

Effects of Gamma Radiation on Tomato Leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract—In present study, it was aimed to evaluate the gamma radiation impacts on tomato leaf miner at different biological stages. The laboratory colony of tomato leaf miner was used to set up the experiments. Different biological stages of the insects (eggs, 4th instars and pupae) were irradiated using Cobalt-60 at doses of 0 (control), 100 Gray (Gy), 200 Gy, 300 Gy and 400 Gy in Cos-44HH-N source, at dose rate of 480 Gy/h. After irradiation, the eggs were incubated until hatching; the mature larvae were reared to complete their developments. Adult emergences from irradiated pupae were also evaluated. The results showed that there were no egg hatching at all tested irradiation doses. Although, the pupal percentages of irradiated mature larvae were 54%, 15% and 8% at doses of 100 Gy, 200 Gy and 300 Gy respectively, there were no adult emergences from irradiated mature larvae. On the other hand, the adult emergences were observed from irradiated pupae, decreased as radiation doses increased along with malformed adult appearance. Male and female individuals were out crossed with laboratory reared adults. Fecundity was correlated with radiation doses.

Keywords—Irradiation, tomato, tomato leafminer, *Tuta absoluta*.

I. INTRODUCTION

THE tomato plant, *Solanum lycopersicum* L., belongs the Solanaceae family, has many pests and diseases during the growing season in the open production areas or in green houses. Tomato plant is known to be originated in Central and South America then distributed throughout the world. One of the most important pests of tomato is the tomato leaf miner or tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) [1]. The pest is originally from South America [2], [3] distributed in Peru, Bolivia, Brazil, Chile, Colombia, Ecuador, Paraguay, Uruguay, Argentina, Venezuela, then now reported in many countries in Europe, in Spain, Italy, France, Holland and Greece [4], [5] also in the Mediterranean Basin [6], in Morocco, Algeria and Tunisia. Tomato leaf miner is an invasive pest in Turkey, reported first in 2009 in Izmir (Urla and Çeşme) and in Çanakkale (Batakovaşı) [7], [8].

The main larval host of tomato leaf miner is tomato, *Solanum lycopersicum*; but, it also feeds on other Solanaceae [9] such as potato (*Solanum tuberosum*), eggplant (*Solanum melongena*) and pepper (*Capsicum* spp.). Another larval host is not cultivated Solanaceae such as, *Solanum nigrum* L. Other hosts are the common bean (*Phaseolus vulgaris* L.) belonging

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to Fabaceae family [10] and some other non-cultivated hosts belonging to Convulvaceae and Chenopodiaceae, Amaranthaceae and Poaceae families [11]-[14].

The tomato leaf miner is present all year around in appropriate climate conditions [15]. The damage is mainly caused by larvae mining on leaves, fruits, buds and stems, thus affecting the photosynthesis and causing 50-100% of yield reduction on tomatoes [16].

There are many strategies for managing the tomato leaf miner in Integrated Pest Management program but mainly the control is based on the broad spectrum insecticides. Several insecticide applications are necessary to reduce tomato leaf miner population in each growing season and this issue may cause appearance of insecticide-resistant field population. Furthermore, insecticide-resistant problems are already reported in Brazil [16] and Argentina [17].

Sterile Insect Technique (SIT) is an alternative control method requiring mass rearing of the species, then application of sterilization (X rays, gamma radiation or chemosterilants) and releasing of sterile insects. It is expected that the released sterile insects mate with species' wild population causing infertile offspring (sterile individuals) and reducing the pest population. SIT has been previously used in many Lepidopteran pests such as the codling moth, *Cydia pomonella* (L.) and the pink bollworm, *Pectinophora gossypiella* (Saunders) [18].

The effects of gamma radiation in moths have been widely studied on *Chilo infuscatellus* Snellen [19], *Chilo auricilius* Dudgeon [20], *Diaphania nitidalis* (Stoll) [21], *Diatraea grandiosella* Dyar, *Eoreuma loftini* [22] *Amyelois transitella* (Walker) [23]. Sterilization effects on *Tuta absoluta* were reported previously in different countries [24], [25].

The objective of the present study is to determine the effects of gamma radiation on different biological stages of laboratory reared the tomato leaf miner.

II. MATERIALS AND METHODS

The initial population of the tomato leaf miner was started by removing about 30-40 adults from a laboratory colony in Insect Molecular Biology Laboratory, at Çanakkale Onsekiz Mart University, Çanakkale, Turkey. Freshly cut tomato leaves were provided for egg laying in adult rearing cages with 10% sugar solution which is dispensed on cotton balls. The eggs were laid individually on tomato leaves in cages then the leaves were coiled with moist cotton (Fig. 1 (a)) and placed in Tupperware® containers (30 × 18 × 7 cm) for egg hatching and larval development (Fig. 1 (b)). Pupae were transferred to adult rearing cages (30 × 17cm) for adult

emergence (Figs. 1 (a), (c)). The colony was maintained at 22 ± 1 °C, 65% RH and 16:8h L:D in the controlled laboratory conditions.



Fig. 1 Laboratory colony of tomato leafminer (a) removing eggs from adult cage, (b) larval development on tomato leaves, (c) pupae transferred to adult cages

The different biological stages of the tomato leafminer (eggs, 4th instars and pupae) were obtained from the laboratory reared colony. The newly laid eggs were collected (n=10) and placed in a sealed 60 x 15mm plastic petri dish (Fig. 2 (a)). The mature larvae (4th instars) were collected with soft forceps by checking the damaged leaves (n=7) (Fig. 2 (b)) and the pupae were also harvested (n=8) from the laboratory colony (Fig. 2 (c)). The experiments were conducted with three replications for each application dose of gamma radiation.

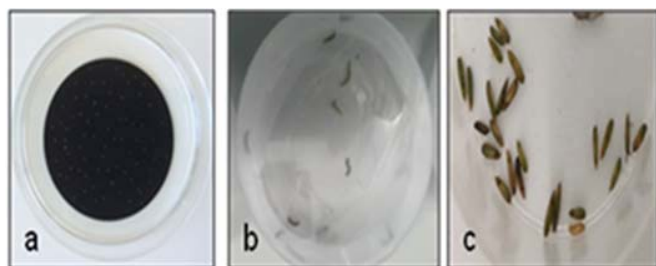


Fig. 2 Different biological stages of tomato leafminer (a) eggs, (b) 4th instars, (c) pupae



Fig. 3 A view of Cobalt-60 in Cos-44HH-N source in TAEK

The gamma radiation was applied in a laboratory of the Center of Sarayköy Nuclear Research and Education of Turkish Atomic Agency Authority (TAEK) in Ankara, Turkey. The tomato leaf miners were irradiated using Cobalt-60 in Cos-44HH-N source, at dose rate of 480 Gy/h (Fig. 3). The eggs and larvae were irradiated at doses of 0 (control), 100 Gy, 200 Gy, 300 Gy. The pupae were irradiated at doses of 0 (control), 100 Gy, 200 Gy, 300 Gy and 400 Gy. After treatment, the eggs were examined daily under Olympus SZX9 stereozoom microscope until hatching. The irradiated larvae were reared to complete their developments and irradiated pupae were monitored until adult emergence. Observations were carried out to determine the percentage of egg hatching, larval to pupal development, the number of pupae and the adult emergence. Adult fecundity was determined by outcrosses of irradiated female with normal males or irradiated male with normal female.

III. RESULTS AND DISCUSSIONS

The effect of gamma radiation on different biological stages of the tomato leaf miner was determined. The egg stages were irradiated with 100 Gy, 200 Gy and 300 Gy doses but there were no egg hatching at all tested irradiation doses. However, approximately 80% of the control eggs, without any treatment, were hatched in about 5.41 ± 1.42 days in the laboratory.

The effect of gamma radiation on mature larvae was indicated in Table I. Irradiated mature larvae survived about 2 to 10 days in the laboratory before pupation. Irradiated tomato leafminer larvae were pupated as 54% at the dose of 100 Gy. The percentages of pupation were 15% and 8% at doses of 200 Gy and 300 Gy respectively. However, pupal eclosions were not successful from irradiated mature larvae in all tested doses (Table I). Control individuals were pupated and pupal duration was 16.24 ± 1.51 days with the success of 58% pupal eclosion.

TABLE I
EFFECTS OF DIFFERENT RADIATION DOSES ON MATURE LARVAE (N= 13, MEAN±SD)

Doses (Gy)	Larval duration (days)	No. of Pupae	Mature larva to pupation (%)	Pupal duration (days)
Control	3.18±1.25	12	92	16.24±1.51
100	10.28±6.92	7	54	-
200	5.50±4.94	2	15	-
300	2.00 ±0.01	1	8	-

The effect of radiation doses on irradiated mature larvae was shown in Fig. 4. Some of the mature larvae after applied 200 Gy radiation were indicated some deformations such as weak pupation (Fig. 4 (a)) or swollen mature larva (Fig. 4 (b)). Although the larvae irradiated with 300 Gy were able to pupate, adult eclosion was not successful (Fig. 4 (c)). These might be caused by gamma radiation.

The effect of gamma radiation on irradiated pupae was indicated in Table II. Pupal durations were decreased as radiation doses increased as compared to the control. The percentages of adult emergence were 67, 42, 17 and 8 at doses of 100 Gy, 200 Gy, 300 Gy and 400 Gy respectively. The adult emergence was the lowest at the dose of 400Gy with

some problems in abdominal appearance and malformed wings (Fig. 5).

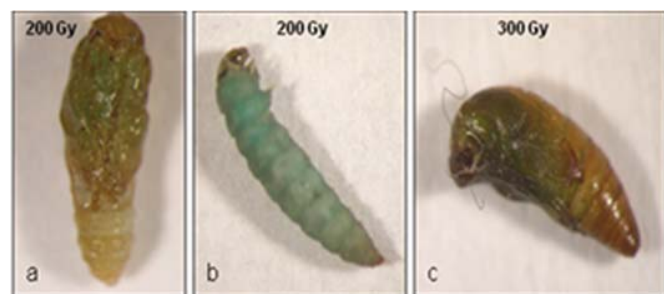


Fig. 4 Effects of radiation doses on irradiated mature larvae (a) deformed pupa, (b) larvae and (c) pupa not eclosed

TABLE II
EFFECT OF DIFFERENT RADIATION DOSES ON PUPAL FITNESS (N=8, EACH TREATMENT HAD 3 REPLICATIONS, MEAN±SD)

Doses (Gy)	Pupal duration (days)	Adult emergence (%)	No. of female pupa	No. of female moth	No. of male pupa	No. of male moth
Control	5.50±1.70	83	13	10	11	10
100	3.87±2.68	67	11	8	13	8
200	2.80±1.47	42	16	7	8	3
300	2.75±1.50	17	13	2	11	2
400	2.00±1.41	8	10	1	14	1



Fig. 5 A possible visible effect of 400Gy radiation dose on irradiated pupa, having malformed wings

The last abdominal segments of irradiated pupae were checked ventrally under the microscope to identify the individuals as female or male [26], [27]. The number of female and male pupae and their eclosions were reported in Table II. After irradiated moths were emerged (Table II), irradiated male and female moths were crossed with laboratory reared adult moths (Tables III, IV). The effect of different radiation doses on male moth fitness was shown in Table III. The fecundity was 77, 82 and 49 eggs at the doses of 100, 200 and 400 Gy respectively. The number of egg hatching was 60 and the percentage of egg viability was 78 on the dose of 100 Gy irradiated male. The number of egg hatching (24) and egg viability (29%) were decreased on the dose of 200 Gy (Table III). There was no mating success on 300 Gy irradiated males. However, 49 eggs were collected from 400 Gy irradiated male cage, there was no egg hatching (Table III).

TABLE III
EFFECTS OF DIFFERENT RADIATION DOSES ON MALE MOTH FITNESS

Doses (Gy)	Irradiated male (♂)	Wild female (♀)	No. of eggs (fecundity)	No. of eclosion eggs	Egg viability (%)
Control	4	2	97	82	85
100	4	2	77	60	78
200	3	2	82	24	29
300	-	-	-	-	-
400	1	2	49	-	0

The effect of different radiation doses on female moth fitness was shown in Table IV. The fecundity was 82 and 27 eggs with the percentages of 59 and 26 eggs viability on the doses of 100 and 200 Gy respectively. There was no mating success on the doses of 300 and 400 Gy irradiated females (Table IV).

TABLE IV
EFFECTS OF DIFFERENT RADIATION DOSES ON FEMALE MOTH FITNESS

Doses (Gy)	Irradiated female (♀)	Wild male (♂)	No. of eggs (fecundity)	No. of eclosion eggs	Egg viability (%)
Control	2	4	123	103	83
100	8	4	82	48	59
200	7	4	27	7	26
300	2	2	0	0	0
400	1	2	0	0	0

The effect of different radiation doses on tomato leaf miner pupa eclosion and adult fitness were shown in Fig. 6. When the pupae were irradiated at dose of 200Gy, the percentage of pupal eclosion was 42 and egg viability was 29 in irradiated male crosses and 26 in irradiated female crosses. The pupal eclosion was very low at the dose of 300 Gy and 400 Gy, so there were no eggs (Fig. 6).

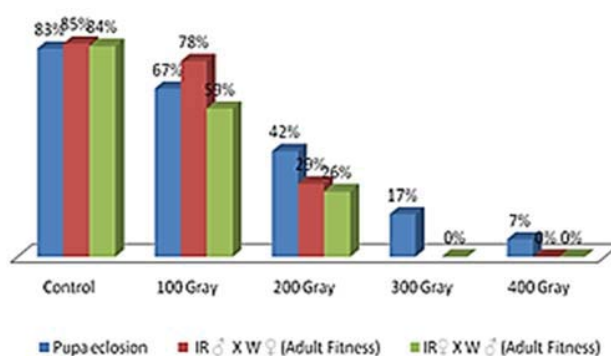


Fig. 6 Compared effects of different radiation doses on pupal eclosion and adult fitness

The SIT technique require the determination of the optimal radiation doses either x-ray or gamma that effectively cause sterility of individuals without any negative effects on their behavior, performance, mating frequency and mating success [19], [25], [26], [29]-[34].

Previous studies [24], [25] indicated that determination of sub-sterility dose for the biological stages is so crucial to avoid negative effects. As reported in this study, laboratory

reared females crossed with irradiated males (300 and 400 Gy) oviposit eggs that was resulted no embryonic development. This is correlated with studies on *Trichoplusia ni* [34], [35] and x-rays effects on *Tuta absoluta* [24] shown that irradiated males crossed with wild-type females resulting no eggs. However, radiation did not have any negative effects on male mating performans on *Cryptophlebia leucotreta* (100-300 Gy) [35], *Plutella xylostella* [37] and *Cactoblastis cactorum* (100-500 Gy) [31]. Moth females are known to be more sensitive to radiation than males. We also observed that females affected more from the same radiation dose than males. Radiation doses between 100 Gy and 300 Gy reduced adult fitness and emergence compared to the control, these results was correlated with previous studies in different moth species [25], [38]-[41]. Doses above 200 Gy suggested causing inherited sterility in males of tomato leaf miner. Dose of 200 Gy is recommended to be suitable for sterility of tomato leaf miner's females [24]. Another study on tomato leaf miner reported that 300 Gy was the lethal dose for pupae [25]. Therefore, the types of radiation source and application method are so crucial and make the difference between the studies.

Presented study indicated that there was a significant effect of gamma radiation on the different biological stages of the tomato leaf miner. However, we can conclude that 300 Gy and above doses were the lethal doses to pupal stages of tomato leaf miner, in order to understand and make sure about the sterility and lethal radiation doses on each biological stage, some additional doses need to be added in further studies.

IV. CONCLUSION

In this study, we reported the radiation effects on the eggs, 4th instars and pupae of tomato leafminer. The Cobalt-60 radiation source, Cos-44HH-N, was used and the application doses were 0, 100 Gy, 200 Gy, 300 Gy and 400 Gy with a dose rate of 480 Gy/h. Irradiated eggs were not hatched at all tested irradiation doses. Irradiated mature larvae were pupated with the percentages of 54, 15 and 8 at the doses of 100 Gy, 200 Gy and 300 Gy respectively, but there was no adult emergence from the irradiated larvae. When the pupae were irradiated, adult emergence was occurred with regard to the radiation doses. Irradiated male and female moths were crossed with laboratory moths. The fecundity and egg viability were also correlated with tested radiation doses.

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