Turkish Journal of Biological Control 2025, 16 (1): 1-7
Türk. Biyo. Mücadele Derg. 2025, 16 (1): 1-7
DOI: 10.31019/tbmd.1694482 ISSN 2146-0035-E-ISSN 2548-1002

Özgün makale (Original article)

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

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

Öz: Bu çalışmada, entomopatojen fungus *Lecanicillium psalliota*e'nin yeşil şeftali yaprakbiti *Myzus persicae*, (Sulzer) (Hemiptera: Aphididae) ve bakla yaprakbiti *Aphis fabae* Scopoli (Hemiptera: Aphididae) üzerindeki patojenik etkinliği laboratuvar koşullarında araştırılmıştır. Fungusun 10^6, 10^7, 10^8 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 10^8 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 10^6, 10^7, and 10^8 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 10^8 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.

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Received (Alınış): 07 Mayıs 2025 Accepted (Kabul ediliş): 04 Haziran 2025

Keywords: Aphid, Biological control, Entomopathogenic fungi, *Lecanicillium psalliota*e

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 ± 5% 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 10^6, 10^7 and 10^8 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±2%

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 $p \leq 0.05$.

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

*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 (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 <

0.001) and 10^7 (F= 52.465, df= 2, P < 0.001) conidia/ml, statisticallly 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 10^8 conidia/ml after a 9-day incubation period. Similarly, in *A. fabae* individuals, the most effective results were observed at the same spore concentration (10^8 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 ± S.E.).

Incubation Period (days)	Spore Concentrations (conidia/ml)			
	Control	10^6	10^7	10^8
3	2.50 ± 2.50b*B**	7.50 ± 4.33bB	7.50 ± 1.44bB	36.25 ± 1.25aC
6	6.25 ± 3.75bAB	23.75 ± 8.99bB	20.00 ± 2.04bB	87.50 ± 2.50aB
9	17.50 ± 4.79cA	72.50 ± 1.44bA	86.25 ± 5.54bA	100 ± 0.00aA

*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 (P<0.05).

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 10^8 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 10^7 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*

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 applcability 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. Addtionally, comprehensive assessments of its non-target effects on natural enemies and mammalian safety, are esential.

Acknowledgements

This study was supported by the TÜBİTAK BİDEB 2209-A University Students Research Projects Support Program. We thank TÜBİTAK for their contributions. We are grateful to Prof. Dr. Fikret Demirci (Ankara University, Turkey) for sharing the fungal cultures.

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