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RESEARCH ARTICLE

Diversity of entomopathogenic micromycetes associated with orchard pest species *Cydia pomonella* (Lepidoptera: Tortricidae) and *Caliroa cerasi* (Hymenoptera: Tenthredinidae)

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Abstract

This study reports the first isolation and characterization of entomopathogenic micromycetes from naturally deceased specimens of two key orchard pests in Uzbekistan: the codling moth *Cydia pomonella* (Linnaeus, 1758) and the cherry slug sawfly *Caliroa cerasi* (Linnaeus, 1758). Comparative analysis revealed distinct fungal communities associated with each species, with *C. cerasi* harboring 15 species of micromycetes and *C. pomonella* hosting 14 species. Dominant genera included *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Cladosporium*, *Metarhizium*, and *Mucor*. The widespread occurrence of these entomopathogenic fungi suggests their dual ecological role as both indicators of potential phytopathogens affecting fruit trees and as natural antagonists of pest insects. These findings provide a foundation for developing targeted biological control strategies against orchard pests while offering insights into plant-pathogen-insect interactions in agroecosystems. The results highlight the potential of native fungal isolates for sustainable pest management in Uzbek orchards.

Keywords
Entomopathogenic fungi, biological control, orchard pests, *Cydia pomonella*, *Caliroa cerasi*, Uzbekistan

Introduction

Insect pests pose significant threats to global agroecosystems due to their increasing resistance to chemical pesticides and their detrimental impacts on biodiversity (Bamisile et al. 2021). Conventional pest control methods, reliant on synthetic chemicals, raise environmental and health concerns, necessitating the development of sustainable alternatives. Among these, entomopathogenic fungi have emerged as promising biocontrol agents, capable of infecting and killing arthropod pests while minimizing ecological disruption (Chandler et al. 2011; Tiwari & Tripathi 2014).

Entomopathogenic fungi, such as *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Metarhizium* spp., naturally regulate insect populations by colonizing their cuticles, leading to disease and death (Świergiel et al. 2016; Wang & Wang 2017). These fungi also exhibit dual roles – suppressing plant pathogens and parasitic nematodes – enhancing their utility in integrated pest management (IPM) (Sharma et al. 2021). Advances in genomic studies have further elucidated their host adaptation mechanisms, enabling targeted biocontrol strategies (Wang & Wang 2017).

Recent innovations include myco-nanopesticides, such as silver nanoparticles derived from fungal metabolites, which demonstrate high efficacy against pests (Bihal et al. 2023). Additionally, microbial biopesticides based on *Bacillus thuringiensis* Berliner and *Metarhizium anisopliae* (Metschn.) Sorokīn are widely used in agriculture (Lacey et al. 2015; Kumar et al. 2019). Field studies highlight the success of fungal agents in reducing pest populations, such as *Cydia pomonella* (codling moth) and *Caliroa cerasi* (cherry slug sawfly), key pests in orchards (Gürlek et al. 2018; Mesquita et al. 2023).

In Uzbekistan, apple and cherry orchards face severe infestations from *C. pomonella* and *C. cerasi*, yet research on native entomopathogenic fungi remains limited. Preliminary studies identified *Aspergillus*, *Fusarium*, and *Alternaria* spp. in these pests (Akhmedova et al. 2024), suggesting untapped potential for biocontrol.

This study aims to isolate and characterize entomopathogenic fungi from naturally deceased *C. pomonella* and *C. cerasi* in Uzbek orchards, evaluate their diversity and prevalence, with a focus on genera like *Metarhizium* and *Beauveria*, and assess their potential as biocontrol agents to support sustainable pest management. By leveraging native fungal strains, this research contributes to eco-friendly pest control strategies, reducing reliance on synthetic pesticides while enhancing orchard productivity.

Materials and methods

Study design and sampling protocol

Modified entomological, microbiological, and mycological approaches were employed to investigate microbial associations in orchard-dwelling insect pests. The study focused on apple and cherry orchards in Uzbekistan, with sampling conducted during May–June in 2023 and 2024.

Nematode extraction and identification

Insect specimens were manually collected from tree trunks, branches, and foliage following a systematic inspection of the study area. Captured specimens were placed in sterile Petri dishes and transported to the Laboratory of Entomophage Ecology and Biomethod Theoretical Foundations at the Institute of Zoology, Academy of Sciences of the Republic of Uzbekistan, for further analysis. Target pest species included *Cydia pomonella* (Linnaeus, 1758) and *Caliroa cerasi* (Linnaeus, 1758).

Species identification was performed using taxonomic keys (Kuchurova & Maksakova 2003), while pest prevalence and distribution were assessed following established methodologies (Zhelokhovtsev 1988; Gilligan et al. 2018; Rakhmonova 2018).

Microbial isolation and cultivation

Surface sterilization and inoculation

Insect specimens were surface-sterilized in 70% ethanol (2–3 min), rinsed in 0.7% saline solution, and homogenized via agitation. Serial dilutions were prepared, and microbiological culturing was conducted in triplicate under aseptic conditions using a FH 1500x laminar flow hood.

Culture conditions and strain isolation

Aliquots were plated on solid media and incubated in a DX 210 precision incubator at 26 ± 0.5 °C for 5–6 days. Emerging fungal colonies were subcultured onto Czapek-Dox agar (CZA) and potato dextrose agar (PDA) for purification and morphological characterization.

Morphological and Taxonomic Identification

Fungal isolates were examined using a BS203 binocular microscope (400× magnification) equipped with a MICDC MOS 5mp USB camera. Taxonomic identification was based on macro- and micromorphological traits (e.g., hyphal structure, sporulation patterns, conidiophore morphology) following standard mycological references (Litvinov 1967; Pidoplichko & Milko 1971; Gorlenko 1976; Bilay 1982).

Results and discussion

From naturally infected cadavers of *Cydia pomonella* and *Caliroa cerasi* collected across Uzbek orchards, we isolated 38 fungal strains representing 6 families, 8 genera, and 15 species. The isolates included members of the Mucoromycetes class (order Mucorales, family Mucoraceae, genus *Mucor* Fresen., 1850), with the most numerous families being Mucoraceae, Aspergillaceae, Cladosporiaceae, Pleosporaceae, Nectriaceae, and Clavicipitaceae. The dominant genera isolated were *Aspergillus* P. Micheli, 1729, *Penicillium* Link, 1809, *Alternaria* Nees ex Wallroth, 1816, *Cladosporium* Link, 1816, *Fusarium* Link, 1809, and *Metarhizium* Sorokīn.

Comparative analysis revealed differences in fungal communities between the two insect hosts. From *C. cerasi* we isolated 11 fungal species, while *C. pomonella* harbored 12 species. Both insect species shared several common fungal species (Table 1). Two potentially entomopathogenic species were identified: *Fusarium solani* (Mart.) Sacc. and *Metarhizium anisopliae* (Metschn.) Sorokīn. The fungal isolates showed characteristic morphological features when cultured on different media, with distinct colony morphologies and microscopic structures that aided in their identification (Table 1). The distribution patterns of these fungi varied between the two insect hosts, suggesting potential host-specific associations in their natural mycobiota.

Table 1. Fungal community composition and characteristics from *Cydia pomonella* and *Caliroa cerasi* cuticles

No	Fungal Species	Host Prevalence (%)		Colony Morphology (PDA, 26 °C)	Microscopic Features
		C. pomonella	*C. cerasi*		
1	*Mucor racemosus* Fresen.	18.2	-	Fluffy white, rapid growth	Non-septate hyphae, globose sporangia
2	*Mucor mucedo* Fresen.	-	12.5	Gray-white, cottony	Columnella present, sympodial branching
3	*Penicillium chrysogenum* Thom	63.6	56.3	Blue-green, velvety	Biverticillate conidiophores
4	*Penicillium canescens* Sopp	27.3	-	Dull green, powdery	Rough-walled stipes
5	*Penicillium digitatum* (Pers.) Sacc.	45.5	37.5	Olive-green, radial furrows	Large phialides

No	Fungal Species	Host Prevalence (%)		Colony Morphology (PDA, 26 °C)	Microscopic Features
		C. pomonella	*C. cerasi*		
6	*Aspergillus fumigatus* Fresen.	-	31.3	Blue-green, suede-like	Columnar conidial heads
7	*Aspergillus niger* Tiegh.	72.7	68.8	Black, powdery	Radiate conidial heads
8	*Aspergillus terreus* Thom	-	25.0	Cinnamon-brown, wrinkled	Compact conidial columns
9	*Aspergillus flavus* Link	54.5	50.0	Yellow-green, granular	Biseriate sterigmata
10	*Cladosporium herbarum* (Pers.) Link	81.8	75.0	Olive-black, velvety	Shield-shaped conidia
11	*Alternaria alternata* (Fr.) Keissl.	36.4	43.8	Gray-black, woolly	Beaked muriform conidia
12	*Fusarium solani* (Mart.) Sacc.	63.6	56.3	White-pink, cottony	Sickle-shaped macroconidia
13	*Fusarium fujikuroi* Nirenberg	18.2	-	Purple-white, sparse	Microconidia in chains
14	*Fusarium oxysporum* Schltdl.	27.3	18.8	White-purple, dense	Foot-shaped basal cells
15	*Metarhizium anisopliae* (Metschn.) Sorokīn	9.1	-	White-green, becoming green	Cylindrical conidia in chains

Note. Host prevalence key: percentage of insect specimens (n=11 for *C. cerasi*, n=12 for *C. pomonella*) yielding each fungal species. Culture conditions: potato dextrose agar (PDA), 26 °C, 5–7 days incubation.

Conclusions

This study represents the first isolation and characterization of entomopathogenic micromycetes from naturally deceased specimens of *Cydia pomonella* and *Caliroa cerasi* collected in Uzbek orchard agroecosystems. Morphological and cultural analyses identified the following predominant fungal species: *Aspergillus* spp. (*A. niger*, *A. terreus*, *A. flavus*), *Penicillium* spp. (*P. chrysogenum*, *P. digitatum*), *Cladosporium herbarum*, *Alternaria alternata*, *Fusarium* spp. (*F. solani*, *F. oxysporum*), and *Metarhizium anisopliae*.

These findings highlight the potential of native fungal strains as biocontrol agents against orchard pests. The results provide a foundational framework for developing targeted biological control products to mitigate agricultural damage caused by these insect species. Further research should focus on evaluating the pathogenicity, host specificity, and field efficacy of these isolates.

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