

## THE POSSIBILITY OF HOST EGGS STORAGE UNDER LOW TEMPERATURE AND ITS ACCEPTANCE BY *Trichogramma evanescens* WOSTWOOD (TRICHOGRAMMATIDAE: HYMENOPTERA) AFTER DIFFERENT PERIODS OF STORAGE.

El Sharkawy, Manal A. A.

Plant Protection Research Institute, ARC, Dokki,, Egypt

### ABSTRACT

The possibility of cold storage of Pink bollworm *Pectinophora gossypiella* (Saunders) and angoumois grain moth *Sitotroga cerealella* (Olivier) eggs, which used as hosts for *Trichogramma evanescens* which reared and investigated under three low temperatures ( $4\pm 1$ ,  $8\pm 1$  and  $10\pm 1^{\circ}\text{C}$ ), also the effect of storage periods on the parasitoid host acceptance were also studied. It was found that storage time was the major factor influencing the number of parasitized *P. gossypiella* and *S. cerealella* eggs. The percentage of emergence, and the percentage of sex ratio, both fitness measures were reduced after storage of 15 days or longer. Both host species could be successfully stored for 15 days on  $10^{\circ}\text{C}$  without detrimental effects on parasitoid acceptance. If the condition obligated to preserve host eggs for longer period it should be stored at  $8^{\circ}\text{C}$ . Cold storage of *P. gossypiella* eggs at  $8^{\circ}\text{C}$  and  $10^{\circ}\text{C}$  for 5 days did not affect parasitization. There were significant differences between the two host species in the percentage of parasitoid emergence and in the percentage of produced females (sex-ratio), where as, there was insignificant difference between the number of parasitized eggs ( parasitoid host acceptance).

### INTRODUCTION

Today, *Trichogramma* species are the most widely used insects as natural enemy in the world, partly because they are easy to mass rear and they attack many important crop insect pests. *Trichogramma* are released to control some 28 different caterpillar pests attacking corn, rice, sugarcane, cotton, vegetables, sugar beets, fruit trees and pine and spruce trees. Most releases are to control corn borers, sugarcane borers and cotton bollworm. Rearing *Trichogramma* requires first rearing an insect, typically a species of moth, to produce eggs in which the wasps will develop. The Angoumois grain moth, *Sitotroga cerealella*, and the Mediterranean flour moth, *Ephesia kuehniella* (Zeller), are easily and inexpensively reared on wheat or other grains and are commonly used to rear *Trichogramma* (Morrison *et al* 1976). In biological control programs which involve the release of a great number of parasites, it is extremely important to preserve the eggs of the hosts for prolonged periods to make better use of the production from a pre established colony, and to have great quantities of beneficial agents available when field release is necessary. The most common method of storing insects or prolonging their longevity is to keep them at low temperatures to reduce the rate of metabolism and activity ( Lating and Eden 1990). Egg parasitoids can successfully develop in host eggs that have undergone

freezing or heating (Wajnberg & Hassan 1994). Eggs from various Heteroptera species can be stored at low temperatures and still be parasitized by Scelionidae species (Orr 1988). Promising results for lepidopterous eggs storage for *Trichogramma* spp. multiplication and use in biological control studies have been reported in India (Dass & Ram 1983) and in China (Morrison 1988). Long-term storage of biological control products may help reduce production costs by allowing discontinuous production schedules. Commercial producers may be required to supply exceptionally large numbers during peak demand periods, with production greatly reduced or halted at other times of the year depending on seasonality of the target pests. Cold storage can permit a more cost-effective production schedule (Glenister and Hoffmann, 1998); it may also be a means to conserve biological control agents when not immediately needed. From the previous studies, it appears the important of host storage for rearing the parasitoids, so the aim of the present study is to (1) determine the suitable degree of temperature for host eggs storage, (2) restricted the longest period for host storage without affecting the acceptance of the parasitoid for this stored host eggs, (3) and to compare between the tolerance of two host species for storage under low temperature.

## **MATEERIAL AND METHODS**

The experiments were carried out in the Integrated Pest Management Laboratory, Bollworms Department, Plant protection Research Institute, ARC, Dokki, Giza, Egypt.

### **Rearing of insects:**

#### **Host rearing:**

Pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) was reared for several generations on modified artificial diet as described by Abd El-Hafez, Alia *et al.* (1982). Ten pairs of freshly emerged moths were confined in a glass chimney cage, inside which a piece of cotton wool previously soaked in 10 % sugar solution was suspended to be renewed every 48 hours for moths nutrition. The top and bottom of each cage were covered with screening mesh kept in position by rubber bands for stimulating egg-laying response in the females. Eggs were deposited through the screening mesh on a piece of paper placed upper and under the cage in open Petri-dishes. Cages were maintained at  $27\pm 1$  °C &  $80 \pm 5$  % R. H. and were examined daily for collecting the pieces of papers that contain eggs.

#### **Rearing of the factitious host, Angoumois grain moth:**

The method of rearing angoumois grain moth *Sitotroga cerealella* (Olivier) was a modification of Hassan (1995), where soft wheat was chosen as the rearing medium. For wheat sterilization, wheat were boiling in water for five minutes, then it was disseminated in sterilized room to dry. After that the grains were loaded into sterilized holding cribs. Six kilograms of wheat were placed in each crib. Six g of 2-days old cleaned eggs of *S. cerealella* was evenly scattered onto the crib, which was laid in a flat horizontal position in the rearing cage. On day 12 the cribs were hung vertically in the emerging

cage, which measure 115 x 60x 200 cm. Moths, emerging from the wheat after 21-28 days, and collected every day and introduced into cylindrical cages (40 X 20 cm.) coated with screen mesh wall through a hole in one end. Females readily oviposited through the cylindrical cage screen into the tray. Eggs were collected every 24 h during oviposition period and sieved through a screen (12 mesh per centimeter) and scales were removed as they passed through the air current of a vacuum system. The optimum conditions for rearing *S. cerealella* are 23-25°C and 60 ± 10% RH. (Laing & Eden (1990) and Hassan (1995)).

#### **Parasitoid rearing**

The parasitoid *T. evanescens* reared on pink bollworm *P. gossypiella* and angoumois grain moth *S. cerealella* eggs for mass production to be used in inundative release programs in the field. In case of rearing the parasitoid on eggs of pink bollworms, host egg sheets (2000-2500 eggs) were exposed to *Trichogramma* adults (100-150 adults) into glass jars (0.4 - liter) provided with filter paper witted by 10% sucrose solution for nutrition and covered with cloth-wrapped cotton kept in position by rubber band.. Where as, in case of rearing the parasitoid on angoumois grain moth eggs, eggs were glued to cards and exposed to *Trichogramma* adults (1 parasitoid : 10 host eggs) in glass jars (1-liter capacity). Egg cards were renewed daily to avoid super-parasitism.

#### **Experimental techniques:**

Pieces of thick paper were varnish with stick material and the obtained *S. cerealella* eggs were sprayed slightly on these papers to make cards of eggs easy to be counted. These cards and the pieces of papers that contain *P. gossypiella* eggs were stored at three degrees of temperatures (4±1°C, 8±1°C & 10 ±1°C ) and 80 ± 5% RH. for the periods of 5, 10, 15, 20, 25, 30, 35, and 40 days. The stored cards were exit from each stored degree after every stored period and cuts into small cards, each card contains 60±5 host eggs. These cards introduced to 20 mated adult female of *T. evanescens* maintained individually in glass vials, these vials divided into three groups for easy handling and transferred to the rearing condition (27±1°C and 80 ± 5% RH). After five days, the number of parasitized eggs/female was determined, however, the percentage of emergence (survivorship) and sex-ratio were calculated after adult progeny emergence.

#### **Statistical Analysis:**

Analysis of variance were done on all data (ANOVA) and when statistical differences existed within a data set, Duncan's multiple range test was used to separate the means (Snedecor & Cochran 1980).

## **RESULTS AND DISCUSSION**

### **a. The effect of cold storage of host eggs on the mean number of parasitized eggs by *T. evanescens* females (parasitoid host acceptance):**

Table (1), showed that there was insignificant difference between the two host species in the degree of parasitoid acceptance for them after storage at low temperatures ( F=1.475, LSD=1.393) as the total mean

number of parasitized eggs after all storage periods were 20.49 & 19.18 eggs/female for *P. gossypiella* and *S. cerealella* eggs, respectively. The best degree for cold storage after 40 days was 8°C for both *P. gossypiella* and *S. cerealella* eggs, as the total mean number of parasitized eggs were 22.78 and 27.86 eggs/female, respectively, whereas, after the same period there were insignificant differences between 4°C and 10°C in case of *P. gossypiella* eggs, whereas, there were significant differences between the two storage temperatures in case of *S. cerealella* eggs as the total mean number of parasitized eggs were 16.46 and 13.22 eggs/ female, respectively. Storage time was the major factor influencing the number of parasitized eggs, as the total mean number of parasitized host eggs reduced gradually as the time of storage increased. The total mean number of *P. gossypiella* parasitized eggs were 38.55, 33.29, 32.75, 13.02, 11.27, 4.75, 1.42 and 2.08 eggs/ females compared with unstored eggs (47.3 eggs/ female) and they were 37.41, 31.29, 29.23, 14.07, 7.78, 7.07, 2.08 and 1.3 eggs/ females for *S. cerealella* eggs compared with 42.4 eggs/ female in control. The parasitoid females did not accept any *P. gossypiella* or *S. cerealella* eggs for parasitism after 30 days in case of storage at 4°C, and after 15 days in case of storing at 10°C. Whereas storing host eggs at 8°C made negligible number of eggs were still acceptable for parasitism until 40 days. After the fifteen days, the best degree for *P. gossypiella* eggs storage was 10± 1°C, as the number of parasitized eggs were 42.7 eggs/ female. Cold storage of *P. gossypiella* eggs at 8°C and 10 °C and storage of *S. cerealella* eggs at 8°C for 5 days did not affect parasitization.

**b. The influence of host eggs storage on the percentage of parasitoid emergence:**

Storage of *P. gossypiella* and *S. cerealella* eggs had a negative impact on the percentage of emerged parasitoid, as it caused significant reduction in the total mean percentage of parasitoid emergence ( 79.86%) compared with 93.35% in control when the stored host was *P. gossypiella* and ( 82.22%) compared with 90.48% when the parasitoid reared on stored *S. cerealella* eggs ( Table 2). There were significant differences between the two host species in their tolerant to storage temperatures ( F= 13.47, LSD= 1.239). There were significant difference between the three storage temperatures in case of *P. gossypiella* eggs, as the mean percentages of parasitoid emergence were 86.57, 69.47 and 91.5 when *P. gossypiella* eggs stored at 4±1°C, 8±1°C and 10±1°C, respectively. Whereas, the storage of *S. cerealella* eggs, the highest percentage of parasitoid emergence was 90.75% when eggs were stored at 10°C, while there were insignificant differences between storage at 4 ±1°C and 8±1°C as the percentage of parasitoid emergence were 79.97 and 80.16%, respectively. Naturally, the total mean percentages of parasitoid emergence were decreased gradually, as the period of storage increased, as they were 90.52, 87.51, 82.49, 78.46, 77.72, 72.57, 43.09 and 34.94% when *P. gossypiella* eggs were stored for 5, 10, 15, 20, 25, 30, 35 and 40 days, respectively compared with control (93.35%), whereas, they were 90.70, 86.44, 77.09, 74.79, 77.08, 84.06, 70.64 and 67.54%, respectively, compared with control (90.48%) in case of stored *S. cerealella* eggs.





This may be due to, the nutritional material gradually dried and became insufficient for the parasitoid nourishing. There were no data about emergence after 30 days in case of storage at 4°C and after 15 days in case of storage at 10 °C in the two stored hosts, on the basis of there were no parasitized eggs after these periods of time. Cold storage of the two host eggs at 10±1°C for 15 days did not affect the percentage of parasitoid emergence.

**c. The impact of host storage on the percentage of produced parasitoid females ( sex-ratio):**

Data in table (3) showed that there were significant differences between the two storage hosts in the percentage of emerged parasitoid females (  $F= 10.433$ ,  $LSD= 1.597$ ), as the total mean percentage of produced females was 58.23 % females when the stored host was *P. gossypiella* eggs, where as, it was 60.54% when the stored host was *S. cerealella* eggs. Also there were significant differences between the three storage temperatures in case of storage *P. gossypiella* eggs. Storage *P. gossypiella* eggs at 10±1°C give the highest percentage of females ( 68.48%), followed by storage at 4±1°C (60.27%), where as, storage at 8±1°C give the lowest percentage of emerged females (52.08%). There were insignificant differences between storage *S. cerealella* eggs at 4±1°C and 10±1°C, as the percentage of produced females were 62.16 and 63.96%, respectively. Where as, there were significant differences between them and storage at 8±1°C (57.76%). Regardless of storage temperature, there were insignificant differences between the control (un stored eggs) and between stored *P. gossypiella* eggs for 5, 10, 15 days, and the percentage of produced females was above 63.05%. After 15 days the percentage of produced females was decreased significantly to 52.09, 58.64, 49.84, 38.94 and 22.96%. Whereas, in case of storage *S. cerealella* eggs, the reduction in the percentage of produced females occurred after 10 days from storage, and it decreased gradually until reached 35.33% after 40 days. There were insignificant differences between the three storage temperatures at all days of storage except for at 15 days, as storage *P. gossypiella* eggs at 10±1°C give the highest percentage of females (76.56%), and it decreased significantly to 65.5 and 56.74% when the storage temperatures were 4±1°C and 8±1°C. As well as, in case of storage *S. cerealella* eggs, there were insignificant differences between the three storage temperatures at all storage days, except for at 5 and 25 days, as the highest percentage of females (72.78 and 56.18%) were obtained when the storage temperature was 4±1°C.

Pratissoli *et al.*, 2003) reported that eggs of some laboratory hosts like *Ephestia kuehniella* Zeller, *S. cerealella*, *Samia cynthia ricini* Boisduval used for mass rearing of *Trichogramma* spp. in different parts of the world have been held under cold temperature conditions for different periods. Pu *et al.* 1988 reported that the refrigerated storage period of host eggs in a chilled or frozen state for use in mass-rearing of *Trichogramma* spp. is generally useful only on a short-term basis. Dass and Ram 1983 stated that *Corcera cephalonica* eggs stored at  $-6 \pm 1^\circ \text{C}$  for 8 days adversely affected biological traits of *Trichogramma* spp. The longest refrigerated storage for a host was four months at 18°C for eri silkworm, *S. Cynthia ricini*. Similarly, storage of *E.*



*kuehniella* eggs for 30 days at  $3\pm 1^{\circ}\text{C}$  resulted in reduced parasitism (60%) in *Trichogramma maxacalii* Voegelé and Pointl, *T. acacioi*, and *T. prtiosum* Riley (Pu *et al.*, 1988). However, in this case, it was reported that continuous rearing of the parasitoids on factitious host eggs reclaimed from frozen storage resulted in low production. Likewise, Voegelé *et al.* (1974) found that *E. kuehniella* 255 eggs could be stored unfrozen up to 2 months at  $4^{\circ}\text{C}$  after which they suggested that an unknown factor was lost that was required for development of *T. brasiliensis* and *T. evanescens* embryos. Ramos & Jimenez (1993) made a novel attempt to extend the useful shelf-life of host eggs, as eggs of another lepidopteran, *Sitotroga cerealella*, were vacuum packed at pressures up to 10 PSI and stored at  $7^{\circ}\text{C}$  for 20 to 60 days. While the effective storage time for this method did not extend past 35 days, a very high rate of parasitism by *Trichogramma* spp. was observed for the eggs treated in this manner. There is specific variation among the *Trichogramma* in the acceptance of cold-stored host eggs. *T. chilostraeae* showed no preference between cold-stored and fresh eggs of *Corcyra cephalonica*, while *T. australicum* would not parasitize eggs that had been refrigerated (Medina & Cadapan 1982). Further, a temporal decline in the acceptability of cold-stored host eggs has been observed for several trichogrammatid and non-trichogrammatid species. The rate of parasitism for *T. exiguum* fell after host eggs of *C. cephalonica* had been stored at  $6^{\circ}\text{C}$  for longer than 8 days (Dass & Ram 1985). Similar results showing a decrease in host acceptability with storage duration were found for *Telenomus remus* with eggs of *Spodoptera litura* and for *Ooencyrtus ennomophagous* with *Clostera inclusa* eggs (Gautam 1987). With *O. ennomophagous*, a situation unrelated to host acceptance was observed when it was offered eggs from a different lepidopteran. Eggs from the geometrid, *Lambdina pellucidaria*, after storage at  $10^{\circ}\text{C}$ , were found suitable for production of *O. ennomophagous*, while no parasitoids emerged from fresh eggs (Drooz 1981). This suggests that cold storage may have inhibited an internal developmental or physiological change in a factitious host eggs that was ordinarily detrimental to development of the parasitoid.

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إمكانية تخزين بيض العائل تحت درجات حرارة منخفضة وتأثير ذلك على قبول  
طفيل الترايكوجراما ايفانسنس له بعد فترات مختلفة من التخزين  
منال عبد المحسن عبد الغنى الشرقاوى  
معهد بحوث وقاية النبات - مركز البحوث الزراعية- الدقى

تم دراسة تأثير التخزين البارد لبيض دودة اللوز القرنفلية و بيض فراشة الحبوب اللذين  
استخدما كعائل تربية لطفيل ترايكوجراما ايفانسنس وقد تم التخزين لهذا البيض على ثلاث درجات  
حرارة هي 4 و 8 و 10° م و تم دراسة تأثير مدة التخزين للعائل على قبول الطفيل لهذا العائل بعد  
فترات مختلفة من التخزين وهي 5 و 10 و 15 و 20 و 25 و 30 و 35 و 40 يوم وقد تبين أن مدة  
التخزين هي العامل الرئيسي الذى يؤثر على عدد البيض المتطفل عليه من كلا العائلين (قبول الطفيل  
للعائل) وأيضا نسبة خروج الطفيل (مناسبة العائل للطفيل) ونسبة الاناث الناتجة للطفيل (النسبة  
الجنسية). وقد تبين أن الطفيل لم يقبل التطفل على أى ببيض بعد التخزين لمدة 30 يوم على درجة 4  
°م وبعد 40 يوم عند التخزين على درجة 8° م وبعد 15 يوم عند التخزين على 10° م. وتبين أن  
درجة 10° م هي أنسب درجة لتخزين العائل وذلك لمدة 15 يوم دون التأثير على قبول الطفيل له.  
تخزين كلا العائلين لمدة خمس أيام على أي من الثلاث درجات حرارة لم يؤثر على قبول الطفيل  
للعائل.

Table (1): The effect of cold storage of host eggs for different periods at 4, 8 and 10°C on the Mean  $\pm$ SD number of parasitized eggs by *T. evanescens*.

Storage temperature	Control	STORAGE PERIODS (DAYS)								Means SD	LSD 5%
		5	10	15	20	25	30	35	40		
<b><i>P. gossypiella</i></b>											
4°C	47.3 <sup>A</sup> $\pm$ 10.03	33.51 <sup>bB</sup> $\pm$ 5.64	37.03 <sup>aB</sup> $\pm$ 8	26 <sup>bC</sup> $\pm$ 7.96	15.6 <sup>aD</sup> $\pm$ 13.8	13.35 <sup>aD</sup> $\pm$ 13.27	10.25 <sup>aD</sup> $\pm$ 16.07	0 <sup>bE</sup> $\pm$ 0	0 <sup>bE</sup> $\pm$ 0	20.34 <sup>b</sup> $\pm$ 18.64	6.724
8°C	47.3 <sup>A</sup> $\pm$ 10.03	40.7 <sup>abA</sup> $\pm$ 11.51	28.97 <sup>bB</sup> $\pm$ 6.66	29.56 <sup>6bB</sup> $\pm$ 6.24	23.45 <sup>aBC</sup> $\pm$ 17.46	20.45 <sup>aC</sup> $\pm$ 16.33	4 <sup>abD</sup> $\pm$ 8.71	4.25 <sup>aD</sup> $\pm$ 9.42	6.25 <sup>aD</sup> $\pm$ 10.37	22.78 <sup>a</sup> $\pm$ 18.57	7.088
10°C	47.3 <sup>A</sup> $\pm$ 10.03	41.45 <sup>aAB</sup> $\pm$ 15.31	33.88 <sup>abB</sup> $\pm$ 12.36	42.7 <sup>aA</sup> $\pm$ 26.12	0 <sup>bC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	18.37 <sup>b</sup> $\pm$ 23.64	8.181
Means SD	47.3 <sup>A</sup> $\pm$ 10.03	38.55 <sup>B</sup> $\pm$ 11.89	33.29 <sup>C</sup> $\pm$ 9.76	32.75 <sup>C</sup> $\pm$ 17.47	13.02 <sup>D</sup> $\pm$ 15.99	11.27 <sup>D</sup> $\pm$ 14.69	4.75 <sup>E</sup> $\pm$ 11.21	1.42 <sup>F</sup> $\pm$ 5.71	2.08 <sup>F</sup> $\pm$ 6.59	20.49 $\pm$ 20.41	3.913
LSD 5%		7.299	5.909	10.240	8.128	7.694	6.683	3.444	3.790	2.259	
<b><i>S. cerealella</i></b>											
4°C	42.4 <sup>A</sup> $\pm$ 5.36	29.21 <sup>bB</sup> $\pm$	28.27 <sup>bB</sup> $\pm$ 13.39	24.4 <sup>bB</sup> $\pm$ 12.74	12.75 <sup>bC</sup> $\pm$ 12.57	6.04 <sup>bD</sup> $\pm$ 5.38	5.12 <sup>bD</sup> $\pm$ 6.18	0 <sup>bD</sup> $\pm$ 0	0 <sup>bD</sup> $\pm$ 0	16.46 <sup>b</sup> $\pm$ 15.52	5.669
8°C	42.4 <sup>B</sup> $\pm$ 5.36	57.4 <sup>aA</sup> $\pm$ 8.31	39.11 <sup>aBC</sup> $\pm$ 15.84	38.82 <sup>aBC</sup> $\pm$ 12.50	29.45 <sup>aC</sup> $\pm$ 29.94	17.3 <sup>aD</sup> $\pm$ 16.42	16.1 <sup>aD</sup> $\pm$ 15.64	6.25 <sup>aE</sup> $\pm$ 11.68	3.9 <sup>aE</sup> $\pm$ 6.30	27.86 <sup>a</sup> $\pm$ 23.04	9.777
10°C	42.4 <sup>A</sup> $\pm$ 5.36	25.63 <sup>0B</sup> $\pm$ 11.15	26.48 <sup>bB</sup> $\pm$ 8.29	24.49 <sup>bB</sup> $\pm$ 6.87	0 <sup>cC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	0 <sup>bC</sup> $\pm$ 0	13.22 <sup>c</sup> $\pm$ 15.90	4.131
Means SD	42.4 <sup>A</sup> $\pm$ 5.36	37.41 <sup>B</sup> $\pm$ 16.61	31.29 <sup>C</sup> $\pm$ 13.87	29.23 <sup>C</sup> $\pm$ 12.83	14.07 <sup>D</sup> $\pm$ 22.08	7.78 <sup>E</sup> $\pm$ 12.18	7.07 <sup>E</sup> $\pm$ 11.71	2.08 <sup>F</sup> $\pm$ 7.26	1.3 <sup>F</sup> $\pm$ 4.03	19.18 $\pm$ 19.46	3.856
LSD 5%	ns	3.125	9.165	6.993	11.873	6.31	6.151	4.270	2.303	2.226	

Means followed by the same capital litter at the same row or by the same small litter at the same column are not significantly different. ANOVA yielded insignificant different between the two host species (LSD= 1.393, F= 1.475 ).

Table (2): The effect of cold storage of host eggs for different periods at 4, 8 and 10°C on the percentage of parasitoid emergence (Mean ±SD ).

Storage temperature	Control	Storage periods (days)								Means SD	LSD 5%
		5	10	15	20	25	30	35	40		
<i>P. gossypiella</i>											
4°C	93.35 <sup>A</sup> ± 3.72	90.18 <sup>aA</sup> ± 8.35	92.53 <sup>aA</sup> ± 7.33	81.9 <sup>abBC</sup> ± 10.61	79.59 <sup>aC</sup> ± 11.92	80.94 <sup>abC</sup> ± 10.42	87.47 <sup>aAB</sup> ± 9.02	-	-	86.57 <sup>b</sup> ± 10.48	6.822
8°C	93.35 <sup>A</sup> ± 3.72	87.98 <sup>aA</sup> ± 6.94	79.17 <sup>bB</sup> ± 10.65	77.16 <sup>bb</sup> ± 13.88	77.33 <sup>ab</sup> ± 12.66	74.5 <sup>ab</sup> ± 9.43	57.68 <sup>bC</sup> ± 11.49	43.09 <sup>D</sup> ± 12.04	34.94 <sup>D</sup> ± 14.65	69.47 <sup>c</sup> ± 21.54	8.233
10°C	93.35 <sup>A</sup> ± 3.72	93.41 <sup>aA</sup> ± 5.74	90.82 <sup>aA</sup> ± 9.64	88.40 <sup>aA</sup> ± 7.67	-	-	-	-	-	91.5 <sup>a</sup> ± 7.49	ns 5.43
Means	93.35 <sup>A</sup> ±	90.52 <sup>A</sup> ±	87.51 <sup>A</sup> ±	82.49 <sup>B</sup> ±	78.46 <sup>C</sup> ±	77.72 <sup>CD</sup> ±	72.57 <sup>D</sup> ±	43.09 <sup>E</sup>	34.94 <sup>E</sup> ±	79.86±	3.639
SD	3.72	7.29	10.89	11.73	12.13	10.3	18.24	12.04	14.65	16.58	
LSD 5%	ns	5.226	6.861	8.116	ns 9.195	7.436	7.728	-	-	2.228	
<i>S. cerealella</i>											
4°C	90.48 <sup>A</sup> ± 2.60	89.58 <sup>abA</sup> ± 8.78	88.82 <sup>aA</sup> ± 6.87	71.97 <sup>bb</sup> ± 12.86	63.41 <sup>aC</sup> ± 11.54	65.08 <sup>bC</sup> ± 9.58	90.45 <sup>aA</sup> ± 4.51	-	-	79.97 <sup>b</sup> ± 14.76	6.669
8°C	90.48 <sup>A</sup> ± 2.60	88.28 <sup>bA</sup> ± 4.39	82.17 <sup>bAB</sup> ± 9.87	69.38 <sup>bd</sup> ± 6.85	86.16 <sup>bA</sup> ± 13.30	89.08 <sup>aA</sup> ± 6.88	77.68 <sup>bBC</sup> ± 13.18	70.64 <sup>CD</sup> ± 14.54	67.54 <sup>D</sup> ± 11.87	80.16 <sup>b</sup> ± 13.2	7.563
10°C	90.48 <sup>AB</sup> ± 2.60	94.23 <sup>aA</sup> ± 7.92	88.34 <sup>abB</sup> ± 8.80	89.93 <sup>aAB</sup> ± 2.41	-	-	-	-	-	90.75 <sup>a</sup> ± 6.87	4.88
Means	90.48 <sup>A</sup> ±	90.70 <sup>A</sup> ±	86.44 <sup>B</sup> ±	77.09 <sup>C</sup> ±	74.79 <sup>C</sup> ±	77.08 <sup>C</sup> ±	84.06 <sup>B</sup> ±	70.64 <sup>D</sup> ±	67.54 <sup>D</sup> ±	82.22±	3.378
SD	2.60	7.57	8.91	12.44	16.84	14.70	11.66	14.54	11.87	10.26	
LSD 5%	ns	5.365	6.309	6.282	9.311	6.004	7.367	-	-	2.069	

Means followed by the same letter at the same row or the same lower letter at the same column are not significantly different ANOVA yielded significant different between the two host species (LSD= 1.239, F=13.470 ). Each total mean ( 79.86& 82.22%) resulted from twenty treatments.

Table: (3) The effect cold storage of host eggs on the percentage of produced females (sex-ratio).

Storage temperature	Control	Storage periods (days)								Means± SD	LSD 5%
		5	10	15	20	25	30	35	40		
<i>P. gossypiella</i>											
4°C	64.88 <sup>A</sup> ± 8.25	64.64 <sup>aA</sup> ± 9.42	64.25 <sup>aA</sup> ± 6.68	65.50 <sup>bB</sup> ± 9.17	52.60 <sup>aBC</sup> ± 18.29	60.35 <sup>aAB</sup> ± 8.39	49.65 <sup>aC</sup> ± 9.68	-	-	60.27 <sup>b</sup> ± 12.26	6.324
8°C	64.88 <sup>A</sup> ± 8.25	64.44 <sup>aA</sup> ± 7.77	62.22 <sup>aAB</sup> ± 11.33	56.74 <sup>bAB</sup> ± 14.76	51.59 <sup>aB</sup> ± 13.31	56.94 <sup>aAB</sup> ± 6.11	50.02 <sup>aBC</sup> ± 16.13	38.94 <sup>C</sup> ± 14	22.96 <sup>D</sup> ± 15.23	52.08 <sup>C</sup> ± 17.56	11.089
10°C	64.88 <sup>B</sup> ± 8.25	69.02 <sup>aB</sup> ± 6.31	63.47 <sup>aB</sup> ± 6.76	76.56 <sup>aA</sup> ± 4.03	-	-	-	-	-	68.48 <sup>a</sup> ± 8.05	5.812
Means	64.88 <sup>A</sup> ± 8.25	66.03 <sup>A</sup> ± 7.95	63.31 <sup>A</sup> ± 8.55	66.27 <sup>A</sup> ± 12.92	52.09 <sup>C</sup> ± 15.58	58.64 <sup>B</sup> ± 7.35	49.84 <sup>C</sup> ± 12.95	38.94 <sup>D</sup> ± 14	22.96 <sup>E</sup> ± 15.23	58.23± 11.7	4.807
LSD 5%	ns	7.281	8.088	9.451	ns	ns	ns	-	-	2.944	
<i>S. cerealella</i>											
4°C	67.6 <sup>A</sup> ± 5.59	72.78 <sup>aA</sup> ± 11.80	67.35 <sup>aA</sup> ± 10.25	60.49 <sup>aB</sup> ± 4.50	58.11 <sup>aBC</sup> ± 4.90	56.18 <sup>aBC</sup> ± 2.07	52.63 <sup>aC</sup> ± 4.17	-	-	62.16 <sup>a</sup> ± 9.58	6.324
8°C	67.6 <sup>A</sup> ± 5.59	68.44 <sup>abA</sup> ± 13.03	66.26 <sup>aA</sup> ± 9.18	61.83 <sup>aAB</sup> ± 6.19	57.76 <sup>aABC</sup> ± 6.68	53.47 <sup>bBC</sup> ± 2.07	58.67 <sup>aABC</sup> ± 15.83	50.46 <sup>C</sup> ± 6.44	35.33 <sup>D</sup> ± 20.49	57.76 <sup>b</sup> ± 14.55	9.94
10°C	67.6 <sup>A</sup> ± 5.59	58.98 <sup>bA</sup> ± 13.82	66.22 <sup>aA</sup> ± 6.88	63.05 <sup>aA</sup> ± 7.67	-	-	-	-	-	63.96 <sup>a</sup> ± 9.44	8.369
Means± SD	67.6 <sup>A</sup> ± 5.59	66.73 <sup>A</sup> ± 13.77	66.61 <sup>A</sup> ± 8.58	61.79 <sup>B</sup> ± 6.13	57.93 <sup>BC</sup> ± 5.71	54.83 <sup>C</sup> ± 2.46	55.65 <sup>C</sup> ± 11.69	50.46 <sup>D</sup> ± 6.44	35.33 <sup>E</sup> ± 20.49	60.54± 8.49	4.234
LSD 5%	ns	11.852	ns	ns	ns	1.943	ns	-	-	2.593	

Means followed by the same litter at the same row or the same lower litter at the same column are not significantly different ANOVA yielded insignificant different between the two host species (LSD= 1.597 F=1.433). Each total mean ( 58.23& 60.54%) resulted from twenty treatments.