Storage possibilities of *Trichogramma pintoi* Voegele on eggs of *Ephestia kuehniella* Zeller

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In this study, the parasitization rates of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs stored at different times at 0, 4 and 8 ° C by *Trichogramma pintoi* Voegele (Hymenoptera: Trichogrammatidae) and the performance of parasitoids obtained from these stored eggs were examined. Studies have been conducted within a 25±1°C temperature % 60-70 relative humidity and 16 hours light 8 hours dark periods per day. In conclusion of the carried observations, low temperature and storing period have an effect on the development period of the stored *Ephestia kuehniella* eggs, emergence rate, adult female parasitoids lifetime, the number of the parasitized eggs and blackening of the parasitized eggs. Although the data obtained from the studied biological characteristics are very close to each other, the performance has been monitored high in storing +8°C temperature compared to 0 and +4°C temperatures. A fall has been observed in all of the biological characteristics when the period of storing is extended. The highest performance is identified in first week at the all three temperature degree.

Key Words: *Trichogramma pintoi*, *Ephestia kuehniella*, Mass rearing, Trichogrammatidae

Parazitlenmemiş *Ephestia Kuehniella* Zeller Yumurtalarında *Trichogramma Pintoi* Voegelé' Nin Depolanma Olanakları Üzerinde Araştırmalar

Bu çalışmada 0, 4 ve 8 °C de değişik sürelerde depolanmış *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) yumurtalarının *Trichogramma pintoi* Voegele (Hymenoptera:Trichogrammatidae) tarafından parazitlenme oranları ve bu depolanmış yumurtalardan elde edilen parazitoidlerin parazitleme performansları irdelenmiştir. Çalışmalar 25±1°C sıcaklıkta %60-70 orantılı nem ve 16 saat aydınlık 8 saat karanlık periyotlarda yapılmıştır. Yapılan gözlemler sonucu, düşük sıcaklık ve depolama süresinin, depolanmış *Ephestia kuehniella* yumurtalarının gelişme süresine, açılma oranına, ergin dişi parazitoid ömrüne, parazitlenen yumurta sayısına ve parazitlenen yumurtaların kararma süresine etkili olduğu belirlenmiştir. Denemelerde, +8°C sıcaklıkta depolamanın 0 ve +4°C sıcaklıklara oranla daha yüksek olduğu gözlenmiştir. Depolama süresi uzadıkça incelenen tüm biyolojik özelliklerde düşüş tepit edilmiştir. En yüksek performansın ise üç sıcaklık derecesinde de birinci haftada olduğu tespit edilmiştir

Anahtar Kelimeler: *Trichogramma pintoi, Ephestia kuehniella*, Kitle üretimi, Trichogrammatidae

Introduction

Trichogramma is a large genus of Hymenoptera parasitoids that attack insects pest, primarily Lepidoptera eggs, and are used worldwide for biological control of agricultural pests in various (Stinner et al., 1974; Hassan, 1993; Pinto and Stouthammer,1994; Yaz and Özder 2016). Worldwide, more than 32 million ha of agricultural and forest land have been treated annually with *Trichogramma* spp. for controlling various insect pests. These parasitoids have been commercially used in China, Colombia, the USA, in various European countries and India (Wajnberg and Hassan 1994; Özder and Kara 2010).

The augmentative release of mass-reared Tichogrammatidae egg parasitoids is a promising method to reduce both egg hatching and subsequent damage by larval feeding. These egg parasitoids are oligophagous, allowing mass rearing in large quantities on a variety of natural hosts (Wajnberg and Hassan 1994; Özder and Kara 2010).

Trichogramma species are mass rearing on *Ephestia kuehnilla* Zeller (Lepidoptera: Pyralidae) *Sitotroga cerealella* Olivier (Lepidoptera: Gelechiidae) *Plodia interpunchtella* Hübner (Lepidoptera: Pyralidae) *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) and *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) in the laboratory. Studies on *Trichogramma* species have mostly been based on the successful construction of mass production of these species and the identification of suitable hosts to be used. In

recent years, in addition to these studies, it has become important to use suitable stored host eggs for mass production of parasitoids (Özder and Kılınçer 1996; Özpınar, 1999; Pitcher et al. 2002; Özder, 2004; Özder and Sağlam 2004; Tezze and Botto, 2004, Kara, 2006).

Material and Methods

*Trichogramma s*pecies were reared on *Ephestia kuehniella* in a climatic cabinet at 25±1°C, 60-70% R.H. and L16:D8. *E. kuehniella* were reared on wheat bran at 25±1°C and 60-70% R.H. Parasitism by *T. pintoi* was measured using 24 hours old eggs sprinkled over a fine gum film on paper stripes, 100 eggs. The paper strips were placed separately in glass vials plugged with cotton. The strips were stored at 0, 4 or 8°C, 60-70% RH and a dark photoperiod. These strips were taken out of storage after 1, 2, 3, 4, 5 and 6 weeks. All the strips were exposed to 10 newly emerged *T. pintoi* for 1 day at 25±1°C 60-70% R.H. and 16:8 h (L:D) photoperiod. Fresh eggs of *E. kuehniella* were exposed to *T.pintoi* adults in glass vials until all female parasitoids died. As a control, eggs of *E. kuehniella* were offered to adults of the parasitoid, which emerged under laboratory conditions. All treatments at all storage periods and temperatures were replicated 10 times. (Özder and Sağlam, 2004; Özder 2004). The observed results that the parasitism rates, the period of blacking time, development periods of *T. pintoi,* emergence of *T. pintoi* adults and mean longevity *T. pintoi* adults.

Statistical Analysis

The effect of time and temperature on the longevity and fecundity of parasitoids was analyzed using ANOVA. Means were compared using Duncan's multiple range tests.

Results and Discussion

Parasitisation rate of *Trichogramma pintoi* on *Ephestia kuehniella*

Duration of eggs storage affected parasitism by *T. pintoi* at all three storage temperatures 0, 4 and 8°C ($p<0,05$). Parasitism increased with decreasing storage time and was highest overall at 8°C (Table 1).

Considering the eggs parasitized on the *E. kuehniella* eggs at 0°C, it is found that the highest parasitizing rate (77.80±0.52) was on the eggs which were stored for 1 week. The eggs, which were stored at 4°C for 1 week and 2 weeks, were found statistically significant. It was determined that the highest parasitizing rate occurred at 8°C comparing with the control group and that the parasitizing performance was higher (Table1).

Table 1. Parasitization rate of *Trichogramma pintoi* on *Ephestia kuehniella* eggs stored at 0, 4or 8 °C (%)*

Temperature	Storage period					
	1 week	2 week	3 week	4 week	5 week	6 week
0 °C	77,80±0.52 aB*	71,26±0.89 abB	69,43±0.45 abB	66,80±0.75 bcB	53,40±0.54 cC	50,81±0.83 cC
4 °C	92,25±0.54 aA	83,20±0.70 abAB	77,68±1.10 bcB	70,24±0.70 cB	65,03±0.88 cdBC	51,20±83 dC
8 °C	94,42±1.51 aA	92,24±0.38 aA	82,35±1.15 abAB	78,54±1.55 bcB	75,52±1.13 cB	71,55±1,56 cB
Control	99,80±0.65 A	99,80±0.65 A	99,80±0.65 A	99,80±0.65 A	99,80±0.65 A	99,80±0.65 A

* The difference between the averages that are shown in the same capital letter in each column and lower case in the same row is statistically insignificant ($p<0.05$).

It was found that the results, obtained from the eggs stored at 4°C, 8°C during one week and between the control group, were statistically not significant (p<0,05). The results, obtained at 8°C during the first 3 weeks, were beyond 80%, and the parasitizing was determined only at 77.80 ± 0.52 rate at 0°C during the first week. There was decreasing in the parasitizing performance at 4°C after the second week. Özder (2004) reported in the study has that cold storage 31 days eggs of *E. kuehniella* min. at 8°C 78.20%, highest at 0 °C 97.8%. 4 and 8 °C storage temperature during the first month of the new parasitoid eggs interference, not the egg parasitoids of *E. kuehniella* found that significant changes in parasitism power (Kılınçer et al. 1990)

***Trichogramma pintoi* of blacking time cold storage *Ephestia kuehniella* eggs at three different cold temperatures**

Considering the research results, it is found that the longest blacking time of parasitoid, which develops on *E. kuehniella*, is at 0°C (p<0,05, Table 2). It is found that as much as the temperature increases in the stored eggs. It was determined the earliest blacking time at 8°C and the statistically same results were obtained with the control group during the first four weeks.

Again, the difference was not found between the averages in the eggs stored at 4°C for six weeks. Blacking time of the interference flour moth eggs *T.turkeiensis* in their work 15, 20, 25 and the average of the order of 30 °C 9:02, 4:08, 4:17 and 3:02 the day, while *T. embryophagum* mean the same temperature in the order of 10.1, 5.21, 4.10 and identified as 3.08 days (Özkan and Gürkan,2001).

Development periods of *Trichogramma pintoi*

It was found that the development periods of parasitoids in the eggs, stored for 1 week at 0°C, 14.00 ± 0.50. The mean development period extended beyond 12.50 ± 0.55 at 4°C after the first 2 weeks; no statistical difference is found in the eggs which are stored at 8°C (Table 3).

The temperature at which the parasitoids show the latest development is 0°C, and indifferent was found between the averages obtained during six weeks. The earliest development period was determined at 8°C, and the results, obtained during six weeks, were not obtained statistically significant (p<0,05). Kılınçer et al. (1990) In parallel with the prolongation of the storage period of up to 2 days at 8°C while the outlet temperature, 4°C, the storage time is very time length output unchanged, the prolongation of the storage period at 0°C, the exit time is reported. Uzun (1994) determined in the study that there was an inverse proportion between the temperature and development period, and as much as the temperature increased, the development period shortened.

Table 2. *Trichogramma pintoi* of blaking time from cold storage *Ephestia kuehniella* eggs at three different cold temperatures (day)*

Temperature	Storage period					
	1 week	2 week	3 week	4 week	5 week	6 week
0 °C	6,05±0,01 aA*	6,12±0.02 aA	6,34±0.01 aA	6,49±0.01 aA	6,55±0.06 aA	6,84±0.04 aA
4 °C	5,02±0.02 aAB	5,24±0.03 aAB	5,30±0.03 aAB	5,55±0.19 aAB	5,62±0.02 aA	5,91±0.02 aA
8 °C	4,48±0.02 aB	4,70±0.03 aB	4,82±0.05 aB	4,96±0.21 aB	5,01±0.03 abAB	5,08±0.08a bAB
Control	4,00±0.01 B	4,00±0.01 B	4,00±0.01 B	4,00±0.01 B	4,00±0.01 B	4,00±0.01 B

* The difference between the averages that are shown in the same capital letter in each column and lower case in the same row is statistically insignificant (p<0.05).

Table 3. Development periods of *Trichogramma pintoi* from cold storage pupae developed in *Ephestia kuehniella* eggs at three different cold temperatures (day)*

Temperature	Storage period					
	1 week	2 week	3 week	4 week	5 week	6 week
0°C	14,00±0.50 aA*	14,03±0.48 aA	14,06±0.51 aA	14,10±0.63 aA	14,58±0.55 aA	14,75±0.51 aA
4 °C	12,50±0.55 aB	12,94±0.65 aAB	13,17±0.47 abA	13,71±0.53 abA0	13,73±0.48 abA	13,84±0.69 abA
8 °C	10,12±43 aBC	10,41±0.44 aBC	10,71±0.4 aB	10,90±0.49 aB	10,97±0.45 aB	10,98±0.50 aB
Control	9,00±0.01 C	9,00±0.01 C	9,00±0.01 C	9,00±0.01 C	9,00±0.01 C	9,00±0.01 C

* The difference between the averages that are shown in the same capital letter in each column and lower case in the same row is statistically insignificant (p<0.05).

Table 4. Percentage of emergence of *Trichogramma pintoi* adults obtained from eggs of *Ephestia kuehniella* stored at 0, 4 or 8°C (day)*

Temperatue	Storage period					
	1 week	2 week	3 week	4 week	5 week	6 week
0 °C	91,65±0.47 aA*	83,43±0.22 bB	82,40±0.44 bB	62,80±0.47 cC	45,70±0.82 dC	32,60±0.48dC
4 °C	96,00±1.15 aA	85,01±0,65 bB	75,53±2.18 cB	74,40±1.04 cB	73,02±2.12 cB	60,20±0.16dB
8 °C	97,12±0.79 aA	81,40±0.53 bB	80,29±1,79bB	68,24±2.05 cBC	62,40±1.75 cB	60,70±0.53cB
Control	98,50±0.89 A	98,50±0.89 A	98,50±0.89 A	98,50±0.89 A	98,50±0.89 A	98,50±0.89A

* The difference between the averages that are shown in the same capital letter in each column and lower case in the same row is statistically insignificant (p<0.05).

Emergence of *Trichogramma pintoi* adults obtained from eggs of *Ephestia kuehniella*

The percentage of *T. pintoi* emerging from host eggs declined with increasing storage time overall temperatures (Table 4). Emergence rates were generally the same over 6 weeks of storage when eggs were held at 0, 4 at 8°C (p<0,05).

During the research, it was found that the emerging rates were 91.65 ± 0.47 at 0°C, 96.00 ± 1.15 at 4°C and 97.50 ± 0.89 at 8°C at three temperatures in the eggs which were stored for 1 week (Table 4). Highest emergence rate was at 8°C and 1 week of storage (p<0,05). Kılınçer et al (1990) parasitising *Ephestia kuehniella* studies regarding the use stored eggs *T. turkeiensis* and *T. embryophagum* by scrambled optimum conditions for storing *Ephestia kuehniella* eggs may be in the 4 and 8 ° C and parasitoids output in eggs stored one during months at these temperatures they stated that the rate was too high. Özder (2004) in a study reported that 0, 4 and 8 ° C store was that *E. kuehniella* derived from eggs have the *T. cacoecia* individual to 8 ° C store has an egg in the first third of the week on the output rate above 83%. *Trichogramma evanescens* interference *E.*

kuehniella eggs at 4 ° C for 10, 20, 30 and have stored for 40 days and stored in the parasitic eggs of the stored eggs with a maximum of 10 days, the adult emergence rate were determined as 91.33% (Karabörk and Ayvaz 2007). Özpınar (1997) reported that 95.13 and 64.39 adult outbreaks were observed in parasitized *E. kuehniella* and *S. cerealella* eggs.

Mean longevity *Trichogramma pintoi* adults obtained from eggs *Ephestia kuehniella*

The longevity of adult *T. pintoi* emerging from stored eggs at all three storage temperatures, in the main, decreased as duration of storage increased (p<0,05, Table 5) (Özder, 2004). The first and second weeks were obtained statistically significant at 0°C and the first and second weeks at 4°C (p<0,05).

Table 5. Mean longevity *Trichogramma pintoi* from eggs of *Ephestia kuehniella* stored at three different cold temperatures (day)*

Temperature	Storage period					
	1 week	2 week	3 week	4 week	5 week	6 week
0 °C	20,4±0.73 aC*	19,7±0.30 abC	18,6±0.37 bC	17,02±0.61 bcC	15,3±0.36 cdC	12,5±0.37 dC
4 °C	22,3±0.21 aBC	21,8±0.20 abBC	20,6±0.30 abBC	19,4±0.87 bcBC	17,4±0.45 cdB	13,6±0.49 dBC
8°C	23,4±0.30 abB	22,1±0.67 abB	21,5±0.34 abBC	20,9±0.50 bcB	18,6±0.65 cB	14,6±0.40 dB
Control	25,9±0.23 A	25,9±0.23 A	25,9±0.23 A	25,9±0.23 A	25,9±0.23 A	25,9±0.23 A

* The difference between the averages that are shown in the same capital letter in each column and lower case in the same row is statistically insignificant (p<0.05).

According to the research results, a significant increasing was found in the extending of longevity upon increasing of temperature, and was statistically significant (p<0,05). It was determined that the longevity were significant at three temperatures during the first two weeks. Gurkan & Ozkan (2001) in their study of *T. turkeiensis* 15, 20, 25 and the average adult lifetime in the order of 30°C; 10.56, 19.74 19.52 and 4.88 days, *T. embryophagum* 15, 20, 25 and 30°C respectively and in the average adult life; 11:32, 18:20, were determined to be 22.96 and 5.86 days. It was suggested that the reason why the parasitoid longevity was longer at the lower temperatures was due to decreasing of metabolic activities (Uzun, 1994). Some biological relations between *Agrotis segetum*, *T. embryophagum* and *T. turkeiensis* Kostadinov on the host *Ephestia kuehniella* and found that the longevity of *T. embryophagum*, obtained from *E. kuehniella* at 15°C, was the mean 9.87 days, and *T. turkeiensis* at 15°C was the mean 11.12 days (Özder and Kılınçer 1996). Özpınar(1997) In a study, performed by *T. evanescens* individuals from *E. kuehniella* and *Sitotroga cerealella* eggs were examined at 26 ± 1 ° C and the female parasitoid life was determined to be 7.70 and 7.98 days, respectively.

Results and Suggestions

In laboratory conditions, mass production of parasitoids is of extreme importance in terms of biocontrol fighting frequency and dose determination. As a result of the study, unparasitized *E. kuehniella* eggs stored at 0, 4 and 8°C were found suitable for *T. pintoi,* in all three temperature, while the duration of storage is increasing parasitization rate decreasing. The experiment have resulted in more than % 80 of the results obtained at 8°C in first week.

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