to continue mating and producing fertile eggs during its whole life span. The decrease in sex pheromone production and perception during the last few days of female and male life is probably attributed to old age which is usually accompanied with much physiological disturbances and decrease or inhibition of many biological processes. The production of'sex pheromone and the responsiveness decrease only during the last two days of their life span (5-7 days). According to Shorey and Gaston (1964), the pheromone production and perception usually begin at age's characteristic of each insect species.

Weatherston and Percy (1968) indicated that the production of pheromone by female *Vitula edmandse* reaches its maximum 2 days after emergence while in the angoumois grain moth, *Sitotroga cerealella,* the age has no influence on the production of pheromone by females (Sower *et al.,* 1973). Kuwahara *et al.* (1968) reported that the sensitivity of male moths to sex pheromone of the female almond moth, *Cadra cautel/a,* increased gradually with their ages up to the seventh day from emergence. Aliniazee and Stafford (1971) stated that two days old females of the omnivorous leaf roller, *Platynata stultana.,* were most attractive to males, while at six days old and older females were almost non attractive.

Also, Nagata *et al.* (1972) reported that pheromone of the female moth of the smaller tea tortix, *Adoxophyes fasciata,* continues at a high level during most of their adult life. Swaby *et al.(* 1987) stated that the age of females of the Douglas-fire tussock moth, *Orgyia pseudotsugata* influenced their attractiveness to males. Females attracted fewer males after the third day. Rafeal and Soroker (1989) observed that maximum levels of pheromone in the American bollworm, *Heliothis armigera* were extracted from 2 to 3 days old females. This results agreement with EI-Sayed (1990) on the Indian meal moth, *P. interpunctella.*

In contrast Payne *et al.* (1969) found that the age did not affect to response of the male cabbage looper, *Trichoplusia ni,* to the sex pheromone. Also Sower *et al.* (1973) proved that extracts prepared from different aged females of the angoumois grain moth, *S. cerealel/a* elicited equal male responses. Szocs and Toth (1979) found that maximum responsiveness of male of the gamma moth *Autographa gamma* is induced in 2-4 day-old males.

1.4. Effect of irradiation on pheromone production bv female and male response:

Results on the effect of different irradiation doses (50,100 and 150 Gy,) on untreated male response to female pheromone extract of 1-2 days old female previously irradiated as full grown pupae are given in Table (4) . All irradiation doses decreased the percentage of male response to female pheromone extract. The obtained data indicate that male responses were drastically affected at 100 and 150 Gy.

Table (4): Response of *A. ipsilon* male to sex pheromone extract from virgin female previously irradiated as full grown pupae with different doses.

Dose (Gy)	Percentage of male response		
	Treatment	Control	Corrected *Exp.
0	68	8	65.5
50	60	7	57.8
100	47	5	44.2
150	41	9	35.2

*** Corrected Experiments = Experiment – Control / 100 - Control**

Table (5) indicated that sub sterilizing doses (50,100 and 150 Gy,) decreased the perception of males to sex pheromone extract of 1-2 days old female. The percentage reduction of treated male response to untreated female pheromone extract was 65.6, 54.9 and 45.0 % at 50, 100 and 150 Gy, respectively. The obtained data indicate that male responses were drastically affected at 100 and 150 Gy.

The sex pheromone production by *S. /ittora/is* females was completely inhibited at a dose of 350 Gy applied to full grown pupae (Hazaa, 1995). Stimman *et al*.(1972) found that irradiated females of the cabbage looper, *Trichoplusia ni,* became less attractive to males as the radiation dose similarly Increased. Fletcher and Glannakakis (1973) reported that pheromone production by the fruit fly, *Dacus tryoni* males decreased with the exposure to 5 k rad applied on the 5th day in puparium. Hendricks (1974) showed that females of the tobacco bud worm moth, *He/iothis virescens,* irradiated as 4th day pupae before eclosion have reduced their attractiveness with a dose of 10 Krad, and considerably greater reduction was obtained with a dose of 50 krad .

Table (5): Response of *A. ipsilon* male previously irradiated as full grown pupae with different doses to sex pheromone of virgin female

Dose (Gy)	Percentage of male response		
	Treatment	Control	Corrected *Exp.
0	71	8	68.5
50	68	7	65.6
100	59	9	54.9
150	51	11	45.0

*** Corrected Experiments = Experiment - Control /1 00 - Control**

Abdu *et al.* (1985) found that production of sex pheromone by virgin females of *T. castaneum,* decreased with the increase of irradiation dose. The effect was very much evident even at the lowest irradiation doses (0.5-1 krad). An almost complete inhibition was reached at a radiation dose of 6 krad. The present result also coincide with the findings by El-Degwi (1990) who indicated that sex pheromone production by female beetles resulted from irradiated pupae of C. *maculatus* decreased as the irradiation dose increased. She concluded that the effect of gamma irradiation on sex pheromone production is an important factor could be recommended in the control programms of C. *maculatus.* The effect will be through preventing two sexes from mating and reproduction.

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تـأثير أشـعة جامـا علـى إنتـاج و إسـتقبال الفرمـون الجنسـي فـى الـدودة القارضـة السوداء *Agrotis ipsilon*

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تتناول هذه الدراسة تأثير ثلاثة جرعات منخفضة من أشعة جاما (٥٠ ، ١٠٠ ،١٥٠جراى) علي إنتـاج و إستقبال الفرمون الجنسي فى الدودة القارضة السوداء.وجد أن هناك تأثير للمذيبات المختلفةالمستخدمة في إستخلاص الفرمون الجنسي من الإناث فى نسبة جذب الذكورحيث كانت أعلى نسبة انجذاب للذكور للمستخلص داى ايثيل ايثير ، يليه مستخلص الكحول الايثيلى ثم الاسيتون واخيرا بترولييم ايثير. كذلك كان لعمر الإناث والذكور تأثير على أنتاج وأستقبال الفرمون حيث بدأت إنـاث الفراشـات فـي إفراز الفرمون منذ خروجها من العذارى وتستمر كذلك خلال حياتها ولكن بمستويات مختلفة حيث كان مرتفعاً خلال الخمسة أيام الأولي في حياتها ووصل الذروة في اليوم الثاني ثم بدأ فى الانخفاض تدريجيا . بدأت استجابة الذكور للفرمون الجنسى عند عمر(٠-١يوم) ثم وصل الى اعلى تركيز له عند عمر(١-٢يوم) ثم بدأ فى الانخفاض تدريجيا و كان اقل تركيز عند عمر(٦-٧يوم).

كان للمعاملة بالإشعاع تأثير علي إنتاج الفرمون الجنسى للاناث واستجابة الذكور له حيث وجد أن إنتاج الفرمون الجنسى يتأثر بالتشعيع حيث يصل إلي أقل معدل له عند الجرعة ١٥٠ جراى بينما لم يتأثر كثيراً عند الجرعة ٥٠ جراى . كما وجد أن استجابة الذكور المشعة للفرمون الجنسي يتأثر تدريجياً بزيادة الجرعة ولكن بدرجة اقل من تأثير الإشعاع على إنتاج الفرمون الجنسى للإناث .