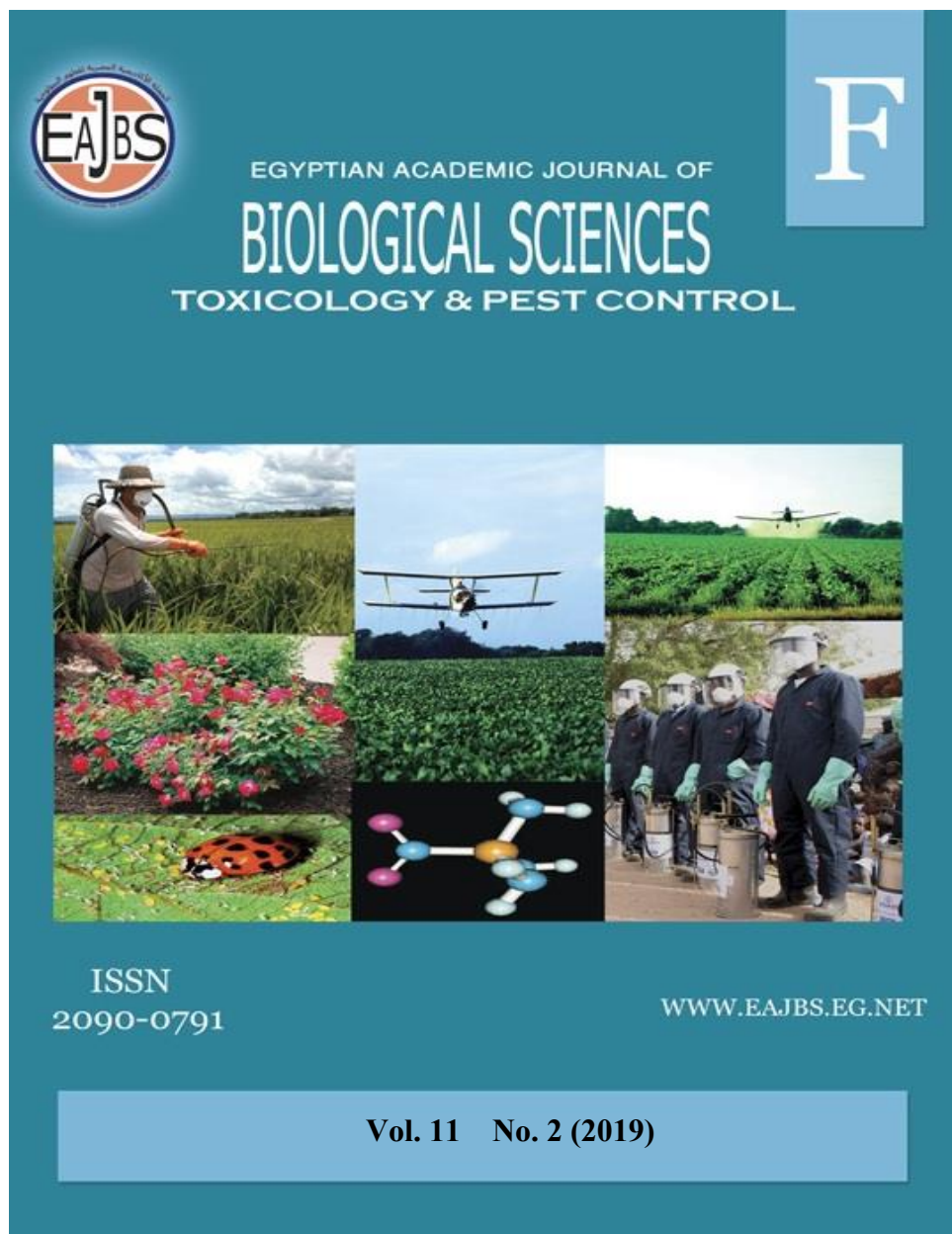


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Applications of Inherited Sterility and *Trichogramma evanescens* to Control Oases Date Moth, *Ephestia calidella*.

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ABSTRACT

Studies were conducted to appraise the effect of sub-sterilizing doses of gamma irradiation (125 and 175 Gy) and egg parasitoid *Trichogramma evanescens* on different egg ages (1, 2 and 3 day) of *Ephestia calidella* resulting from irradiated full grown pupae male with two sub-sterilizing doses (125 and 175 Gy). The results revealed that the first generation (F₁) moths were more sterile than parent (P₁) moths in the two doses applied. While the hatching percentage of eggs in the F₁ males was higher than in the F₁ females in the two tested doses. Moreover, at the dose of 125 Gy, the percentages of hatched eggs were 27.7 and 33.3% in the F₁ males and F₁ females, respectively and decreased to 12.7 and 20% in the F₁ males and F₁ females, respectively in the case of 175 Gy. The percentage of parasitism on *E. calidella* eggs by *T. evanescens* was significantly influenced by the dose of radiation given to male parents and age of host eggs. Also, the highest rate of parasitoid eggs was recorded at 24h, while the lowest rate was recorded at 72h old in the descendants of both irradiated and non-irradiated parents. Female-biased sex ratios were observed at all treatments in whether parental or F₁ generation. The data indicated that the host eggs of the F₁ generation of irradiated *E. calidella* were less effective for parasitization by *T. evanescens* females than the host eggs descendant of the parents and the control treatments. These results could lead to maximize the integrated *E. calidella* control with gamma irradiation and *T. evanescens* parasitism.

INTRODUCTION

Oases date moth, *Ephestia calidella* (Guenee) (Lepidoptera: Pyralidae) is considered a serious pest infesting dates in field and stores. Because larvae infest dates, particularly ripe dates causing fruit drop, the insect is capable of re-infesting fallen dates or non-harvest dates remained on palm trees after harvesting (Mikhaiel and Boshra 2006). Control of lepidopteran pests worldwide is achieved almost entirely through the use of synthetic insecticides. This dependence on insecticides has contributed to the development of insecticide resistance in many of the most serious pests (Denholm 1999, Ishaaya 2001 and Cui 2006). Production of natural enemies in an efficient and economical way is a precondition for the biological control program. Interesting using biological

control has increased and has long been recognized as an important tool in the suppression of insect pests. *Trichogramma* species (Hymenoptera: Trichogrammatidae) are widely used egg parasitoids for biological control of insect pest species on different crops through augmentation and release. It is a natural enemy of many harmful lepidopteran insect pest species on crops and vegetables (Jalali and Singh, 1993). *Trichogramma* has been used against the lepidopteran pests of cotton, cabbage, apple and tomato (Smith, 1996). *Trichogramma* species are the most studied groups of egg parasites. Their universal use in biological control programs is due to their efficiency and easy breeding under laboratory conditions (Parra & Zucchim 2004). *Trichogramma* species are successfully used against many insect pests on different systems of crops and pests (Li 1994). The main advantages of *Trichogramma* from other natural enemies are the time of their short generation, the economic way of mass production. (Jervis *et al.* 2001) and females begin to lay eggs shortly after emergence (Chassain and bouletreau 1991). The release of sterile or partially sterile insects with biological control agents is known to have synergistic effects to suppression the population when applied simultaneously (Bloem, et al. 1998). This sterile insect showed an impact on the pupal stage, while the biological control agents target mostly the immature stages, and reproducing on the F₁ offspring in inherited sterility releases Sallamet *al* (2000). This work aims to provide an overview of examining eggs oviposited by the females of *E. calidella* that mated with irradiated male pupae and their F₁ progeny which may be less or more equally suitable than irradiated one for parasitization by *T. evanescens* females to assess the potential of using combined releases of partially sterile *E. calidella* and *T. evanescens*.

MATERIALS AND METHODS

Origin of Hosts:

The main stock culture of the Oases dates moth, *Ephestia calidella* was obtained from heavy infested dates (collected from Siwa and El-baharia oases) and reared on artificial media according to Waites and Gothif (1969) in the laboratory of the Biological Applications Department, Inshas, Egypt. The insect stock culture was maintained at 28±2°C and 70±5% R.H. To obtain large numbers of pupae, the last instar larvae, which are ready to pupate, were collected, sexed and isolated in glass jars provided with fine tissue paper.

Irradiation Technique:

Full-grown male pupae of *E. calidella* (10 day-olds) were irradiated with two sterilizing doses 125 and 175 Gy using Cobalt 60 irradiator at Cyclotron Project-Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, with the dose rate of 0.55 Gray / second. Immediately after adult emergence, irradiated males (I♂) from each dose rate were paired with non-irradiated (U♀) females (I♂ x U♀), also non-irradiated males paired with non-irradiated females (U♂ x U♀) as control. Three Replicates were used for each combination with five pairs for each replicate. Newly hatched larvae (F₁ progeny) resulting from irradiated P₁ males were reared in groups on rearing media until the last instars. Then full-grown larvae were collected sexed and kept separately in glass jars for pupation. Three replicates were set-up for the following matting: at each dose U♀ x F₁♂, F₁♀ x U♂ moth pairs were allowed to mate and lay F₁ eggs for the two tested doses. The number of laid eggs that fall through wire mesh was counted for each pair. The percentage of reproduction control (PRC) was calculated using the equation of Rizvi *et al* (1980) :

$$PRC = (V_1 - V_2) / V_1 \times 100$$

Where V₁ = eggs laid by non-irradiated females V₂ = eggs laid by irradiated females.

The relative index (RI) for egg viability was calculated using the equation.

$$RI = (X1 - X2) / X1 \times 100$$

Where X1= mean the number of the viability of eggs from non-irradiated females
X2= mean number of viability of eggs from irradiated females.

Source of Parasite:

The initial culture of *Trichogramma evanescens* started with parasitized egg masses of the Angoumois grain moth, *Sitotroga cerealella* obtained from Biological Control Research Department Plant Protection Research Institute, ARC, Egypt. These eggs were placed in glass tubes (2.5x12cm), closed with muslin cloth-wrapped cotton plugs. As the adults began to emerge, sheets of gummy cardboard (2x 6cm) containing *E. calidella* eggs were introduced to serve as host; and parasitized host eggs were kept in the rearing room at 24±1°C and 70±5% RH. For studying the effects of host eggs resulted from irradiated parental males with the two sub-sterilizing doses, The parasite *T. evanescens*, of 1, 2 and 3-day old on the *E. calidella* eggs (50 eggs) were collected from F₁ generation and control. A batch of 50 host eggs of each tested dose and age.F₁ crosses or non-irradiated was glued onto sheets of cardboard with a water solution of nontoxic glue. The cards were placed separately in glass tubes (6 cm × 1.5 cm) containing newly emerged a couple of *T. evanescens* and stopper with cheesecloth-wrapped cotton plugs. Three replicates were used for each treatment. After 24h of exposure to the parasite, eggs were removed and placed in clean tubes. After 4 days, the eggs were detected in black indicating that the parasite larvae inside the host egg had turned into a virgin. The number of adults appeared and adult output was determined on the host eggs. The percentages of female adults and sex ratio were estimated according to Jardak, et al. (1979).

Statistical Analysis:

The data were analyzed using an ANOVA analysis technique and the mean separation was determined by the multiple-range test in Tukeys at P <0.005.

RESULTS AND DISCUSSION

Full-grown male pupae of *E. calidella* were irradiated with two sub-sterilizing doses of gamma radiation, 125Gy and 175Gy, and then mated with the non-irradiated females. The egg hatch was decreased by increasing the doses and the reduction was appeared in the 175Gy (27.3 and Relative index 68.7) when compared with control (90.3 and Relative index-) (Table 1). The egg hatch was decreased by increasing the doses and the reduction was appeared in the 175Gy when compared with control.

The percentage of egg hatching was more decreased significantly among the F₁ progeny than parents and F₁ males inherited more sterility than did in F₁ females. The egg hatching at the dose level 125Gy was 32.7% among P₁ generation while it was recorded (27.3% and 33.3%) among F₁ males and F₁ females, respectively.. The relative index was increased obviously at all tested doses. The reproductive rate of female was significantly decreased as a consequence of radiation treatment. Similar result was agree with Carpenter *et al* (1987). A greater reduction in reproductive performance was observed at the 175Gy dose than the 125Gy dose. A similar result was observed by Hight *et al.* (2005) on *Plutella xylostella*, Boshra (2007) on *Sitotroga cerealella*, and, Abd El-ghfar *et al.* (2012) on *E. cautella*. Hu *et al.* (2012) confirmed these results on *Helicoverpa armigera* and on *P. xylostella*, Conopomorpha *sinensis*; Farghaly *et al.* (2014) on *Corcyra cephalonica*, Akin and Hanife (2016) on *Tuta absoluta*. On the other hand, the success of parasitoid for *T. Evanescens* was recently out when three different ages of *E. calidella* eggs were resulting from exposing the parents to the two tested doses. The data in (Fig 1) showed that the parasitism percentages on *E. calidella* eggs by *T. evanescens*

was significantly decreased by increasing the applied doses and the age of egg host. Moreover, the biological effects of this parasite on the different ages of *E. calidella* eggs that obtained from irradiated male have significantly deviated from the untreated one. Furthermore, 3 -day old eggs showed the least suitability for successful development of the parasitoid as compared with the other two younger ages (Fig 1). The percentages of parasitism on host eggs at 125Gy were (90.7, 35.3 and 24.6 %) on the three ages of eggs, respectively. In general; there was a significant interaction between the two doses of radiation and the three ages of *E. calidella* eggs (Fig1.) In (Fig 2), the obtained results revealed the emergence percentage of parasites was declined sharply with increasing the egg age; it was 90% in the control and 43.1 in the dose 175Gy. The mean number of female parasitoids produced was affected by an interaction between the dose of radiation and the age of *E. calidella* eggs (Fig3).

Table (1): Effect of two substerilizing doses of gamma irradiation on reproduction and progeny development of male *Ephestia calidella*.

Treatment	Doses (Gy)	Crosses Male: Female	Fecundity /female (Mean \pm S.E.)	% PRC	% Hatchability (\pm S.E)	% RI
Parent (P)	0.0	U δ X U ϕ	256.30 \pm 0.90a	-	90.30 \pm 3.41a	-
	125	I δ X U ϕ	139.00 \pm 0.59b	44.6	32.70 \pm 0.34b	64.01
	175	I δ X U ϕ	109.70 \pm 1.23d	57.2	27.30 \pm 0.9bc	68.7
Offspring (F1)	0.0	U δ X U ϕ	257.00 \pm 0.34a	-	85.70 \pm 0.034a	-
		F1 δ X U ϕ	108.00 \pm 0.34d	58.01	27.30 \pm 0.53bc	68.1
	125	U δ X F1 ϕ	117.70 \pm 0.34c	54.2	33.30 \pm 0.51b	61.5
		F1 δ X U ϕ	095.60 \pm 0.34f	62.8	12.70 \pm 0.34d	85.3
	175	U δ X F1 ϕ	098.70 \pm 0.34e	61.6	20.00 \pm 0.59cd	76.7
		F1 δ X F1 ϕ				
df	-	-	14	-	14	-
Pr	-	-	0000 ***	-	0000 ***	-
F.value	-	-	6105.04	-	350.43	-

Means followed by the same letter in each column for each generation are not significantly different at $P \leq 0.005$

PRC=Percent reduce from the control RI =Relative index
U=Non irradiated female I=Irradiated male

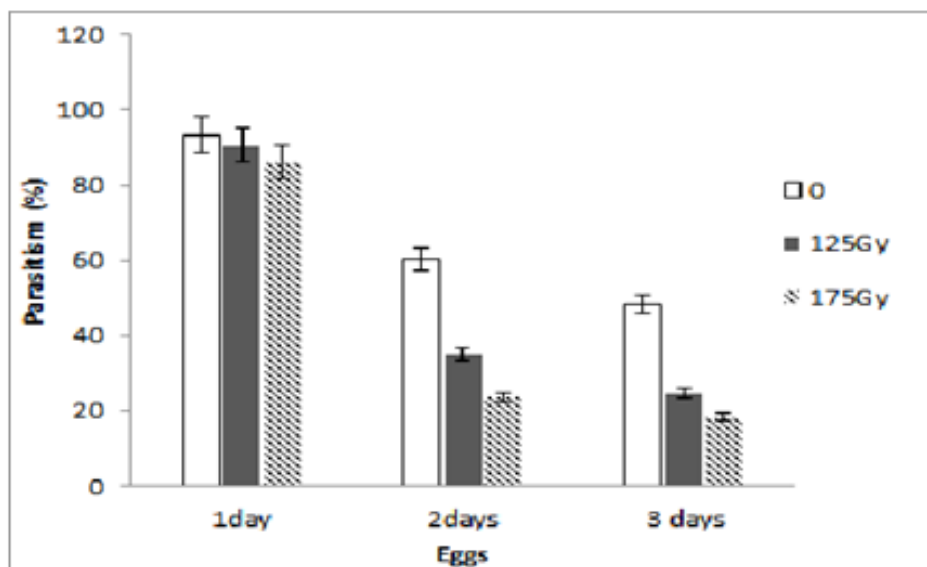


Fig.1. Parasitism (%) of *T. evanescens* on three different ages of *E. calidella* eggs resulted from irradiated pupae with different doses of gamma radiation (Parent generation).

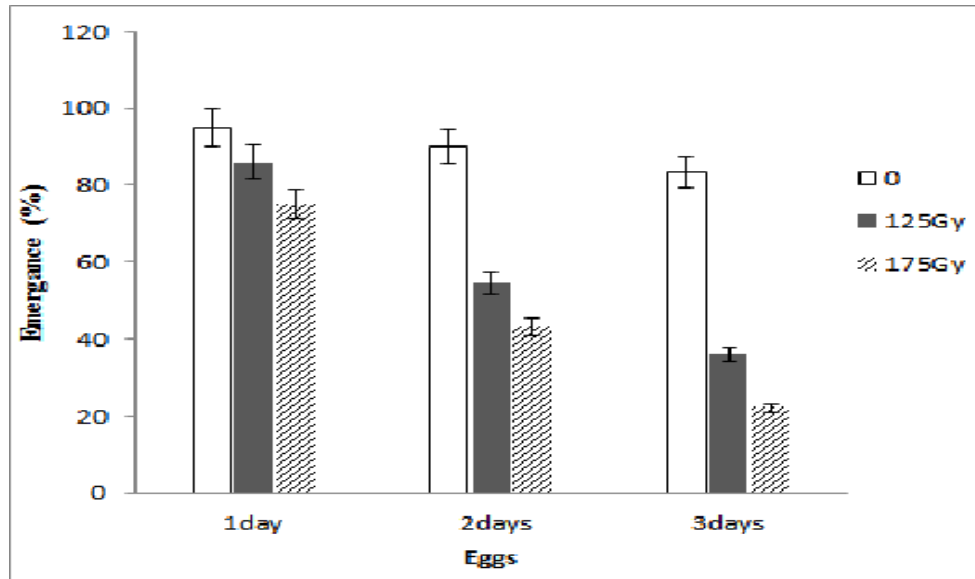


Fig.2. Adult emergence (%) of *T. evanescens* that parasitise on three different ages of *E. calidella* eggs from sub-sterilizing doses of gamma radiation (Parent generation)

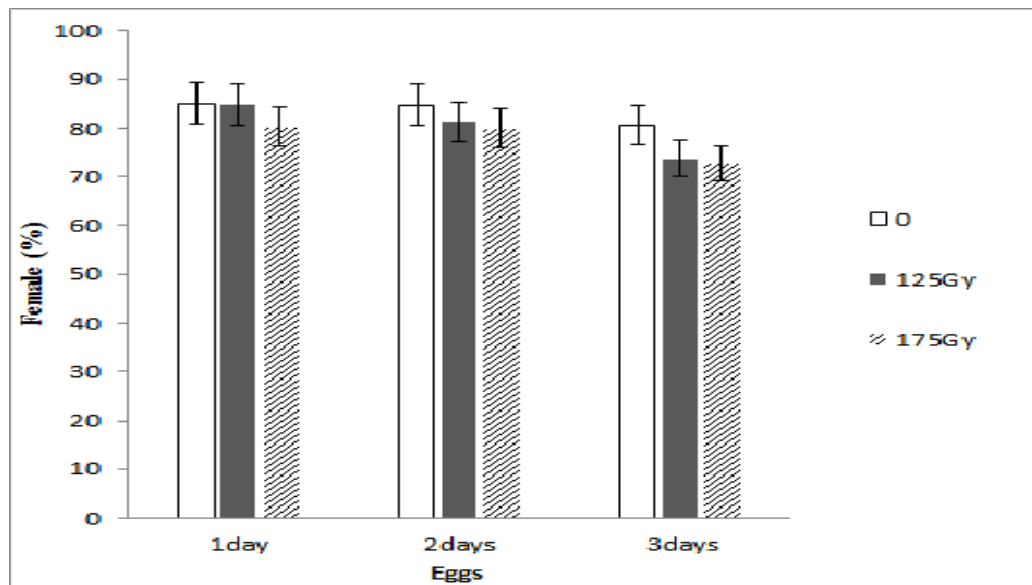


Fig.3. Female (%) of *T. evanescens* that resulted from the parasitism on three different ages of *E. calidella* eggs previously irradiated with sub-sterilizing doses of gamma radiation (Parent generation).

Radiation delayed the host eggs development and enhanced the suitability for parasitization of certain dipteran and lepidopteran insects by Fatima *et al.* (2009), Hamed *et al.* (2009) and Zapater *et al.* (2009) These results contradict those of Brower (1982) who reported that the percentage of female parasites resulting from *T. pretiosum* parasitism on eggs produced by *Plodia interpunctella* parents irradiated with radiation or non-irradiated more than males. While, they agree with those reported by Harwalker *et al.* (1989) who reared *T. brasiliensis* (Ashmead) on eggs laid by irradiated sterile females of potato tuber moth, Calvin, *et al.* (1997) when *T. pretiosum* females were exposed to fertile and infertile eggs of *Diatraea agrana diosella* Dyar and Carpenter, *et al.* (2004) who

found that the number of parasitized eggs was lower than the control when *T. cryptophlebiae* parasitized eggs of irradiated false codling moth. The data in Fig (4) indicates that the reduction in the percentage of parasitism was more pronounced in F₁E. *calidella* male than that in F₁female and the 3-day old egg of host. The percentage of wasp's emergence was decreased in the parasitized host eggs in the subsequent days by increasing the dose Fig (5). The highest number of parasitized host eggs was reported on 1– day old eggs, and then decreased gradually during the successive two days. The percentages of parasitism were recorded (64.7 and 68.3 %) on the 2- day -old egg produced from the crosses F₁♂X U♀ and U♂X F₁♀, respectively, at dose 175Gy as compared with its control (62.0%) Fig (4). Also, the difference between the percentages of emerged parasitoids adults was significantly influenced by the type of cross, dose of radiation and host egg age of *E. calidella*. The percentage of *T. evanescens* wasps' emergence was (92.3%)of parasitoid by cross F₁♂X U♀ at the dose 125 Gy on the 1-day- old of host egg Fig (5). The percentage of progeny wasps resulted from the aforementioned mating of parasitoid was strongly skewed in favor of females by all doses of hosts used and the deviation from the expected ratio increased with increasing the dose Fig (6). The present results indicated that in the F₁ progeny the host cross, dose of radiation and host egg age significantly influenced the mean percentage of parasitized eggs. Also, the results of the present study showed that the parasitoid preferred newly laid eggs and host suitability decreased as host age increased. In *T. principium* parasitization, the behavior was stable when young (preferred) and old (less preferred) grain moth eggs *Sitotroga cerealella* were offered in sequence (Reznik, *et al.* 1997). Ruberson and Kring(1993)and Tadaka *et al.* (2000)indicated that the of host eggs for parasitization by *Trichogramma* sp. is reduced significantly with the boost in their age. Thus, an adequate amount of fresh eggs are conserved to be used as hosts for production of parasitoids Also, Fatima *et al.* (2009) found that *S. cerealella* eggs were used as hosts for *T. chilonis* and the suitability of non-irradiated host eggs decreased as the age of the eggs increased, with no success in parasitization of eggs older than 4 days of age.

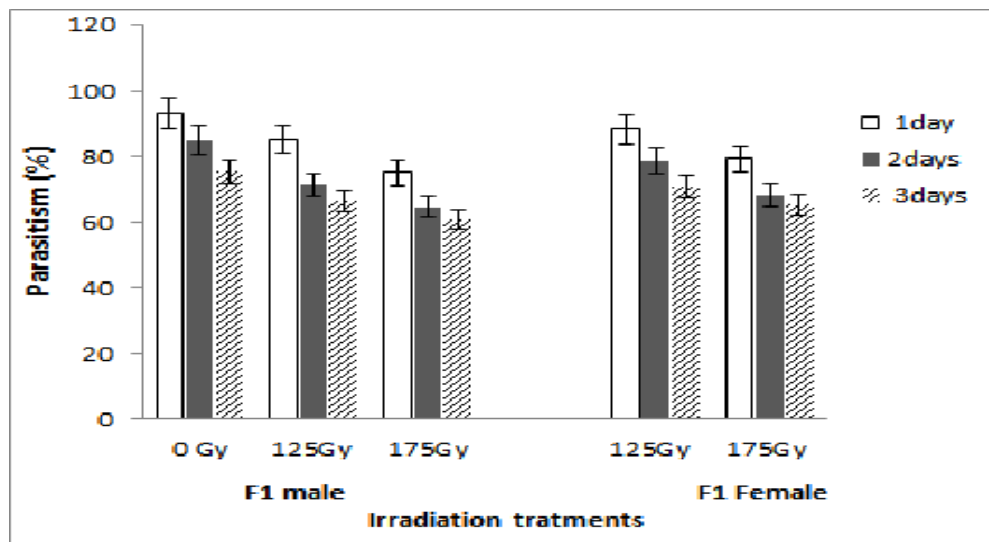


Fig.4.Parasitism (%) of *T. evanescens* on three different ages of *E. calidella* eggs resulted from irradiated pupae with different doses of gamma radiation (F₁ generation)

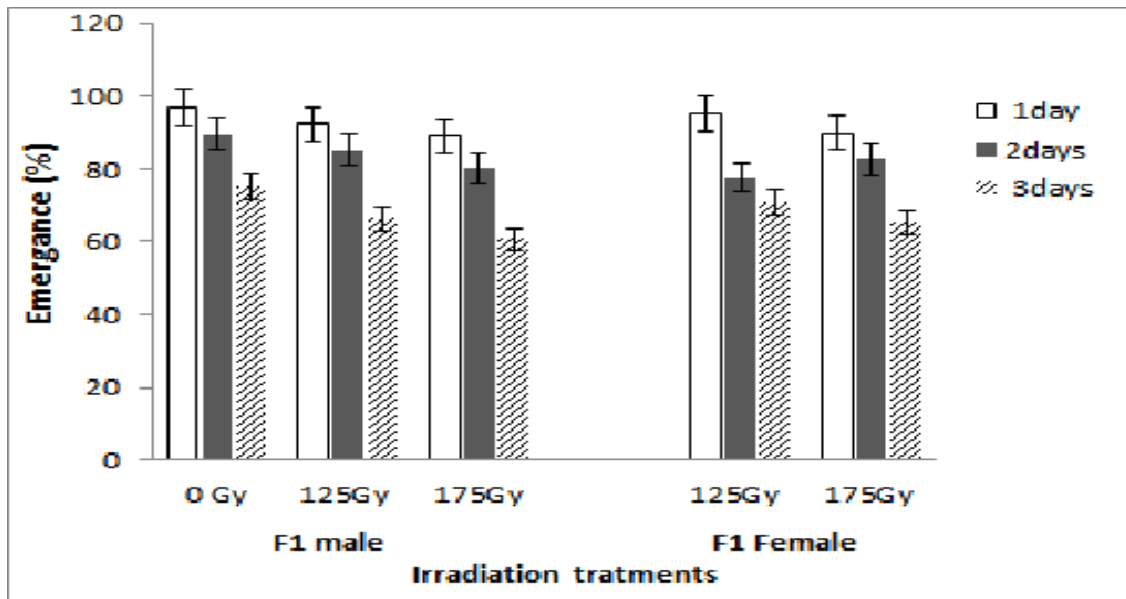


Fig.5.Adult emergence (%) of *T. evanescens* that parasitise on three different ages of *E. calidella* eggs from sub-sterilizing doses of gamma radiation (F₁ generation).

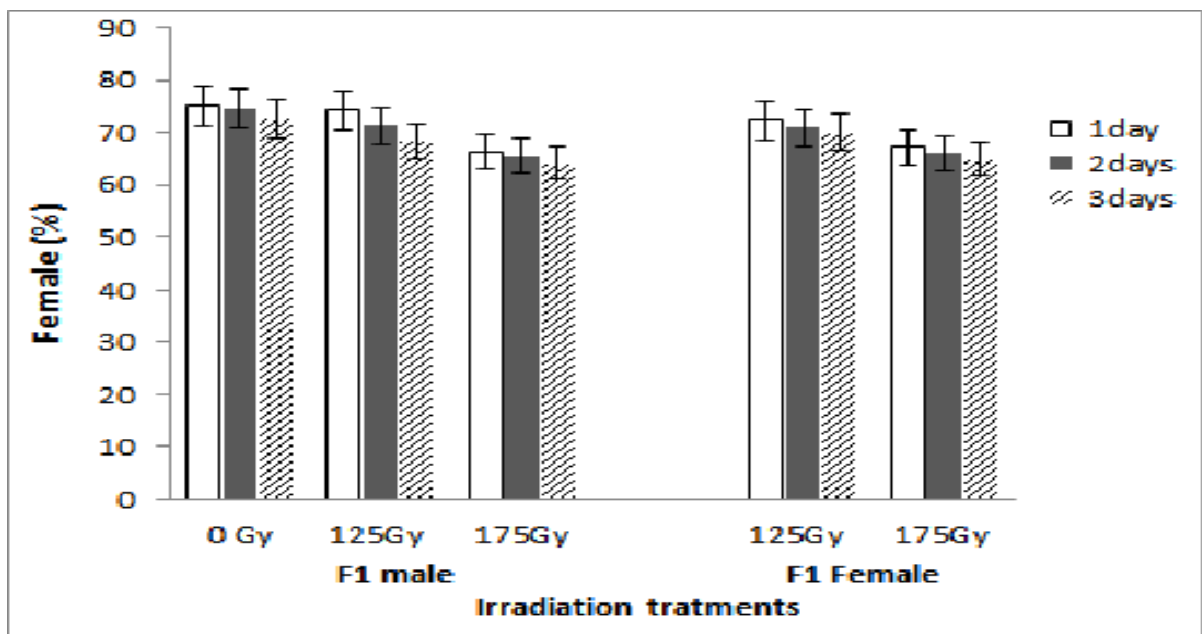


Fig.6.Female (%) of *T. evanescens* that resulted from the parasitism on three different ages of *E. calidella* eggs previously irradiated with sub-sterilizing doses of gamma radiation(F₁ generation).

Conclusion

In conclusion, these experiments indicated that the dose of 125Gy would be appropriate for irradiation of *E. calidella* males that intended to be used. Although eggs from irradiated male pupae of *E. calidella* were found to be suitable as hosts, *T. evanescens* demonstrated a preference for the eggs from non-irradiated ones.

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ARABIC SUMMARY

"تطبيقات لعقم المورث وطفيل التريكوجراماافنسينس لمكافحة فراشة بلح الواحات افستياكالديلا"

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1-هيئة الطاقة الذرية -مركز البحوث النووية
2-مركز البحوث الزراعية -قسم وقاية نبات

اجريت الدراسات لتقييم تأثير جرعات تحت المعقمة لأشعة جاما (125 و175 جراى) وطفيل التريكوجراما افنسينس البيضى على أعمار مختلفة من البيض تتراوح بين (1 و2 و3 أيام) لفراشة بلح الواحات افستياكالديلا الناتج من تشجيع ذكور العذارى كاملة النمو للجرعتين تحت المعقمة. أوضحت النتائج ان فراشات الجيل الأول (F₁) أكثر عقمًا من الأباء (P₁) فى الجرعتين المختبرتين بينما كان نسبة الأنخفاض فى نسبة الفقس لدى الذكور F₁ أعلى من الإناث فى الجرعتين المختبرتين. وكانت نسبة الأنخفاض فى فقس البيض عند الجرعة 125 جراى 27.7 و 33.3 % فى ذكور F₁ و أناث F₁ على التوالي وانخفضت إلى 12.7 و 20 % فى ذكور F₁ و أناث F₁، على التوالي فى حالة Gy175. تأثرت النسبة المئوية للتطفل على بيض فراشة البلح بواسطة طفيل التريكوجراما معنويا حسب الجرعة المعطاه لذكور الأباء وعمر البيض وكان اعلى معدل للتطفل حدث بعد 24 ساعة وأقل عند 72 ساعة لكل من البيض المعامل وغير معامل. وكانت نسبة الجنسة متحيزة للإناث الطفيل فى كل المعاملات سواء فى جيل الأباء او الجيل الأول. وأشارت البيانات إلى أن البيض المتطفل عليه بواسطة طفيل التريكوجراما الناتج من الجيل الأول F₁ أقل بالمقارنة لبيض الأباء P₁ والبيض الغير معامل لفراشة بلح الواحات. هذه النتائج يمكن أن تؤدي إلى تعظيم دور مكافحة المتكاملة فى السيطرة على فراشة بلح الواحات بأستخدام التشجيع الجامى مع طفيل التريكوجراماافنسينس .