Ormancılık Araştırma Dergisi
Turkish Journal of Forestry Research
2025, 12:2 , 221-227

DOI: https://doi.org/10.17568/ogmoad.1780823
Koruma/Conservation
Araştırma makalesi/Research article

Assessing *Musca domestica* larvae as an alternative nutrient source for the mass rearing of *Calosoma sycophanta* in biocontrol

Biyolojik mücadelede *Calosoma sycophanta*'nın kitlesel üretimi için alternatif besin kaynağı olarak *Musca domestica* larvalarının değerlendirilmesi

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Geliş tarihi *(Received)*
10.09.2025
Kabul Tarihi *(Accepted)*
17.11.2025

Sorumlu editör (*Corresponding editor*)
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Atıf *(To cite this article)*: Serttaş, A., Aslan Bıçkı, Ü., Çetin, H., Yolcu, H., İ., Uyan, M., (2025). Assessing Musca domestica larvae as an alternative nutrient source for the mass rearing of *Calosoma sycophanta* in biocontrol, Ormancılık Araştırma Dergisi, 12(2), 221-227
DOI: https://doi.org/10.17568/ogmoad.1780823

Abstract

In this study, we investigated the potential use of housefly *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae) larvae as an alternative feed for mass-rearing *Calosoma sycophanta* (Linnaeus, 1758) (Coleoptera: Carabidae), a species utilized in biological control against forest pests. Our research focused on the egg production efficiency of *Calosoma* adults when fed housefly larvae and the growth and development of first-instar *Calosoma* larvae under laboratory conditions. We compared these groups with *Calosoma* individuals fed on pine processionary moth (PPM), *Thaumetopoea wilkinsoni* (Tams, 1926) (Lepidoptera: Thaumetopoeidae). The results indicated a significant increase in weight for first-instar *C. sycophanta* larvae after 16 days: a 52.9-fold increase when fed PPM and a 16-fold increase when fed housefly larvae. In terms of length growth, PPM-fed larvae demonstrated a 4.52-fold increase, whereas housefly-fed larvae showed a 3.06-fold increase. Although the larvae consumed M. domestica larvae, the adult *Calosoma* did not produce eggs, and the larvae did not pupate. In contrast, *Calosoma* adults fed with *T. wilkinsoni* produced eggs, with their larvae successfully maturing to adulthood. Fifty percent of larvae fed with PPM and eighty percent fed with houseflies survived to 16 days. The findings suggest that while *Calosoma* larvae raised on houseflies are smaller and lighter than those fed with PPM, feeding them houseflies for a minimum of two weeks and subsequently releasing them in their larval stage could aid in the biological control of PPM.

Keywords: Alternative prey, *Calosoma sycophanta*, housefly, mass production

Öz

Çalışmada, orman zararlılarına karşı biyolojik mücadelede kullanılan *Calosoma sycophanta* (Linnaeus, 1758) (Coleoptera: Carabidae)'nın kitlesel üretimi için alternatif besin kaynağı olarak ev sineği *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae) larvalarının potansiyel kullanımı araştırılmıştır. Araştırma, ev sineği larvalarıyla beslenen *Calosoma* erginlerinin yumurta üretim verimliliği ile birinci dönem *Calosoma* larvalarının laboratuvar koşullarındaki büyüme ve gelişimine odaklanmıştır. Bu gruplar, çam kese böceği (ÇKB) (*Thaumetopoea wilkinsoni* (Tams, 1926)) (Lepidoptera: Thaumetopoeidae) ile beslenen *Calosoma* bireyleriyle karşılaştırılmıştır. Sonuçlar, 16 gün sonunda birinci dönem *C. sycophanta* larvalarında anlamlı bir ağırlık artışı olduğunu göstermiştir: ÇKB ile beslenenlerde 52,9 kat, ev sineği larvalarıyla beslenenlerde ise 16 kat artış kaydedilmiştir. Boy uzunluğundaki büyüme açısından ise ÇKB ile beslenen larvalarda 4,52 kat ev sineği larvalarıyla beslenenlerde ise 3,06 kat artış gözlenmiştir. Larvalar *M. domestica* larvalarını tüketmiş olsalar da *Calosoma* erginleri yumurta üretmemiş ve larvalar pupaya geçememiştir. Buna karşılık, *T. wilkinsoni* ile beslenen *Calosoma* erginleri yumurta üretmiş ve bu yumurtalardan çıkan larvalar başarıyla ergin evreye ulaşmıştır. ÇKB ile beslenen larvaların %50'si, ev sineği larvalarıyla beslenenlerin ise %80'i 16. güne kadar yaşamıştır. Bulgular, ev sineği larvalarıyla beslenen *Calosoma* larvalarının ÇKB ile beslenenlere kıyasla daha küçük ve hafif olduğunu, ancak en az iki hafta ev sineği larvalarıyla beslendikten sonra larva döneminde doğaya salınmalarının ÇKB'ye karşı biyolojik mücadelede fayda sağlayabileceğini göstermektedir.

Anahtar Kelimeler: Alternatif av, *Calosoma sycophanta*, ev sineği, kitlesel üretim

1. Introduction

Insect damage holds a significant place among the destruction caused by insects, fungi, and other organisms in Turkish forests. Due to global climate change, which has led to increased populations and expanded distribution areas of forest pests, biological control methods have become a primary strategy in combatting these threats (Pureswaran et al., 2018). As a result, the proportion of mechanical, biological, and biotechnical control methods within overall pest management strategies in Türkiye increased from 81% in 2009 to 99.5% during the 2018-2022 period, by the Strategic Plans of the General Directorate of Forestry (GDF, 2016; 2023).

Thaumetopoea pityocampa (Denis & Schiffermüller, 1775) and *T. wilkinsoni* (Tams, 1926), both belonging to the family Thaumetopoeidae (Lepidoptera), are commonly referred to as pine processionary moths (PPMs). These moths feed on the needles of *Pinus brutia* Ten. (Pinales: Pinaceae), impeding tree growth, causing economic losses, and reducing the trees' resistance to other stressors. Furthermore, the larvae's setae can trigger allergic reactions in humans and other mammals, posing public health risks. These allergic reactions, characterized by red, itchy swellings on the skin, especially after direct contact with the larvae, can detrimentally impact the health of both animals and humans (Semiz et al., 2006; Bonamonte, 2013).

C. sycophanta (Linnaeus, 1758) (Coleoptera: Carabidae), a notable predator of lepidopteran pests, was first documented in England during the early 20th century (Burgess, 1911). Its effectiveness as a predator, in both adult and larval stages, has established its use as a biological control agent against various lepidopteran pests (Weseloh, 1988). Research has been conducted on its predatory effects on numerous species, including *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae) (Weseloh, 1985, 1990; Weseloh et al., 1995; Evans, 2009), *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Lymantriidae) (Evans, 2009), and the pine processionary moths *T. pityocampa* and *T. wilkinsoni* (Kanat and Özbolat, 2006).

Mass production of *C. sycophanta*, utilized as a predator, is conducted in laboratories under the General Directorate of Forestry in Türkiye to control pine processionary moth (PPM) populations. During this process, PPM larvae serve as the primary food source for both adult and larval stages of *C. sycophanta*. However, this practice has led to health concerns for the staff, as the setae of PPM larvae can cause allergic reactions in laboratory personnel, creating difficulties in staff recruitment for mass production tasks. To address this issue, identifying an alternative, non-allergenic food source for *C. sycophanta* during its production phase is crucial. Such a solution would safeguard employee health and enhance labor conditions, ensuring the sustainability of biological control efforts against PPMs.

This research aimed to explore the feasibility of using housefly (*Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae)) larvae as an alternative food source to pine processionary moth larvae in the mass production of *C. sycophanta*.

2. Material and Methods

2.1. Test insects

Adult *C. sycophanta* and *T. wilkinsoni* larvae were collected from Turkish red pine (*P. brutia*) trees in the forests of Isparta province, while the third instar larvae of *M. domestica* were cultivated in the laboratory. Specifically, 40 male and 20 female *C. sycophanta* adults were randomly collected from these pine trees from nests containing *T. wilkinsoni* larvae. The collected adults were transported to the laboratory within 6 hours using 5-liter plastic containers, ensuring they remained unharmed.

Subsequent trials with *C. sycophanta* as an alternative food source were carried out at the entomology laboratory of the Southwest Anatolia Forest Research Institute in Antalya, Türkiye. Here, *C. sycophanta* adults were maintained under laboratory conditions with moist soil at a temperature of 24 ± 2°C, relative humidity of 60 ± 10%, and a 16:8 h light: dark photoperiod (Kanat and Özbolat, 2006).

2.2. Adult feeding and egg-laying (oviposition) tests

To determine oviposition rates, adult *C. sycophanta* were divided into two groups, considering the male-to-female ratio. Following the recommendations of Serttas and Cetin (2013), who suggested that placing two or three males with each female would yield the maximum number of eggs, each container was stocked with two males and one female. The findings of that study guided the sex ratio applied in this experiment. For the control group, ten containers housed *C. sycophanta* adults that were exclusively fed 4th and 5th instar *T. wilkinsoni* larvae. Conversely, in the experimental group, another ten containers were set up where 3rd instar housefly larvae were provided as the food source.

Adult houseflies, intended for use in feeding tests, were captured at an animal shelter, their natural habitat, using a sweep net. They were then transported to the laboratory in tulle cages (20x20x20 cm) using an air-conditioned vehicle to ensure their well-being during transit. To sustain the flies, cotton soaked in water, along with milk and a 10% sugar water solution, was provided in the cages. Upon arrival at the laboratory, species identification of the collected flies was conducted using the keys provided by Buck et al. (2009) and other Diptera identification resources.

House flies were cultured in the laboratory within tulle cages under controlled conditions: a temperature of 24 ± 2°C, relative humidity of 60 ± 10%, and a 12:12 h light-dark photoperiod. Adequate nutrition (bran, milk, and sugar) and water were supplied to support individual development and maintain a healthy culture. The 3rd instar larvae produced under these conditions were then utilized as a food source.

Plastic containers measuring 14x20x8 cm were employed for the alternative food trials. To ensure proper ventilation, small holes were drilled into the container lids. Additionally, the soil placed within these containers was sterilized by heating it at 120°C for 2 hours. For the feeding experiments, the food quantities were standardized as follows: A minimum of three PPM larvae (4th and 5th instar) per container, or an equivalent weight of 1.1061 grams of housefly larvae per container, based on the average weight of one PPM larva being 0.3687 grams.

In the experiments, fresh housefly larvae were provided daily as food for the *C. sycophanta* adults and larvae (Figure 1).

Figure 1. *C. sycophanta* adults consuming housefly larvae (Photo: A. Serttaş)
Şekil 1. *C. sycophanta* erginlerinin ev sineğinin larvalarıyla beslenmesi

Both adult and larval *C. sycophanta* readily accepted and consumed the offered housefly larvae. Notably, the *C. sycophanta* individuals consumed almost all the provided PPM larvae. However, when fed with housefly larvae, they did not consume the entirety of the provided food, indicating that the provided amount was adequate and ensuring the beetles did not experience hunger. Uneaten food was removed the following day and replaced with fresh provisions. Egg laying was monitored, and egg counting commenced five days post-trial initiation, with data collection occurring every 3-5 days.

2.3. Feeding and development tests of larvae obtained from eggs

Eggs obtained from *C. sycophanta* adults, which were nourished with PPM larvae, were individually isolated and placed into rearing containers. Upon hatching, the first-instar larvae were exclusively fed either PPM larvae or housefly larvae. Initial measurements of weight and body length were taken prior to feeding. Subsequently, these measurements were repeated at 4- to 5-day intervals to calculate the average growth in weight and length.

In each production container, three *C. sycophanta* larvae were placed and assigned to one of two dietary groups: One group was fed PPM larvae, and the other was provided with third-instar housefly larvae. Each group consisted of 30 individuals, totaling 60 larvae under observation. The development of these larvae was meticulously monitored to ascertain whether they successfully reached the pupal stage and subsequently matured.

2.4. Data analysis

Statistical analysis was conducted on the collected data to assess parameters such as the mean weight gain, length increase of larvae, and their maturation rates during the feeding tests. The SPSS 17.0 software was employed for this analysis. The data were first subjected to logarithmic transformation to ensure normal distribution and homogeneity of variance. Subsequently, an ANOVA was performed to detect any significant differences in the data. Post-hoc comparisons were conducted using Duncan's Multiple Range Test (DMRT) with a significance level set at $p \leq 0.05$.

3. Results

3.1. Comparison of *C. sycophanta* adults feeding on pine processionary moth and housefly larvae

The experimental results revealed that the 10 female *C. sycophanta* adults, which were fed with PPM larvae, produced a total of 201 eggs, averaging 20 eggs per female. The egg-laying process for these *C. sycophanta* adults in the control group, fed with PPM larvae, spanned approximately 20 days. In contrast, no eggs were obtained from the female *C. sycophanta* adults that were fed with housefly larvae during the same period.

3.2. Comparison of *C. sycophanta* larvae feeding on PPM and housefly larvae

Measurements of larval development were concluded 16 days post-initiation, coinciding with the larvae's tendency to initiate pupation. The results indicated that the *C. sycophanta* larvae nourished with PPM larvae exhibited more significant increases in both weight and length compared to those fed housefly larvae. Specifically, the initial average weight of larvae consuming housefly larvae was 0.0243 grams, which increased to 0.3890 grams at the final measurement.

In contrast, larvae fed with PPM larvae started with an average weight of 0.0227 grams and reached 1.2019 grams, thereby gaining an average of 0.8119 grams more than their counterparts fed on housefly larvae. It was determined that first instar *C. sycophanta* larvae had experienced a 52.9-fold increase in weight when fed with PPM and a 16-fold increase when fed with houseflies after 16 days (Table 1). Similarly, length measurements demonstrated comparable initial sizes for both groups, yet larvae consuming PPM larvae achieved an average increase of approximately 1.5 cm more than those fed housefly larvae by the end of the observation period (Tables 1 and 2).

Table 1. Statistical comparison of larval body size growth by days and diet
Tablo1. Larva boy artışının günlere ve besin kaynaklarına göre istatistiksel karşılaştırması

Body size of *Calosoma* larvae (cm)					
	1st day	5th day	9th day	12th day	16th day
Larvae fed with pine processionary moth larvae	1.00 a^{x} A^{y} (n= 30) (SE= 0.01)	1.87 b A (n= 26) (SE= 0.02)	3.04 c A (n= 19) (SE= 0.02)	3.97 d A (n= 16) (SE= 0.03)	4.52 e A (n= 15) (SE= 0.02)
Larvae fed with housefly larvae	0.99 a A (n= 30) (SE= 0.02)	1.57 b B (n= 28) (SE= 0.02)	2.23 c B (n= 26) (SE= 0.02)	2.62 d B (n= 26) (SE= 0.02)	3.03 e B (n= 24) (SE= 0.02)

^{x}If the lowercase letters in a row are different, there is a statistical difference (Duncan p≤0.05). ^{y}If the capital letters in a column are different, there is statistical difference (Duncan p≤0.05).
n= The number of individuals measured. SE= The standard error.

Table 2. Statistical analysis of larval weight gain over time and across different diets
Tablo 2. Larva ağırlık artışının günlere ve besin kaynaklarına göre istatistiksel karşılaştırması

Weight of *Calosoma* larvae (gr)					
	1st day	5th day	9th day	12th day	16th day
Larvae fed with pine processionary moth larvae	0.022 a^{x} A^{y} (n= 30) (SE= 0.002)	0.127 b A (n= 26) (SE= 0.01)	0.412 c A (n= 19) (SE= 0.03)	0.911 d A (n= 16) (SE= 0.07)	1.20 e A (n= 15) (SE= 0.09)
Larvae fed with housefly larvae	0.024 a A (n= 30) (SE= 0.003)	0.090 b B (n= 28) (SE= 0.01)	0.208 c B (n= 26) (SE= 0.05)	0.297 d B (n= 26) (SE= 0.01)	0.389 e B (n= 24) (SE= 0.02)

During the alternative food trials, 60 *C. sycophanta* larvae were evenly split into two groups. In the group fed PPM larvae, 15 out of 30 larvae succumbed before pupating, while the remaining 15 successfully pupated, and 13 of these matured into adults. Among these successful adults, there were 7 females and 6 males, leading to a maturation rate of 86%. Conversely, in the group fed housefly larvae, 6 out of 30 larvae died, and although the remaining 24 attempted to pupate, none successfully matured. Subsequent observations revealed that these larvae failed to fully transition into the pupal stage and eventually perished inside their encasements (Figure 2). Thus, larvae nourished with housefly larvae did not complete their life cycle or reach adulthood.

Although both feeding groups began with 30 larvae each, only 15 larvae in the PPM-fed group and 24 in the housefly-fed group survived until day 16. A statistically significant difference in body size increase was noted between the two groups over the days, with the PPM-fed larvae consistently showing greater growth ($p \leq 0.05$). By day 16, compared to the initial measurements, the PPM-fed larvae's length had increased by 4.52 times, whereas the housefly-fed larvae's length had increased by only 3.06 times. Furthermore, the PPM-fed larvae were significantly larger than the housefly-fed larvae on all subsequent days after the start of the experiment ($p \leq 0.05$), with size differences ranging between 1.19 and 1.5 times larger (Table 1).

Figure 2. *C. sycophanta* larvae failed to pupate after being fed housefly larvae (Photo: A. Serttaş)
Şekil 2. Ev sineğinin larvalarıyla beslenen ve pupaya dönüşemeyen *C. sycophanta* larvaları

A statistically significant difference in weight gain was noted between the larvae fed with PPM larvae and those fed with housefly larvae over the course of the study, with both groups showing continuous weight increases ($p \leq 0.05$). By day 16, compared to their initial weights, larvae fed with PPM larvae had experienced a 54.5-fold increase, while those fed with housefly larvae saw their weights increase by 16.2-fold. Comparing the weight gains of larvae fed with the two distinct diets, it was found that those consuming PPM larvae were significantly heavier than their housefly-fed counterparts on all measurement days following the initiation of the experiment, except for the starting day ($p \leq 0.05$). Specifically, PPM-fed individuals were 1.41 to 3.0 times heavier than those fed housefly larvae (Table 2).

4. Discussion and Conclusion

Our research demonstrated that *C. sycophanta* adults and larvae readily accepted and consumed housefly larvae as an alternative food source, with observed mating behaviors in both male and female adults fed this diet. However, while adults fed with PPM larvae produced eggs, those fed with housefly larvae did not.

Larvae given PPM larvae successfully progressed to the pupal stage and matured into adults. In contrast, despite completing their initial larval stages and the dormant period, *C. sycophanta* larvae fed with housefly larvae failed to undergo complete metamorphosis. This could be attributed to insufficient nutritional content or an inability to accumulate necessary reserves for metamorphosis from the housefly larvae, which might lack certain critical components present in PPM larvae.

It was found that *C. sycophanta* larvae nourished with PPM larvae exhibited greater increases in both weight and length compared to those fed with housefly larvae. Specifically, larvae consuming PPM demonstrated an average weight gain of 0.8119 grams more than those fed on housefly larvae. Correspondingly, an increase in length was also noted, with PPM-fed larvae growing 1.5 cm longer than their counterparts on the housefly diet. Statistical analysis of the data confirmed significant differences in both length and weight gains between the two groups.

Various researchers have explored alternative nutrient sources, including different prey species (e.g., *Ephestia kuehniella* Zeller 1879, *Lymantria dispar* L. 1958, *Spodoptera littoralis* Boisdual 1883, *Thaumetopoea solitaria* Freyer 1838) and food types (e.g., liver, fresh grapes), for laboratory cultivation of *C. sycophanta*. However, successful development of *C. sycophanta* was only achieved with *L. dispar* and *T. solitaria* as food sources (Ceylan et al., 2012; Kanat and Özbolat, 2006; Weseloh, 1993). Challenges were noted with other food types: *E. kuehniella* larvae were too small and escaped from the feeding containers, while liver caused adherence issues and mortality among *C. sycophanta* adults, rendering these options unsuitable for mass production.

Both the larvae and the adult stage of *C. sycophanta* are excellent hunters, capable of consuming a variety of prey, particularly pine processionary caterpillars. In feeding experiments performed in a laboratory setting, it is hypothesized that larvae nourished with houseflies, showing over 2.5 times growth within 12 days, could fulfill their nutritional requirements by consuming diverse prey. This varied diet would support their successful transition to the pupal stage and subsequent maturation once released into the wild.

This study evaluated the potential of housefly (*M.*

domestica) larvae as an alternative food source for rearing *C. sycophanta*, a biological control agent. While both the larvae and adults of *C. sycophanta* readily accepted and consumed housefly larvae, significant differences were observed in reproductive and developmental outcomes when compared to the traditional prey, PPM larvae. Specifically, adults fed on PPM larvae successfully reproduced, whereas those fed on housefly larvae did not produce eggs. Similarly, larvae fed on PPM larvae progressed to pupation and matured into adults, whereas those fed on housefly larvae failed to complete their development.

These findings underscore the critical role of appropriate nutrition in the lifecycle of biological control agents and suggest that, while housefly larvae can serve as a food source for *C. sycophanta*, they do not support the complete lifecycle requirements of this predator. The implications for biological control practices include the need for further research to identify alternative non-allergenic food sources that fulfill the nutritional needs of *C. sycophanta*, ensuring effective mass production and deployment in pest management programs. These results also highlight the importance of considering nutritional content and developmental outcomes in selecting alternative prey for rearing entomophagous species in biological control settings.

The incorporation of houseflies into the mass rearing process of *C. sycophanta*, a key agent in the biological control of forest pests, alongside the pursuit of pioneering alternative food sources, plays a crucial role not only in augmenting the production process's efficiency but also in enhancing the health and safety of GDF employees. This strategy is instrumental in lowering the workforce's exposure to allergens present in the caterpillars conventionally utilized in breeding programs.

Investigating alternative nutritional sources, including houseflies and other viable substitutes, is a vital step in mitigating health hazards associated with the rearing environment. By embracing such innovative feeding practices, the sector can significantly advance in terms of operational safety and efficiency, underscoring the importance of continuous improvement in the cultivation of biological control agents. Our study is important in this respect for the biological control activities of Turkish foresters.

Acknowledgements

This article has been prepared based on the final report of the research project titled "Investigation of the Possibilities of Using *Musca domestica* L. (Housefly Larvae) as an Alternative Food in the Production of *Calosoma sycophanta* L." numbered '19.4703 / 2019-2020 ' supported by the General Directorate of Forestry, and conducted by theSouthwest Anatolia Forest Research Institute.

Authors' contributions

Main idea/Planning: A. Serttaş, Ü. Aslan Bıçkı, H. Çetin, Data collection and analysis: A. Serttaş, Ü. Aslan Bıçkı, H. Çetin, Literature review: A. Serttaş, Ü. Aslan Bıçkı, H. Çetin, H. İ. Yolcu, M. Uyan, Review and correction: A. Serttaş, Ü. Aslan Bıçkı, H. Çetin

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