



## Studies on the response of normal and irradiated pink bollworm males towards pheromone source

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Sterile Insect Technique (SIT) is the right eco-friendly method to overcome the pink bollworm *Pectinophora gossypiella* (Saunders) menace. In SIT attractiveness of released moths to their natural counterpart is most important as such this ability was examined in our study. For this, newly emerged five virgin female gamma irradiated with 0–250 Gy were used. These females were placed inside small plastic vial provided with aeration and suspended inside the delta traps by providing 10% honey solution. Traps were placed in the field during evening. Moth catches in each trap were counted and removed daily and these observations continued up to 5 days. In a same manner irradiated males were released separately inside the field cage to know its attractiveness to virgin female. The results indicated that attraction of male moths to irradiated virgin females decreased significantly with increasing doses of radiation i.e., at 200 and 250 Gy. The number of males captured per female was greatest on day 2<sup>nd</sup> due to its peak sexual activity, i.e., 25.60, 23.05, 19.45, 14, 9.4 and 5.9 moths at 0, 50, 100, 150, 150, 200, and 250 Gy, respectively. Gamma radiation also affected the irradiated males to find their natural mates at a higher dose; however, the maximum number of 5.30, 5.95 and 5.10 irradiated male moths were attracted to the natural pheromone secreted by female moths at 50, 100 and 150 Gy respectively, compared to the 6.80 moths at 0 Gy. The results on attractiveness of irradiated male moths towards virgin female-baited traps were considered a good measure of male competitiveness.

**Key words:** *Pectinophora gossypiella*, Gamma radiation, competitiveness, Sterile Insect Technique

## Introduction

Cotton (*Gossypium hirsutum* L.) is the most important commercial crop producing natural fiber; this white gold is being grown in more than 111 countries as a commercial crop across the world. India is the largest producer of cotton globally; this crop holds significant importance for the Indian economy and the livelihood of Indian cotton farmers. India ranks first in cotton cultivation with 120.69 lakh hectares with an annual production of 340.62 lakh bales and a mean productivity of 469 kg/ha [3]. In Karnataka, cotton is being cultivated in 8.97 lakh hectares with a pro-

duction of 21.48 lakh bales and productivity of 407 kg/ha [15]. Despite the promising scenario in cotton, several biotic and abiotic factors are responsible for the reduction in yield and quality deterioration of cotton in India. Among the biotic factors, insect pests are major in India.

Among the biotic problem, bollworms such as *Helicoverpa armigera* (Hub.), *Earias vittella* (F) and *Pectinophora gossypiella* (Saunders) are important [22]. The first two species of bollworms ruled cotton production before the introduction of *Bt* cotton in 2002. But in recent years, pink bollworm has emerged as a threat to cotton cultivation across the India.

The pink bollworm (PBW), *Pectinophora gossypiella* (Saunders), is one of the key pests of cotton, which damages the seeds and fiber, thereby causing economic losses. The cotton pink bollworm, *Pectinophora gossypiella* (Saunders), was originally reported from India in 1842. It feeds on cotton, okra, hibiscus and a few other species of *Malvaceous* plants. The incidence of pink bollworm goes unnoticed by the farmers since young larvae enter the cotton boll during the developing stage and remain inside the seeds. Its damage will be seen only when bad-opened bolls with damaged seeds are found at the harvesting stage. As cotton is used for both fiber and seed oil the damage is twofold.

After the introduction of *Bt* cotton, farmers reaped the benefits of high lint yield and major bollworm control until 2010. But, since 2010 pink bollworm has slowly increased its pest status. PBW resistance to Cry1Ac was first confirmed in four districts of Gujarat [11]. Studies conducted clearly indicated that the PBW developed resistance to cry toxins deployed in Bollgard II [16]. Surveys conducted across India showed progressive increases in the survival rate of PBW larvae in green bolls of *Bt*-II cotton  $F_1$  hybrid varieties [21]. The pink bollworm damage to *Bt* cotton varies across India, In south zone, highest larval recovery and green boll damage due to PBW was recorded at Raichur (10.9–50.83%). In central zone, the green boll damage (%) due to PBW ranged from 0.0–40.0 and maximum damage was observed at Akola and in north zone the green boll damage (%) due to PBW was ranged from 0.50–11.25 and maximum damage was recorded at Sirsa [15].

All the efforts were failed to prevent pink bollworm further losses including conventional pesticides; the pest exasperate and caused 40–80% damage in different parts of the country [16]. In these incongruous situations, such as resistance to chemicals and *Bt* toxins by PBW, farmers need an alternative eco-friendly control measures for cotton pink bollworm. Mass trapping [4, 5], mating disruption by SPLAT-PBW [24] are currently promising eco-friendly technologies in *Bt* cotton eco-system to manage pink bollworm. Along with these, sterile insect technique (SIT) is also gaining equal importance. In sterile insect technique, exposed gamma radiation make insects reproductively sterile by causing germ-cell chromosome fragmentation that leads to dominant lethal mutations, resulting in imbalanced gametes, the inhibition of mitosis and the ultimate death of the embryo.

Now, the modified version of the sterile insect technique, i.e.,  $F_1$  sterility technique, is widely used across the world to target lepidopteran pests. In  $F_1$  sterility male parents are exposed to a sub-sterilizing dose of radiation and are released into fields to mate with wild females of the pest species, Thus, the resulting  $F_1$  progeny are more sterile than the irradiated parent. The modification over original SIT technique is mainly because to optimize radio tolerance in lepidoptearans. These, lepidoptearans are radio resistant compared with most other

insects. Possible molecular mechanisms responsible for the high radio resistance in lepidoptera might be due to their holokinetic chromosomes nature [18].

Further, because the sterile  $F_1$  progeny developed under field conditions, the detrimental effects of laboratory rearing, handling, and irradiation are eliminated and the biological rhythms of the  $F_1$  generation are in synchrony with those of the wild population [17]. All studies have shown that  $F_1$  sterility is compatible with other pest control tactics [7, 8, 19, 20].

The pragmatic application of  $F_1$  sterility or inherited sterility technique has been studied for many economically important lepidopteran species.  $F_1$  sterility is successfully used to eradicate the pink bollworm along with the transgenic *Bt* cotton in USA [25]. USA scientist joined the forum to eradicate the pink bollworm in Mexico and China [25]. This technique is being practiced in limited field conditions in Egypt [12]. In India, S. Hanchinal et al. [14] studied the effect of various gamma radiation dose (50–250 Gy) on reproductive biology of pink bollworm and selected 150 Gy as an appropriate ionizing dose to be employed for  $F_1$  sterility technique. G. Akshatha [2] who studied the impact of gamma radiation on the reproductive behaviour of pink bollworm and reported that 150 Gy is sub-sterilizing dose for pink bollworm males wherein the same dose causes complete sterility in females, Further, this 150 Gy did not downstream the mating quality of the released males and contributes 100 % sterility in the all the  $F_1$  out crosses. Hence, ability of the both irradiated male and female (0–250 Gy) to attract their natural counterpart play an important role in population suppression especially when both sexes are irradiated and released. As such this ability was examined in our studies. Here the effect of both female and male age on their attractiveness to natural counterpart was also determined here.

## Materials and Methods

### Rearing of *P. gossypiella* on semi synthetic diet

The individual field collected larvae were reared on an artificial diet in bioassay trays with 158 cavities and covered with a perforated cap until pupation. The fresh diet was supplemented whenever necessary. Sex differentiation of the pink bollworm was done both in the larval and pupal stages, as mentioned by [10] for pairing adult moths. After adult emergence, they were collected and released in oviposition jars of 45×30×30 cm (l×b×h) cm size containing cotton twigs with terminal leaves and squares inserted in a small plastic container with a 10 per cent sucrose solution as adult food and also for egg laying. The bottom of the twigs was immersed in water to retain the turgidity of the tissue. Cotton twigs were changed once every three days in the oviposition jars; cut-off twigs were transferred to a transparent plastic container covered with black cloth and tightly fastened with a rubber band for egg

hatching. And this setup was maintained for up to 7–10 days. Once eggs passed their incubation period, the hatched first instar larvae were transferred individually into rearing trays containing a semi synthetic diet [10] and covered with a perforated cap.

The final instar larvae were later transferred individually to a plastic vial with perforations on the cap for aeration. A fresh diet was supplemented whenever necessary until pupation. Larvae were maintained in the growth chamber by maintaining the temperature at  $27\pm 0.5^{\circ}\text{C}$ , relative humidity of  $65\pm 5\%$  and photoperiod of 14L:10D until pupation. This culture was utilized for further investigations into radiobiological studies under the laboratory conditions mentioned above.

#### Sex differentiation in pink bollworm

The determination of sexes based on external characteristics was essential for the mass-rearing of pink bollworms. The identifying characters of pupae are mainly based on the position of the genital and anal openings. Males and females were differentiated in the larval stage itself based on the presence of a pair of dark-colored testis in the 7<sup>th</sup>–8<sup>th</sup> abdominal segments of male. Whereas in female it is absent.

#### Irradiation technique and sterilization process of pink bollworm

Most commonly used radiation source, cobalt-60 ( $\text{CO}^{60}$ ) was used for the exposure of mature pupae through Gamma Chamber-5000.

#### Irradiation unit-Gamma Chamber-5000: Working principle

Gamma Chamber-5000 is a compact self-shielded cobalt-60 gamma irradiator providing an irradiation volume of approximately 5000 cc. The material for irradiation placed in sample chamber located in the vertical drawer inside the lead flask. This drawer can be moved up and down with the help of a system of motorized drive which enables precise positioning of the sample chamber at the center of the radiation field. Radiation field is provided by a set of stationary cobalt-60 sources placed in a cylindrical cage. The sources are doubly encapsulated in corrosion resistant stainless steel pencils and are tested in accordance with international standards. Two access holes of 8 mm diameter are provided in the vertical drawer for introduction of service sleeves for gases, thermocouple, etc. A mechanism for rotating/stirring samples during irradiation is also facilitated. The lead shield provided around the source is adequate to keep the external radiation field well within permissible limits.

#### Response of the normal and irradiated pink bollworm population towards the pheromone source

The ability of irradiated male and females to attract their natural counterpart play an important role in population suppression especially when both sexes are irradiated and released.

#### Response of normal pink bollworm males towards irradiated females pheromone source

For this study, mature female PBW pupae were irradiated at 0, 50, 100, 150, 200 and 250 Gy (Plate 6) and these pupae were kept separately. Newly emerged females from each dose were used as bait in Delta traps separately. Here five irradiated virgin females were confined inside small plastic vial (perforated for aeration) and suspended inside the traps for each dose. The females were provided with a 10% honey solution on a cotton wick and this honey was provided every alternate day by using syringe. Traps were hung at the level of plant canopy and placed in the field in the evening. Moth catches in each trap and in respective replications were counted. and removed daily and these observations continued up to 8 days. The effect of gamma dose and female age on their attractiveness to male moths was also determined.

#### Response of irradiated pink bollworm males towards normal female pheromone source

The cage experiment was conducted to study the response of gamma irradiated male moths to natural pheromone released by the female moths. This study was conducted under shade net and each blocks were separated by net. Cotton plants were raised in a 360 m<sup>2</sup> area as per the package of practices. During the flowering stage of the crop, the crop was covered with nylon nets of size 25×30m<sup>2</sup>, as such, six blocks were made inside the shade net. The native population of PBW remaining inside the field cages was monitored using gossypure-baited traps and inspection of cotton fruiting bodies on a weekly basis. Monitoring was continued until no individuals were present in the gossypure baited trap. Later, five normal virgin females were confined inside small plastic vial (perforated for aeration) and suspended inside the traps instead of gossypure for each dose. The females were provided with a 10% honey solution on a cotton wick and this honey was provided every alternate day by using a syringe. Traps were hung at the level of the plant canopy and placed in the shade net house in the evening, Meantime, 100 PBW male adults irradiated at 0, 50, 100, 150, 200 and 250 Gy were released separately in each nylon net containing five virgin normal females suspended inside the trap. Here irradiated male moths captured in each trap were counted and removed daily.

### **Results and discussion**

#### Studies on the response of the normal and irradiated pink bollworm moths towards the pheromone source

The ability of the irradiated females and males to attract their natural counterparts plays an important role in population suppression especially when both sexes are irradiated and released. As such, this ability was examined in the present studies.

**Table 1.** Response of normal pink bollworm males to irradiated female pink bollworm as a pheromone source

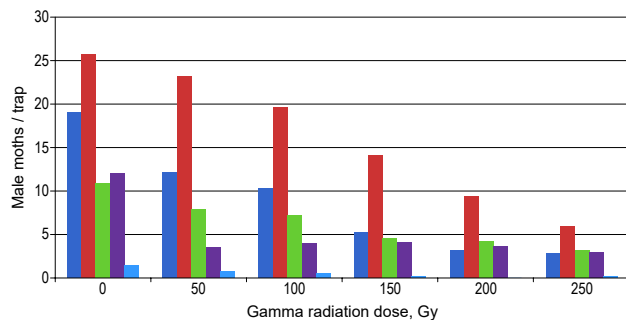
Treatments	Number of male moths attracted to irradiated virgin female				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
T <sub>1</sub> : 0 Gy	18.90 (4.40) <sup>c*</sup>	25.60 (5.11) <sup>c*</sup>	10.80 (3.36) <sup>b*</sup>	11.95 (3.53) <sup>b*</sup>	1.40 (1.38) <sup>b*</sup>
T <sub>2</sub> : 50 Gy	12.10 (3.55) <sup>b</sup>	23.05 (4.85) <sup>c</sup>	7.85 (2.89) <sup>b</sup>	3.55 (2.01) <sup>a</sup>	0.70 (1.10) <sup>a</sup>
T <sub>3</sub> : 100 Gy	10.20 (3.27) <sup>b</sup>	19.45 (4.47) <sup>b</sup>	7.05 (2.75) <sup>b</sup>	4.00 (2.12) <sup>a</sup>	0.55 (1.02) <sup>a</sup>
T <sub>4</sub> : 150 Gy	5.20 (2.39) <sup>a</sup>	14.00 (3.81) <sup>b</sup>	4.40 (2.21) <sup>a</sup>	4.05 (2.13) <sup>a</sup>	0.10 (0.77) <sup>a</sup>
T <sub>5</sub> : 200 Gy	3.15 (1.91) <sup>a</sup>	9.40 (3.15) <sup>a</sup>	4.05 (2.13) <sup>a</sup>	3.65 (2.04) <sup>a</sup>	0.00 (0.71) <sup>a</sup>
T <sub>6</sub> : 250 Gy	2.80 (1.82) <sup>a</sup>	5.90 (2.53) <sup>a</sup>	3.10 (1.90) <sup>a</sup>	2.85 (1.83) <sup>a</sup>	0.05 (0.74) <sup>a</sup>
SE, m±	0.25	0.17	0.23	0.23	0.15
CD @ 5%	0.75	0.87	0.67	0.69	0.45
CV, %	16.28	14.05	18.12	19.52	20.20

Note. \* — figures in the parentheses are  $\sqrt{X+0.5}$  transformed values.

**Response of normal pink bollworm males towards irradiated female**

The mean number of PBW males captured in each trap baited with virgin females irradiated at different doses was shown in table 1. Our results showed that male moths responded to all the female exposed to different doses radiation. However, female attractiveness was significantly reduced at higher doses of 200 and 250 Gy. The responses of males to traps baited with untreated control females were significantly higher than for traps baited with irradiated females. Female age also affected the male moths capture. Male moths captured in each trap baited with females irradiated at different doses of irradiation were drastically reduced when calling females were older than two days.

The number of male moths captured at 24 hours after the sleeve funnel traps were installed in the field was 18.90, 12.10, 10.20, 10.11, 8.51, and 2.8 moths at 0, 50, 100, 150, 200, and 250 Gy exposed females, respectively. Whereas maximum male moths were captured on the second day, i.e., 25.60, 23.05, 19.45, 14, 9.4 and 5.9 moths at 0, 50, 100, 150, 200, and 250 Gy,

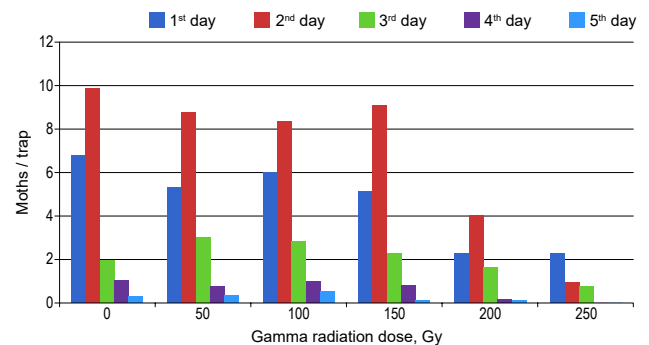


**Fig. 1.** Mean number of pink bollworm male moths captured at different day intervals towards the virgin females irradiated at different doses

**Table 2.** Response of irradiated pink bollworm males to normal female as a pheromone source in the caged condition

Treatments	Number of irradiated male moths attracted to virgin female				
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	4 <sup>th</sup> day	5 <sup>th</sup> day
T <sub>1</sub> : 0 Gy	6.80 (2.70) <sup>b</sup>	9.90 (3.22) <sup>c</sup>	4.10 (2.14) <sup>b</sup>	1.05 (1.24)	0.30 (0.89)
T <sub>2</sub> : 50 Gy	5.30 (2.41) <sup>b</sup>	9.10 (3.10) <sup>c</sup>	3.00 (1.87) <sup>b</sup>	0.80 (1.14)	0.35 (0.92)
T <sub>3</sub> : 100 Gy	5.95 (2.54) <sup>b</sup>	8.75 (3.04) <sup>c</sup>	2.85 (1.83) <sup>b</sup>	1.00 (1.22)	0.50 (1.00)
T <sub>4</sub> : 150 Gy	5.10 (2.37) <sup>b</sup>	8.79 (3.04) <sup>c</sup>	2.25 (1.66) <sup>a</sup>	0.85 (1.16)	0.10 (0.77)
T <sub>5</sub> : 200 Gy	2.30 (1.67) <sup>a</sup>	4.05 (2.13) <sup>b</sup>	1.65 (1.47) <sup>a</sup>	0.15 (0.81)	0.10 (0.77)
T <sub>6</sub> : 250 Gy	2.25 (1.66) <sup>a</sup>	0.95 (1.20) <sup>a</sup>	0.80 (1.14) <sup>a</sup>	0.00 (0.71)	0.00 (0.71)
SE, m±	0.19	0.29	0.22		
CD @ 5%	0.57	0.88	0.68	NS	NS
CV, %	6.49	7.69	9.16		

respectively. The mean number of male moths captured 72 hours after the female exposed in trap ranged from 10.80 to 3.1 moths at 0–250 Gy. On the 4<sup>th</sup> and 5<sup>th</sup> day, the mean number of male moths captured ranged from 11.95 to 2.85 and 1.40 to 0.05 at 0–250 Gy, respectively. Higher dose of 150, 200 and 250 Gy affected the calling behavior of irradiated females kept in the trap even for the first two days (fig. 1). In general, when radiation dosages increased, male moths' attractiveness to irradiated virgin females declined noticeably. Male moths reacted more quickly to untreated virgin females than to females that had received radiation. On the second day, there were more males captured per female. It might be due to the peak mating activity for both treated and native moths began at 23:00 hours after emergence and peak sexual activity was recorded during the early morning (02:00–03:00) as documented by the number of mating pairs collected from mating tables containing treated, native or mixed source clipped-wing females revealed that mating activity for both treated and native PBW moths began at 23:00 hours. Peak sexual activity was recorded during the early morning (02:00–03:00) [1].



**Fig. 2.** Number of irradiated male moths attracted to the virgin females kept in the trap under field cage conditions

The results are in concurrent with the results of Hendricks and Garcia indicated that virgin female pink bollworms irradiated with 25 krad were somewhat less attractive than untreated virgin females, traps containing 15 live irradiated females were exposed in the field for 10 days and averages of 4.49 and 3.90 moths were caught for untreated and irradiated females, respectively. In contradictory to the present results, H. M. Flint et al. [13] reported that trap tests with female pink bollworms irradiated with doses of 0 or 25 krad indicated no reduction of their attractiveness when compared with untreated female, they supported their findings as, attractiveness of continuous mass reared insects are more compared to the native insects, the difference was mainly witnessed when their fecundity was reduced at higher dose like 250 Gy. The results are in agreement with the N. Ahmad et al. [1] who reported that attraction of pink bollworm male moths to irradiated virgin females decreased significantly with the increasing doses of radiation (0–200 Gy). The highest number of male moths was captured at 48 hours after the release. Female age was also affected the male moth capture. Captures of male moths was drastically reduced when calling females were older than two days.

#### Studies on the response of irradiated pink bollworm males towards normal female pheromone source in the caged condition

Gamma radiation affected the irradiated males to find their mates at a higher dose; however, the maximum number of 5.30, 5.95 and 5.10 irradiated male moths were attracted to the natural pheromone secreted by female moths at 50, 100 and 150 Gy respectively, compared to the 6.80 moths at 0 Gy. At higher doses of 200 and 250 Gy, 2.30 and 2.25 moths were attracted 24 hours after the release. It indicated that an increase in the dose of irradiation affected male moths orientation towards virgin female moths.

The maximum attraction of the released irradiated male moths towards the natural female was noticed on the 2<sup>nd</sup> day, i.e., 8.75, 8.30, 9.10, 4.05 and 0.95 at 50, 100, 150, 200 and 250 Gy, respectively compared to the untreated control, where it was 9.90 moths. 72 hours after the release, irradiated male moths attracted towards the virgin females ranged from 4.10 to 0.80 moths at 0–250 Gy (fig. 2). The number of irradiated male moths captured in virgin female-baited traps was considered a good measure of male competitiveness. On the 4<sup>th</sup> and 5<sup>th</sup> days after the release, the number of irradiated male moths attracted to the virgin females differed non-significantly among all the dosages, including the untreated control (table 2). Our present findings suggested that the responses of males irradiated at sub-sterilized doses up to 150 Gy, towards pheromone traps in the field cages were not different from non-irradiated control moths. Adequate responses of sub sterilized male moths towards pheromone baited traps are one of the important parameters that provide indications for successful mating. Similarly,

J. E. Carpenter et al. [9] conducted study on *H. zea*, observed that males captured in pheromone traps were considered measures of male competitiveness and no significant differences were found between non-irradiated and 100 Gy-treated males. The results are in line with S. Bloem et al. [6] who examined the inherited sterility in the Codling moth (*Lepidoptera: Tortricidae*), reported that males treated with 100 Gy responded to calling virgin females in larger percentages than those treated with 200 and 250 Gy. Similarly, R. K. Seth [23] examined the mating behavior of irradiated *Spodoptera litura* (*Lepidoptera: Noctuidae*) males and their F<sub>1</sub> progeny for use of inherited sterility in pest management approaches, reported that response of males irradiated at sub-sterilized doses of 100 and 130 Gy towards pheromone traps or virgin female baited traps in field cages were not different from non-irradiated control moths.

The response of both normal males and irradiated males towards virgin irradiated females and normal females depends on the age of the female moth and also the dose. Attractiveness was found to be highest at 2<sup>nd</sup> after the release and the dose range above 150 Gy was significantly reduced the attractiveness of both sexes.

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## Дослідження реакції нормальних і опромінених самців рожевої совки на джерело феромонів

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Метод стерилізаційної боротьби з комахами (*Sterile Insect Technique*, SIT) — це екологічний метод подолання загрози рожевої совки *Pectinophora gossypiella* (Saunders). У SIT привабливість випущених метеликів для природних представників їхнього виду є найважливішою, тож ми дослідили цю здатність у нашій роботі. Для цього використали п'ять незайманих самок, які щойно вилупилися. Їх опромінували гамма-променями 0–250 Гр, поміщали в невеликий пластиковий флакон з аерацією та залишали всередині дельта-пасток, забезпечуючи 10% розчин меду. Пастки були розміщені на полі протягом вечора. Улов молі в кожній пастці щодня підраховували та видаляли. Ці спостереження тривали до 5 днів. Аналогічно опромінених самців випускали окремо всередину польової клітки, щоб визначити їхню привабливість для незайманої самки. Результати показали, що привабливість самців молі для опромінених незайманих самок значно зменшилася зі збільшенням доз радіації, тобто за опроміненень 200 і 250 Гр. Кількість спійманих самців на одну самку була найбільшою на 2-й день через пік її статеві активності, тобто 25,60; 23,05; 19,45; 14; 9,4 і 5,9 місяця за опромінення 0, 50, 100, 150, 150, 200 і 250 Гр відповідно. Гамма-випромінювання також впливало на те, що опромінені самці знаходили своїх природних партнерів за вищих доз; однак максимальна кількість 5,30; 5,95 і 5,10 опромінених самців метеликів була приваблена природним феромоном, виділеним самками молі, за 50, 100 і 150 Гр відповідно порівняно з 6,80 метеликами за 0 Гр. Результати щодо привабливості опромінених самців молі до пасток із незайманими самками вважалися хорошим показником конкурентоспроможності самців.

**Ключові слова:** *Pectinophora gossypiella*, гамма-випромінювання, конкурентоспроможність, метод стерилізаційної боротьби з комахами