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Corresponding address:

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E-mail: bimude@cu.edu.tr

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Özgün makale (Original article)

Lethal effects of *Lecanicillium psalliotae* (Hypocreales: Cordycipitaceae) on *Myzus persicae* (Sulzer) and *Aphis fabae* Scopoli (Hemiptera: Aphididae)

Ayşe Müge DURMAZ^{1*}, Murat MUŞTU²

Lecanicillium psalliotae (Hypocreales: Cordycipitaceae)'nin Myzus persicae (Sulzer) ve Aphis fabae Scopoli (Hemiptera: Aphididae) üzerindeki öldürücü etkileri

Öz: Bu çalışmada, entomopatojen fungus *Lecanicillium psalliotae*'nin yeşil şeftali yaprakbiti *Myzus persicae*, (Sulzer) (Hemiptera: Aphididae) ve bakla yaprakbiti *Aphis fabae* Scopoli (Hemiptera: Aphididae) üzerindeki patojenik etkinliği laboratuvar koşullarında araştırılmıştır. Fungusun 10⁶, 10⁷, 10⁸ konidi/ml konsantrasyonları yaprakbitlerinin 2. dönemine inoküle edilmiştir. Entomopatojen fungus inoküle edilen yaprakbitleri 3., 6., ve 9. günlerde kontrol edilerek ölü bireyler sayılmıştır. Konsantrasyon yoğunluğu arttıkça her iki yaprakbiti türü için de ölüm oranlarının arttığı belirlenmiştir. *Myzus persicae* nimflerinde en yüksek ölüm oranı, %95 ile 10⁸ konidi/ml spor yoğunluğu ve 9 günlük inkübasyon süresinde elde edilmiştir. Benzer şekilde, *A. fabae*'de de aynı konsantrasyon ve inkübasyon periyodunda %100 ölüm oranıyla en etkili sonuç elde edilmiştir. Elde edilen bulgular, spor yoğunluğu ve inkübasyon süresinin *L. psalliotae*'nin entomopatojenik etkinliğinde belirleyici olduğunu göstermekte ve bu entomopatojen fungusun biyolojik mücadele açısından potansiyelini desteklemektedir.

Keywords: Biyolojik mücadele, Entomopatojen fungus, *Lecanicillium psalliota*e, Yaprakbiti

Abstract: In this study, the pathogenic efficacy of the entomopathogenic fungus *Lecanicillium psalliotae* was evaluated against the green peach aphid *Myzus persicae* (Sulzer) and the black bean aphid *Aphis fabae* Scopoli (Hemiptera: Aphididae) under laboratory conditions. Second-instar nymphs of the both aphid species were treated with fungal suspensions at concentrations of 10⁶, 10⁷, and 10⁸ conidia/ml. Mortality was recorded on the 3rd, 6th, and 9th days, post-inoculation. Results demonstrated that mortality rates increased with higher conidial concentrations in both aphid species. In *M. persicae*, the highest mortality rate (95%) was observed at 10⁸ conidia/ml after nine days. Similarly, *A. fabae*, exhibited 100% mortalityunder the same treatment conditions. The findings suggest that both conidial concentration and incubation period are key factors affecting the pathogenicity of *L. Psalliotae*, highlighting its potential as a promising biological control agent for aphid management.

ORCID ID (Yazar sırasıyla): 0009-0009-7974-6449; 0000-0001-9428-9236

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¹ Graduate School of Natural and Applied Sciences, Erciyes University, 38039 Kayseri, Türkiye

² Faculty of Agriculture, Department of Plant Protection, Erciyes University, 38039 Kayseri, Türkiye

^{*} Sorumlu yazar (Corresponding author): a.mugedurmaz@gmail.com

Keywords: Aphid, Biological control, Entomopathogenic fungi, Lecanicillium psalliotae

Introduction

The green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), is a cosmopolitan pest that feeds on over 400 plant species across more than 50 families in both agricultural fields and greenhouses (Blackman & Eastop 1984). It causes damage by feeding on fresh shoots and secreting, toxic substances during feeding, as well as by producing honeydew, which facilitates the development of sooty mold (Özdemir & Toros 1997).

Similarly, the black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae) is capable of infesting over 200 plant species (Barnea et al. 2005). It feeds on phloem sap also excretes honeydew, promoting sooty mold formation that interferes with photosynthesis (Shannag 2007). Furthermore, *A. fabae* acts as a vector for various plant pathogens, contributing to the spread of diseases (Garzo et al. 2004).

Due to their high reproductive capacity and tendency to inhabit concealed areas such as the undersides of leaves, aphids are often managed with chemical control methods. However, the extensive use insecticide has led to resistance development in aphid populations and has caused collateral damage to non-target organisms (Metcalfe 1989; Ay et al. 2007; Özdemir & Salman 2021). Furthermore, the uncontrolled use of chemical pesticides results in residue accumulation on plants, posing risk to human and environmental health, and raising concerns regarding food safety.

Given the negative consequences associated with chemical pesticide use, the development of alternative control strategies is increasingly necessary. One promising alternative is the use of biopesticides which are derived from various natural sources, such as plants, animals, microorganisms, and minerals. Among microbial agents, entomopathogenic fungi have gained attention due to their natural abundance, ease of laboratory cultivation stands out as an essential group of microorganisms, and commercial production potential (Eken & Demirci 1997).

Lecanicillium psalliotae (Hypocreales: Cordycipitaceae) is an entomopathogenic fungus known produce red pigmentation on Potato Dextrose Agar (PDA), attributed to the production of oosporins (Wainwright et al. 1986). Species within the genus Lecanicillium are recognized for their suppressive effects on aphids and other agricultural pests (Jung et al. 2006).

Although various studies have been conducted in Türkiye to identify pathogens suitable for biological control, their practical application in pest management remains limited. Moreover, the pathogenic potential of *L. psalliotae* against aphid species has not been adequately explored. This study aims to determine the lethal effects of *L. psalliotae* on two economically important aphid species *M. persicae* and *A. fabae*, under laboratory conditions

Materials and Methods

Host plants

To establish a laboratory culture of M. persicae, bell pepper ($Capsicum\ annuum$, cv Akman) seeds were sown in seedling trays and regularly watered to promote germination. When the seedlings reached the four-leaf stage, they were transplanted into plastic pots (15 cm depth, 18 cm diameter) and maintained in growth chambers under controlled environmental conditions (25 ± 1 °C, $60\pm5\%$ relative humidity, and a 16:8 h light: dark photoperiod). For A. fabae culture, dry bean ($Phaseolus\ vulgaris$, cv. 'Aras') seeds were sown at a depth of 3–4 cm in plastic pots of the same dimensions and grown under identical environmental conditions in growth chambers.

Aphid rearing

Field-collected specimens of *M. persicae* and *A. fabae* were taxonomically identified by Assoc. Prof. Işıl Özdemir (Kocaeli University, Faculty of Agriculture, Department of Plant Protection). Stock colonies were initiated by transferring the aphids onto the previously cultivated pepper and bean plants once the plants reached the 7–8 leaf stage. Aphid rearing was conducted in climate chambers maintained at 25±1 °C, 60±5% relative humidity, and a 16:8 h light: dark photoperiod.

Preparation of fungal inoculum

The fungal isolate used in this study, *Lecanicillium psalliotae* (KK-8), originally isolated from adult *Eurygaster* spp. as part of a master's thesis project (Accession No: AB360367.1 at NCBI) supported by the Scientific Research Projects Unit of Ankara University. The isolate is preserved in the entomopathogenic fungi stock culture collection at the Biological Control Laboratory, Faculty of Agriculture, Erciyes University. The isolate was cultured on Potato Dextrose Agar (PDA) medium for two weeks. Spore suspensions were then prepared at concentrations of 10⁶, 10⁷ and 10⁸ conidia/ml in sterile distilled water containing 0.02% Tween 80. Spore concentrations were determined using a Thoma hemocytometer under a light microscope.

Effects of the entomopathogen on Myzus persicae and Aphis fabae

To maintain leaf humidity, the bottom halves of 55 mm diameter Petri dishes, each equipped with a mesh-covered ventilation opening, were filled with 1% water agar. Clean pepper or bean leaf discs were placed on the agar surface with the abaxial (lower) side facing upward. Ten adult *M. persicae* or *A. fabae* individuals were introduced onto each leaf disc. After 24 hours, all adults were removed, leaving only first-instar nymphs. The nymphs were monitored daily until they molted into second instar. At that point, 20 uniformly aged second-instar nymphs were retained per petri dish, and excess individuals were removed.

Previously prepared *L. psalliotae* (KK-8) spore suspensions at concentrations of 10^6 , 10^7 , and 10^8 conidia/ml were applied to the aphid infested leaf discs using a handheld sprayer, with 1 ml of suspension sprayed per leaf in fine droplets. The treated Petri dishes were than transferred to humidity chambers maintained at $80\pm2\%$

relative humidity within a climate-controlled growth chamber set at 25±1 °C with a 16:8 h light: dark photoperiod.

Aphid mortality was recorded on the 3rd, 6th, and 9th days post-application using a stereomicroscope. Control groups were treated with sterile distilled water containing 0.02% Tween 80. The experiment was conducted with four replicates per treatment and the control. Mortality data were arcsine-transformed before being subjected to analysis of variance (ANOVA), differences among the means were assessed using Tukey's test at significance level of $p \le 0.05$.

Results and Discussion

The lethal effects of different spore concentrations of *L. psalliotae* (10^6 , 10^7 , 10^8 conidia/ml) on *M. persicae* varied significantly across the incubation periods (3, 6, and 9 days) (Table 1). Mortality rates increased with both higher spore concentrations and longer incubation periods. Specifically, the highest mortality rate (95%) was observed at concentration of 10^8 conidia/ml after 9-days. Statistical analysis revealed significant differences between the spore concentrations of 10^7 and 10^8 conidia/ml compared to both 10^6 conidia/ml treatment and the control group, particularly on the day 6 (F= 11.961, df= 3, p= 0.001) and day 9 (F= 15.195, df= 3, p< 0.001).

Table 1. Effects of different concentrations and incubation periods of *Lecanicillium* psalliotae on the percentage mortality of Myzus persicae (Mean + S.E.).

Incubation	Spore Concentrations (conidia/ml)			
Period				
(days)	Control	10^{6}	10^{7}	10^{8}
3	0.00 ± 0.00 cB**	10.00 ± 10.00 bcA	48.75 ± 20.25 abA	77.50 ± 12.50 aA
6	$0.00 \pm 0.00 bB$	$20.00\pm20.00bA$	71.25 ± 11.62 aA	$88.75 \pm 11.25 aA$
9	11.25 ± 5.15 bA	33.75 ± 17.37 bA	85.00 ± 5.00 aA	95.00 ± 5.00 aA

^{*}Lowercase letters in the same row indicate no statistically significant difference according to the Tukey test (P<0.05).

When the lethal effects of different spore concentrations (10^6 , 10^7 , 10^8 conidia/ml) and incubation periods (3, 6, and 9 days) of *L. psalliotae* on *A. fabae* examined, it was found that mortality rates significantly increased with both higher spore concentrations and longer incubation periods (p < 0.05) (Table 2). The highest mortality was recorded on day 9 at the spore concentration of 10^8 conidia/ml. At this concentrationstatistically significant differences in mortality were observed compared to other spore concentrations and the control group on, day 3 (F = 14.131, df = 3, p < 0.001), day 6 (F = 33.301, df = 3, p < 0.001), and day 9 (F = 52.583, df = 3, P < 0.001). Furthermore, on the 9th day of incubation, the spore concentrations of 10^6 and 10^7 conidia/ml also showed statistically significant differences in mortality compared to the control.

As the incubation period of *L. psalliotae* increased, its lethal effect on *A. fabae* also increased (Table 2). At spore concentrations of 10^6 (F = 24.774, df = 2, $P < 10^6$

^{**}Uppercase letters in the same column indicate no statistically significant difference according to the Tukey test (P<0.05).

0.001) and 10^7 (F = 52.465, df = 2, P < 0.001) conidia/ml, statistically significant differences in mortality were observed between the 6th and 9th days of incubation. In contrast, at a concentration of 10^8 conidia/ml, statistically significant differences in mortality were detected across all three incubation periods (F = 457.840, df = 2, P < 0.001).

A significant increase in mortality rates was observed in both pest species as the spore concentration and incubation period of *L. psalliotae* increased. In *M. persicae* individuals, the highest mortality rate, reaching 95%, was achieved particularly at a concentration of 10⁸ conidia/ml after a 9-day incubation period. Similarly, in *A. fabae* individuals, the most effective results were observed at the same spore concentration (10⁸ conidia/ml) and incubation period, with mortality rates showing statistically significant differences compared to other concentrations and the control group.

Table 2. Effects of different concentrations and incubation periods of *Lecanicillium* psalliotae on the percentage mortality of *Aphis fabae* (Mean \pm S.E.).

Incubation	Spore Concentrations (conidia/ml)			
Period (days)	Control	10^{6}	107	108
3	2.50 ± 2.50b*B**	7.50 ± 4.33 bB	7.50 ± 1.44 bB	36.25 ± 1.25aC
6	$6.25 \pm 3.75 bAB$	23.75 ± 8.99 bB	$20.00\pm2.04bB$	$87.50 \pm 2.50 aB$
9	17.50 ± 4.79 cA	72.50 ± 1.44 bA	$86.25 \pm 5.54 \text{bA}$	$100 \pm 0.00 aA$

^{*}Lowercase letters in the same row indicate no statistically significant difference according to the Tukey test (*P*<0.05).

**Uppercase letters in the same column indicate no statistically significant difference according to the Tukey test

Berber & Birgücü (2020) evaluated two isolates of Beauveria bassiana against M. persicae and reported that the mortality rates increased with both spor concentrations and longer exposure durations. Similarly, Özçelik et al. (2013) tested Isaria farinosa and Purpureocillium lilacinum at a concentration of 10⁸ conidia/ml under different humidity levels against M. persicae, and observed that the efficacy of both entomopathogens improved with increasing humidity, yielding promising results for biological control. For A. fabae, Arıcı et al. (2012) demonstrated that the application of Fusarium subglutinans under controlled conditions significantly reduced population levels within two weeks. Senthil Kumar et al. (2015) studies the impact of L. psalliotae on thrips at a spore concentration of 10⁷ conidia/ml and reported a mortality rate of 62.9% after 10 days, compared to only 7.5% in the control group. Beyond its entomopathogenic activity L. psalliotae has also shown potential as a biological control agent against nematodes. Pérez-Anzúrez et al. (2024) reported that this species produces nematocidal compounds effective against *Haemonchus* contortus, a common parasite of small ruminants. Similarly, Gan et al. (2007) demonstrated that a chitinase gene obtained from L. psalliotae was effective in killing the eggs of the root-knot nematode Meloidogyne incognita. Other species within the Lecanicillium genus have also proven effective in pest management. Lecanicillium lecanii significantly reduced the reproductive capacity of Aphis

^{**}Uppercase letters in the same column indicate no statistically significant difference according to the Tukey test (P<0.05).

gossypii (Gurulingappa et al. 2011) while *Lecanicillium muscarium* is commercially utilized for the control of aphids and whiteflies (Anonymous 2025).

In conclusion, it has been determined that *L. psalliotae* exhibits a high level of virulence against both *M. persicae* and *A. fabae*, with mortality increasing in response to high sporulation density and longer incubation periods. These results highlight its strong potential as an effective entomopathogen for use in biological control programs. Previous studies on related species and isolates have similarly reported successful outcomes against different pests. This supports the potential of *L. psalliotae* as a biological control agent with a broad spectrum of activity. Furthermore, its combined nematophagous and entomopathogenic capabilities enhance its applicability in integrated pest management (IPM) strategies by offering control over multiple pest groups. However, before *L. psalliotae* can be commercially utilized or fully integrated into IPM systems, its efficacy under greenhouse and field conditions must be evaluated. Additionally, comprehensive assessments of its non-target effects on natural enemies and mammalian safety, are esential.

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Özgün makale (Original article)

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

Handan KUBAT¹, Mehmet MAMAY^{1*}

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

Oz: 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*

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

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¹ Harran University, Agricultural Faculty, Department of Plant Protection, 63050, Şanlıurfa, Türkiye

^{*} Sorumlu yazar (Corresponding author): mehmetmamay@hotmail.com ORCID ID (Yazar sırasıyla): 0000-0002-0372-8684; 0000-0003-3723-5228

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 ($27 \times 37 \times 7$ 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 $25\pm1^{\circ}$ C, $65\pm5\%$ 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 $25\pm1^{\circ}$ C, $65\pm5\%$ 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.

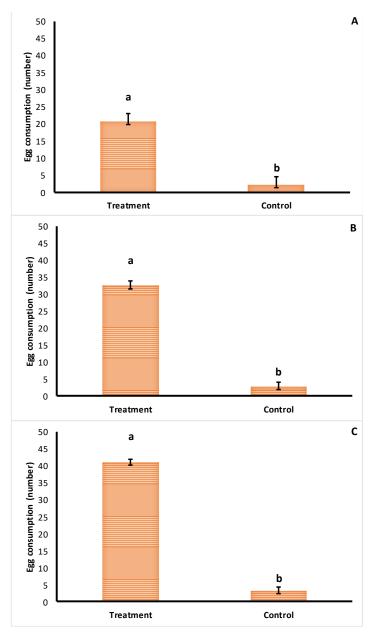


Figure 1. The effect of different hunger periods (A: 12-hour, B: 24-hour, C: 36-hour) on adult-egg cannibalism of *Oenopia conglobata*. (The average data was used and the differences was determined by independent t test)

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).

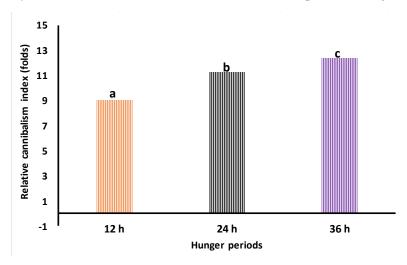


Figure 2. The effect of different hunger periods on relative cannibalism index of egg consumption (F = 37.84; p < 0.05)

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.

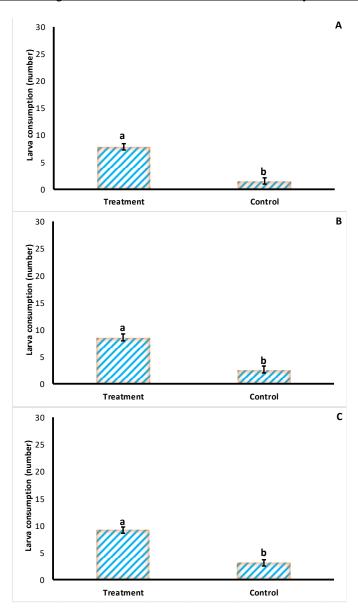


Figure 3. The effect of different hunger periods (A: 12-hour, B: 24-hour, C: 36-hour) on adult-larva cannibalism of *Oenopia conglobata* (The average data was used and the differences was determined by independent t test)

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.

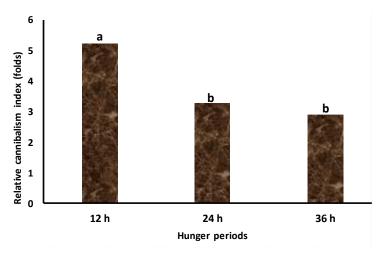


Figure 4. The effect of different hunger periods on cannibalism rate of larva consumption (F = 1.2517; p < 0.05)

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.

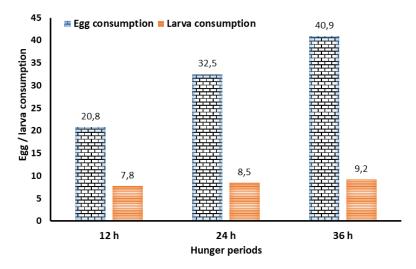


Figure 5. The effect of different hunger periods on egg and larva consumption by adults of *Oenopia conglobata*

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.

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- 1. Makale; Microsoft Word programında (MS Word 2000 veya üzeri versiyonu), Times New Roman karakterde, 11 punto, tek satır aralığında ve normal karakterde yazılmalıdır.
- 2. Eserler, standart A4 kağıdına ve sayfa yapısı; üst ve alt bilgiler dâhil üstten ve alttan 4.5 cm, sol ve sağ 4.0 cm boşluk bırakılarak sayfanın sağ kenarı hizalı biçimde yazılmalı ve şekil ve çizelgeler ile birlikte 16 sayfayı geçmemelidir.
- Makalenin ilk sayfasında üst bilgi olarak sola dayalı, 10 punto, normal karakterde; Türkiye Biyolojik Mücadele Dergisi Turkish Journal of Biological Control ISSN 2146-0035
 - ifadeleri yazılmalı ve altında da 14 punto tek satır boşluk bırakılmalıdır.
- 4. Türkçe eserler; "Başlık, Yazar adı-soyadı, İngilizce başlık ve Abstract, Keywords, Öz, Anahtar kelimeler, Giriş, Materyal ve yöntem, Bulgular ve tartışma, Sonuç (istenirse), Teşekkür (istenirse), Kaynaklar"
- 5. İngilizce eserler; "Title, Author's name, Türkçe başlık, Öz, Anahtar kelimelerler, Abstract, Keywords, Introduction, Materials and methods, Results and discussion, Conclusion (optional), Acknowledgement (optional), References" ana başlıklarından olusmalıdır.
- 6. Derleme eserlerde ise, "Abstract, Öz ve Giriş" bölümlerinden sonra uygun bölüm başlıkları verilebilir.
- 7. Eserin başlığı hangi dilde yazılıyorsa bold ve 14 karakterde, sola yaslı, tamamı küçük harf (sadece özel isimlerin baş harfleri büyük), tek satır aralığında yazılmalı, başlıkta verilen latince isimler italik yapılmalıdır.
- 8. Başlıktan sonra 11 punto bir satır boşluk bırakıldıktan sonra yazarların açık adları unvan belirtilmeden küçük harflerle (baş harfi büyük), soyadları ise büyük harflerle, sola yaslı, birden fazla yazar adı arasında virgül ve bir boşluk olacak şekilde 11 karakterde bold olarak yazılmalıdır. Eser ve yazar adlarına "Ekle → Başvuru → Dipnot" takip edilerek numara verilmeli ve ilk sayfanın sonunda bunlara ait bilgiler, sorumlu yazarın e-mail adresi ile alınış ve kabul ediliş ifadeleri 9 karakterde yazılmalıdır.
- 9. Yazar adlarından sonra 11 punto bir satır boşluk bırakılarak eserin ikinci dildeki başlığı 11 karakterde, sola yaslı ve bold olarak yazılmalıdır.
- 10. Abstract ve Öz başlıkları 12 karakter, bold, paragraf girintisi yapılmadan iki nokta (:) konduktan sonra aynı satırdan başlayarak, metin kısmı 10 karakterde, tek satır aralığı ile yazılmalı ve 150 kelimeyi geçmemelidir.
- 11. "Keywords ve Anahtar kelimelerler (bold)" Abstract ve Öz metinlerinden sonra 6 nk boşluk bırakılarak sola yaslı ve 10 karakterde yazılmalıdır.
- 12. Eserin; 2, 4, 6, 8 gibi çift nolu sayfalarında üst bilgi olarak makale başlığını kısaca ifade eden bir cümle sağa yaslı; yine 3, 5, 7, 9 gibi tek nolu sayfalarında ise sol tarafta derginin Türkçe ve İngilizce açık adı ve sağ tarafta yazar adı (Öztürk & Karacaoğlu veya Uygun et al. gibi) ile derginin yıl, cilt ve sayı numarası 10 karakterde normal ve sonrasında 10 punto bir satır boşluk olacak şekilde yazılmalıdır.

- 13. Ana bölüm başlıkları; sola yaslı, bold, ilk harfleri büyük ve 13 karakterde yazılmalı, başlıklardan önce 11 punto tek satır, sonra 6 nk boşluk bırakılmalıdır. Alt başlık varsa 12 karakterde, sola yaslı, ilk harfi büyük diğerleri küçük, bold olarak yazılmalıdır.
- 14. Eserin tüm metin kısmı 11 karakterde, normal, iki yana yaslı, tek satır aralığında, ilk paragrafta girinti yok, ancak ara paragraflarda ise 0.5 cm girinti olmalı ve paragraflar arasında boşluk bırakılmamalıdır.
- 15. Fotograf ve grafikler "Şekil", sayısal değerleri içeren tablo ve çizelgeler ise "Çizelge" olarak belirtilmeli ve Şekil 1, Şekil 2 veya Çizelge 1, Çizelge 2 gibi ardışık olarak numaralandırılmalıdır. Şekil başlıkları şeklin altında, öncesinde 6 nk boşluk ve çizelge başlıkları ise çizelgenin üstünde sonrasında 6 nk boşluk olmalı, normal, 10 karakterde olacak şekilde ve tek satır aralığında yazılmalıdır. Eğer varsa, çizelge dipnotları çizelge altında, normal, sola yaslı ve 8 karakterde kısa ve öz olarak verilmelidir.
- 16. Türkçe hazırlanan eserlerde, İngilizce "Figure" ve "Table" başlıkları ayrıca verilmelidir.
- 17. Her iki dilde de yazılan eserde kaynaklara ilişkin bildirimler metin içerisinde "yazar ve yıl" sırasına göre yapılmalı, metin içindeki açıklama ve yazar sayısına bağlı olarak bildirim "Uygur (2008), Ulusoy & Kazak (2009), Aysan et al. (2010)," örneğinde olduğu gibi veya bildirimin sonunda tamamı parantez içinde olacak şekilde verilmelidir Örneğin; (Karut 2008; Ulusoy & Öztürk 2009; Elekçioğlu et al. 2010).
- 18. Eser metninde organizmaların bilimsel adları ilk geçtiği yerde "Author" adı ile birlikte açık, daha sonra cins adı kısaltılmış olarak yazılmalı ve gerek metin ve gerekse kaynaklar da "italik" olmalıdır. Ana ve alt başlıklar ile çizelge ve şekil başlıklarında ise, Author adı verilmeden açık yazılmalıdır.
- 19. Kaynaklar listesi ilk yazarın soyadına göre, numara verilmeden alfabetik olarak, 10 karakterde, tümü küçük harf (özel isimler hariç), 0.5 cm asılı ve tek satır aralığında yazılmalıdır. Tek veya daha fazla yazarlı eserlerin bildiriminde son yazardan önce "&" işareti kullanılmalıdır. (Örn.: Öztürk N. 2011., Karut K. & S. Satar 2009., Uygun N., S. Satar & M. Karacaoğlu 2010.). Dergilerin isimleri açık ve italik, diğer kaynakları normal karakterde açık olarak yazılmalıdır. İnternetten alınan kaynakların ise ayrıca web adresleri ile erişim tarihleri de belirtilmelidir (Örn.: Erişim tarihi: 10 Ocak 2010).

Dergi

Öztürk N. & M.R. Ulusoy 2003. Mersin ili kayısılarında saptanan zararlılar. *Alatarım Dergisi*, 2 (2): 21-26.

Pruszynski S. & W.W. Cone 1973. Biological observations of *Typhlodromus athiasae* Porath and Swirski (Acari: Phytoseiidae) on hops. *Annals of the Entomological Society of America*, 66: 47-51.

Kongre veya sempozyum:

Karut K. & E. Şekeroğlu 1999. *Chrysoperla carnea* (Stephens) yumurtalarımn laboratuvar koşullarında depolanma olanaklarının araştırılması. Türkiye 4. Biyolojik Mücadele Kongresi Bildirileri, 26-29 Ocak 1999, Adana, 203-210.

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Tez:

Şenal D. 2006. Avcı böcek *Chilocarus nigritus* (Fabricius) (Coleoptera: Coccinellidae)'un bazı biyolojik ve ekolojik özellikleri ile doğaya adaptasyonu üzerinde araştırmalar. Doktora tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Balcalı-Adana, 127 s.

Kitap:

Uygun N. 1981. Türkiye Coccinellidae (Coleoptera) Faunası Üzerinde Taksonomik Araştırmalar. Çukurova Üniversitesi Ziraat Fakültesi Yayınları, Yayın No: 157, 111 s

Kitaptan bir bölüm:

Elekçioğlu İ.H. & U. Gözel 2001. Turunçgillerde zararlı nematodlar ve entegre mücadelesi (Editör: N. Uygun, Türkiye turunçgil bahçelerinde entegre mücadele, zararlılar-nematodlar-hastalıklar-yabancıotlar). TÜBİTAK-TARP Türkiye Tarımsal Araştırma Projesi Yayınları, Ankara, 61-69.

Internet:

Neden Biyolojik Mücadele? URL: http://www.biyolojikmucadele.org.tr (Erişim tarihi: 24 Nisan 2008).

Yazarı belli olmayan yayınlar:

Anonymous 2008. Türkiye'de çilek üretimi. T.C. Başbakanlık Devlet İstatistik Enstitüsü, Yayın No: 1577, Ankara.

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For Meetings and symposiums:

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For Books:

Chant, D.A. & J.A. McMurtry 2007. Illustrated keys and diognoses for the genera and subgenera of the Phytoseiidae of the world (Acari: Mesostigmata). Indira Publishing House, West Bloomfield, 219pp.

For Book Chapters:

Elekçioğlu İ.H. & U. Gözel 2001. Turunçgillerde zararlı nematodlar ve entegre mücadelesi (Editör: N. Uygun, Türkiye turunçgil bahçelerinde entegre mücadele, zararlılar-nematodlar-hastalıklar-yabancıotlar). TÜBİTAK-TARP Türkiye Tarımsal Araştırma Projesi Yayınları, Ankara, 61-69.

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