

Sterile Insect Technique Against Date Moth, *Ectomyeloisceratoniae* Zeller 1881, in Tunisia: Research of Gamma Radiation Sterilising Dose

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Abstract— The Carob Moth *Ectomyeloisceratoniae*, is a polyphagous pest that causes damage to many crops in Tunisia, mainly dates and pomegranates, where losses can reach 20 and 90% respectively. The female lays eggs outward the fruit, then L1 larvae enter and continue their life cycle up to the stage L5 in the fruits. Pupation occurs inside the fruit for dates and in soil for pomegranates, which makes their control difficult. Biological control is the most successful approach in this two cultures, several control techniques have been mastered to maintain the population below economic threshold; as the releases of *Trichogramma* (*Trichogrammacacociae*) or other larval parasitoids (*Habrobraconhebetor*) and the mass trapping. The Sterile Insect Technique, proved its effectiveness on other Lepidoptera such as codling moth, was also selected to be used against date moth in Tunisia.

In order to establish the gamma radiation dose that sterilize females and partially sterilize males of date moth, many tests were conducted in the laboratory. The doses chosen for the experiment were: 100, 150, 200, 250 and 300 Gy. The biological parameters were assessed for all combinations (irradiated male x irradiated female) (un-irradiated male x irradiated female) (irradiated male x un-irradiated female) and (control) for the parent, F1 and F2 generations. The results of fecundity, fertility, egg hatch and adult emergence of the date moth suggest that the most effective radiation dose to sterilize female and partially sterilize male is 250 Gy. This dose allowed no emergence of adults for all combinations of the F1 generation, except for crossing control.

Keywords— Carob moth, F1 sterility, Irradiation, Lepidoptera, Sterile Insect Technique

The innovative pest control tactics and strategies for modern agriculture aims to reduce farmer's reliance on pesticides as the primary means for crop protection by considering the use of the sterile insect technique (SIT) to control or perhaps eradicate pests.

The sterile insect technique (SIT) is a species-specific and sustainable method of insect control that has been used successfully over the past decades in eradication campaigns against a variety of pests particularly, the New World screwworm fly in the USA, Mexico, Central America and Panama [1], and against several fruit fly species such as the Mediterranean fruit fly *Ceratitis capitata* in California and Florida, USA (e.g. [2], [3]) and Mexico [4]. Lately, the SIT has also been used with great success against several Lepidopteran pests such as the codling moth *Cydia pomonella* (L.) in the Okanagan Valley of Canada [5], the false codling moth *Thaumatotibia leucotreta* (Meyrick) in South Africa [6], and the Australian painted apple moth *Teiaanartoides* Walker in New Zealand [7].

In addition, SIT may work synergistically with other socially acceptable tools that target different life stage of a pest species, such as biological control targeting eggs and larvae [8], bio pesticides such as *Bacillus thuringiensis* (Bt), and mating disruption [9]. The SIT relies on the mass rearing, sterilization and release of large numbers of insects (e.g. [10], [11]). One of the most important prerequisites for the success of SIT is to have the ability to sterilize both sexes without seriously affecting male behavior, particularly competitiveness and longevity [12].

Lepidoptera species are more radio-tolerant than many other insect orders (e.g. [13], [14]) and those high doses of radiation required to achieve full sterility may reduce their competitiveness and performance in the field [15]. One approach to circumvent the negative effects associated with the high radio-resistance of lepidopteran pests is using an approach called inherited or F1 sterility. This genetic phenomenon of Lepidoptera is characterized by the development of dominant lethal genes in offspring from partially sterile parents. This is expressed by higher sterility in the F1 generation than their parents, lower fecundity, longer larval development times, higher mortality, and a skewed F1 sex ratio in favor of males (e.g. [13], [16], [17]).

The date moth or carob moth *Ectomyeloisceratoniae* (Zeller, 1881) (Lepidoptera: Pyralidae) is a polyphagous pest that attacks a wide range of agricultural crops such as pomegranates (*Punicagranatum*) and dates (*Phoenix dactylifera*) [18], various types of citrus fruits e.g. lemon, orange, pistachio nuts (*Pistaciavera*) (e.g. [19], [20]), walnuts (*Juglansregia*) [21], almonds (*Prunusdulcis*) [22] as well as other non-economic plants of a wide range of plant families [23]. In Tunisia, *E. ceratoniae* is the major insect pest of dates both in the field and in storage [24] with annual infestation rates of 20% of the harvestable crop [25]. The date industry is extremely important for the Tunisian economy as it represents 5% of the total value of agricultural production and 16% of the total value of agricultural exports in Tunisia. Tunisian dates are ranked the 4th in terms of quantity of exported produce and the first in terms of foreign exchange earnings [25].

Different approaches to control date moth have been relied to maintain the population below economic threshold; as the releases of use of natural enemies (*Habrobraconhebetor* (Say) and *Phanerotomaflavitestacea* (Kohl) has been tested on an experimental basis in date and pomegranate orchards and resulted in some high levels of parasitism [26] and the mass trapping.

Therefore, additional control tactics can be integrated with existing ones and that are effective and friendly to the environment such as SIT.

The study presented here was designed to evaluate the effect of increasing doses of gamma radiation on parental adults (fecundity, fertility, egg hatching, emergency and longevity) and to assess radiation-induced inherited effects on F1 and F2 progeny. These results can provide guidance to initiate a full-sterility or F1 inherited sterility in an area-wide integrated pest management (AW-IPM) approaches against the date moth.

II. MATERIALS AND METHODS

A. Insect Rearing

Insects used in the experiments were obtained from rearing colony in the Laboratory of Plant Protection at the National Agronomic Institute of Tunisia (INAT) from infested field-collected dates and pomegranates. The moths were reared on an artificial diet based on wheat bran (90%), sugar (18%), salt mixture (3%), yeast (5%), lysine (0.3%), methyl paraben (0.2%), vitamin C (1%), aureomycine (3%), glycerine (18%) and distilled water (10%) [27]. Males and females adults were placed in plastic boxes (20 × 15 × 10 cm) furnished with a cotton soaked in sugar and water solution for feeding, and waxed paper for laying eggs. After that, eggs were transferred to other boxes containing artificial diet to continue their development until the emergence of adults. The rearing conditions 27 ± 1°C, a photoperiod of LD (Luminosity: Darkness) 16:8 and 65 ± 5% relative humidity.

B. Irradiation Procedure

Newly emerged virgin adults are segregated by sex and collected in small plastic boxes (8 x 3.5 x 4 cm) before irradiation. Then, they are transported in a cooler (4°C) to be irradiated. Both male and female adults were irradiated separately. The irradiations were conducted at the National Center of Nuclear Sciences and Technologies of SidiThabet (CNSTN) by using a Cobalt60 Gamma cell source. The irradiations were carried out with 5 doses of 100, 150, 200, 250 and 300Gy. For each irradiation dose, adults were paired into 4 combinations: : Irradiated Female with Irradiated male (IF X IM), Irradiated Female with Untreated Male (IF X UM), Untreated Female with Irradiated Male (UF X IM) and Untreated Female with Untreated Male (control) (UF X UM). Only the parental population was irradiated.

C. Effect of Gamma Radiation on Biological Parameters of the Date Moth

The object of our study was to assess the impact of 5 gamma doses 100, 150, 200, 250 and 300 Gy on fecundity (number of laid eggs), fertility (number of pink eggs), hatching adult emergence, and longevity of irradiated adults of Carob moth and their offspring (Parents, F1, and F2). After irradiation, male and female were mated in petri dishes with cotton soaked in sugar and water solution for feeding, and waxed paper for laying eggs.

The males (n = 25) and females (n = 25) were paired in ratio of 1:1 and were allowed to mate and lay eggs on the wax paper into petri dishes until they had died. For each combination and irradiation dose, the mating was under standard rearing conditions. Eggs were counted

daily until no more eggs were produced. Eggs were considered fertilized when they had a pink color.

The egg hatching larvae of each mating combination and each radiation dose were counted, transferred to big plastic (10 x 25 x 30 cm) boxes and placed on diet to continue their development and incubation at the rearing conditions. The number and sex of emerging adults was recorded until no more adults emerged from diet.

Following egg hatch larvae of each mating combination and each radiation dose were counted, transferred to big plastic (10 x 25 x 30 cm) boxes and placed on diet to continue their development and incubation at the rearing conditions. The number and sex of emerging adults was recorded until no more adults emerged from diet. For the first (F1) and second (F2) generations, male and female from each dose and combination were mated with parental and un-irradiated adults of the opposite sex. Methods for the F1 and F2 generations were similar to the parental generation except that only number of eggs and hatching were recorded for the F2.

D. Statistical Analysis

The effect of irradiation on egg production and egg hatch was assessed with a one way-ANOVA, using SPSS statistical program (version 16.0). The comparisons of means were performed through the Duncan test at the 5% threshold.

The correlation of irradiation doses with fecundity, egg hatch and adult emergence was quantified using a polynomial model fitted to egg production and egg hatch data, respectively.

III. RESULTS AND DISCUSSION

The effect of different doses of gamma radiation applied to parental males and females (P) of *E. ceratoniae* on various parameters of the P, F1 and F2 progeny are described in the following paragraphs.

1) Effects of Gamma Radiation on Fecundity

Increased radiation dose significantly and consistently decreased fecundity (fig 1-3). The average of egg production per female in the P generation varied from 87 eggs/female in the control to 75 eggs/ female for all crosses at 300 Gy. Radiation effect on fecundity was more pronounced in F1 and F2 generations than P generation.

Results indicated that females were more sensitive to radiation treatment than males. For instance, irradiated females crossed with males produced fewer eggs than

un-irradiated females. Finally, the fecundity declined in the different crosses and doses of radiation.

Statistical analysis showed that there is a difference between control and the different crosses in parental generation but there is no difference between different doses of radiation.

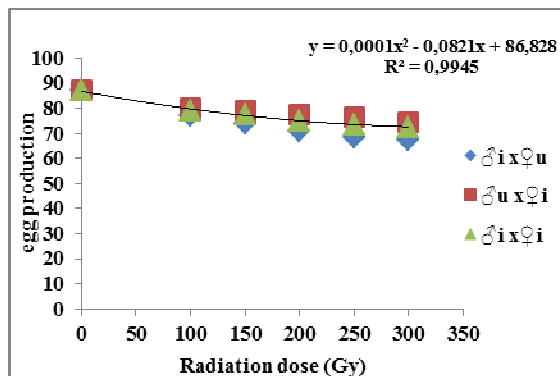


Fig.1: Effect of gamma radiation on the fecundity of parental generation

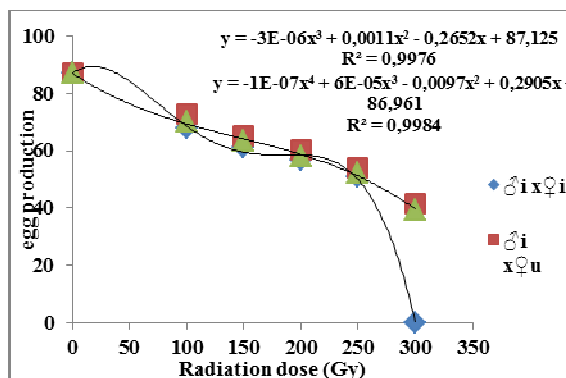


Fig.2: Effect of gamma radiation on the fecundity of F1 generation

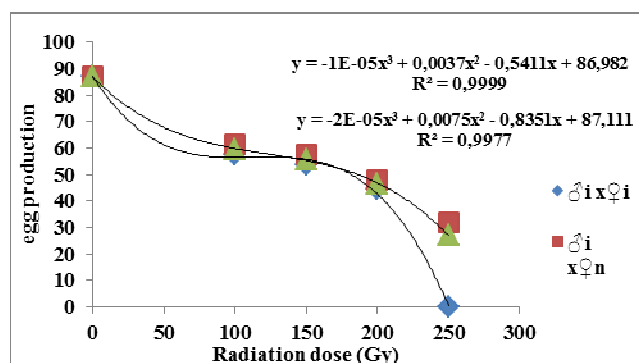


Fig.3: Effect of gamma radiation on the fecundity of F2 generation

Declining parental fecundity and fertility of date moth with increasing dose of irradiation were reported on other studies in Austria. However, some authors

reported that radiation did not affect the fecundity of either irradiated or un-irradiated females mated with irradiated males in P, F1 and F2 generations (e.g. [28]-[31]). Reported studies proved that fecundity of *Plutellaxylostella* (L.) females mated to irradiated males was equal to that observed in the untreated controls in the F1 and F2 generations (e.g. [32], [33]).

The female moths were more radio sensitive than the male, as it is typical for Lepidoptera (e.g [16], [34]-[36]), although not always the case [17].

2) Effect of Gamma Radiation on Fertility

The results revealed that the fertility was affected by radiation dose for all generations and crosses (fig 4-6). The percentage of fertile eggs recorded in the F1 generation was 0% for irradiated females mated with irradiated males at the dose of 300 Gy.

Statistical analysis indicated a significant difference between radiation doses and different type of crosses. The fertility of adults has been severely affected by irradiation in offspring. Indeed, it was noted that the percentage of fertile eggs decreases even for low intensities of gamma radiation. These results suggest that the deleterious effects in the P generation were still present in the F2 population.

Moreover, the results of our study showed that the deleterious effect on the fertility was inherited from parents. On the other hand, ref. [35], [37] revealed that the intensity of the applied dose of irradiation does not affect fertility of un-irradiated females mated with treated males. But, a severe atrophy was observed in ovarian cells of the treated females with very high doses of gamma radiation; thus inducing a reduction in fertility [38].

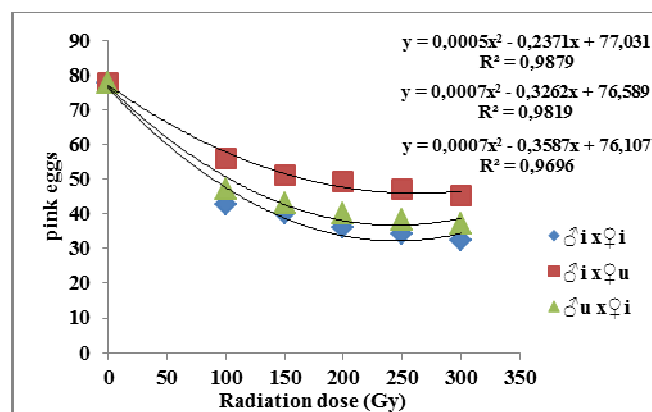


Fig.4: Effect of gamma radiation on the fertility of parental generation

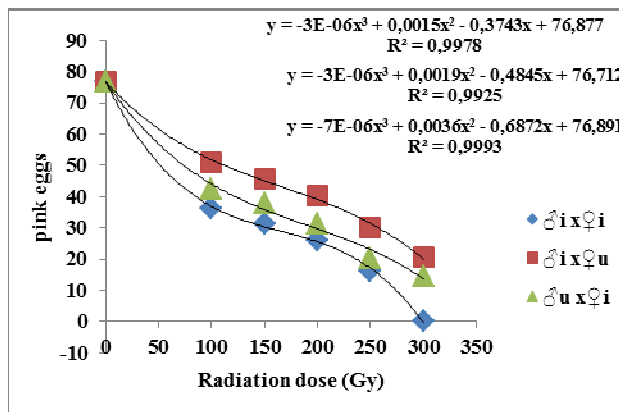


Fig.5: Effect of gamma radiation on the fertility of F1 generation

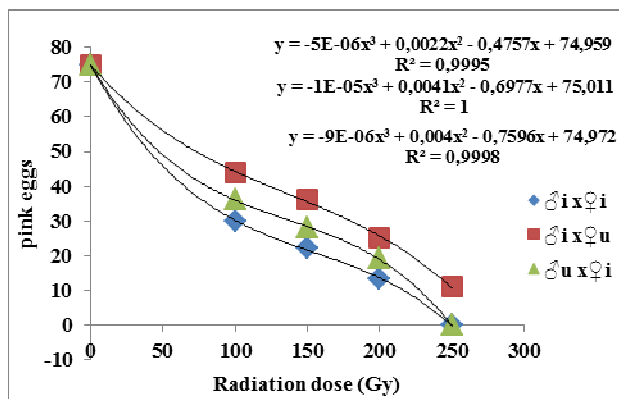


Fig.6: Effect of gamma radiation on the fertility of F2 generation

3) Effect of Gamma Radiation on Egg Hatch

We observed that an irradiation dose of 250 Gy administered to parental females and males resulted in some egg hatch in F1 generation but importantly no egg hatch in F2 generation. Statistical analysis showed significant differences in hatchability between control and different crosses in parental generation (fig7-9). The percentage of F1 and F2 progeny that survived to adulthood was inversely related to the dose of radiation administered to the parents.

The females of P generation that were treated with the dose of 250 Gy and mated with irradiated males produced eggs but the hatch rate was only 10%. However, no egg hatch was observed for this crossing at the F1 generation.

The percentage of hatching eggs depends on the intensity of radiation dose and different crosses. Indeed, the results revealed that hatching is affected even at low doses. For the same dose of radiation, the crossings where females are irradiated recorded the lowest level of hatching eggs.

For high dose of radiation, effects on F1 adults were greater than the effect on P adults which were

irradiated and decreased egg hatch for F1 adults was more pronounced than for P adults.

In addition to that, eggs hatching in the crossing of irradiated females were reduced compared with normal females. Therefore, no adults were produced at 250 Gy.

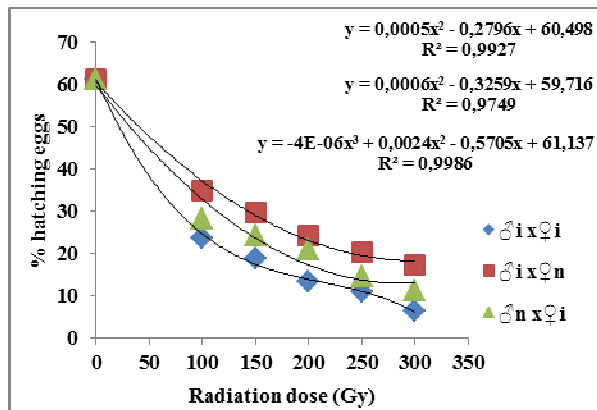


Fig.7: Effect of gamma radiation on the egg hatch of parental generation

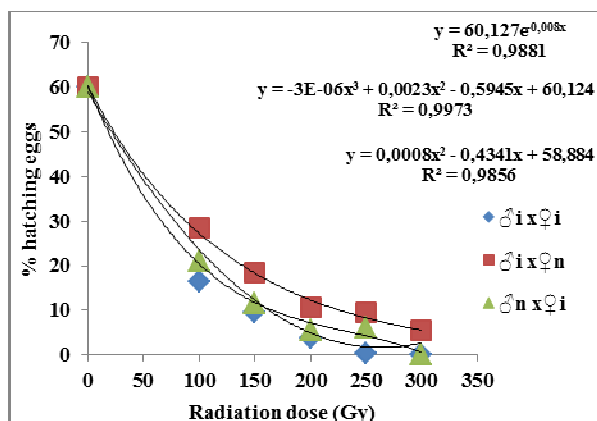


Fig.8: Effect of gamma radiation on the egg hatch of F1 generation

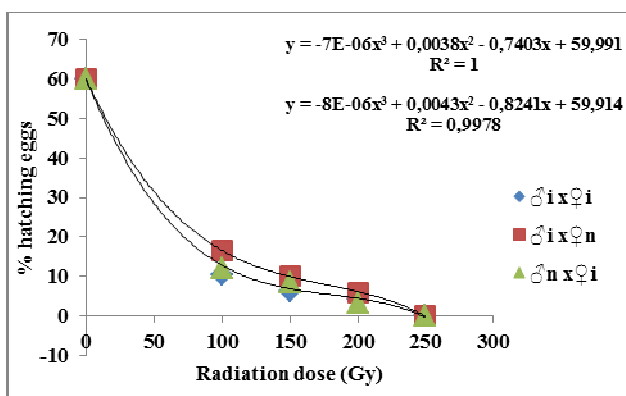


Fig.9: Effect of gamma radiation on the egg hatch of F2 generation

4) Effect of Gamma Radiation on Adult's Emergence

In fact, there was a significant difference between the adult's emergence in control and different crosses in P generation; more the dose increase more the emergence is lower (Figures 10, 11 and 12).

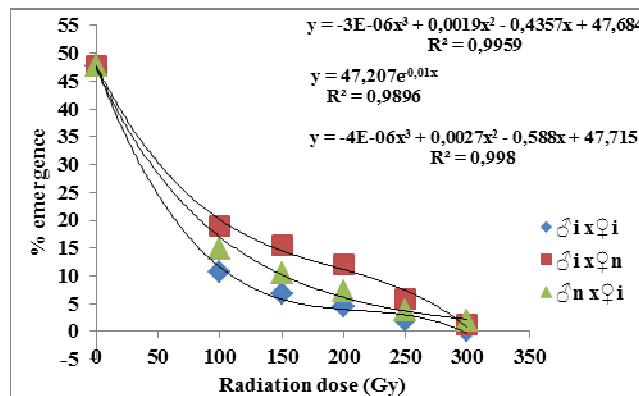


Fig.10: effect of gamma radiation on adult emergence of parental generation

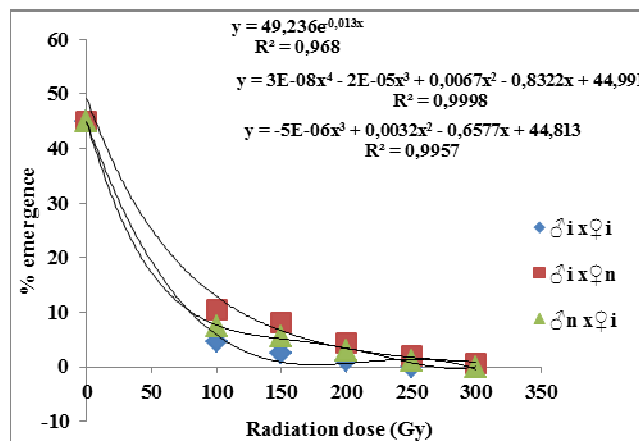


Fig.11: effect of gamma radiation on adult emergence of F1 generation

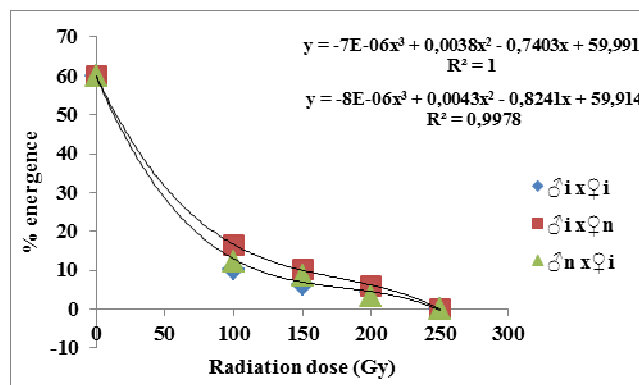


Fig.12: Effect of gamma radiation on the adult emergence of F2 generation

The emergence rate decreased from 60% in the control to 12 and 5.8% for doses of 200 and 250 Gy in the crossing of irradiated males with un-irradiated females. For the dose of 300 Gy no emerged adults were registered in F1 generation and for the F2 generation, no adult's emergence was observed for 250 Gy.

5) Effect of Gamma Radiation on Adult Longevity

It was noted that the longevity decreased with the different doses and with the different generations (Figure 13). The results exposed a decrease in longevity particularly for irradiated crossing.

However, statistical analysis showed a significant difference between control and different crosses related to this parameter.

The longevity of females from un-irradiated males with irradiated females reached 5.6 and 5.4 days respectively at doses of 200 and 250 Gy. However, male longevity ranged from 5.5 to 5.3 days. These results suggest that female's longevity was slightly longer compared to males. The offspring F1 and F2 generations presented a short lifespan compared with the control population.

We observed that an irradiation dose of 300 Gy administered to parental generation results in some egg hatch in F1, but no emerged adults. The percentage of hatching eggs laid by the F1 adults was affected by the dose of radiation administered to parental generation. Radiation effects on the F1 were higher than the effects on P adults for the different crosses. Ref. [16] reported that survival of Lepidopteran F1 progeny from irradiated parents was poor.

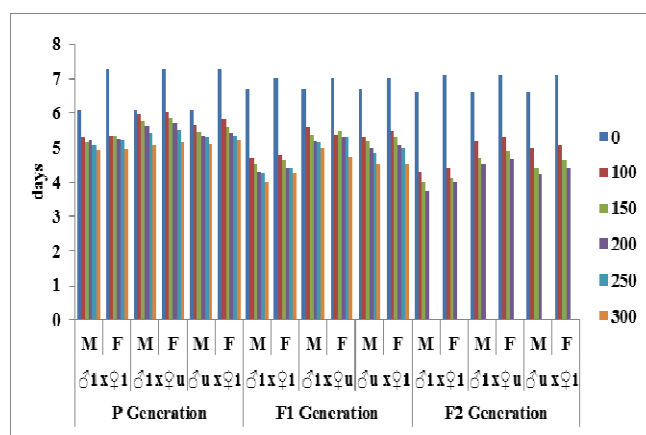


Fig.13: Effect of gamma radiation on adult longevity of P, F1 and F2 generations

The longevity of date moth was affected by increasing radiation doses. Similar results were obtained when studying the effects of radiation on male longevity of

ringworm of the potato. Indeed, ref. [39] has shown that the longevity was not affected by the application of irradiation. On the other hand, the impact of radiation is closely related to developing stage, sex and the intensity of applied radiation [40].

IV. CONCLUSIONS

Our results have shown that for the different populations, an irradiation dose of 250 Gy administered to *Ectomyeloisceratoniae* moths induced total sterility in the females and more than 95% of sterility in the males. Parental fecundity and fertility were negatively affected by increasing dose of irradiation.

Many authors described aspects of inherited sterility in different species of Lepidoptera. These aspects include differential sensitivity to radiation between males and females in the parent generation, F1 male and female offspring that are more sterile than the irradiated parents, a biased male/female ratio in produced progeny and reduced sperm quality in the F1 generation (e.g. [13], [29]).

Successful application of SIT/F1 in Lepidoptera is achieved by selecting a treatment dose of radiation that fully sterilizes females, to avoid increasing host plant damage, while only partially sterilizing males to maintain mating competitiveness and produce F1 progeny highly sterile when mated with wild females [41]. Our results have shown that an irradiation dose of 250 Gy administered to *E. ceratoniae* adults induced total sterility in females and sub-sterility in males. Parental result was congruent with previous reported studies on other Lepidoptera [29].

In addition, progeny of irradiation of parents at sub-sterilising doses have been shown to be more sterile than that of the parents (e.g. [8], [16]) and it has been found that the release of partially sterile Lepidoptera offered greater suppressive control in the field than the release of fully sterile insects (e.g. [8], [13], [31]).

More studies are required in order to fully describe the biology of the carob moth, to improve the mass rearing conditions and larval diet, and finally to use the F1 inherited sterility towards an insect pest management program against this pest.

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