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IRRADIATION OF PINK BOLLWORM LARVAE BY Co-60 AND Cs-137 SOURCES AND THEIR EFFECTS ON VIABILITY, REPRODUCTIVITY AND STERILITY OF SUBSEQUENT P_1 AND F_1 PROGENY

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The irradiation of mature (cut out) larvae at 35 Gy from Co-60 and Cs-137 resulted in reduced and delayed pupation. Co-60 caused significantly more lethality than Cs-137. The fecundity of the females both P_1 and F_1 emerging from irradiated mature larvae was drastically reduced for Co-60 as well as for Cs-137 treatment. The female moths were comparatively more prone to gamma radiation damage than the males. Larval survival and adult emergence were dose dependent following irradiation of mature pink bollworm larvae at 40-65 Gy from Cs-137 alone. The fecundity of mature P_1 and F_1 progeny and percent of males in F_1 generation reduced with the increased doses. Complete sterility was recorded in crosses treated male x treated female (TMxTF) at all doses. The effect of larval irradiation on mature progeny of F_1 adults was also reduced in crosses involving either F_1 males or F_1 females. The doses tested for mature larvae were too high and are not suitable for the control of pink bollworm through F_1 sterility.

Key words: Gamma iradiation, Pink bollworm larave, Radiation sources, Effect on P_1 andF_1 progeny.

Introduction

The ability of sterile insects to compete with field populations is a prime consideration in the development of insect control programmes in the sterile insect release technique. Any reduction in competitive ability increases the cost of the programme since large releases may be required to compensate for this lack. In lepidopteran species partial sterility in the treated P_1 males is associated with high levels of sterility in the F_1 generation. Bartlett and Buler [1]] found that F_1 female progeny of irradiated females (15 & 20 krad) paired with untreated males were more than 90% sterile. Cheng and North [2] and Graham *et al.* [3] concluded that lower doses of radiation should be considered for pink bollworm that would be used in sterile insect releases and doses as low as 5-10 krak should be tested. Flint *et al.* [4] reported that moths treated with 10 krad were more effective in suppressing the native population than moths treated with 20 krad.

In pink bollworm, *Pectinophora gossypiella* (Saunders), egg production was reduced when female moths were mated with irradiated males [5]. Females mated with irradiated male showed propensity for multiple mating [2]. This indicated that irradiated male moths did not elicit normal reproductive behaviour. Bartlett *et al.* [6] reported that irradiation of pink bollworm eggs did not yield competitive adults. The work on the effect of gamma radiation on the larvae of pink bollworm particularly using two different gamma radiation sources i.e. Co-60 (1.33 Mev.) and Cs-137 (0.66 Mev.) has been lacking. Therefore, the present studies were undertaken to determine the comparative radiation effects of two gamma radiation sources on mature larvae of pink bollworm and their subsequent P_1 and F_1 progenies.

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Materials and Methods

Pink bollworm larvae were reared on wheat germ medium [7] to build up a homogenous laboratory stock of larvae to determine the effect of radiation on the reproduction in the resulting adults and their progeny. We used the Phoenix APHIS strain of pink bollworm moths from the USDA, APHIS Pink Bollworm Mass Rearing Facility, Phoenix, Arizona.

Effect of radiation source on mature larvea and subsequent P_1 and F_1 progeny. Mature (cut out) larvae obtained from the laboratory culture were irradiated at a dose of 35 Gy in a Co-60 gamma cell irradiator (does rate 35 Gy/min.) and a Cs-137 Gammator M at the dose rate of 22.58 Gy/min. to compare the effect of radiation source on mature larvae. The time to pupation and survival of larvae to pupation were recorded.

Upon pupation, the pupae were sexed and crossed in the following combinations in oviposition cages as described by Bartlett and Wolf (7).

Treated males (TM) x untreated females (UTF).
Treated males (TM) x treated females (TF).
Untreated males (UTM) x treated females (TF).
Untreated males (UTM) x untreated females (UTF) check.

Each combination was comprised of 8 sigle pairs per replicate and each test was replicated thrice. Total egg production, number of eggs hatched and number of mature larvae were recorded for each mating.

The F_1 adults emerging from each P_1 cross of Cs-137 and Co-60 treated moths were crossed separately to determine the effect of the source of irradiation to the larval stage on fecundity, fertility and number of mature larvae/female of the F_1 progeny. The crosses made were as follows:

F_1 M x UTF (Untreated female)

F_1 M x F_1 F

UTM (Untreated male) x F_1 F

UTM x UTF (check)

Where F_1M and F_1 F stand for male and female moths respectively obtained from P_1 irradiated as mature larvae.

The eggs were collected from different crosses for 14-days. There were 10 pairs in each treatment with three replications.

Another set of experiment was conducted to study the effects of irradiation on mature larvae by Cs-137 alone at doses ranging from 40-65 Gy with an increment of 5 Gy. Other procedures to evaluate the effects on subsequent P_1 and F_1 generations following irradiation of larvae were same as described earlier. The data was subjected to analysis of variance and chi square where appropriate.

Results and Discussion

Effect of radiation sources on mature larvae and subsequent generations. The results of irradiation on pupation of mature pink bollworm larave exposed to 35 Gy from Co-60 and Cs-137 sources respectively (Table 1) indicated that pupation was delayed in both cases. Furthermore, larval survival to the pupal stage was also reduced significantly by gamma radiation, however, Co-60 irridiation was significantly more lethal (66% pupation) than Cs-137 (81% pupation) as compared with control (96% pupation).

The effect on reproduction (fecundity) in P_1 moths following larval irradiation for the two different sources of radiation (Table 2) showed that egg production was drastically reduced in crosses TM x TF when compared with untreated moths (check). The fecundity of the treated female when confined with untreated male was significantly less than untreated female crossed with treated male either with Co-60 or Cs-137 gamma radiation. Hence female moths were comparatively more affected by gamma radiation than the males. The larvae from the corsses UTM x TF of Co-60 radiation failed to mature which further confirmed the high sensitivity of female moths to radiation. The fertility of the adults was lower in crosses involving treated females than in crosses with untreated ones, and this reduction was more obvious in Co-60 treated females. The percentage of mature larvae hatched from the eggs was also reduced more when treated females were paired either with normal or treated males in Co-60 or Cs-137.

Egg production, fertility and number of mature larvae/female of F_1 progeny obtained from adults following larval irradiation with Co-60 and Cs-137 were affected significantly

TABLE 1. EFFECTS ON PUPATION FOLLOWING IRRADIATION OF MATURE LARVAE AT 35 GY WITH CO-60 AND CS-137.

Source of irradiation	No.of mature larvae irradiated	No.of days to pupation	No. of pupae	Percent pupation**
Cs-137	263*	5	212	81b
Co-60	256*	7	170	66 c
Check(no treatment)	235	3	225	96 a

* Pupation was delayed; ** Significant at 5% level.

TABLE 2. EFFECT OF LARVAL IRRADIATION AT 35 GY (CO-60 AND CS-137 SOURCES) ON EGG PRODUCTION, FERTILITY AND LARVAL SURVIVAL OF P_1 GENERATION.

Crosses	Mean number of eggs laid/female	Fertility (% of control)	Mature larvae (% of control)
Cs-137 IRRADIATION			
UTM x UTF(Check)	129.9 a	100.00 a	100.00 a
TM x UTF	118.3 b	74.14 b	97.14 a
TM x TF	21.9 c	31.03 c	37.71 c
UTM x TF	20.9 c	65.52 b	57.14 b
Co-60 IRRADIATION			
UTM x UTF(check)	129.9 a	100.00 a	100.00 a
TM x UTF	95.4 b	65.52 b	77.14 b
TM x TF	2.1 c	39.65 c	0.00 d
UTM x TF	4.0 c	29.31 c	37.26 c

Each figure is an average of three replicates with 8 pairs/replicate; Means with same letters are statistically non-significant ($P \geq 0.05$).

TABLE 3. EGG PRODUCTION, HATCH PERCENTAGE AND LARVAL SURVIVAL OF F_1 PROGENY OBTAINED FROM ADULTS EMERGED FROM LARVAL IRRADIATION WITH CS-137 AND CO-60 IRRADIATORS (35 GY).

Radiation source	Crosses	Total eggs laid a/	Fertility (% of control)	No.of mature larvae/female
Cs-137	UTM x UTF (check)	114.6 c	100.00 a	14.95 a
	F_1M x F_1F	2.1 d	0.00 c	0.00 c
	F_1M x UTF	161.4 b	8.7 b	1.86 b
	UTM x F_1F	194.7 a	15.8 b	3.32 b
Co-60	UTM x UTF (check)	114.6 b	100.0 a	14.95 a
	F_1M x F_1F	0.0 d	0.0 c	0.00 c
	F_1M x UTF	35.7 c	35.1 b	1.93 b
	UTM x F_1F	221.3 a	42.1 b	15.93 a

a/ Each figure is an average of 3 replicates with 10 pairs/replicate; Means with same letters are statistically non- significant ($P \geq 0.05$).

by source of radiation (Table 3). The egg production was significantly reduced by Cs-137 radiation in crosses $F_1M \times F_1F$ whereas eggs failed to hatch and complete sterility was recorded in Co-60 radiation treatment. Untreated females paired with F_1 males of Cs-137 treated larave laid significantly more eggs than untreated (check) females, however, hatch percentage was drastically reduced. Complete sterility was recorded in crosses $F_1M \times F_1F$ in both the radiation treatments from Co-60 and Cs-137. The number of mature larvae/female was also reduced in all the crosses involving F_1 adults from treated crosses.

Effect of Cs-137 radiation on mature larvae and subsequent generations. Larval survial and adult emergence was dose dependent following irradiation of mature pink bollworm larvae at various doses of gamma radiation from Cs-137 (Table 4). Larval survival was reduced as the doses of gamma radiation increased. Females were significantly more susceptible to radiation than males. Structural deformities such as crumpled wings, twisted legs and reduced body size at 40 Gy and above doses were observed.

Fecundity, percent mature P_1 progeny and percent of male progeny in the F_1 generation following larval irradiation (Table 5) was dose dependent. As the dose to mature larvae increased, average egg production and percent mature progeny decreased. Egg production was reduced more drastically in the cross UTM x TF than the corss TM x UTF. The sex ratio was skewed in favour of males in TM xUTF crosses at all doses except 40 Gy. Complete sterility was recorded when treated males were paired with treated females at all the doses tested and F_1 moths were not available for this cross at 45 Gy and higher doses.

The results on egg production, number of mature progeny/female and percent males in the F_2 generation obtained from the progeny of F_1 moths are presented in Table 6. Egg production was reduced significantly in crosses UTM x F_1 F at both the radiation doses, however, 45 Gy was significantly more lethal to F_1 females than 40 Gy. The effect of larval irradiation on mature progeny of F_1 adults was also reduced considerably in corsses involving either F_1 males or females. The number of F_2 males was less than the F_2 females in all crosses except UTM x F_1 F at 45 Gy, however, a consistent effect is not apparent.

The effects of radiation sources on the mature larvae and resulting P_1 and F_1 generations were significantly more lethal when exposed to Co-60 than Cs-137 source. This is due to higher energy and penetration power of gamma rays from Co-60 (1.33 Mev.) than Cs-137 (0.66 Mev.) which caused more lethal effects. Esbaide *et al.* [8] reported that Co-60 was more effective than accelerated electrons against pupae and adults of *Sitophilus* spp. infesting maize.

TABLE 4. EFFECT OF DIFFERENT RADIATION DOSES ON LARVAL SURVIVAL AND ADULT EMERGENCE FOLLOWING CS-137 IRRADIATION OF LARVAE OF PINK BOLLWORM. DATA RECORDED AFTER 17 DAYS FOLLOWING IRRADIATION OF MATURE LARVAE.

Does Gy)	Total larvae irradiated		No.of dead larvae		No. of adults emerged Male		Female	
	Male	Female	Male	Female	Normal	Deformed	Normal	Deformed
0	89	178	2	4	81	6	170	4
40	65	69	12	22	37	16	25	22
45	88	66	56	37	15	17	11	18
55	48	53	39	32	7	13	2	8
65	60	42	56	38	3	10	1	3
			$X^2 = 0.892$		$X^2 = 35.22$		$X^2 = 113.62$	

TABLE 5. FECUNDITY, SEX RATIO AND LARVAL SURVIVAL OF ADULTS OF P_1 GENERATION FOLLOWING LARVAL IRRADIATION (CS-137).

Dose (Gy)	Crosses	X Eggs/ female	Mature progeny (% of control)	% males in F_1
0	UTM x UTF	136.7 b	100.00 a	53.68 b
40	TM x UTF	98.6 c	35.57 b	50.94 b
	TM x TF	0.0	0.00	0.00
	UTM x TF	2.0 e	0.67 e	0.00
45	TM x UTF	149.8 a	16.11 d	58.33 b
	TM x TF*	0.0	0.00	0.00
	UTM x TF	4.2	0.00	0.00
55	TM x UTF	95.5 c	28.19 c	76.19 a
	TM x TF*	0.0	0.00	0.00
	UTM x TF	0.0	0.00	0.00
65	TM x UTF	37.7 d	12.75 d	57.89 b
	TM x TF*	0.0	0.00	0.00
	UTM x TF	0.0	0.00	0.00

* Moths were not available. 10 pairs/mating in each cross replicates thrice; Means with same letters are statistically non-significant ($P \geq 0.05$).

TABLE 6. EGG PRODUCTION AND ADULT PROGENY OF F_1 MOTHS OBTAINED FROM P_1 MALE PARENTS IRRADIATED AS MATURE LARVAE.

Dose (Gy)	Crosses	X Eggs/ female	No. of mature progeny/female	% Males in F_2
0	UTM x UTF*	136.70 a	13.6 a	53.68 b
40	F_1M x UTF**	86.60 c	10.8 a	50.15 bc
	F_1M x *F_1F*	46.75 d	4.7 b	45.74 c
	UTM x F_1F**	50.13 d	6.6 b	53.53 b
45	F_1M x UTF+	103.10 b	9.9 a	50.50 b
	UTM x F_1 F+	3.20 e	0.8	62.5 a

+ One replicate; * Average of two replicates; ** Average of three replicates; NB. Each replicate had 10 pairs. 'Means with same letters are statistically non-significant ($P \geq 0$).

The doses of gamma radiation tested against pink bollworm larvae to induce sterility in the resulting adults and their

F_1 progeny had significant effect on pupation, adult susceptibility to radiation and reproduction of adults. Bartlett and Lewis [9] irradiated last instar (cut out) larvae at doses of 2-32 krad of Co-60 gamma radiation and none of the doses tested had a significant effect on pupation of the treated larvae but the percentage of morphologically normal adults was reduced by more than 85% when doses exceeded 4 krad for the males or 2 krad to the females.

The reproductive ability of the F_1 progeny from treatments of 35 Gy was reduced significantly when compared control (untreated) moths. These results regarding the inherited sterility are in close conformity to those reported by Bartlett and Lewis [9].

Complete sterility was recorded when treated females were paired with either treated or untreated males indicating the higher sensitivity of females to radiation at larval stage. However, when adult males from treated larvae were mated to untreated females, the fecundity and fertility decreased with the increased doses. Bartlett and Lewis [9] reported that no reproduction occurred in any cross involving treated insects if the dose to either mate exceeded 5 krad. In the present studies females treated with 45 Gy laid a few eggs which failed to hatch. This indicated that oogenesis was drastically affected by gamma radiation. However, Bartlett and Lewis [9] reported no reproduction if the female of a cross was treated at any dose exceeding 2 krad.

The results on the reproduction of adults resulting from the irradiated larvae showed that adult from 35, 40 and 45 Gy performed mating well (spermatophore transfer), however, fecundity and fertility reduced significantly to produce F_1 progenies. Therefore, such higher doses are not feasible for use in the control of pink bollworm through F_1 sterility technique.

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