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Biological characteristics of the egg-larval parasitoid *Chelonus oculator* (Fabricius, 1775) (Hymenoptera: Braconidae) on the potato tuber moth *Phthorimaea operculella* (Zeller, 1873) (Lepidoptera: Gelechiidae) at different temperatures

Patates güvesi *Phthorimaea operculella* (Zeller, 1873) (Lepidoptera: Gelechiidae) üzerinde yumurta-larva parazitoiti *Chelonus oculator* (Fabricius, 1775) (Hymenoptera: Braconidae)'nin farklı sıcaklıklarda biyolojik özellikleri

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ABSTRACT

This study was conducted to determine some biological properties of the potato tuber moth *Phthorimaea operculella*, a new host of the egg-larval parasitoid *Chelonus oculator* at different temperatures. The emergence rate, development time, longevity, adult weight, and sex ratio of *C. oculator* were assessed at three different temperature levels (20±1 °C, 25±1 °C, 30±1 °C), 65±5% humidity and 16:8 light: dark conditions. Forty potato tuber moth eggs, aged 0-24 hours, adhered to the filter papers, were placed into the tubes, and presented to the parasitoids for parasitization for a day. At the end of this period, the parasitized eggs were allowed to develop at the specified temperatures. The study found the highest emergence rate was found at 30 °C, with 20.5%. The longest development period occurred at 20 °C, determined as 49.00±2.00 days in females and 52.27±1.64 days in males. The longest longevity of the parasitoid was found to be 41.71±2.29 and 45.73±3.75 days in males and females, respectively. The highest adult weight was observed at 25 °C for both males and females. The sex ratio was found to be in favor of males as the temperature decreased. It is thought that these results can be used for the biological control of potato tuber moth in the field and storage conditions for release studies.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a crucial agricultural product used in human nutrition worldwide and serves as an industrial plant. Additionally, it is utilized for animal nutrition, while its factory wastes are repurposed as fertilizer.

One of the most important pests affecting potatoes is the potato tuber moth (PTM), *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). This pest also targets primarily potatoes and other Solanaceae plants such as tomatoes,

tobacco, eggplant, and various weeds. The primary host of the PTM is the potato, causing substantial losses by feeding on tubers. Adults lay their eggs under the leaves, on shoots and buds, and around the eyes of the tubers during the harvest period. The larva emerging from the egg feeds by opening regular galleries on the leaves and branches, and by opening irregular galleries in the tuber. These galleries, which have a hard surface, are filled with white excrement. In case of infection of the tubers, the loss increases up to 100% if no control is made. Damaged tubers are infected by bacterial and fungal infections so current damage increases more. This pest deteriorates the edibility and seed properties of the potato, resulting in weight and quality loss. The pest that infects the tuber in the field before the harvest continues to reproduce if it finds suitable conditions in the stores and increases its damage (Anonymous 2008).

Survey, biology, and chemical control studies to PTM were carried out in warehouses and laboratory conditions in our country. In the control of PTM, it is desirable to apply primarily cultural control methods (such as earthing up, deep planting, irrigation and early harvest, irrigation and not leaving potato tubers in the field after harvest). However, since these cultural methods are not taken and the irrigation is stopped close to the harvest, the tubers are raised to the surface, and PTM adults lay eggs on these tubers.

In Türkiye, potatoes enter storage contaminated with pests. The main damage of the PTM occurs in storages. Therefore, it is crucial to control the PTM before the potatoes arrive in storage. Chemicals have harmful effects on the ecosystem, so alternative methods should be employed. The most up-to-date and sustainable of these methods is biological control. There have been no biological control application of this pest in our country. In the survey studies, *Bracon (Habrobracon) variegator* Spinola (Hymenoptera: Braconidae), *Temelucha decorata* (Grav) (Hymenoptera: Ichneumonidae) and *Diadegma pulchripes* (Kokujev) were identified as parasitoids of the PTM (Has et al. 1999).

The koinobiont, endoparasitoid, solitary egg-larval parasitoid *Chelonus oculator* (F.) (Hymenoptera: Braconidae) is another natural enemy of the PTM (Özkan et al. 2013). *C. oculator* was first obtained from the *Spodoptera littoralis* Boisid. (Lepidoptera: Noctuidae) culture brought from the cotton cultivation areas of Adana province in 1998 (Özmen et al. 2002).

C. oculator lays one egg on the host eggs. It is known that the parasitoid lays its eggs in the egg of the PTM, and it completely consumes the host larva by feeding the first and second larval stages inside the host and the third stage

outside the host larva. In the host diversity studies, it was determined that the PTM is among the natural hosts of the parasitoid. However, little is known about the biology of *C. oculator* on its new host, the PTM (Tunca et al. 2011).

Many studies on the biology of *C. oculator* on different hosts were determined and it was concluded that this parasitoid can be used as an effective biological control agent. However, since the PTM is a new host of the parasitoid, there are gaps in the understanding of the parasitoid's biology on this pest. In this study, the effects of different temperatures on some biological properties of *C. oculator* grown on *P. operculella* were investigated.

MATERIALS AND METHODS

Rearing of insect cultures

Insect cultures used in the study were grown in climate cabinets and climate rooms (25±1 °C, 65±5% RH, and 16:8 h L:D photoperiod). The cultivation of PTM followed the method of Visser (2004) and Maharjan and Jung (2011), with modifications. Potato tubers, collected from the field during harvest, were brought to the laboratory to ensure the emergence of PTM. Potato tubers and adult moths were placed in plastic growing containers (13.5 x 18 cm), and the container was covered with gauze. Adult moths emerging from contaminated potatoes were collected with the help of an aspirator and taken into empty plastic containers for laying eggs, and the containers were covered with gauze. The filter paper was placed on the net, and glass Petri dishes of the same size were placed on the filter papers. Honey was applied to the edges of the plastic containers for feeding the adults. Subsequently, the filter papers containing the host eggs were collected, and the host eggs were used in the experiments.

The parasitoid *C. oculator* population was obtained from the culture grown in the Ankara University Plant Protection Department. 0-24 h *P. operculella* eggs were presented to the parasitoid for 24 hours. Parasitized eggs were transferred to plastic containers containing potato tuber. The emerged adult parasitoids were used for the experiments.

Biological aspects of C. oculator

The trials were carried out in the climatic chamber at three different temperatures (20±1 °C, 25±1 °C, 30±1 °C), with 65±5% RH, 16:8 L:D photoperiod at the Biological Control Laboratory of Plant Protection Central Research Institute in 2018. In the experiments, 0-24 hour parasitoids, fed with honey and mated, were used. Parasitoids were taken into glass tubes and 0-24 hour-old 40 PTM eggs on filter paper,

were presented to parasitoids to parasitize. The parasitized eggs were removed from tubes and placed into containers containing potato tubers, and their development was noted daily. The emergence rate, development time, sex ratio, adult weight, and longevity of the parasitoid were determined. To measure the adult weight, the emerging adult parasitoids were kept in aluminum foil in an oven at 60 °C for five days. The parasitoids' dry weights were measured with the help of analytical balance. Experiments were set up with 10 replications.

Statistical analysis

The analysis was performed in the Minitab 18 package program. The difference between the means was evaluated using one-way ANOVA, with the Tukey test applied within 0.05 error limits (P<0.05).

RESULTS

It was determined that the emergence rate of the parasitoid increased with the increase in temperature, and this increase was statistically found significant (df =2, F=25.02, P=0.000). The emergence rate at 30 °C was found to be higher than at other temperatures (Table 1).

Table 1. Effect of temperature on the emergence rate of the *Chelonus oculator*

Temperature (°C)	Emerged parasitoids (number)		Parasitoid emergence rate (%)
	♂	♀	
20	15	3	4.5 C*
25	31	15	11.5 B
30	57	25	20.5 A

* The difference between the means with different letters in the same column is statistically significant according to the Tukey test (P≤0.05)

Temperature effect on the development time of the parasitoid

It was concluded that temperature significantly affects the development time of both female and male individuals, with the longest development time observed at 20 °C (F_{male} =345.55, P=0.000, df=2; F_{female} =110.01, P=0.000, df=2). Although female individuals developed in a shorter time than male individuals at 20 °C, the difference was not found to be significant (df =2, F₂₀=0.37, P=0.695). The development time of both male and female individuals was found to be at least 25 °C. However, the development of female individuals emerged at 25 °C and 30 °C took longer than male parasitoids and the difference was found to be significant (F₂₅ =5.87, P=0.004, df=2; F₃₀ =7.92, P=0.001, df=2) (Table 2).

Table 2. Effect of temperature on the development time of *Chelonus oculator*

Temperature (°C)	Development time (days)		
	♂	♀	♂+ ♀
	Mean± SE	Mean± SE	Mean± SE
20	52.27±1.64 A*a**	49.00±2.00 Aa	51.72±1.42 Aa
25	25.71±0.31 Cb	28.00±0.63 Ca	26.46±0.33 Cb
30	29.07±0.39 Bb	31.76±0.39 Ba	29.89±0.33 Bb

* Differences between means with different capital letters in the same column are significant according to the Tukey test (P≤0.05)

**Differences between means with different lowercase letters in the same row are significant according to the Tukey test (P≤0.05)

Temperature effect on the longevity of the parasitoid

The study concluded that temperature affects the longevity of both female and male individuals (F_{male} =84.89, P=0.000, df=2; F_{female} =40.10, P=0.000, df=2). The difference between the longevity of males and females emerging at the same temperature was statistically insignificant (F₂₀ =0.91, P=0.413, df=2; F₂₅ =0.46, P=0.634, df=2; F₃₀ =2.9, P=0.058, df=2) (Table 3).

Table 3. Effect of temperature on the longevity of *Chelonus oculator*

Temperature (°C)	Longevity (days)		
	♂	♀	♂+ ♀
	Mean± SE	Mean± SE	Mean± SE
20	33.40±4.65 A*a**	18.67±4.71 Ba	30.94±4.13 Ba
25	41.71±2.29 Aa	45.73±3.75 Aa	43.02±1.97 Aa
30	9.26±1.15 Ba	14.28±1.65 Ba	10.79±0.97 Ca

* Differences between means with different capital letters in the same column are significant according to the Tukey test (P≤0.05)

**Differences between means with different lowercase letters in the same row are significant according to the Tukey test (P≤0.05)

Temperature effect on the adult weight of Chelonus oculator

The analysis indicates that temperature significantly affects adult weight in both males and females (df=2, F_{male} =14.16, P=0.000; df=2, F_{female} =21.15, P=0.000). According to the results, the average adult weight was higher at 25 °C. On the other hand, it was determined that the average adult weight of female parasitoids was higher than the average adult weight of male parasitoids at all three temperatures (Table 4).

Table 4. Effect of temperature on adult weight of *Chelonus oculator*

Temperature (°C)	adult weight (mg)		
	♂ Mean± SE	♀ Mean± SE	♂+ ♀ Mean± SE
20	0.89±0.20 B*a**	1.00±0.17 Ba	0.91±0.17 Ba
25	1.25±0.11 Ab	1.79±0.18 Aa	1.43±0.10 Aab
30	0.70±0.03 Ba	0.80±0.06 Ba	0.73±0.03 Ba

* Differences between means with different capital letters in the same column are significant according to the Tukey test ($P \leq 0.05$)

**Differences between means with different lowercase letters in the same row are significant according to the Tukey test ($P \leq 0.05$)

Effect of temperature on the sex ratio of *Chelonus oculator*

In the study, the sex ratio was calculated over total male and female individuals in different temperature applications. As a result of the experiments, the sex ratios of the individuals exiting from different temperatures were respectively (male:female) 5:1; 2.1:1; It was found in favor of male individuals in all three temperature degrees, 2.28:1 (Table 5).

Table 5. Effect of temperature on the sex ratio of parasitoid

Temperature (°C)	♂ Number of individuals (pieces)	♀ Number of individuals (pieces)	♂/ ♀ Male:Female (M:F)
20	15	3	5: 1
25	31	15	2.1: 1
30	57	25	2.28: 1

DISCUSSION

In biological control studies, determining the relationship between the parasitoid and the host is essential. It is observed that the increase in temperature leads to an increase in the emergence rate. According to the results, the higher emergence rate at 30 °C than at other temperatures suggests that the parasitoid can adapt more easily to higher temperatures.

In the study, temperature emerged as significant a factor affecting the development time of *C. oculator*. The longest development time was found at the lowest temperature, 20 °C. It was found that the development period of females was longer than males at 25 °C and 30 °C, and shorter at 20 °C,

but the difference was not significant. Different temperatures can influence the development time of *Chelonus species* in different hosts. Rao et al. (1979) reported a development time of 23.5 days for *C. blackburni* in the PTM. The development time of *C. blackburni* on *P. operculella* was found 25.8±1.6 days at 24±2 °C by Kumar and Ballal (1990). The variation may be attributed to the difference in the host nutrient utilization rate of the pre-adult stages of the parasitoid at each temperature. Although it is known that an increase in temperature shortens the development of parasitoids, it is thought that this difference between the temperature and the development time of the parasitoid is related to the compatibility of the parasitoid with the host.

The longevity of *C. oculator* at 30 °C was found to be lower in both male and female individuals compared to the other two temperatures. Medina et al. (1988) obtained that *Chelonus sp. nr. curvimaculatus* males live 16.5 days and females live 20 days at 20 °C. Kolaib et al. (1987) investigated the longevity of *Chelonus inanitus* (Linnaeus, 1767) (Hymenoptera: Braconidae) at different temperatures (10, 15, and 20 °C). They found that female parasitoids lived 39.5 days and males lived 45.2 days at 10 °C; at 15 °C males lived 36.9 days and females lived 33.2 days; longevity was found 23.5 days for male parasitoids and 19.4 days for females at 20 °C. Qureshi et al. (2016) reported that temperature significantly affects longevity. They stated that the longevity of *C. murakatae* lasts longer at low temperatures. They reported that life expectancy is longer because metabolic activities slow down at low temperatures and that the life span of the female is especially important for the continuation of the generation. Determination of longevity is important in terms of biological control. The longer life of male parasitoids increases the encounter rate with more female individuals, so more females are provided to mate. The long life of female parasitoids means that they encounter more hosts and can parasitize more. When the obtained results are examined, the reason for the decrease in the life span of the parasitoid as the temperature increases can be explained by the increase in the metabolic activities of the parasitoid depending on the temperature. Also, it can be said that the longest longevity is found at 25 °C for both female and male parasitoids, and this temperature is the most suitable temperature for the above-mentioned reasons.

Temperature is a factor affecting adult weight in parasitoids. The adult weight of female parasitoids was found to be higher than that of male individuals at all temperatures tested. It can be said that the most suitable temperature degree in terms of the excess number of eggs in the ovarioles of females is 25 °C. Adult weight can be both an advantage and a disadvantage in

parasitoids. It may mean that female individuals have more eggs in the ovarioles. Also, the heavier the female parasitoid, the more restricted its host-seeking behavior will be. Thus, adult weight can turn into a disadvantage. Yassin Ali (2013) found that temperature increase positively affects the adult weight of *C. oculator*. Kumar and Ballal (1990) reared *C. blackburni* on six laboratory hosts (*Galleria mellonella*, *P. operculella*, *Corycra cephalonica*, *Sitotroga cerealella*, *S. litura*, *Achroia griseella*) and found no difference in terms of adult weight on *C. cephalonica*, *P. operculella* and *A. griseella*.

Another biological feature that is affected by different temperatures is the sex ratio. Özmen (2004) found that *C. oculator* sex ratio on *E. kuehniella* 2.5:1 (male:female) at 25 °C. Tunca (2005) determined the sex ratio of *C. cautella*, the host of *C. oculator*, at 15 °C, 20 °C, 25 °C, and 30 °C and found that males were more common at three temperatures except 15 °C. In our study, the sex ratio was found in favor of males at all three temperatures. In general, a high rate of male parasitoids is an undesirable situation in terms of biological control. Especially considering in terms of mass production, 20 °C is considered to be inappropriate. However, in insects that reproduce with arrhenotoky, such as *C. oculator*, the female individual must mate. The higher the number of males will increase the chance of mating, so it will be advantageous in maintaining the parasitoid generation. On the other hand, the high reproduction of *C. oculator* and the high ratio of male individuals can be considered as an advantage in biological control.

In this study, which was conducted for the first time to determine the biology of *C. oculator* on PTM at three different temperatures, it was observed that *C. oculator* could parasitize this host and develop successfully, despite its first encounter with PTM, and successfully completed its life in emerging adult parasitoids. It is thought that *C. oculator* can suppress the moth population in biological control against PTM, and it should be decided whether it can be used after adjusting the release dose.

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Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Bu çalışma, farklı sıcaklıklarda yumurta-larva parazitoiti *Chelonus oculator*'un yeni bir konukçusu olan patates güvesi *Phthorimaea operculella* üzerindeki bazı biyolojik özelliklerini belirlemek amacıyla yapılmıştır. Çalışmada 20±1 °C, 25±1 °C, 30±1 °C sıcaklıklarda %65±5 oranlı nem ve 16:8 aydınlık:karanlık ışıklanma koşullarında *C. oculator*'un çıkış oranı, gelişme ve yaşam süresi, cinsiyet oranı ve ergin ağırlığı belirlenmiştir. Filtre kâğıtlarına yapışık halde bulunan 0-24 saat yaşlı kırk adet patates güvesi yumurtası tüpler içerisine konarak bir gün boyunca parazitlenmesi için parazitoidlere sunulmuştur. Bu sürenin sonunda parazitlenen yumurtalar belirtilen sıcaklıklarda gelişime bırakılmıştır. Çalışmada, en fazla çıkış oranı 30 °C'de %20.5 olarak bulunmuştur. Gelişme süresinin 20 °C'de dişi bireylerde 49.00±2.00 gün, erkek bireylerde 52.27±1.64 gün olduğu bulunmuştur. Ergin ömrü erkek ve dişilerde sırasıyla 41.71±2.29 ve 45.73±3.75 gün olarak saptanmıştır. Ergin ağırlığı en fazla 25 °C'de tespit edilmiştir. Cinsiyet oranının sıcaklık azaldıkça erkekler lehine olduğu belirlenmiştir. Elde edilen bu sonuçların patates güvesinin biyolojik mücadelesinde tarla ve depo koşullarında kullanılabileceği düşünülmektedir.

Anahtar kelimeler: biyolojik mücadele, *Chelonus oculator*, gelişme süresi, çıkış oranı, yeni konukçu

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