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Cold storage and gamma irradiation of *Sitotroga cerealella* Olivier eggs (Lepidoptera: Gelechiidae) in relation to the success of parasitism by *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae)

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Abstract

The performance of parasitism by the egg parasitoid, *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) on eggs of Angoumois grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) was investigated under cold storage and gamma irradiation treatments of the host eggs. Cold storage treatment could improve the parasitoid mass rearing techniques and reduced the costs of biological control programs, while gamma irradiation might be used as a supplementary support at the times of high demand. The suitability of the *S. cerealella* eggs, stored at $-20\text{ }^{\circ}\text{C}$ for 0.5, 1, or 2 h. as a host for *T. evanescens* was evaluated. The sensitivity of *S. cerealella* eggs to gamma irradiation treatments and the acceptability of irradiated eggs for parasitism by *T. evanescens* females for the parental P and F₁ generations were examined. The results revealed that parasitism was drastically reduced more than adult's emergence and sex-ratio (% of females) after cold storage periods of *S. cerealella* eggs. Moreover, the parasitism percentages were relatively reduced to (97.1, 96.1, 93.03, and 92.7 %) after irradiating the *S. cerealella* eggs at 40, 60, 80, and 100 Gy, respectively than the control (97.3% emergence). The percentages of emergence and females' percent were slightly decreased by gamma irradiation doses, while, equal preferred by the F₁ generation of parasitoid that produced from irradiated *S. cerealella* eggs.

Keywords: Irradiation, Cold storage, Eggs, *Sitotroga cerealella*, *Trichogramma evanescens*

Background

Trichogramma spp. is among the most important natural enemies used widely around the world to control the lepidopteran insect pests. They have a wide host range and can be easily be mass produced (Li, 1994). Augmentative release of the egg parasitoid to manage the stored product pests is a promising technique as a bio-control agent (Grieshop et al. 2006). The utilization of *T. evanescens* against lepidopteran stored-product pests has gradually increased in many regions for the

control of the Mediterranean flour moth *Ephestia kuehniella* Zeller, the warehouse moth *Ephestia elutella* (Hübner) and the Indian meal moth *Plodia interpunctella* (Hübner) (Prozell and Schoeller, 1998). The costs of mass rearing of *Trichogramma* spp. on natural hosts are relatively high, so, the eggs of the grain moth, *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) are often used as an alternative host (Vieira and Tavares, 1995). Storing large numbers of *Trichogramma* and host eggs is desirable as the field requirements can vary, in order to face a fluctuating demand. The low temperature has been applied to kill the host eggs before being exposed to the egg parasitoid, (Bradley et al. 2004). In addition, the ultra violet light (UV) irradiation treatment

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has been suggested to kill eggs of insect hosts of *Trichogramma* spp. (Voegelé et al. 1974 and Goldstein et al. 1983). However, the UV radiation is a weak penetrating power, and it makes the UV treatment less applicable than ionizing radiation, while, Gamma radiation has very high penetration ability and could be used successfully to kill the host eggs (Brower 1982; Ayvas and Tuncbilek 2006 and Ayvaz et al. 2008). The irradiated and un-irradiated eggs with gamma radiation were equal acceptable for parasitism by *Trichogramma* spp. (Saour 2004 and Ayvaz et al. 2008). The radio sensitivity of the eggs has to be investigated, in order to determine the appropriated irradiation dose. In lepidopteran insects, combined treatments; release of partial sterile insects and *Trichogramma* may prove practical and would be a potential control strategy.

The objectives of this study were to determine the effects of cold storage periods and gamma radiation rates of *S. cerealella* eggs on the performance of *T. evanescens*, in order to assess the potential of using both treatments for increasing the efficacy of using *Trichogramma* parasitoids.

Materials and methods

S. cerealella rearing

A colony of *S. cerealella* was originally obtained from the Center of Bio-organic Agricultural Services (CBAS), Aswan Governorate, Egypt. *S. cerealella* was reared and maintained at 26 ± 1 °C and $85 \pm 5\%$ R.H. Mass rearing technique was carried out as described by Abdel-Hameid (2018).

Rearing of *T. evanescens*

T. evanescens, used in this study, was originally obtained from CBAS. The parasitoid was reared on the eggs of *S. cerealella* glued on small carton cards, each carrying about 500 eggs. Rearing was carried out by exposing the egg-cards of *Sitotroga* to the parasitoid in cages covered with cotton-cloth.

Effect of cold storage

One-day-old *S. cerealella* eggs, mounted on cards, were separately placed in glass vials (1.6 cm diameter \times 10 cm height) to be killed by cold storage at (-20 °C) for 0.5, 1, and 2 h. Storage was carried out in a deep freezer. The frozen eggs were introduced into glass vials to the parasitoid adults (3 egg cards, carrying *S. cerealella* eggs + 1 card of the parasitized eggs). The emerged adults were placed, individually, in glass vials. Percentages of emerged adults, the % parasitism and sex-ratio (% of females), were recorded. For each storage period, 50 replicates and a control were used.

Effect of irradiation

For studying the acceptance of irradiated *S. cerealella* eggs for parasitism by *T. evanescens* females, 24-h-old eggs were used. Egg cards carrying *S. cerealella* eggs were exposed to gamma radiation dosages in a gamma cell supplied with a Co-60 source rounded the cylindrical irradiation chamber (Issledovatel Gamma Irradiator, Technabexport Co. Ltd. USSR) located at Cyclotron Project-Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, with a dose rate of 0.55 Gy/s. Five replicates of 500 eggs, each was irradiated at different doses (0, 40, 60.80, and 100 Gy). The treated eggs were transferred to normal rearing conditions and introduced into the glass vials to be exposed to the parasitoid (3 egg cards" carrying *S. cerealella* eggs + 1 card of the parasitized eggs).

Parasitism percent per card was scored 5 days after incubation. The number of emerging parasitoid adults was recorded, and the percentages of parasitism and females were calculated. A new *S. cerealella* eggs card was introduced to the newly emerged wasps (F_1 generation) that emerged from irradiation treatments. Percentages of parasitism, adult emergence, and females' percentage were determined in each treatment.

Statistical analysis

The statistical analysis of data was conducted using ANOVA technique using SPSS Ver. 19.0. The significance between means was determined by the multiple-range test (Duncan at $P < 0.05$). Data were designed according to Steel et al. (1997).

Results and discussions

S. cerealella eggs (whether treated by irradiation or cold storage) showed acceptable rates for parasitism by *T. evanescens* females (Tables 1, 2 and 3).

Effect of cold exposure periods

When the *S. cerealella* eggs were stored at -20 °C, the parasitism percentages significantly reduced to (21.50, 18.54, and 10.55%) at the storage periods, 30, 60, and 120 min, respectively than the control (97.10 %) (Table 1). As shown in the same table, the high numbers of the parasitoid adult emergences were recorded at the storage treatment of 30 min than those recorded in 60- and 120-min cold storage. While, there was insignificant difference in the percentage of females within the 3 storage treatments (30, 60, and 120 min). Statistical analysis of data showed highly significant differences in percentages of parasitism and emergence, among the different cold storage treatments. The results indicated that the 3 storage periods applied (30, 60, and 120 min) to *S. cerealella* eggs sharply declined the acceptability of *T. evanescens* parasitism, while the longer periods 60 and 120 drastically reduced the adult emergence percentages of parasitoid. However, the

Table 1 Effects of different cold storage periods of *Sitotroga cerealella* eggs at -20°C on *Trichogramma evanescens* parasitism, emergence rate, and sex ratio

Storage (hour)	No. of exposed eggs (mean \pm S.E)	% of parasitism	% of emergence	Sex-ratio (% of females)
Control	4286.00 \pm 0.33	97.10 \pm 0.10a	97.50 \pm 0.07a	97.70 \pm 0.33a
1/2 h.	3556.66 \pm 0.35	21.50 \pm 0.85b	80.40 \pm 0.33b	87.30 \pm 0.33b
1 h.	3553.33 \pm 0.58	18.54 \pm 0.58b	67.50 \pm 0.80c	86.33 \pm 0.88b
2 h.	3307.33 \pm 0.58	10.55 \pm 0.85c	63.10 \pm 0.11c	85.33 \pm 0.33b
P		0.0000	0.0000	0.0000
F		820.00532	51.363718	118
df		11	11	11

a, b, & c: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter.

female percentages were relatively similar in the 3 storage period treatments. These results agree with Karabörklü and Ayvaz (2007) who found that the parasitization and emergence of *T. evanescens* adults reduced depending on the storage periods of *E. kuehniella* and *S. cerealella* eggs. Moreover, they found that the storage temperature and periods decreased the adult longevity of the parasitoid in case of *S. cerealella* more than in *E. kuehniella* eggs. Also, it was reported that the cold treatment of host eggs reduced the *Trichogramma* performance (parasitism rate, emergence, and sex ratio) (Bradley et al. 2004). The present results contradict with those of Karabörklü and Ayvaz (2007) who found that the percentage of sex ratio of *T. evanescens* that emerged after cold storage of the host eggs was relatively similar to the control treatment. The parasitoid *T. evanescens* had a high tolerance to temperatures and was used to control *Plodia interpunctella* and *Cadra cautella* (Walker) in storage to protect the stored products (Scholler and Hassan 2001).

Irradiation of *S. cerealella* eggs

Irradiation of 1–24 h. old eggs with 40, 60, 80, and 100 Gy doses, slightly affected the performance parameters of *T. evanescens*. The parasitism percentages were relatively reduced (97.1, 96.1, 93.03, and 92.7%) in 40, 60, 80, and 100 Gy, respectively than the control treatment (97.3%) (Table 2). The reduction of parasitism was

significant in (60, 80, and 100 Gy) than the 40 Gy and control treatments. The same trend was recorded in the percentages of emerged adults, where they were recorded, at the 4 rate treatments being, significantly reduced to (93.23 and 91.5% at 80 and 100 Gy), respectively than (96.96, 95.16, and 97.5%) in 40, 60 Gy and control, respectively. As shown in the same table, although, the females' percentage resulted from the eggs exposed to 100 Gy dose reached (90.0%), it was significantly reduced than the recorded (97.66, 95.3%) at the control and 40 Gy, respectively and insignificantly decreased than that recorded (93 and 91%) at 60 and 80 Gy. In similar studies, Brower (1982) reported that equal parasitization was recorded by *T. pretiosum* on the eggs from un-irradiated and irradiated Indian meal moth *P. interpunctella* adults with 150 Gy. This finding may be due to a high radio-tolerance of *P. interpunctella* than to the other insect species (Brower, 1975). Similarly, Saour (2004) found that irradiated potato tuber moth, *Phthorimaea operculella* (Zeller), with 150 Gy and non-irradiated parental crosses influenced by the *Trichogramma* parasitoid at the same rate. The present results contradict with those of Cossentine et al. (1996) on codling moth *Cydia pomonella* and Carpenter et al. (2004) on false codling moth *Cryptophlebia leucotreta*, who found significant declines in the performance of *T. platneri* and *T. cryptophlebiae*,

Table 2 Effects of gamma irradiation of *S. cerealella* eggs (different rates) on *T. evanescens* parasitism, emergence rate and sex ratio (parental, P generation)

Treatment	No. of exposed eggs (mean \pm S.E)	% of parasitism	% of emergence	% sex ratio (female)
Control	6123.3 \pm 0.33	97.3 \pm 0.06a	97.5 \pm 0.067 a	97.7 \pm 0.33a
40 GY	5511.7 \pm 0.88	97.1 \pm 0.52a	96.96 \pm 0.62a	95.3 \pm 0.33b
60 GY	4897.7 \pm 0.33	96.1 \pm 0.37ab	95.16 \pm 0.82ab	93.0 \pm 0.58c
80 GY	4797.3 \pm 0.67	93.03 \pm 0.58ab	93.2 \pm 0.57bc	91.0 \pm 0.58c
100 GY	4285 \pm 0.100	92.7 \pm 0.95ab	91.5 \pm 0.64c	90.0 \pm 0.58c
P		0.0104	0.0001	0.0000
F		5.9284953	20.574855	40.318182
df		14	14	14

a, b, & c: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter.

Table 3 Effects of gamma irradiation of *S. cerealella* eggs (different rates) on *T. evanescens* parasitism, emergence rate and sex-ratio (F1 generation)

Dose (Gy)	No. of exposed eggs (mean \pm S.E)	% of parasitism	% of emergence	% sex- ratio (female)
Control	6123.3 \pm 0.33	96.80 \pm 0.06a	98.0 \pm 0.07a	94.00 \pm 0.33a
40 Gy	5511.7 \pm 0.88	96.70 \pm 0.52a	97.5 \pm 0.07a	92.33 \pm 0.33a
60 Gy	4897.7 \pm 0.33	96.10 \pm 0.37a	97.20 \pm 0.62a	85.30 \pm 0.58ab
80 Gy	4797.3 \pm 0.67	95.90 \pm 0.58a	97.13 \pm 0.62a	84.6 \pm 0.57ab
100 Gy	4285.0 \pm 0.10	95.24 \pm 0.95a	96.57 \pm 0.57a	81.67 \pm 0.58b
P		0.2919ns	0.5172ns	0.0115
F		1.43608	0.865078	5.74014
df		14	14	14

a, b, & c: There is no significant difference ($P > 0.05$) between any two means, within the same column have the same superscript letter.

respectively, when the host adults were irradiated by gamma radiation. Also, Mikhael et al. (2019) found that both sub-sterilizing doses (125 and 175 Gy) and egg ages had a negative impact on the parasitism with *T. evanescens* on *E. calidella* eggs.

The data in Table 3 presented that the performance parameters of F₁ generation of *T. evanescens* adults that emerged from irradiated *S. cerealella* eggs were relatively similar with un-irradiated ones. The results showed that the percentages of parasitism and emergence rates were insignificantly different than those recorded in the control treatments. While, the female percentages of *T. evanescens* F₁ previously emerged from irradiated host with 60, 80, and 100 Gy were significantly decreased to 85.30, 84.6, and 81.67%, respectively than (94.00 and 92.33%) of 40 Gy and control treatments, respectively. These findings on the effect of fertile and sterile host eggs on *T. evanescens* may help for lepidopterous pests' management in both field and storage conditions. The obtained results are in line with Voegelé et al. (1974) who found that irradiated eggs of *E. kuehniella* with the UV irradiation were equally preferred by *T. evanescens* with non-irradiated ones. In contrast, Mikhael et al. (2019) reported that there were preferences in parasitism when the eggs from irradiated *E. calidella* were used as host to *T. evanescens* and un-irradiated ones.

Conclusion

Cold storage periods of *S. cerealella* eggs as host significantly, reduced the parasitism, emergence rates, and female percentage of *T. evanescens*. Furthermore, some biological parameters were slightly reduced by irradiated *S. cerealella* eggs with gamma irradiation doses. These findings in particular could be utilized to improve the quality of mass-reared *T. evanescens*.

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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NFA put the idea of research and prepare a plan for the search and responsible for *S. cerealella* rearing. EIRM performed the samples preparation of the *S. cerealella* and *T. evanescens*, and was a major contributor in writing the manuscript, collecting references and responsible for publishing and participating in the steps of carrying out the research. MAL studying the effect of cold storage and making statistical analysis. SWAA studying the effect of irradiation by (Issledovatel Gamma Irradiator, Techsnabexport Co. Ltd. USSR) located at Cyclotron Project-Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt. All of the authors of this manuscript contributed equally to the design and/or execution of the experiments described in the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable

The study was conducted on insect species that are abundant in the ecosystem and does not require ethical approval.

Consent for publication

All authors consent to publish this paper. The manuscript has not been published in completely or in part elsewhere.

Competing interests

The authors declare that they have no competing interests.

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