Turkish Journal of Biological Control 2025, 16 (1): 8-22
Türk. Biyo. Mücadele Derg. 2025, 16 (1): 8-22
DOI: 10.31019/tbmd.1697618 ISSN 2146-0035-E-ISSN 2548-1002

***Özgün makale* (*Original article*)**

The effect of different hunger periods on the cannibalistic behavior of the predatory ladybird *Oenopia conglobata* L. (Coleoptera: Coccinellidae)

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Farklı aç kalma sürelerinin avcı böcek *Oenopia conglobata* L. (Coleoptera: Coccinellidae)'nın kanibalistik davranışlarına etkisi

Öz: Kanibalizm faydalı böceklerin kitle halinde ekonomik olarak yetiştirilebilmesini olumsuz etkileyen faktörlerden birisidir. Bu nedenle faydalı böceklerin kanibalistik davranışlarının bilinmesi önem arz etmektedir. Bu çalışma, predatör *Oenopia conglobata* L. (Coleoptera: Coccinellidae)'nın laboratuvar koşullarında verimli şekilde üretilebilmesi için farklı aç kalma sürelerinin kanibalistik davranışlarına etkisini belirlemek amacıyla yürütülmüştür. Bu amaçla 12, 24 ve 36 saat aç bırakılan *O. conglobata* erginlerinin yumurta ve larva üzerinde kanibalistik davranışlarının belirlenmesi için 10 tekerrürlü denemeler kurulmuştur. Farklı sürelerde (12, 24, 36 saat) aç bırakılmış *O. conglobata* erginlerinin 24 saatin sonunda sırasıyla ortalama 20.8, 32.5 ve 40.9 adet, ek besin verilen kontrol grubunda ise sırasıyla ortalama 2.3, 2.9 ve 3.3 adet yumurta tükettiği ve sırasıyla 9, 11 ve 12 kat kanibalistik davranış sergilediği belirlenmiştir. Aynı sürelerde aç bırakılmış *O. conglobata* erginlerinin açlık sürelerine göre sırasıyla ortalama 7.8, 8.5 ve 9.2 adet, kontrol grubunda ise sırasıyla ortalama 1.5, 2.6 ve 3.2 adet larva tükettiği ve buna göre Kanibalizm oranının ise sırasıyla 5, 3 ve 2 kat olduğu belirlenmiştir. Erginlerin günlük tükettikleri ortalama yumurta sayısı larva sayısına oranla daha yüksek olmuştur. Elde edilen bulgular, açlığın *O. conglobata* ergin bireylerinde yamyamlık davranışını anlamlı düzeyde artırdığını ve açlık süresi uzadıkça tüketilen yumurta ve larva sayısının da arttığını açıkça ortaya koymaktadır. Sonuç olarak *O. conglobata*'nın insektaryum koşullarında kitlesel üretimi yapılırken tüketeceklerinden fazla miktarda besin verilerek aç kalmalarının önüne geçilmelidir. Hatta mümkünse kanibalizmin en fazla görüldüğü dönemler izole edilerek yetiştirilmelidir.

Anahtar sözcükler: *Oenopia conglobata*, Biyolojik mücadele, Kitle Üretim, Kanibalizm, *Ephestia kuehniella*

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Received (Alınış): 12 Mayıs 2025 Accepted (Kabul ediliş): 27 Haziran 2025

Abstract: Cannibalism is a key factors that negatively impacts the economic mass rearing of beneficial insects. Understanding the cannibalistic behavior of beneficial insects is crucial in framework of biological pest control. This study was conducted to determine the effect of different starvation periods on the cannibalistic behavior of the predatory beetle *Oenopia conglobata* L. (Coleoptera: Coccinellidae) under laboratory conditions, in order to improve its mass rearing efficiency. For this purpose, experiments with 10 replications were established to observe the cannibalistic behavior of adult *O. conglobata* subjected to starvation for 12, 24, and 36 hours, using conspecific eggs and larvae as potential prey. The average number of eggs consumed after 24 hours was 20.8, 32.5, and 40.9 for the 12, 24, and 36-hour starvation groups, respectively. In contrast, the corresponding values in the control group in which supplemental food was provided, were significantly lower: 2.3, 2.9, and 3.3, respectively. Egg cannibalism rate in starved groups were approximately 9-fold, 11-fold, and 12-fold higher than in their respective controls. Similarly, the average number of larvae consumed was 7.8, 8.5, and 9.2, across the increasing starvation durations while in the control group, these values were 1.5, 2.6, and 3.2, respectively. Larval cannibalism rates were approximately 5-fold, 3-fold, and 2-fold higher in the starved groups compared to controls. Additionally, adults consumed more eggs than larvae on a daily average basis. The results clearly demonstrate that starvation significantly increases cannibalistic behavior in adult *O. conglobata*, with longer starvation periods leading to higher consumption rates of both eggs and larvae. In conclusion, to reduce the adverse effects of cannibalism during mass rearing under insectarium conditions, adequate food must be supplied. Furthermore, rearing protocols should, where feasible, include the isolation of developmental stages most prone to cannibalism to enhance production efficiency.

Key words: *Oenopia conglobata*, Biological control, Mass rearing, Cannibalism, *Ephestia kuehniella*

Introduction

Agricultural production is a critical sector for meeting the increasing food demands of the global population. Therefore, efficient and sustainable farming practices form one of the cornerstones of agricultural policies. However, one of the most significant challenges faced by agriculture is the presence of pests that limit agricultural productivity. Pests, particularly insects, are organisms that threaten crop production and cause substantial yield losses. Globally, approximately 20–30% of agricultural production is lost annually due to pest damage (Oerke 2006; Chakraborty & Chattopadhyay 2011). Hence, effective pest control is vital for enhancing agricultural productivity.

Traditional pest control methods, such as the widespread use of chemical pesticides, pose serious risks to both the environment and human health, and also contribute to the development of resistance among pest species (Devine & Furlong 2007; Sayyed et al. 2010). Considering these issues, researchers have increasingly turned to more environmentally friendly and sustainable control strategies. In this context, biological control has become an increasingly preferred method for managing pests. Biological control involves the use of natural enemies such as predators, parasitoids, and entomopathogens to regulate pest populations, offering

an effective strategy without disrupting ecological balance (Zhang et al. 2008; Gurr & You 2016; Sönmez & Mamay 2018). In terms of sustainable agriculture, biological control represents a promising approach due to its environmental compatibility (Uygun et al. 2010; Mamay & Mutlu 2019; Özgen et al. 2022).

One of the primary approaches in biological control against agricultural pests is the mass rearing and release of natural enemies under insectarium conditions (Eroğlu 2016; Mamay & Dusak 2023). In natural agricultural ecosystems, the native populations of predators and parasitoids may not be sufficient to suppress pest populations below economic threshold levels. Therefore, mass rearing and augmentation of these beneficial organisms are necessary to effectively reduce pest densities (Van Lenteren 2000).

An essential aspect of biological control involves the large-scale mass rearing of beneficial insects and their release into natural habitats to manage pest populations. This strategy supports natural pest suppression while reducing dependence on chemical pesticides. However, successful release and reproduction of beneficial insects require a thorough understanding of their behaviors and ecological interactions (Mamay & Mutlu 2019; Özgen et al. 2022; Mamay & Dusak 2023).

The mass rearing of beneficial insects used in biological control plays a crucial role in the success of these methods. However, it is essential to accurately understand the behavioral traits of predatory insects used in such strategies. Among these traits, cannibalism may emerge as a survival strategy in predatory species (Elgar & Crespi 1992; Mamay & Dusak 2023). In this regard, cannibalistic behaviors observed in some predatory insect species present an important concern for biological control strategies. Cannibalism is defined as the consumption of individuals of the same species and tends to become more pronounced under environmental stressors such as hunger and population density. In predator insects, cannibalism may emerge as a survival mechanism when food is scarce (Fox & Morrow 1981; Elgar & Crespi 1992). Understanding this behavior is therefore crucial, as it can directly impact the effectiveness of species used in biological control. For species produced in mass, it is imperative to determine whether they exhibit cannibalistic tendencies (Eroğlu 2016; Mamay & Dusak 2023). In particular, understanding the effect of starvation duration on this behavior is necessary for the successful mass rearing and release of beneficial insects. Moreover, recognizing cannibalistic tendencies in biological control species is critical for optimizing production and deployment processes (Mamay & Dusak 2019; Mamay & Dusak 2023). A clear understanding of cannibalism can contribute to improved success in mass rearing programs and minimizing economic losses (Eltez et al. 1996).

Several studies have shown that cannibalism is common among predatory beetles, particularly in species belonging to the Coccinellidae family within the order Coleoptera. For example, species such as *Coccinella septempunctata* L. and *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) (Khan & Yoldaş 2018a; Khan & Yoldaş 2018b), *Coccinella undecimpunctata* L. and *Cydonia vicina nilotica*

Muls. (Bayoumy & Michaud 2015), *Propylea dissecta* Mulsant and *Coccinella transversalis* Fabricius (Pervez et al. 2006), *Harmonia axyridis* (Yasuda & Ohnuma 1999), *Coleomegilla maculata* De Geer (Cottrell & Yeargan 1998), *Adalia bipunctata* (Agarwala 1991), and *Oenopia conglobata* L. (Coleoptera: Coccinellidae) (Mamay & Dusak 2023) have been identified as exhibiting cannibalistic behaviors.

Oenopia conglobata is a predatory beetle species that feeds on agricultural pests such as aphids and is commonly found in cultivated fields. It holds significant importance in biological control efforts (Mamay & Mutlu 2019; Sabuncu et al. 2021; Özgen et al. 2022; Mamay & Dusak 2023). However, during the mass rearing and release of this species, potential cannibalistic behaviors must be taken into consideration. While the species has great potential in biological control, its behavioral characteristics, particularly in relation to mass rearing and release, require close attention. Cannibalistic behavior in *O. conglobata*, shaped by starvation and environmental conditions, may influence its effectiveness in pest control applications. Therefore, the intensity of cannibalism in this species, as influenced by starvation periods, and its possible implications for biological control strategies represent an important area of study.

This study aims to investigate the cannibalistic behavior of *O. conglobata* under varying periods of starvation. Given that previous research by Mamay & Dusak (2023) confirmed the presence of cannibalism in this species, this study builds on the hypothesis that starvation will increase cannibalistic tendencies. Assuming that behavioral responses related to cannibalism intensify under starvation, the study explores how different starvation durations affect the cannibalistic behaviors of this predatory beetle. In this context, better understanding of the role of cannibalism in biological control strategies and its effects on the mass rearing of beneficial insects will contribute to the efficient and economical mass rearing of natural enemies of pests.

Materials and Methods

Rearing of *Ephestia kuehniella*

Ephestia kuehniella was reared in a climate-controlled chamber maintained at 25±1°C, 65±5% relative humidity, and a photoperiod of 16:8 (L:D) hours. A diet consisting of a 2:1 mixture of flour and bran by weight was used as the rearing medium (Bulut & Kılınçer 1987; Mamay et al. 2022a).

The flour-bran mixture was placed in plastic containers and sterilized in an oven at 60°C for three hours. The containers were not damaged at this temperature and during this time. After cooling in a refrigerator, the substrate was transferred to plastic trays (27×37×7 cm) at a thickness of 0.5 cm. Approximately 75 mg of *E. kuehniella* eggs were scattered into each tray, which was then covered with muslin

cloth (Mamay et al. 2022b).

After 35-40 days, adults emerging from the medium were collected using an aspirator and transferred into oviposition jars with wire-meshed sides. Eggs were collected from these jars over a period of three days.

Rearing of *Oenopia conglobate*

Oenopia conglobata was reared under controlled conditions at 25±1°C, 65±5% relative humidity, and a 16:8 (L:D) hour photoperiod. Eggs of *E. kuehniella* were used as the food source for both larval and adult stages of *O. conglobata*.

Transparent plastic jars with a 1.5-liter capacity, opened on both sides and covered with fine muslin, were used for rearing adults. The jars contained black cardboard strips to which *E. kuehniella* eggs were affixed with the help of distilled water. Crumpled tissue paper was placed inside the jars to serve as a substrate for oviposition.

Every two days, the jars were examined and tissues with *O. conglobata* eggs were transferred to new jars containing *E. kuehniella* egg strips. Larvae hatching from the eggs were reared in these jars until adulthood. Adults aged 0–24 hours, obtained from the insectarium colony, were used in the experiments.

The effect of different hunger periods on the cannibalistic behavior of *Oenopia conglobate*

The effect of different hunger periods on adult-egg cannibalism

Adult individuals of *O. conglobata* aged 0–24 hours, obtained from the insectarium stock culture, were starved for 12, 24, and 36 hours, respectively. After starvation, they were individually placed in plastic containers (5 × 5.5 cm) for experimentation. The trials were conducted at 25±1°C, 65±5% relative humidity, and a 16:8 (L:D) hour photoperiod.

To assess egg cannibalism, 50 conspecific eggs were placed in each container with a single starved adult regardless of gender. No additional food (i.e., *E. kuehniella* eggs) was provided. Each treatment combination was replicated 10 times. After 24 hours, each container was inspected, and the number of eggs consumed out of the initial 50 was recorded.

As controls, adults subjected to the same starvation periods (12, 24, and 36 hours) were also provided with 50 conspecific eggs along with an excess amount (100 eggs) of *E. kuehniella* eggs. These control groups were also replicated 10 times. After 24 hours, the number of consumed *O. conglobata* eggs was recorded to determine cannibalism in the presence of an alternative food source.

By comparing egg consumption between treatment and control groups, the rate of adult-egg cannibalism under different hunger periods was determined.

The effect of different hunger periods on adult-larva cannibalism

Adults of *O. conglobata* aged 0–24 hours from the stock culture were starved for 12, 24, and 36 hours, then individually placed in plastic containers (5 × 5.5 cm). The trials were carried out under controlled environmental conditions of 25±1°C, 65±5% relative humidity, and a 16:8 (L:D) photoperiod.

To determine adult-larva cannibalism, 30 first-instar larvae of *O. conglobata* were introduced into each container housing a single starved adult. No additional food was supplied. This experimental setup was replicated 10 times for each starvation period.

In the control groups, starved adults (12, 24, and 36 hours) were offered the same number of larvae (30) along with an excess quantity of alternative food (100 *E. kuehniella* eggs). These combinations were also replicated 10 times.

Both treatment and control groups were observed after 24 hours, and the number of larvae consumed by the adults was recorded. Based on these observations, the degree of adult-larva cannibalism was calculated.

Statistical Analysis

Independent T-Test was performed to compare the cannibalistic interactions of adults on eggs and larvae at each starvation duration with their respective control groups. Normality in the data was tested by Shapiro-Wilk normality test, which indicated a normal distribution. To assess the interaction between starving duration and prey, Analysis of Variance (ANOVA) was conducted to assess the effect of different starvation periods on the cannibalistic behavior toward eggs and larvae. Tukey's Honestly Significant Difference (HSD) test was used as a post-hoc multiple comparison method to identify which groups differed significantly. All statistical analyses were conducted using JMP Pro 13 statistical software (Jones and Sall 2011; JMP 2016). Relative cannibalism index was calculated as the ratio of the number of prey (egg or larva) consumed in the treatment group to the number consumed in the corresponding control group.

Result and Discussion

The effect of different hunger periods on the adult-egg cannibalism of *Oenopia conglobata*

The data obtained from the experiments investigating the egg cannibalism of *O. conglobata* adults following starvation periods of 12, 24, and 36 hours are presented in Figure 1.

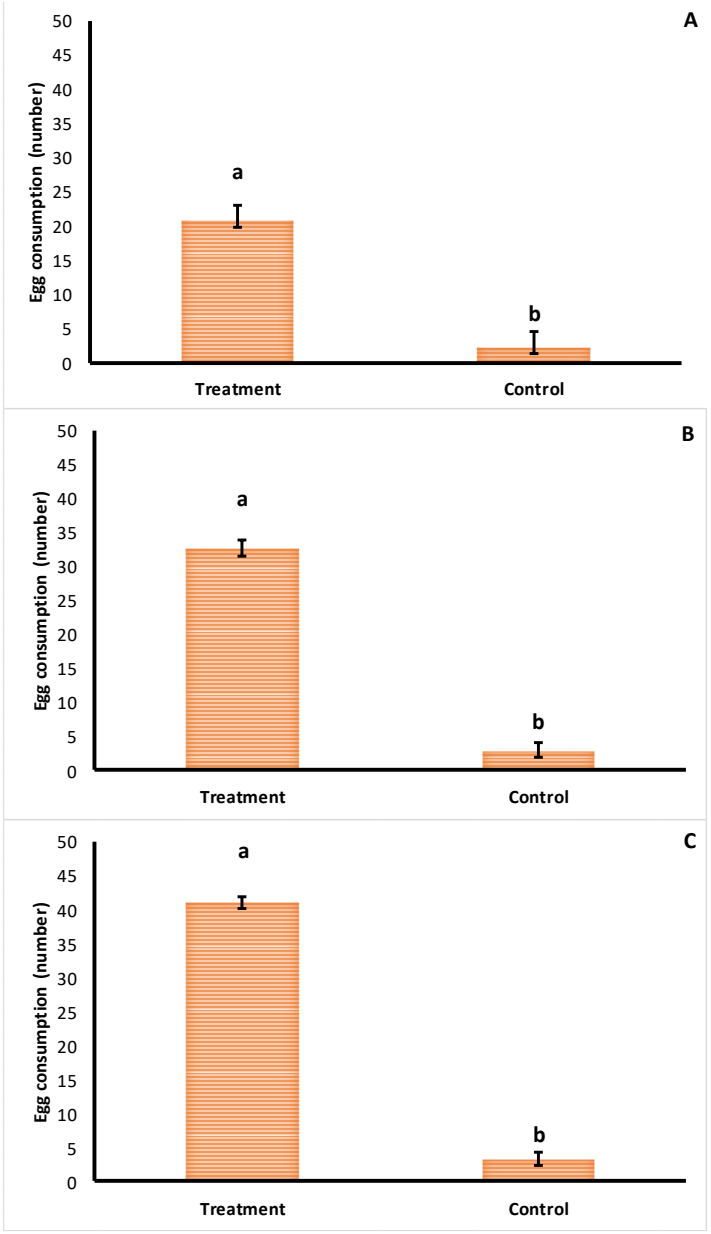

Figure 1. The effect of different hunger periods (A: 12-hour, B: 24-hour, C: 36-hour) on adult-egg cannibalism of *Oenopia conglobata.* (The average data was used and the differences was determined by independent t test)

The data obtained from the experiments on the egg cannibalism of *Oenopia conglobata* adults after being starved for 12, 24, and 36 hours without supplemental food revealed that, after 24 hours, they consumed an average of 20.8, 32.5, and 40.9

O. conglobata eggs, respectively (Figure 1). In the control group, where additional food was provided, the individuals consumed an average of 2.3, 2.9, and 3.3 *O. conglobata* eggs for the same starvation periods. According to the T-test, a statistically significant difference was found between the treatment and control groups ($p < 0.05$).

When the cannibalistic behavior of *O. conglobata* adults on their own eggs across different starvation periods was evaluated, it was determined that starvation duration significantly affected the cannibalistic behavior ($F = 37.84$; $p < 0.05$) (Figure 2).

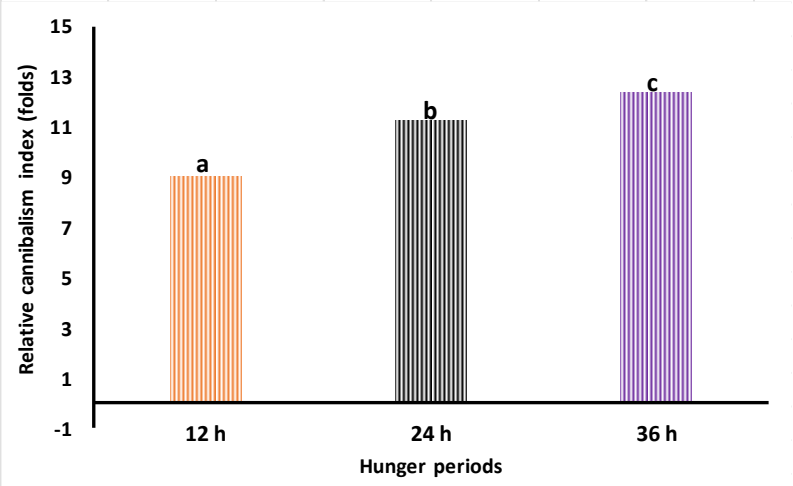

Figure 2. The effect of different hunger periods on relative cannibalism index of egg consumption ($F = 37.84$; $p < 0.05$)

The relative cannibalism index, which expresses the ratio of the number of eggs consumed in the treatment groups to the number consumed in the control group when the predator adults were starved for 12 hours, was determined to be 9 times higher. When the starvation period was extended to 24 and 36 hours, the cannibalism rate was calculated to be 11 and 12 times higher, respectively. These results indicate that as the starvation period increases, the cannibalism rate also rises (Figure 2).

The effect of different hunger periods on the adult-larva cannibalism of *Oenopia conglobata*

The results obtained from the studies conducted to determine the cannibalistic behavior of *O. conglobata* adults on larvae are presented in Figure 3.

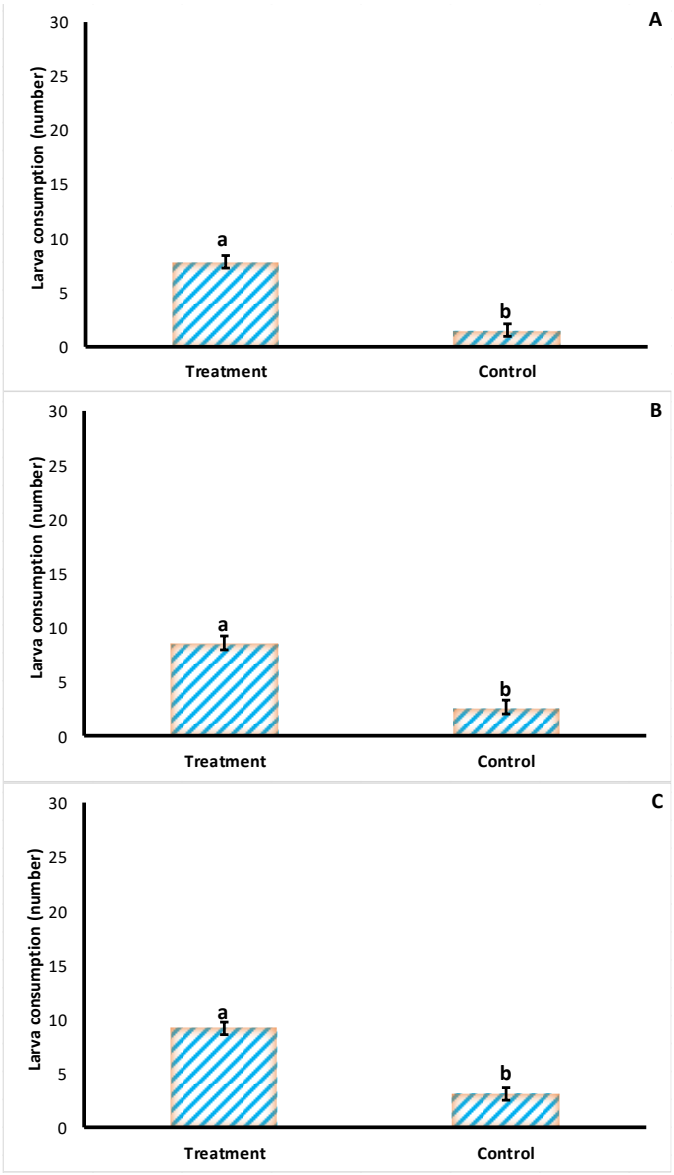

Figure 3. The effect of different hunger periods (A: 12-hour, B: 24-hour, C: 36-hour) on adult-larva cannibalism of *Oenopia conglobata (*The average data was used and the differences was determined by independent t test)

When 30 first-instar larvae were provided to *O. conglobata* adults that had been starved for 12 hours, an average of 7.8 larvae were consumed after 24 hours, while only 1.5 larvae were consumed in the control group, where additional food was provided during the same period (Figure 3). According to the independent t-test, a statistically significant difference was found between treatment and control groups

($p<0.05$). In trials involving larvae, when the predator adults were starved for 24 hours, the number of larvae consumed was 8.5 in the treatment group, while the control group consumed only 2.6 larvae. After 36 hours of starvation, the number of larvae consumed was 9.2 and 3.2 in the treatment and control groups, respectively, as counted after 24 hours (Figure 3). The independent t-test indicated a statistically significant difference between the treatment and control groups at both the 24-hour and 36-hour starvation durations ($p<0.05$). The results show that as the starvation period increases, the number of larvae consumed also increases (Figure 3). The number of larvae consumed by *O. conglobata* adults increased with longer starvation periods. Statistical analysis revealed no significant difference in cannibalism on larvae between the different starvation periods ($p>0.05$). The lack of difference in cannibalism between starvation periods is believed to be due to the fact that, regardless of the starvation duration, the adults' nutritional or energy requirements were met by consuming a certain number of larvae.

The comparison of *O. conglobata* adults' cannibalistic behavior on larvae with different starvation periods is shown in Figure 4.

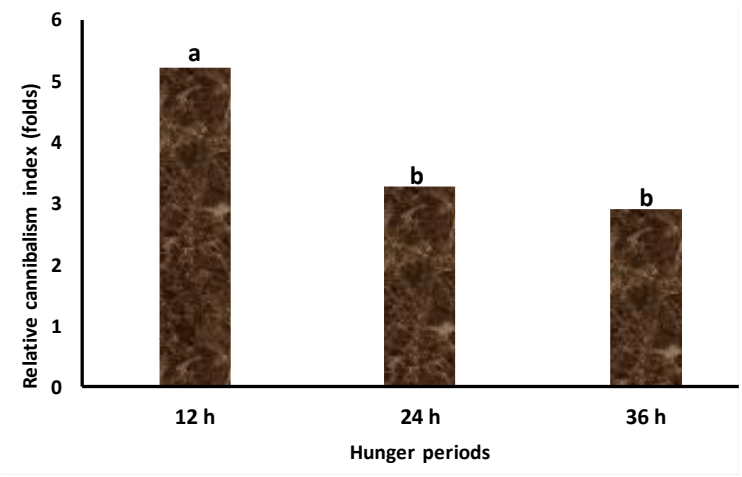

Figure 4. The effect of different hunger periods on cannibalism rate of larva consumption ($F = 1.2517$; $p < 0.05$)

The cannibalism rate of *O. conglobata* adults on larvae was 5.2 times higher in the 12-hour starvation conditions, and 3.3 and 2.9 times higher in the 24 and 36-hour starvation conditions, respectively (Figure 4). An interesting observation regarding the cannibalism rate on larvae was that, although the number of larvae consumed increased with longer starvation periods, the cannibalism rate did not increase proportionally and even decreased. This is thought to be due to the fact that, as the starvation period extended, adults in the control group, who had a greater need for

food or energy, preferred to consume larvae to quickly meet their needs rather than engage with the additional food (*E. kuehniella* eggs) provided.

The consumption of both eggs and larvae by *O. conglobata* adults under different starvation durations is shown in Figure 5.

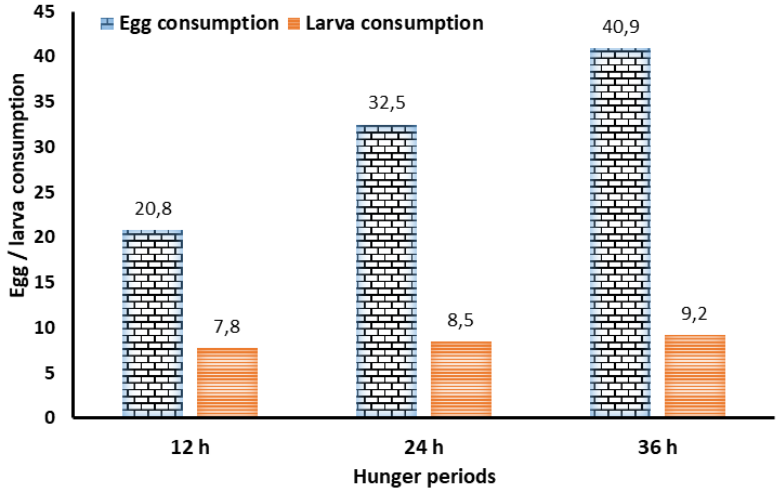

Figure 5. The effect of different hunger periods on egg and larva consumption by adults of *Oenopia conglobata*

Figure 5 shows that when *O. conglobata* adults are starved for the same durations, the number of eggs consumed was higher than the number of larvae consumed across all starvation periods. This is thought to be due to the fact that larvae have higher nutritional content or provide more energy to adults compared to eggs. Because 1st instar larvae are offered as prey to adults, it is not thought that the reason they are consumed in smaller numbers than eggs is because they defend themselves like older larvae. Considering the consumption quantities, it was determined that *O. conglobata* adults exhibited cannibalistic behavior on both eggs and larvae, with a preference for consuming eggs over larvae (Figure 5).

When all data obtained from the study were evaluated together, it was concluded that as the starvation period of *O. conglobata* adults increased, both egg and larva consumption increased. Additionally, the number of eggs consumed was higher compared to larvae for the same starvation duration. A significant finding was that the cannibalism rate on eggs was higher than that on larvae. The data obtained suggest that *O. conglobata* adults should not be starved during mass rearing. In fact, Agarwala (1991) reported that in the absence of aphids, *A. bipunctata* larvae and adults exhibit cannibalistic behavior on conspecific eggs, which helps them survive longer. Similarly, in a study conducted to determine the cannibalistic behavior of *C. septempunctata*, it was found that the cannibalism on conspecific eggs was inversely

proportional to the availability of aphids as food. When aphids were absent or their numbers were insufficient, the cannibalism rate increased (Khan & Yoldaş, 2018a). In line with this, Osawa (1992) reported that when food is abundant, *H. axyridis* shows low levels of cannibalism on its own eggs. In another study, it was reported that even when sufficient food was available, *H. variegata* adults exhibited high levels of cannibalism on their own eggs (Khan & Yoldaş, 2018b).
Similar to the findings of this study, it has been reported that insects exhibit different cannibalistic behaviors during different biological stages, and that the cannibalistic behaviors of different genders may also vary. For example, Mamay & Dusak (2024) reported that female and male *O. conglobata* individuals consumed more eggs than larvae. The researchers found that the predator consumed more first instar larvae compared to fourth instar larvae, and that as larval stages progressed, both the number of larvae consumed, and the cannibalism rate decreased. Khan & Yoldaş (2018a) reported that *C. septempunctata* adults consumed more first instar larvae, which parallels the results of the current study that uncovered the cannibalistic behavior of *O. conglobata*. The same researchers also reported similar findings for the cannibalistic behaviors of *H. variegata* (Khan & Yoldaş, 2018b).

The results of this study are supported not only by studies conducted on species from the Coccinellidae family but also by studies on the cannibalistic behavior of predators from various orders and families. Tommasini et al. (2002) found that cannibalism occurred in the predators *Orius insidiosus* and *Orius laevigatus* (Hemiptera: Anthocoridae), but *O. insidiosus* consumed more first instar nymphs. The researchers reported that the cannibalistic behavior of both species on young nymphs was inversely proportional to food abundance. Similarly, Michaud (2003) found that cannibalism between adults and larvae of the predators *Cycloneda sanguinea*, *Olla v-nigrum*, and *H. axyridis* occurred at different rates specific to the species, and even when sufficient food was available, cannibalism was observed to some extent in all three species. All of these studies provide results that support the findings of our research.

Conclusion

It has been determined that when the adult individuals of *O. conglobata*, are starved for different periods, they exhibit at least 9 times more cannibalism on their own eggs. Similarly, it has been found that *O. conglobata* adults also consume 1st instar larvae when starved for different durations. When compared to the control group, it was understood that providing sufficient food significantly decreased cannibalism, but did not completely eliminate it, depending on the duration of starvation.

Therefore, insufficient food, high population density, and long starvation periods, which have a significant impact on cannibalism, should be avoided in the mass rearing of beneficial insects. Based on the results of this study, the following recommendations are made: I) an adequate amount of food should be provided

during mass rearing of beneficial insects under laboratory conditions, II) frequent prey controls should be conducted to prevent starvation, III) if possible, the periods when cannibalism is most prevalent should be isolated during rearing, and IV) especially due to the high rate of cannibalism on eggs by adult predators, it is strongly advised that egg harvesting be done daily during mass rearing.

Acknowledgement

This study was financially supported by the Harran University Scientific Research Projects Coordination Unit (Project Number: HÜBAP-19036). This manuscript is produced from Handan Kubat MSc Thesis.

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