International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:11, No:10, 2017

# Mass rearing and Effects of Gamma Irradiation on the Pupal Mortality and Reproduction of Citrus Leaf Miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae)

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***Abstract*—**Citrus leaf miner (*Phyllocnistis citrella* Stainton) is native to Asia and one of the most serious pests of Iran's citrus nursery stocks. In the present study, the possibility of insect mass rearing on four various citrus hosts and the effects of gamma irradiation on the pupal mortality and reproduction of this pest were studied. Trifoliate orange and grapefruit showed less infection, while the number of pupae in Valencia oranges and sweet lemons cages was so high. There was not any significant difference between weight of male and female pupae among different citrus hosts, but generally the weight of male pupae was less than females. Use of Valencia orange or sweet lemons seedlings in especial dark emergence and oviposition cages could be recommended for mass rearing of this pest. In this study, the effects of gamma radiation at doses 100 to 450 Gy on biological and reproductive parameters of the pest has been determined. The results show that mean percent of pupal mortality increased with increasing doses and reached to 28.67% at 450 Gy for male pupae and 38.367% for female pupae. Also, the mean values of this parameter were higher for irradiated female, which indicated the higher sensitivity of this sex. The gamma ray irradiation from 200 and 300 Gy caused decrease in male and female adult moth longevity, respectively. The eggs were laid by emerged females, and their hatchability was decreased by increasing gamma doses. The fecundity of females in both combinations of crosses (irradiated male × normal female and irradiated female × normal male) did not differ, but fertility of laid eggs by irradiated female × normal male affected seriously and the mean values of this parameter reached to zero at 300 Gy. The hatchability percentage of produced eggs by normal female × irradiated male at 300 Gy was 23.29% and reached to less than 2 % at 450 Gy as the highest tested dose. The results of this test show that females have more radio-sensitivity in comparison to males.

***Keywords*—**Citrus leaf miner, *Phyllocnistis citrella*, citrus hosts, mass rearing, sterile insect technique.

## I. Introduction

CITRUS fruits constitute a big share in the trade of fruits both in the world and local markets of Iran. Total citrus production of the world has been reported to be 122,976 thousand tons in 2010 and Iran produced 2,628 thousand tons in the same year, ranked 11th producer in the world [1]. Citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), is a serious pest in all of citrus producer countries. This pest is native to Asia and can be found throughout most parts of this continent, also it is present in Australia, Africa, the Mediterranean area of Europe, Caribbean Islands, Central America, Mexico, and South America [2]. The pest was first found in southern and northern parts of Iran in 1961 and 1994, respectively [3].

Adults can deposit 50 eggs during their life cycle. Larvae have four instars (three instars and a prepupal stage) and then they enter to pupal stage, which occurs within the mine [4], [5]. Adults live for only about one week. Their entire life cycle requires 14 to 50 days depending on temperature. This pest is almost found throughout the year and completes 5-13 generations in a year [2], [5], [6]. Newly hatched larvae immediately enter the leaf and start feeding by creating shallow tunnels in flushes of seedling and budded shoots in citrus nurseries [2]. Also, these larvae have been linked to the severity of citrus canker (*Xanthomonas axonopodis* pv. *citri*), a serious disease of citrus [7].

During recent years many insecticides are continuously applied to control of *P. citrella* at short-term intervals led to induce resistant problems and eliminate many natural enemies so outbreak of CLM. A sterile insect technique (SIT) has been initiated to control of this pest population and reduces the harmful effects of insecticides in citrus orchards of Iran. The SIT involves rearing large numbers of the pest target species, exposing them to gamma rays to induce sexual sterility and then releasing them into the wild. The sterile males compete with wild males to mate with wild females that resulting no offspring [8]. So, the aims of this study were investigating of citrus host preference for mass rearing of CLM and determination of proper doses of CLM moths' sterility to evaluate possibility of using SIT against this pest.

## II. Materials and Methods

### *A. Collection of Samples and Transfer to Laboratory*

Wild CLM in pupal stage were collected from rolling edge of leaves at the end of created mines, from young citrus trees (*Citrus sinensis* var. Thomson Navel) of Dashte Naz agro-industrial company in Mazandaran province (36.6° N, 52.1° E; 16 m a.s.l.) (Fig. 1) in the north of Iran and all infected leaves

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were transferred to laboratory in plastic bags.

### *B. Laboratory Rearing of CLM*

Since there was not sufficient study on mass laboratory rearing of CLM, regarding biological characteristics and miner behavior of this pest, it was very important to establish an accurate laboratory mass rearing technique which includes the following steps: A number of pupae collected from the citrus trees will be put in the "pupal holding cage" (50 cm×50×50) till emerging adult moths. These cages were covered with black cardboards, and emerging moths were attracted to the light and were collected in transparent containers installed in front of the cages.

Collected adult moths (200) were transferred to specially designed "oviposition cages" (105 cm×70×95) to put eggs on the existing flushes of citrus seedling in the cages for two days. There were 20-24 pots of *Citrus sinensis* var. Valencia seedlings (30 cm tall).

All cages and the citrus seedlings infested with CLM eggs are incubated in the rearing room at atmospheric condition of 27±5 °C and 80±10% RH and 16h photo phase. The pupae collected from infested leaves were used in biological study, determining effect of irradiation or used to continue mass rearing.

### *C. Host Citrus Plants*

During different experiments, citrus was either grown from seed or obtained as seedlings from Dashte-Naz nurseries in Mazandaran province; four different citrus seedlings consist of *Citrus sinensis* var. Valencia, *Poncirus trifoliate*, *Citrus paradise* and *Citrus aurantifolia* var. Sweet Lime were ordered from Dashte-Naz nurseries. Seedlings with about 50 cm tall were planted in plastic pots (15 cm in diameter) and kept in greenhouse. These seedlings were used for host preference test and irrigated usually 2 times per week.

For the other tests, *Citrus sinensis* var. Valencia seeds were planted using the method described by Smith and Hoy [9] with minor modifications. For this purpose, the seeds were planted in a 1:1 mixture of Vermicompost and sand in a cavity seedling tray (Hummert International, Earth City, MO) containing 96 (2.5cm ×2.5×6) cavities. Each seedling with 3-4 leaves was transferred to a little plastic pot (8 cm in diameter). Seedlings with 30 to 50 cm height were ready to be used as hosts for the CLM.

### *D. Host Preference Study*

Four different citrus seedling: Valencia orange (*C. sinensis* var. Valencia), trifoliate orange (*P. trifoliate*), grapefruit (*C. paradise*), and Sweet lemon (*C. aurantifolia* var. Sweet Lime) were placed inside each oviposition cage to evaluate the effect of different citrus hosts on the infestation. Then, 200 moths (1:1 male: female) were released in each cage. Some factors such as total number of pupae in each cage, number of the infested leaves in each pot, number of pupae in each infected leaves and weight of the male and female pupae were investigated in each citrus host.

### *E. Radiation Biology Study*

The male and female pupae were separated according to the morphology of the last abdominal segment (Fig. 2). The female pupae show a long last segment (presumably a fusion of segments IX and X) bearing two long hairs, whereas males exhibit a shorter pygidium without any hair [10]. Both male and female CLMs were irradiated separately at 0 (untreated), 100, 150, 200, 250, 300, and 350 Gy doses of gamma radiation whether at pupal stage. A Co60 source was used to irradiate the pupae. Irradiated male and female pupae of each treatment were confined in separate cylinder container for adult emergence. After emergence, treated females (TF), treated males (TM), untreated females (UF), and untreated males (UM) were placed in the cages in the following combinations: (i) UF x UM, (ii) TM x UF and (iii) TF x UM.

Data on pupal mortality, longevity of both male and female moths, total number of eggs per females and egg hatch in each replication were recorded.

Fig. 1 Infected leaves of Dashte Naz agro-industrial company citrus orchards

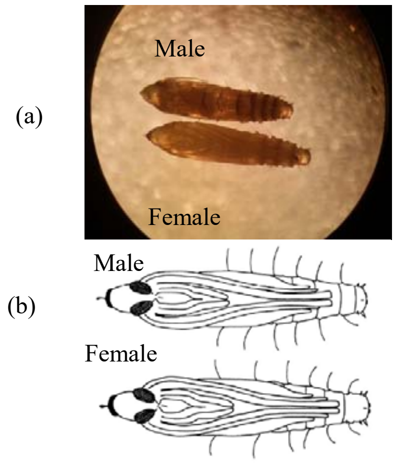


Fig. 2 Male and female Pupae of *Phyllocnistis citrella*: Female last abdominal segment is longer and bears two long hairs lacking in males (a) Original, (b) taken from [10]

## III. Results

### *A. Host Preference*

The total number of pupae in pots was low in trifoliate orange and grapefruit (24 and 53 pupae, respectively), while this amount was very abundant in Valencia orange and Sweet lemon (594 and 526 pupae respectively, Table I); The number of the infested leaves in each pot was significantly (P< 0.01) different from about seven infested leaves in Valencia orange and Sweet lemon to 1-2 leaves in trifoliate orange and grapefruit.

The number of pupae in each infested leaf was significantly different too (P<0.01). There was average of three pupae in each leaf of Valencia orange and sweet lemon, whereas about one pupa was observed on a leaf of trifoliate orange or grapefruit. Although there was not any significant difference between the weight of the male and female pupae in different hosts, the weight of male pupae (0.15-0.18 mg) was less than that of female pupae (0.19-0.27 mg).

TABLE I
EFFECT OF DIFFERENT CITRUS HOSTS ON TOTAL NUMBER OF PUPAE IN A CAGE, NUMBER OF THE INFESTED LEAVES IN EACH POT, NUMBER OF PUPAE IN EACH INFECTED LEAF, SEX RATIO (MALE:FEMALE) AND THE WEIGHT OF MALE AND FEMALE PUPAE OF *CLM*

| Citrus species | No. of pupae in a cage | No. of infested leaves in a pot | No. of pupae in an infected leaf | Sex ratio | Weight of male (mg) | Weight of female (mg) |
|---|---|---|---|---|---|---|
| ***P. trifoliate*** | 24 | 0.79±0.14 c* | 1.10±0.07 b | 1: 1.1 | 0.15±0.02 a | 0.19±0.03 a |
| ***C. sinensis* var. Valencia** | 594 | 7.12±0.24 a | 2.94±0.06 a | 1: 1.3 | 0.18±0.01 a | 0.27±0.01 a |
| ***C. paradise*** | 53 | 2.0±0.24 b | 1.29±0.06 b | 1: 1.2 | 0.17±0.02 a | 0.22±0.02 a |
| ***C. aurantifolia* var. Sweet Lime** | 526 | 6.7±0.29 a | 3.10±0.08 a | 1: 1.4 | 0.18±0.01 a | 0.25±0.01 a |

* Means followed by the same letter in each column are not significantly different at p<0.05.

### *B. Radiobiology*

Analysis of variance of CLM pupal mortality showed that the applied doses, sex of pupae, and interaction effect between these two factors had significant effect on this biological characteristic (P≤0.01). The mortality percentage of 5-day-old pupae irradiated with different gamma radiation doses and the abnormal adults emerged from irradiated pupae were measured as an important factor involved in adequate emergence of high quality sterile insects using SIT (abnormal adults emerged from irradiated pupae were calculated as a dead pupa because of their inability to fly and mating).

The mean percentage of pupal mortality and emerged abnormal adults increased with increasing doses (28.67% and 38.67% at 450 Gy for male and female respectively compared to 0.00% and 0.67% in the control) (Table II). There were differences between the control treatment and doses of 300 Gy and higher for this character, and the mean percentage of female pupal mortality was higher in comparison with male in different doses (P≤0.05, Table II). This result shows the adverse effect of irradiation is more pronounced on the female than on the male pupae.

TABLE II
EFFECT OF DIFFERENT DOSES OF GAMMA RADIATION APPLIED TO 5-DAY-OLD CLM PUPAE ON THE PUPAL MORTALITY

| Dose (Gy) | % Abnormality after emergence and Pupal Mortality (±SD) | |
|---|---|---|
| | Male | Female |
| **0** | 0.00 (0.00) d* | 0.67 (1.15) e |
| **100** | 2.00 (2.00) d | 1.33 (0.15) e |
| **200** | 2.00 (0.00) d | 2.67 (1.15) e |
| **300** | 7.33 (1.15) c | 10.67 (1.15) d |
| **350** | 10.80 (8.33) c | 20.00 (2.00) c |
| **400** | 16.67 (2.31) b | 26.00 (4.00) b |
| **450** | 28.67 (3.06) a | 38.67 (1.15) a |

* Means followed by the same letter in each column are not significantly different at p<0.05.

Fecundity, fertility, and longevity of adults of both sexes, when 5-day-old pupae were irradiated in various crosses are shown in Table III. The number of laid eggs per female decreased significantly (p≤0.05) as the radiation dose increased (30.41 and 30.23 eggs at 450 Gy for irradiated female and male respectively compared to 45.00 and 44.27 eggs in the control). Furthermore, the longevity of adults decreased with increasing doses (3.52 and 4.41 days at 450 Gy compared to 5.67 and 7.11 days in the control for male and female respectively). The mean percentage of hatched eggs resulted from both crosses decreased significantly as the radiation dose increased (P≤0.01).

This value markedly decreased when untreated males mated with females irradiated as 5-day-old pupae and reached to zero at 300 Gy (this value was less than 1% at 200 Gy). Increased radiation dose decreased the number of hatched eggs laid by untreated female mated with treated male significantly (from 100 Gy) and consistently. This value reached less than 2% and 4% at 400 and 350 Gy respectively in comparison with 90.28% in control. The results of the effect of irradiation on the fertility of the female *P. citrella* irradiated as pupae indicated that females generally are much more sensitive to radiation than males of the species.

Analysis of variance on the mean longevity of adult moths developed from irradiated pupae at various gamma irradiation doses showed the significant effect (P≤0.01) of radiation on both males and females moth longevity and a significant decrease was observed at doses higher than 100 and 300 Gy for female and male moths respectively compared with control (3.52 and 4.41 days at 450 Gy compared with 5.67 and 7.11 in control respectively for males and females).

## IV. DISCUSSION

In current study, Valencia orange showed the highest infestation of CLM so it seems this variety is one of the most susceptible citrus hosts and could be recommended for mass production programs of *P. citrella*. The results of current study showed that *P. trifoliate* had the lowest rate of infestation to CLM and it could not be recommended as host in mass rearing of this pest. Kharrat and Jerraya [11] released 40, 80, and 160 pairs of moths on 25-30 saplings of *P. trifoliate* (20-40 cm tall) as citrus host in three cages (1.5m×1m×1.5m) to mass production of CLM. They determined high mortality rate of young larvae with increasing of moth density although the number of egg deposited by each female did not change significantly in different density of moths (56-61 eggs per female). So, unsuitable host could be the reason of high mortality of *P. citrella* and low efficiency of mass production in this study.

In the present study, the effects of different doses of gamma radiation (from 100 to 450 Gy) on some biological parameters of CLM have been measured with the aim of evaluating the radiation induced sterility of the pest.

TABLE III
EFFECT DIFFERENT DOSES OF GAMMA RADIATION ON NUMBER OF EGGS/FEMALE, EGG HATCHABILITY AND ADULT LONGEVITY OF CLM MOTHS WHEN IRRADIATED AS 5 DAY-OLD PUPAE

| Mean of fecundity and fertility (± SD) | | | | | |
|---|---|---|---|---|---|
| Dose (Gy) | | Eggs/Female (±SD) | Egg hatch (%) (±SD) | Adult longevity (Days) | |
| | | | | Male | Femle |
| IM×UF | UM×UF | 44.27(0.67) a* | 90.28(1.85) a | 5.67(0.37) a | - |
| | 100 | 41.90(2.59) ab | 73.66(6.79) b | 5.38(0.17) a | - |
| | 200 | 37.97(1.59) bc | 43.99(5.05) c | 5.65(0.08) a | - |
| | 300 | 36.72(3.05) c | 23.29(3.50) d | 4.92(0.16) a | - |
| | 350 | 37.67(1.52) bc | 13.55(1.55) e | 4.01(o.48) b | - |
| | 400 | 31.19(6.54) d | 3.30(0.78) f | 3.81(0.58) b | - |
| | 450 | 30.23(1.17) d | 1.28(1.39) f | 3.52(0.97) b | - |
| UM×IF | UM×UF | 45.00 (0.67)a | 90.28(1.85) | - | 7.11 (0.87) a |
| | 100 | 39.17 (0.76)b | 4.25(1.47) | - | 7.23 (0.74) a |
| | 200 | 38.24 (0.74)b | 0.87(1.51) | - | 5.88 (0.92) b |
| | 300 | 35.89 (4.79)b | 0.00(0.00) | - | 5.55 (0.39) bc |
| | 350 | 36.48 (3.39 b | 0.00(0.00) | - | 5.65 (0.16) b |
| | 400 | 30.86 (1.58)c | 0.00(0.00) | - | 4.79 (0.28) bc |
| | 450 | 30.41 (0.68)c | 0.00(0.00) | - | 4.41 (0.42) c |

U: unirradiated, I: irradiated, M: male, F: female
*Means followed by the same letter in each column are not significantly different at $p<0.05$.

The effect of different doses of gamma radiation on 5-day-old male and female pupae caused an increased mortality by increasing doses and maximum mortality occurred at the highest applied dose (450 Gy). The mean values of this parameter for irradiated females were higher than males indicating the higher sensitivity of this sex.

Fu et al. [12] observed that the adult emergence of *Conopomorpha sinensis* from irradiated mature pupae decreased with increasing dose and reached to 76% and 66% at the doses of 250 and 300 Gy respectively compared with >93% in control.

The pupal and adult mortality and deformity of *Agrotis ipsilon* increased significantly by increasing gamma radiation doses when male and female pupae were irradiated with 50 and 100 Gy [13].

According to Ramesh et al. [14], the percentage of normal emerged adults of *Spodoptera litura* irradiated as 5-day-old pupae at dose of 70 Gy was 75.5% compared with 83.3% in control (decreased by about 8%), and this dose caused 13.4% emerged abnormal moths with severe body deformation.

In the study of Dhoubi and Abderhamane [15] on effect of gamma radiation (200-600 Gy) on different-aged Pupae of *Ectomyelois ceratoniae*, irradiation resulted in a decrease of adult emergence depending on dose and age. The highest hatching percentage belonged to the oldest pupae, when 8-9-day-old pupae were irradiated with 500 and 600 Gy, 30% and 10% normal adults emerged, respectively. In this study, male and female pupae were not separated with respect to their radio-sensitivity.

Tothova and Marec [16] analyzed the chromosomal aberrations performed in male progeny of gamma-irradiated males of Lepidoptera; this research confirmed that males are more able to survive with a higher number of chromosomal breaks than females. This can justify higher female mortality emerged from irradiated pupae in our study. However, Katiyar [17] reported that the adverse effect of irradiation (600 Gy) is more pronounced on the male than female pupae of coffee leaf miner, *Leucoptera coffeella* Guer.

Results of effects of gamma radiation on longevity and reproductive parameters of CLM irradiated as pupae indicated that moth longevity, fecundity, and fertility were reduced with increasing doses of radiation. The number of eggs (fecundity) of female moths between two various combinations of crosses was not significantly different. The percentage of hatching eggs (fertility) resulted from untreated males mated with females (irradiated as pupae or adult moths) was significantly reduced and reached to zero at the dose of 300 Gy, and this value was less than 2% and 3% when normal females mated with irradiated males at 450 Gy. As it was stated in relation to sex dependence radio-sensitivity, females were much more sensitive to gamma radiation than males. The lifespan of males and females CLM irradiated during pupal stages was significantly shortened. The results of this investigation showed that the adult longevity of male and female moths irradiated as 5-day-old pupae decreased significantly from 350 and 200 Gy respectively. “

Daguang et al. [18] reported that the fecundity and fertility of irradiated *H. armigera* females mated with normal males were significantly reduced, and females treated with 300 Gy were completely sterile. No significant difference in female fecundity was observed when normal females mated with irradiated males. No detrimental effects on female fecundity were recorded for *H. armigera* in all various combinations of crosses while fertility of females mated with irradiated males was only 64% of control [19]. When pupae of *Ectomyelois ceratoniae* were treated with 400 or 500 Gy, the number of eggs and percentage of hatching eggs resulted from various combinations of crosses between treated and untreated moth were drastically reduced [15]. The results of another study on this species suggested that the most effective radiation dose to sterilize female and partially sterilize male was 250 Gy. This dose allowed no emergence of adults for all combination of F1 generation [20]. The study on radiobiology of *Grapholita molesta* showed that a dose of gamma radiation between 400-500 Gy induced almost complete male sterility (93-97%) without any deteriorative effects on adult longevity, while the dose that causes complete female sterility was 150 Gy [21].

Walton et al. [22] reported a significant interaction of dose and various combinations of crosses on fertility of *Eldana Saccharina* and concluded that treated females were more radio-sensitive than males. The percentage of hatching eggs was 3.11% and 0.04% when males and females were irradiated and crossed with non-irradiated counterparts.

In the most insects, the adult females are sensitive to radiation equally or more than the males and require a lower dose rate to be sterilized. Mitotically active reproductive cells (MARC) sensitivity in female insects is further complicated by the presence of nurse cells that are subject to injury. Nurse cells are extremely radiosensitive when they are undergoing endomitosis. Thus, females are in general more radio-sensitive (when sterility is taken as the index of the radio-sensitivity) [23].

There are different species dependence reports on longevity of Lepidopterean adults emerged from irradiated pupae at various gamma radiation doses.

Daguang et al. [18] observed no significant decrease in male and female *Helicoverpa armigera* moth longevity when pupae sexed and irradiated 1-2 days before adult emergence with 200, 250 and 300 Gy of gamma radiation, while Rahman et al. [24] found that irradiation of pupae at 100 and 150 Gy significantly reduced longevity of *Spilosoma oblique* adults, particularly of males.

## V. Conclusion

Based on the results obtained from the present study, Valencia orange is one of the most susceptible citrus hosts and could be recommended for mass production programs of *P. citrella*. The doses higher than 400 and 300 Gy could be suggested as proper sterilizing doses of male and female *Phyllocnistis citrella* Stainton, respectively.

## Acknowledgment

The authors are grateful to Mr. Yousefnia in Dashte Naz Co. for collecting and transferring of *P. citrella* and Infested citrus leaves. This study was supported by Iran National Science Foundation (INSF) with grant number of 92039201.

## References

[1] FAO, 2013. FAO Statistical Yearbook 2013, World food and agriculture. Food and Agriculture Organization of the United Nations. Rome. 289 pp.

[2] E. E. G. Cardwell, K. E. Godfrey, D.H. Headrick, P. A. Mank, J. E. Pena, "Citrus leaf miner and citrus peelminer," University of California. ANR Publication. 8321, pp. 1-12, 2008.

[3] E. Ebrahimi, M. Malekzadeh, Z. Yefremova, "Parasitoid wasps of Phyllocnistis citrella (Lep., Gracillariidae) in Iran," Plant Pathol. Entomol., vol. 76, pp. 81-92, 2008.

[4] G. A. C Beattie, D. Smith, Citrus leafminer, N.S.W. Agriculture No: 42. AB. 4. Rydalmere, N.S.W., Australia, 1993, pp 12.

[5] J. B. Heppner, "Citrus leafminer, Phyllocnistis citrella, in Florida (Lepidoptera: Gracillaridae: Phyllocnistidae)," Fla. Entomol., vol. 78, no.1, pp. 183-186, 1995.

[6] S. A. S. Ba-Angood, "On the biology and food preference of the Citrus leaf miner, Phyllocnistis citrella Stainton (Gracilariidae, Lepidoptera) in PDR of Yemen," Zeitschrift Fur Angewandte Entomologie, vol. 86, pp.53-57, 1978.

[7] M. C. Chagas, J. R. P. Parra, T. Namekata, J. S. Hartung, P. T. Yamamoto, "*Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and Its Relationship with the Citrus Canker Bacterium *Xanthomonas axonopodis* pv *citri* in Brazil," Neotrop. Entomol., vol. 30, pp. 55-59, 2001

[8] V. A. Dyck, H. Hendrichs, A. S. "Robinson, Sterile Insect Technique Principles and Practice," in *Area-Wide Integrated Pest Management*, Espringer, Netherlands. 2005.

[9] J. M. Smith, M. A. Hoy, "Rearing Methods for Ageniaspis citricola (Hymenoptera: Encyrtidae) and Cirrospilus quadristriatus (Hymenoptera: Eulophidae) Released in a Classical Biological Control Program for the Citrus Leafminer Phyllocnistis citrella (Lepidoptera: Gracillariidae)," Florida Entomologist, vol. 78, no.4, pp. 600-608, 1995

[10] J. A. Jacas, A. Garrido, "Differences in the morphology of male and female phylloconistis citrella (Lepidoptera: Gracillaridae)," Fla. Entomol., vol.79, pp. 603-606, 1996.

[11] S. Kharrat, A. Jerraya, "Rearing parasitoids by mass production of citrus leafminer larvae, Phyllocnistis citrella (Lepidoptera: Gracillariidae)," Entomologia Generalis, vol. 28, no.2, pp. 115-120, 2005.

[12] H. Fu, F. Zhu, Y. Deng, Q. Weng, M. Hu, T. Zhang, "Development, Reproduction and Sexual Competitiveness of *Conopomorpha sinensis* (Lepidoptera: Gracillariidae) Gamma-Irradiated as Pupae and Adults," Fla. Entomol., vol, 98, pp.66-72, 2016

[13] H. M. Salem, M. A. Foudo, A. A. Abas, W. M. Ali, A. Gabarty, "Effects of gamma irradiation on the development and reproduction of the greasy cutworm, *Agrotis ipsilon* (Hufn.)," J Radiat. Res. Appl. Sci. vol. 7, pp. 110-115, 2014.

[14] K. Ramesh, A. K. Garg, R. K. Seth, "Interaction of substerlizing gamma radiation and Thiodicarb treatment for management of the tobacco caterpillar *Spodoptera litura*." Phytoparasitica, vol. 30, pp. 7-17. 2002.

[15] M. H. Dhouibi, C. T. Abderahmane, "The effect of substerilizating doses of gamma radiation on the pupae of the carob moth *Ectomyelois ceratoniae* (Lepidoptera: pyralidae)," in *Evaluation of Lepidoptera population suppression by radiation induced sterility*, IAEA-TECDOC 1083. International Atomic Energy Agency, Vienna, Austria, 2002, pp. 43- 45.

[16] A. Tothova, F. Marec, "Chromosomal principle of radiation-induced F1 sterility in *Ephestia kuehniella* (Lepidoptera: Pyralidae)," Genome, vol, 44, pp. 172-184, 2001

[17] K. P. Katiyar, "Studies on the biology and sterilization of the coffee leaf miner, *Leucoptera coffeella* Guer," in *The application of nuclear energy to agriculture. Annual Report*, AT (30-1)-2043, 1967, pp. 111-122.

[18] L. Daguang, L. Xiaohui, H. Jiangguo, W. Endong, H. Qiulan, L. Yongjun, "Cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae): Large scale rearing and the effect of gamma radiation on selected life history parameters of this pest in china," in *Evaluation of Lepidoptera population suppression by radiation induced sterility*, IAEA-TECDOC 1083. International Atomic Energy Agency, Vienna, Austria, 2002, pp. 23- 27.

[19] V. R. Ocampo, J. B. Leon, "The effects of radiation on the biology and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae)," in

*Evaluation of Lepidoptera population suppression by radiation induced sterility*, IAEA-TECDOC 1083. International Atomic Energy Agency, Vienna, Austria, 2002, pp. 29- 36.

[20] S. Chakroun, M. Skander, K. G. Lebdi, "Sterile insect technique against date moth, *Ectomyelois ceratoniae* Zeller 1881, in Tunisia: Research of gamma radiation sterilizing dose," Int. j. eng. sci. technol. (IJSET). Vol. 3, pp.55-62, 2014.

[21] N. Genchev, "Suppression of oriental fruit moth (*Grapholita molesta*, Lepidoptera:Tortricidae) population using the sterile insect technique," in *Evaluation of Lepidoptera population suppression by radiation induced sterility*, IAEA-TECDOC 1083. International Atomic Energy Agency, Vienna, Austria, 2002, pp. 49- 60.

[22] A. J. Walton, D. E. Conlong, M. F. Addison, "Parental and F1 sterility of *Eldana Saccharina* Walker (Lepidoptera:Pyralidae)" Proc. S. Afr. Sug. Technol. Ass. 84, pp. 281-286, 2011.

[23] A. Bakri, N. J. Heather, J. Hendrichs, I. Ferris, "Fifty years of radiation biology in entomology: lessons learned from IDIDAS," Ann. Entomol. Soc. Am. Vol. 98, pp. 1-12, 2005.

[24] R. Rahman, M. M. Rahman, S. Islam, R. Huque, 2002. "Observation on the growth parameters of *Spilosoma oblique* (Lep: Arctiidae) reard on artificial diets and reproductive component of this irradiated pest," in *Evaluation of Lepidoptera population suppression by radiation induced sterility*, IAEA-TECDOC 1083. International Atomic Energy Agency, Vienna, Austria, 2002, pp. 7- 14.