

Original Research Paper

Standardisation of gamma irradiation dose for Sterile Insect Technique to manage South American tomato moth [*Phthorimaea (Tuta) absoluta* (Meyrick)]

Sridhar V.^{1*}, Rami Reddy P.V.¹, Vidyashree L.K.¹, Sree Chandana P.¹
and Hadapad Ashok B.²

¹ICAR-Indian Institute of Horticultural Research, Bengaluru - 560089, India

²Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai - 400085, India

*Corresponding author Email : Sridhar.V@icar.gov.in

ABSTRACT

Laboratory studies were conducted to standardise optimum dose of gamma irradiation to induce sterility in males of South American tomato moth [*Phthorimaea (Tuta) absoluta* (Meyrick)], a major pest on tomato. Among the different irradiation doses tested, 150 Gy was found to be effective to induce male sterility in *P. absoluta*, where sterile males exhibited mating competitiveness, significantly reduced fecundity and hatchability in F₁ and F₂ generations. Five days old pupae were found ideal for exposing to gamma irradiation to induce male sterility in *P. absoluta* that could be used for area wide management using SIT.

Keywords : Gamma-radiation, IPM, *Phthorimaea absoluta*, sterile insect technique, tomato

INTRODUCTION

Sterile Insect Technique (SIT) is a method of pest control involving area-wide inundative release of sterile insects to inhibit reproduction in field population of same species (FAO, 2007). SIT involves mass rearing of target insect species and then exposed to different radiation (X-rays, gamma radiation) making the insect sterile. The sterile insects are then released in the field, which mate with same species of wild population resulting in infertile offspring. In order to release insects in a SIT programme, different amounts of sterility might be inflicted upon them. Moths and butterflies (Lepidoptera) exhibit several peculiar cytogenetic and cytological characteristics that distinguish them from other insects causing high resistance of ionizing radiation. These play crucial role in the mechanism of inherited sterility (IS). The characteristics include female heterogamety, holokinetic structure of chromosomes and dichotomous spermatogenesis. Thus, it is suggested to apply sub-sterilizing doses in lepidopterans as it showed better competitiveness and also transmit sterility to F₁ progeny higher than the parents.

After the first report of the incidence of *P. absoluta* from Karnataka, India (Sridhar et al., 2014), it has further spread to different tomato growing areas of the country (Hadapad & Hire, 2019) and had significantly impacted the tomato production. There are many

strategies advocated for the management of *P. absoluta*. But farmers practice of overreliance on insecticides is resulting in environmental risks combined with resistance development due to its strong reproductive potential and short generation cycle (Sridhar et al., 2019). As an alternative, SIT can be used for the management of *P. absoluta* on tomato, which can be integrated with other IPM tools for its eco-friendly management. Research on SIT for lepidopteran pests is scanty in India and hence the present study was conducted to standardise the age of pupa and gamma irradiation dose required to induce male sterility in *P. absoluta*.

MATERIALS AND METHODS

Nucleus culture of *Phthorimaea absoluta* was collected from the tomato fields of ICAR-Indian Institute of Horticultural Research, Bengaluru and reared in the tomato based semi synthetic diet.

Exposing pupae of *P. absoluta* to gamma irradiation

Two and five days old pupae were exposed to five gamma irradiation at 100, 125, 150, 200 and 300 Gy doses in completely randomised block design with three replications each containing 100 male pupae and a control without any irradiation during 2021 to 2023. Extent of healthy and malformed adult emergence after irradiation of two and five days old pupae was



recorded. The data was subjected to ANOVA, after suitable statistical transformation.

From the emerged adults, two irradiated and untreated males were mated with each of the wild female (2:1 ratio) and were transferred to wooden ‘oviposition cages’ (105×70×95 cm) having tomato plants (cv. Arka Rakshak), provided with honey swab (50%) as food for the moths. The caged plants were replaced with new plants at three days intervals and were examined for number of eggs laid by a female. On hatching, larvae were reared on semi-synthetic diet. Pupae were further used for biological studies to determine the effect of irradiation or to continue mass rearing. The data on different parameters like adult emergence (%), adult malformation, adult longevity, fecundity when mated with untreated female, larval period and pupal period were recorded.

Effect of gamma irradiation on F₁ and F₂ progeny’s egg laying and hatching

After ascertaining the effective dose of gamma irradiation (150 Gy), in order to assess the hereditary impact of sterility on egg laying and hatching on F₁ and F₂ generations, a separate laboratory experiment was carried out with different cross combinations of FM (fertile male), IM (irradiated male), WF (fertile female, wild) and IF (irradiated female). Six replicates (petri dishes) were used for each cross combination. Various combinations included were IF x FM, WF x IM and WF x FM. In

both generations, female fecundity and eggs hatched were observed and subjected to ANOVA.

RESULTS AND DISCUSSION

Effect of gamma radiation on two days old pupae of *P. absoluta*

The lowest adult emergence was observed with 300 Gy (33.34%) and highest in 100 Gy (60.12%) as against 93.34% in control. The deformity ranged from 6.7% to 22.7% within the doses and no deformity was observed under control. Longevity of adults ranged 3.03±0.11 to 5.3±0.26 days at 300 and 100 Gy, respectively, as against 7.27±0.20 days in control (Table 1). Adult emergence and longevity decreased with increased doses, while, extent of deformity enhanced. Highest adult emergence and deformity (60.12% and 22.70%) was recorded in lowest dose (100 Gy) and highest dose (300 Gy), respectively.

There was a differential response in terms of larval and pupal period of *P. absoluta* within the doses (Table 1). The larval period decreased with the increase in the doses, while, there was an increase in pupal period. Up to 58.6% pupation was observed in 150 Gy dose. The lowest larval period was observed in 300 Gy (4.67 days) and highest in 100 Gy (10.34 days) as against 11.2 days in control. Pupal period ranged 7.34 days (100 Gy) to 10.6 days (300 Gy) as against 6.34 days in control. However,

Table 1 : Effect of gamma radiation doses on two days old pupae of *P. absoluta*

| Gamma irradiation (Gy) | Adult emergence* (%) | Deformity* (%) | Longevity of adult male) (days)#^ | Larval period (days)#^ | Pupation* (%) | Pupal period (days)#^ |
|------------------------|----------------------|------------------|-----------------------------------|------------------------|------------------|-----------------------|
| 100 | 60.12 (50.84) | 6.70 (15.02) | 5.30 (2.31) | 10.34 (3.22) | 62.60 (52.32) | 7.34 (2.71) |
| 125 | 57.35 (49.23) | 13.40 (21.48) | 5.06 (2.25) | 9.60 (3.11) | 60.78 (51.23) | 7.67 (2.77) |
| 150 | 56.25 (48.59) | 14.70 (22.56) | 4.94 (2.22) | 7.70 (2.77) | 58.60 (49.94) | 7.90 (2.81) |
| 200 | 50.67 (45.32) | 17.40 (24.67) | 3.5 (1.87) | 6.20 (2.51) | 50.67 (55.39) | 8.60 (2.94) |
| 300 | 33.38 (35.30) | 22.70 (28.48) | 3.03 (1.74) | 4.67 (2.16) | 38.60 (38.42) | 10.60 (3.23) |
| Control | 93.45 (75.24) | 0.00 | 7.27 (2.70) | 11.20 (3.35) | 84.40 (66.75) | 6.34 (2.52) |
| CD @ 1% | 2.19 | 0.59 | 0.11 | 1.61 | 1.03 | 3.35 |
| CV % | 1.73 | 1.91 | 1.92 | 0.12 | 0.79 | 0.24 |

^ mean of three replications; * figures in parenthesis are *arc sin* values; # figures in parenthesis are square root values

pupation ranged from 38.6% (300 Gy) to 62.6% (100 Gy) as against 84.4% in control.

Effect of gamma irradiation on five days old pupae of *P. absoluta*

Gamma irradiation of five days old pupae caused a reduction in adult emergence percentage. The adult emergence was significantly decreased as dose increased *i.e.* 66.60, 58.70, 57.34, 53.34 and 31.34% at 100, 125, 150, 200 and 300 Gy, respectively. Per cent malformed adult moths were 11.67, 13.40, 14.70, 16.43 and 18.70 with 100, 125, 150, 200 and 300 Gy (Table 2 and Fig. 1), respectively, as against no malformed adults in the control.

The lowest adult emergence was observed in irradiated doses of 300 Gy (31.34%) and highest in 100 Gy (66.6%) as against 93.34% in control (Table 2). Deformity ranged from 6.7% to 22.7% in 300 Gy and 100 Gy, respectively. Longevity of adults ranged from 3.17 days (300 Gy) to 5.6 days (100 Gy) as against 7.65 days in untreated control (Table 2).

Effect of irradiation doses on larval period, pupal period and per cent pupation

The study revealed similar results to that of two days old pupae by following decreased and increased trend

of larval and pupal period with increase in doses. About 60.80% pupation was observed in 150 Gy (Table 2).

Lowest larval period was observed in 300 Gy (4.90 days) and highest in 100 Gy (10.34 days), as against 11.34 days in control. Pupal period ranged from 7.60 days (100 Gy) to 10.70 days (300 Gy) as against 6.34 days in control. Pupation and larval period had decreased with increase in the dose of gamma radiation, where, pupation ranged from 38.67% (300 Gy) to 62.7% (100 Gy) as against 84.34% in control (Table 2).

Age and gamma radiation dose

Treating five days old *P. absoluta* pupae with 150 Gy was more desirable than two days old pupae in terms of mating competitiveness (laboratory observation), though the impacts are comparable in terms of different biological parameters. The F₁ generation of 150 Gy treated population had shown superior effect on its biological cycle, where egg laying was 78.33/ female out of which 51.33 larvae hatched, of them 31.67% entered pupation and 26.26 pupae emerged into adults, indicating that the treatment 150 Gy was highly suitable for SIT for *P. absoluta* management (Table 3). These findings are in line with Fezza et al. (2021) who observed that irradiation of older pupae

Table 2 : Effect of different gamma radiation doses on five days old *P. absoluta* male pupae

| Gamma irradiation (Gy) | Adult Emergence* (%) | Deformity* (%) | Longevity of adult male) (days)#^ | Larval period (days)#^ | Pupation* (%) | Pupal period (days)#^ |
|------------------------|----------------------|------------------|-----------------------------------|------------------------|------------------|-----------------------|
| 100 | 66.60 (54.71) | 11.67 (19.96) | 5.60 (2.37) | 10.34 (3.21) | 62.70 (52.35) | 7.6 ± 0.69 (2.76) |
| 125 | 58.70 (50.02) | 13.40 (21.44) | 4.5 (2.10) | 9.34 (3.06) | 61.30 (51.52) | 7.34 ± 0.19 (2.71) |
| 150 | 57.34 (49.23) | 14.70 (22.56) | 4.27 (2.06) | 9.10 (3.02) | 60.80 (51.25) | 8.6 ± 0.34 (2.93) |
| 200 | 53.34 (46.92) | 16.43 (23.91) | 3.76 (1.92) | 6.50 (2.56) | 53.50 (47.02) | 9.34 ± 0.19 (3.06) |
| 300 | 31.34 (34.05) | 18.70 (25.61) | 3.17 (1.78) | 4.90 (2.22) | 38.67 (38.46) | 10.7 ± 0.75 (3.28) |
| Control | 87.34 (69.18) | 0.00 (0.00) | 7.65 (2.77) | 11.34 (3.37) | 84.34 (66.73) | 6.34 ± 0.25 (2.52) |
| CD @ 1% | 1.58 | 1.25 | 0.04 | 0.09 | 1.03 | 0.14 |
| CV % | 1.25 | 4.01 | 0.69 | 1.25 | 0.81 | 1.94 |

^mean of three replications; * figures in parenthesis are arc sin values; # figures in parenthesis are square root values

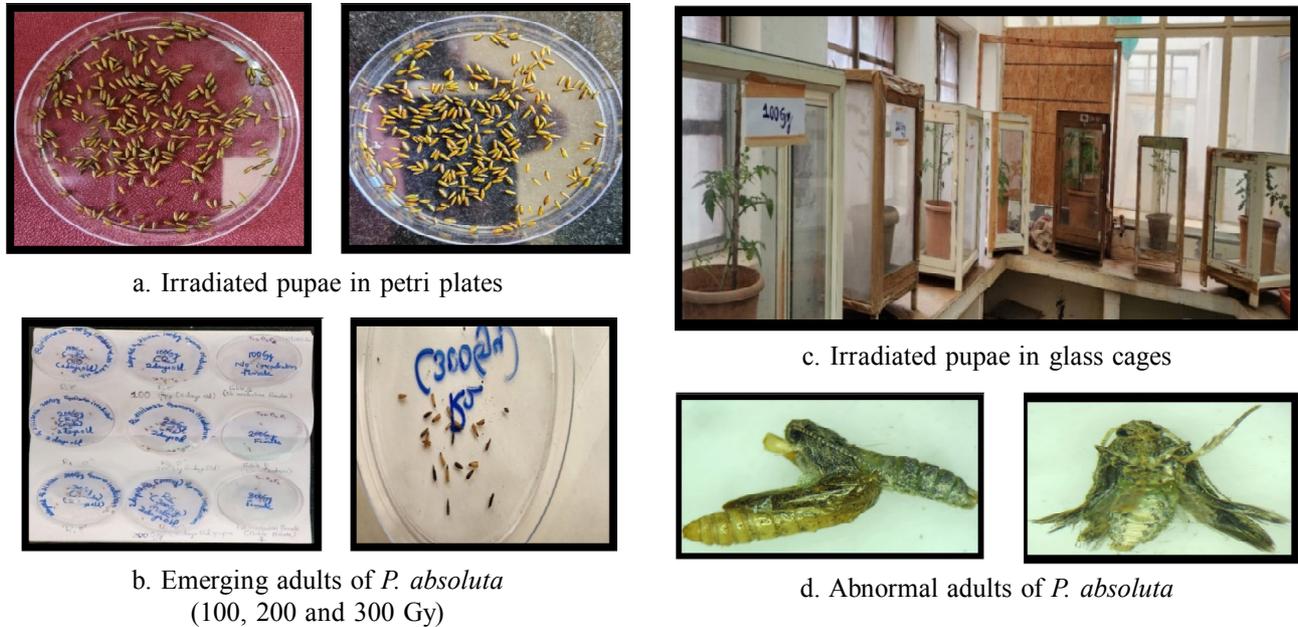


Fig. 1 : Experimental material and abnormal adults of *P. absoluta* after gamma radiation

Table 3 : Effect of gamma radiation doses on F₁ egg laying, hatching and adult emergence

| Gamma irradiation (Gy) | No. of eggs# / female | No. of eggs hatched# / female | Hatching* (%) | No. of larvae# pupated/ female | Pupation* (%) | Adult emergence* (%) |
|------------------------|-----------------------|-------------------------------|---------------|--------------------------------|---------------|----------------------|
| 100 | 97.67 (9.88) | 75.33 (8.68) | 77.13 (61.44) | 67.67 (8.23) | 69.28 (56.34) | 58.70 (50.01) |
| 125 | 87.33 (9.35) | 64.67 (8.04) | 74.05 (59.39) | 51.33 (7.16) | 58.78 (50.06) | 47.33 (43.48) |
| 150 | 78.33 (8.85) | 51.33 (7.16) | 65.53 (54.05) | 31.67 (5.63) | 40.43 (39.49) | 34.04 (35.69) |
| 200 | 64.33 (8.02) | 32.67 (5.72) | 50.78 (45.45) | 21.67 (4.66) | 33.68 (35.47) | 26.94 (31.28) |
| 300 | 40.33 (6.35) | 17.67 (4.20) | 43.80 (41.44) | 9.33 (3.05) | 23.14 (28.75) | 16.53 (23.99) |
| Control | 194.67 (13.95) | 163.67 (12.79) | 84.08 (66.50) | 155.33 (12.46) | 79.79 (63.29) | 70.55 (57.13) |
| CD @ 1% | 0.14 | 0.18 | 1.35 | 0.15 | 1.06 | 0.65 |
| CV % | 0.71 | 1.14 | 1.21 | 1.06 | 1.15 | 0.79 |

* figures in parenthesis are arc sin values; # figures in parenthesis are square root transformed values

resulted in healthy adult with good flying ability and mating competitiveness. Various stages of *P. absoluta* and their durations observed were presented in Table 3.

Effect of gamma radiation doses on F₁ egg laying, egg hatching and emergence

Fecundity

Highest fecundity/female was observed with 100 Gy (97.67 eggs/female), followed by 125 Gy (87.33 eggs/

female), 150 Gy (78.33 eggs/female), 200 Gy (64.33 eggs/female) and lowest in 300 Gy (40.33 eggs/female) as against 194.67 eggs/female in control.

Egg hatching

The highest egg hatching and hatch was observed in 100 Gy (75.33 and 77.13%), followed by 125 Gy (64.67 and 74.05%), 150 Gy (51.33 and 65.53%), 200 Gy (32.67 and 50.78%) and lowest in 300 Gy (17.67 and 43.80%) as against 84.08% hatching in control.

Pupation

Maximum pupation was observed in 100 Gy (69.28%) followed by 125 Gy (58.78%), 150 Gy (40.43%), 200 Gy (33.68%), while, lowest pupation was recorded in 300 Gy (23.14%) as against 79.79% pupation in control.

Adult emergence

The *P. absoluta* adult emergence was lowest in highest dose of 300 Gy (16.53%) and was highest in lowest dose 100 Gy (58.70%), as against 70.55% in control.

Effect of gamma radiation on egg laying in F₁ and F₂ generations

Lowest number of eggs laying and hatching was observed in the cross where irradiated male was involved in both F₁ and F₂ (Table 4).

Among the doses (100 and 300 Gy) tested, 150 Gy was significantly effective with reduced fecundity and hatchability both in F₁ and F₂ generations (Table 4). Present findings are in line with Tate et al. (2007), where F₁ generation of Cactus moths (*Cactoblastis cactorum*) were the test insect. Similar results were found with Saour & Makee (1997), Carpenter et al. (2001) and Makee & Saour (2004) with different test insects. *Labesia botrana* adults responded to increasing doses of gamma radiation with a decline in female fecundity and male fertility. Walton & Conlong (2016) reported African sugarcane borer, *Eldana saccharina*, laboratory-reared irradiated females were significantly less fertile than males and almost completely sterile at 200 Gy. Based on studies, effective irradiation dose requirement was different in various target insect species which may be due to age of pupae, laboratory culture, fitness of the insect etc.

Comparing the results of *L. botrana* male fertility with those obtained from *Cydia pomonella* (Bloem et al., 2007) and *Thaumatotibia leucotreta* (Carpenter et al., 2007), *L. botrana* was more radio-resistant than these two tortricid species (400 vs 350 Gy to obtain full sterility). However, *L. botrana* was less resistant to radiation than the potato tuber moth, *P. operculella* (Saour & Makee 1997) and *C. cactorum* (400 vs 500 Gy) (Carpenter et al., 2001).

Successful application of SIT/F₁ in European grapevine moth was achieved by Saour (2014), by selecting a irradiation dose of 150 Gy to *L. botrana* resulting in ~61% reduction in fertility of irradiated males mated with unirradiated females and only 20% of eggs hatched for F₁ males that mated with unirradiated females. In the present study also, the males irradiated with 150 Gy were competitive in mating like normal adults and sterile males resulted in reduced fecundity and hatchability in both F₁ and F₂. The effects of irradiation and IS on the reproduction were similar to those described for other insect species of Lepidoptera, i.e. reduced survival of larvae, the delay in the developmental time from F₁ neonate to adult, and the shift of sex ratio in favor of males in the F₁ generation.

Horner et al. (2020) also reported that the development of inherited sterility by applying SIT against the codling moth, *C. pomonella* resulting in significant reduction in its population suppression. Simmons et al. (2021) implemented the SIT to suppress populations of the European grapevine moth, *L. botrana*. These studies supported the present findings of effective reduction through SIT applications which can be a part of IPM as well.

Table 4 : Influence of 150 Gy dose of gamma irradiation on 5 days old male pupae on egg laying and hatching in F₁ and F₂ of *P. absoluta*

| Cross Combination | Eggs/Female (Nos.) | | Mean eggs/Female ± SD (No.) | Hatched (Nos.) | | Mean eggs hatched ± SD (Nos.) |
|-------------------|--------------------|----------------|-----------------------------|----------------|----------------|-------------------------------|
| | F ₁ | F ₂ | | F ₁ | F ₂ | |
| IF x WM | 79.3 | 36.3 | 57.8 ± | 59.3 | 25.9 | 42.6 ±16.70 |
| WF x IM | 68.5 | 19.6 | 44.1 ± | 38.5 | 14.2 | 26.3±12.15 |
| WF x FM | 147.3 | 87.1 | 117.2± | 139.1 | 84.3 | 111.7±27.40 |
| SEM± | 0.83 | 0.54 | 0.68 | 0.75 | 0.80 | 0.78 |
| CD @ 1% | 1.76 | 1.43 | 1.59 | 1.68 | 1.73 | 1.70 |
| CV % | 0.84 | 1.56 | 1.22 | 0.96 | 2.16 | 1.56 |

IF: irradiated females, WF: wild female, IM: irradiated males, WM: wild males

It is worth noting that the fecundity of F_1 females obtained in present study from male parents irradiated with 150 Gy and mated with unirradiated females was not significantly different from that of irradiated as also reported by Makee & Saour (1997) and Bloem et al. (2003) in F_1 progeny of *P. operculella* and *C. leucotreta*, respectively.

The SIT-based programs have been especially successful against dipteran pests. However, the SIT applicability for controlling lepidopteran pests has been challenging mainly due to their high resistance to the ionizing radiation that is used to induce sterility (Marec and Vreysen, 2019). Releasing of sub sterile *P. absoluta* males in field cages at a 15:1 substerile (200 Gy-treated pupae) to untreated male ratio caused a significant decline in larval production as compared with that control (Cagnotti et al., 2016).

In almost all SIT sterility programs against lepidopterans, both males and females are mass-reared, irradiated, and then released into the targeted area because no practical method is available to separate the adult moths by gender (Bloem et al., 2007). Moreover, the irradiated moths are released continuously from the beginning of the season, thus the possibility of crosses involving 150 Gy-irradiated males with F_1 females and F_1 males with their female counterparts could occur. The results of the study showed that the fertility of unirradiated males crossed to F_1 females did not differ significantly from that of the cross between F_1 males mated with unirradiated females, which suggests that F_1 females inherited the deleterious effects from their irradiated male parents. High values of unhatched eggs and sterility index were obtained when F_1 males were mated with either F_1 or unirradiated females and when 150 Gy-irradiated males were mated with F_1 females.

CONCLUSION

Among the different doses of gamma radiation tested against male pupae of *P. absoluta*, 150 Gy was effective for inducing sterility, where sterile males resulted in reduced fecundity and hatchability in F_1 and F_2 . Five days old pupae of *P. absoluta* were more suitable for irradiation. These studies helps in evaluation of SIT under confined and area wide IPM.

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