รังสีชักนำให้เกิดการเป็นหมันในรุ่นลูกในหนอนเจาะสมอฝ้าย, *Helicoverpa armigera*

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บทคัดย่อ

เพาะเลี้ยงหนอนเจาะสมอฝ้าย, *Helicoverpa armigera* ด้วยอาหารเทียม ศึกษาการเจริญเติบโต 4 รุ่นติดต่อกันไม่พบความแตกต่างในทางสถิติ ฉายรังสีดักแด้เพศผู้อายุ 9±1 วัน ที่ปริมาณรังสี 0, 50, 100, 150 และ 200 เกรย์ พบว่า การเป็นหมันมีความแตกต่างในทางสถิติตั้งแต่ 150 เกรย์ขึ้นไป การเป็นหมันในรุ่นลูกมีความแตกต่างกันในทางสถิติและสูงกว่ารุ่นพ่อแม่ทุกปริมาณรังสี การแข่งขันผสมพันธุ์ของตัวเต็มวัยพบว่า ตัวผู้ฉายรังสีและตัวผู้ในรุ่นลูกที่ได้จากตัวผู้ฉายรังสี 150 เกรย์ มีความสามารถแข่งขันผสมพันธุ์กับตัวผู้ปกติในการผสมพันธุ์กับตัวเมียปกติได้

Radiation Induced the First Filial(F_1) Sterility in Cotton Bollworm, *Helicoverpa armigera*

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ABSTRACT

The cotton bollworms, *Helicoverpa armigera* were reared on the artificial diet in the laboratory. The development of this species were not significant differences in each of 4 successive generations. The irradiation of mature male pupae at 0, 50, 100, 150 and 200 gray showed that the sterility was significantly different at the dose above 150 gray. The sterility of F_1 progeny was significant differences, F_1 progeny were more sterile than the treated male parent at all dose. Labolatory mating competitiveness indicated that P_1 moths from irradiated male pupae at 150 gray and their F_1 male moths were fully competitive against untreated males in mating with untreated females.

INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Lepidoptera : Noctuidae) is a well known and serious key pest of economically important crops. It is a highly polyphagous species that can attack a diverse array of plant species in many plant families. At least 60 cultivated and 67 uncultivated plants have been reported to be host-plants of the species. Recently, *H. armigera* has been recorded on over 103 species of plants of which 61 species are crops including legumes, cereals, cotton, vegetable and other crops[1]. *H. armigera* has also demonstrated successful development of resistance to a range of insecticides, which has led to field failure in many crops[2]. Development of resistance by *H. armigera* to pyrethroids has recently been reported in many countries and shown to be influenced by genetic, ecological, behavioral and agronomic factors.[3,4] High fecundity is a major characteristic of the *armigera* species combining with a short generation period which contributes to high capacity for population growth.[5] *H. armigera* can easily maintain population and become serious problem in various cropping system in many parts of the world.

Knipling first demonstrated the potential advantage of inherited sterility over the sterile insect technique (SIT) through the use of population models. North & Holt[6] calculated the theoretical depression of *Trichoplusia ni* (Hubner) populations resulting from a single release of partially sterile males at a 1:1 or 9:1 sterile male/ wide male ratio. They projected that 92% control could be obtained for three generations. Similar conclusions were expressed for *Galleria mellnella* (L.)[7] , *Ephestia cautella*(Walker)[8] and *Spodoptera frugiperda* (J.E. Smith)[9]. Objectives of the study were

1. To study the efficiency of laboratory rearing *H. armigera* on artificial diet.
2. To study the effects of substerilizing doses of gamma radiation and inherited sterility on the insect reproduction.
3. To study effects of substerilizing doses of gamma radiation on mating competitiveness.

MATERIALS AND METHODS

1. Laboratory rearing on artificial diet

The culture was started with eggs and larvae which were reared on artificial diets in the laboratory. The composition of the diet is shown in Table 1A. The adults emerging from pupae were paired in oviposition cages (Lantern globe, 12x12.5 cm, covered with paper towel fixed with a rubber band and placed on the plastic basin containing water). A 10% honey solution was provided as food and paper towel was employed and covered with lid as an oviposition substrate. Eggs were removed daily and kept in the plastic container (9cm x 18cm) until hatching occurred. The larvae, after hatching, were reared on the artificial diet. The observations on various biological parameters were recorded from laboratory rearing at $27\pm2^{\circ}C$, $70\pm10\%$ relative humidity (RH) and 10:14 (light:dark) photoperiod.

Developmental data for 4 successive generations on egg hatchability, larval period, pupal recovery, pupal weight and moth emergence were recorded.

2. Radiation effects on mature pupae

Mature male pupae (9 ± 1 days) from the laboratory culture were irradiated with 50,100,150 and 200 Gy at dose rate of 33.6 Gy / min in a ^{60}Co gamma irradiator (Gamma Beam 650). The similar group of pupae was held as a control. Treated and untreated pupae were held in the same conditions previously described. Data on moth emergence, moth deformation and longevity were recorded.

2.1 Production of parents

The sterility of parent moths was recorded from the following crosses for each radiation dose:

1) UTF x UTM (Control)
2) UTF x TM

Where UT stands for untreated, T stands for treated, M stands for male and F stands for female. Each treatment was replicated using oviposition cages with five pairs of moths per cage per replication.

2.2 Production of F_1 progeny

The moths used in this experiment were obtained from the parents (P_1) crosses of the respective radiation doses. The F_1 moths of each dose were crossed separately in the following combinations to record their sterility, fecundity and longevity:

1) UTF x UTM (Control)
2) UTF x F_1M
3) F_1F x UTM

Where F_1F stands for females and F_1M stands for males obtained from P_1 irradiated as mature male pupae. The procedures adopted to record sterility, fecundity and longevity were similar to those described earlier for production of parents. The data were analysed by the analysis of variance (ANOVA) and the means compared by Duncan's new multiple range test at p=0.05.

3. **Mating competitiveness**

Mating competitiveness of irradiated males and F_1 male progeny was studied. The male moths and F_1 male moths from male parents irradiated with 150 Gy as mature pupae, normal male moths and normal female moths were caged in oviposition cages. Data from four treatments showed the ratio of irradiated or F_1 males : normal male : normal female were 0:1:1 , 1:0:1 , 1:1:1 and 3:1:1. Each cage was provided with a 10% honey solution and paper towel served as an oviposition site for the female. The eggs were collected daily and the number of hatched eggs in each population was recorded. The competitiveness value and expected egg hatch rates were computed as described by Fried.[10]

RESULTS

1. Laboratory rearing

Table 1.1 Duration of various development stages of the cotton bollworm, *Helicoverpa armigera* under laboratory condition (27$\pm$2°C, 70$\pm$10 %RH and 10:14 (L:D) photoperiod)

Development stages	Range (d)	Mean $\pm$ S.D (d)
Egg :	2-3	2.41 $\pm$ 0.05
Larva : Instar 1	2-3	2.25 $\pm$ 0.44
Instar 2	1-3	1.95 $\pm$ 0.39
Instar 3	1-3	2.00 $\pm$ 0.92
Instar 4	1-3	2.00 $\pm$ 0.86
Instar 5	3-6	4.55 $\pm$ 0.89
Pupa :	10-12	10.78 $\pm$ 0.83
Adult : male	16-35	29.0 $\pm$ 3.82
female	10-34	17.6 $\pm$ 6.25
No. eggs/female	-	1,356.15 $\pm$ 437.96

<u>Table 1.2</u> Development of cotton bollworm, *Helicoverpa armigera* for 4 successive generations reared on an artificial diet at 27±2°C, 70±10% relative humidity(RH), 10:14 (light:dark) photoperiod

Gen	Mean±S.D				
	Egg hatchability (%)	Larval period (d)	Pupal recovery (%)	Pupal weight (g)	Moth emergence (%)
1	73.20±9.38 a	14.80±1.76 a	86.37±6.24 a	0.321±0.31a	94.37±2.24 a
2	71.72±5.63 a	13.96±0.39 a	87.30±1.67 a	0.327±0.01a	96.59±0.01 a
3	69.05±9.23 a	15.90±0.62 a	89.20±0.62 a	0.315±0.02a	95.74±0.02 a
4	72.77±8.11 a	14.02±0.59 a	82.15±0.59 a	0.316±0.01a	95.83±0.01 a

Means in columns followed by the same letter are not significantly different at 5% level.

2 <u>Radiation effects on mature pupae</u>

<u>Table 2.1</u> Effects of gamma radiation on moth emergence, moth deformation, longevity and sterility of cotton bollworm, *Helicoverpa armigera*, following irradiation of mature pupae

Dose (Gy)	Moth emergence (%)	Moth deformation (%)	Longevity (d)	Sterility (%)
0	99.17 a	1.56 a	13.35 c	27.04 a
50	97.50 a	3.54 ab	10.20 ab	30.49 a
100	98.75 a	2.91 ab	9.45 a	33.12 a
150	97.92 a	5.39 ab	11.65 bc	48.84 b
200	99.06 a	8.33 b	11.10 ab	62.73 c

Means in columns followed by the same letter are not significantly different at 5% level.

Table 2.2 Survival and male progeny of F_1 progeny of irradiated cotton bollworm, *Helicoverpa armigera*, from male parents irradiated as mature pupae

Dose to P_1 male	No. larvae implanted for rearing	Pupal recovery (%)	Male progeny (%)
0	1,080	78.25 a	52.58 a
50	245	74.00 a	50.38 a
100	332	73.75 a	51.03 a
150	232	70.75 a	63.85 ab
200	378	51.50 b	77.22 b

Means in columns followed by the same letter are not significantly different at 5% level.

Table 2.3 Effects of gamma radiation on fecundity, longevity and sterility of the F_1 progeny from male parents of cotton bollworm, *Helicoverpa armigera*, irradiated as mature pupae

Dose	Fecundity	Longevity (d)		Sterility (%)	
(Gy)	(Eggs/female)	Male	Female	Male	Female
0	1,356 a	29.00 c	17.60 a	26.17 a	26.17 a
50	1,519 a	26.13 bc	18.00 a	52.77 b	52.75 b
100	1,347 a	24.90 ab	17.55 a	92.01 cd	84.76 c
150	1,292 a	26.35 bc	17.15 a	96.84 d	98.91 d
200	1,085 a	22.55 a	17.19 a	100.0 d	100.0 d

Means in columns followed by the same letter are not significantly different at 5% level.

3 **Mating competitiveness**

Table 3 Mating competitiveness of irradiated males of cotton bollworm, *Helicoverpa armigera*, with 150 Gy and their F_1 males

Cross ratio		Egg hatch (%)		Competitiveness value [2/]
TM : UTM : UTF [1/]		Observed	Expected [2/]	
P_1	0 : 1 : 1	52.67	-	-
	1 : 0 : 1	27.10	-	-
	1 : 1 : 1	34.16	39.88	1.17
	3 : 1 : 1	27.75	33.49	1.20
F_1	0 :1 : 1	57.65	-	-
	1 : 0 : 1	0	-	-
	1 : 1 : 1	19.34	28.82	1.49
	3 : 1 : 1	6.90	14.41	2.08

Means in columns followed by the same letter are not significantly different at 5% level.

[1/] TM for treated males, UTM for untreated males, UTF for untreated females.

[2/] The competitiveness value and expected egg hatch rates were computed as described by Fried (1971).

DISCUSSION AND CONCLUSION

The development of laboratory rearing is one of the major prerequisites leading to the application of sterile insect release methods for the control of target insect species. In the present studies, the cotton bollworm was successfully reared on the artificial diet. The diet was improved of a medium on vitamin stock and wheat germ. Composition of the diet was shown in Table 1A. The development of this species were not significantly different on egg hatchability, larval period, pupal recovery, pupal weight and moth emergence in each of 4 successive generations(Table 1.2). The results were in close conformity with Sutantawong[(11)] , who developed a method for mass-rearing of the cotton bollworm (Table 1.2) with better results than the those of Sutantawong. The difference might be differ of vitamin stock and the replacement of tetracycline hydrochloride with wheat germ. The vitamin mixture was similar to that developed in earlier work[(12)],which came out as commercial product, "Bodivitin". Composition of the vitamin solution is presented in Table 1A.

An important consideration in using artificial diet is the contamination of rearing medium by microorganism. Contamination was usually traceable to poor technique or mite infestations. Good sanitation practices were sufficient to hold contamination at low levels when the antimicrobial agents were included in the diet. In addition, the larvae were separated before reaching the 3 rd instar. Large larvae of *H. armigera* are cannibalistic. Efforts are in progress to furthurly improve the diet while keeping in view the economics of rearing.

The effect of radiation on mature male pupae of cotton bollworm, *H. armigera* when old pupae were treated with doses ranging from 50 to 200 Gy found moth emergence for both treated and untreated pupae to be significantly different while pupal irradiation showed a higher incidence of moth deformation at dose of 200 Gy when compared with untreated insects. Moth deformation was positively correlated with radiation doses. The above results were similar to those reported by Henneberry and Clayton[13] on pink bollworms, *P. gosspiella*, (Saunders) when old pupae were treated with 50 to 150 Gy of doses. Longevity of P_1 moth following radiation of mature male pupae varied significantly with radiation doses. Longevity of P_1 moth decreased with increasing radiation doses (Table 2.1). The results were also similar to those reported by Qureshi *et al.*[14] on pink bollworm when old pupae were treated with doses ranging from 50 to 200 Gy but contrast to that reported by Henneberry and Clayton.[13] The difference remained unexplained, but might be related to quality of insects. The results indicated that pupal irradiation may be a viable option if pupal harvesting techniques can be developed that are compatible with other mass rearing procedures. The sterility from matings of treated males at doses of 50 to 200 Gy with untreated females was considerably higher than that from the reciprocal matings. The sterility slightly increased with increasing radiation doses. Similar results were also reported by Henneberry and Clayton[13], Qureshi *et al.*[14] on pink bollworms, *P. gosspiella*, Sutrisno *et al.*[15] on diamondback moth, *Plutella xylostella* when old pupae were treated with doses ranging from 50 to 200 Gy. They suggested that the sensitivity differential between males and females might be an advantage if a single dose of radiation would partially sterilize males and completely sterilize females. The sterility probably occurred because of the lack of eupyrene sperm transferred by irradiated males during mating.[16]

The effects of radiation on inherited sterility of cotton bollworm, *H. armigera* were similar to those described for other species of Lepidoptera. The results of this study were concluded that higher doses of irradiation reduced survival of F_1 larval to adult stages. The number of survival larvae decreased as the doses applied to P_1 males increased. At all doses, the number of F_1 larvae that survived to emerge as adults was lower than the control. The number of survival of F_1 larva to adult was significant lower than control at the dose of 200 Gy. The sex ratio of the emerging F_1 adults was also skewed in favor of males, and significantly more males were obtained than in controls when the P_1 males were treated with dose of 200 Gy (Table 2.2). The similar results reported by LaChance *et al.* [17] on pink bollworm; they reported that the survival to adult stage of F_1 larvae at all doses were significantly lower than the control and the sex ratio of the emerging F_1 adults was also skewed in favor of males and significantly more males were obtained than the control when P_1 males were treated with dose of 50 Gy and above. Carpenter *et al.*[18] similarly reported on fall armyworm when irradiated P_1 adults with dose of 100 Gy.

Knipling[19] and LaChance *et al.*[17] reported that the F_1 progeny were either fully or partially sterile depending on the doses of irradiation received by the male parent. The F_1 progeny were more sterile than the treated P_1 male parent regardless of the doses to the male parents, and the F_1 males were usually more sterile than the F_1 females. The studies showed the similar characters of the F_1 progeny. The sterility of F_1 progeny was significantly different from that of untreated insects (Table 2.3). Furthermore, the average eggs hatch of F_1 males treated with doses of 50 to 150 Gy was higher than that in F_1 females. Similar results were also reported by Omar and Mansor[20], Sutrisno *et al.*[15] on diamondback moth; the sterility of F_1 progeny were more sterile than the parents at all doses when male pupae were treated with 50 to 250 Gy of doses. They concluded that lower doses of irradiation should be considered for diamondback moth that would be used in sterile insect releases and that doses as low as 150 to 200 Gy should be tested.

Fecundity of F_1 females were not significantly different from that of untreated insects. The results was in contrast to the report of Qureshi *et al.*[14] They reported that fecundity of F_1 females of pink bollworm obtained from male parent irradiated as mature pupae with doses of 50 to 150 Gy were reduced as compared with untreated insects. This difference probably occurred because of the number of F_1 female moths were not equal number in the design.

Longevity of F_1 female was not significantly different while that of F_1 males was significantly different from untreated insects. F_1 males from 200 Gy irradiated parents lived significantly shorter than F_1 males from untreated parents. The results also agreed with those reported by Henneberry and Clayton[13], Qureshi *et al.*[14] on F_1 progeny of pink bollworms, *P.*

gosspiella obtained from male parent irradiated as mature pupae with doses of 50 to 150 Gy. All these changes on the reproduction of F_1 progeny must be related to altered genetic information inherited from the treated male parent.

The studies support results obtained with other species of Lepidoptera: an irradiated sperm that does not contain a dominant lethal mutation and permits production of a viable larva was definitely not free from genetic changes that debilitate the progeny inheriting an irradiated genome, i.e., reduced survival of larvae, the shift of sex ratio in favor of males in the F_1 generation, and the lower reproductive ability of the F_1 males. All these changes must related to the altered genetic information inherited from the treated male parent.

The mating competitiveness of irradiated parental males and their F_1 male are presented in Table 3.3. The average egg hatch in cages with irradiated parental males all treatments (ratios) were not significantly different but competitiveness showed higher value because the parental males were treated a low doses which induced partial sterility. Therefore, no apparent effect on the sterility of parental males when crossed with normal females. However, the sterility of F_1 males was significantly reduced for all treatments when compared with the control. The competitiveness value of between 1.17 and 2.08 showed that the irradiated males and F_1 males could be fully competitive. Similar results were reported by Sallam and Ibraahim[21] on cotton leafworm, *Spodoptera littarali*, they reported that mating competitiveness of males irradiated with 100 or 150 Gy and their F_1 male progeny were fully competitive with untreated males in mating with untreated females and released into field cages caused significant reductions in population. Carpenter *et al.* [22] also reported that the effects of reduced competitiveness of F_1 males varied depending upon the release ratio and number of nights which the females were active in mating and oviposition. However, the results of this study were obtained under laboratory conditions only.

Still, the results of efficiency of artificial diet and effects of gamma radiation on mating and reproduction of mature pupae and the inherited effects on mating reproduction of their F_1 progeny were also studied under laboratory conditions. Therefore, before final judgement is made, it is necessary to study the inherited effects on mating reproduction of F_1 progeny in the field that the partially sterile cotton bollworm and their F_1 progeny can establish populations in the field.

is made, it is necessary to study the inherited effects on mating reproduction of F_1 progeny in the field that the partially sterile cotton bollworm and their F_1 progeny can establish populations in the field.

In addition, the F_1 generation can be reared in the field. This may be important because the rearing of most lepidopterous species was costly. It is likely that substantial cost reductions may be gained by combining the use of F_1 sterility with various methods of control. Resistant crop cultivars should be planted if available. However, high level populations of lepidopterous pests may first be reduced by using pathogens, parasites or predators, and then prevented from rebuilding to damaging levels by means of F_1 sterility.

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APPENDIX

Table 1A Composition of artificial diet used for rearing *Helicoverpa armigera*

Ingredients	Amount
Bean meal	260 g
Dried baker yeast	20 g
Methyl parahydroxybenzoate	5 g
Sorbic acid	3 g
Ascorbic acid	6 g
Wheat germ	6 g
Choline chloride	1 g
Formaldehyde	4 ml
Vitamin stock	20 ml
water	1,500 ml

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อรรถพล นุ่มหอม จิราวรรณ แย้มประยูร และ อภิพร อดุลพิชิต
คณะเทคโนโลยีการแปรรูป สถาบันเทคโนโลยีแห่งเอเชีย ถนนพหลโยธิน คลองหลวง ปทุมธานี 12120
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บทคัดย่อ

เมื่อนำกุ้งแห้งความชื้นสูง (35 เปอร์เซ็นต์) ม าฉายรังสีแกมมาระดับต่ำที่ 2 และ 4 กิโลเกรย์ พบว่า หลังจากการฉายรังสี ความชื้น Aw ค่าความเป็นกรดด่างไม่เปลี่ยนแปลง แต่ค่าความหืนเพิ่มมากขึ้น และความเป็นสีแดงลดลง แต่ผู้บริโภคไม่สามารถแยกความแตกต่างทางประสาทสัมผัสได้ เมื่อทดสอบ อายุการเก็บรักษาพบว่าการฉายรังสีสามารถลดปริมาณเชื้อราและยีสต์ และยืดอายุการเก็บรักษาจาก 10 วัน (ตัวอย่างที่ไม่ฉายรังสี) เป็น 35 และ 49 วัน เมื่อฉายรังสี 2 และ 4 กิโลเกรย์ตามลำดับ นอกจากนี้ จากการทดสอบทางประสาทสัมผัสพบว่าผู้บริโภคชอบตัวอย่างที่ฉายรังสีมากกว่า

Extending Shelf Life of Semi-Dried Shrimp with Low Dose Irradiation

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ABSTRACT

Semi-dried shrimp (35% moisture content) was irradiated at 2 and 4 kGy. The results showed that product moisture content, water activity and pH did not change. Lipid peroxidation was increased and redness was reduced. However, there was no significant difference of sensorial properties. With storage study, irradiation could reduce initial yeast and mold load and consequently shelf life of semi-dried shrimp could be extended to 35 and 49 days with 2 and 4 kGy of irradiation compared to 10 days with non-irradiated samples. Panelists preferred irradiated samples.