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Pathogenicity trials reveal *Phaeoacremonium fraxinopennsylvanicum* being pathogenic on *Agaricus bisporus*

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Abstract

Phaeoacremonium (*Togniniaceae*) species are known to inhabit a wide range of substrates, including soil, woody hosts, arthropods, and humans. Recently, *Phaeoacremonium fraxinopennsylvanicum* has been isolated from the caps of *Flammulina velutipes* sensu lato in Iran; however, the pathologic relevance of the isolates on cap fungi remains unknown. The present study aimed to evaluate the pathogenicity of *P. fraxinopennsylvanicum* originating from wild-grown *F. velutipes* in the Arasbaran forest, as well as *P. minimum* originating from grapevine (*Vitis vinifera*) on *Agaricus bisporus* caps and excised grapevine shoots. The results of pathogenicity trials revealed that *P. fraxinopennsylvanicum* was pathogenic on detached *A. bisporus*, while no disease symptoms developed on grapevine shoots. *Phaeoacremonium minimum* was incapable of causing disease on *A. bisporus*. The data presented in the study reveal cap fungi as a possible new reservoir host for *Phaeoacremonium* spp.; therefore, extensive sampling of cap fungi is required to explore the fungicolous habit of *Phaeoacremonium* species further.

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Introduction

The genus *Phaeoacremonium* (Togniniales, *Togniniaceae*), with *Phaeoacremonium parasiticum* as the type, primarily comprises fungal species responsible for trunk diseases in woody host plants globally (Crous et al., 1996). *Phaeoacremonium* species occur on a wide spectrum of substrates such as soil, woody-hosts, arthropods, humans, etc. While most of the species in this genus are known as the causal agents of trunk diseases in different taxonomic groups of woody plants, especially on grapevine (causing Petri and esca diseases); other species occur as endophytes in vascular tissues of woody and non-woody plant species such as *Lactuca canadensis* and *Dactylis glomerata* (Spies et al., 2018; Sohrabi et al., 2020; Lim et al., 2025). A number of species have been reported as etiologic agents of phaeohyphomycosis in humans or associated with insect's larvae or other arthropods (Lim et al., 2025). Since the description of the genus, the number of species has increased to 75 (www.indexfungorum.org; accessed on 9 June 2025). Even though *Phaeoacremonium* species have been reported from diverse ranges of substrates, there is no data available on fungicolous habit among the members of this genus. The reports are limited to the occurrence of *P. inflatipes* on perithecia of *Hypoxylon truncatum* in the United States (Mostert et al., 2005); with unclear interaction/relation with *H. truncatum*. The present study was aimed to evaluate pathogenicity of *P. fraxinopennsylvanicum* isolates recovered from hymenia of *Flammulina velutipes* in Arasbaran forests (Torbati & Arzanlou, 2020), on *Agaricus bisporus* in sliced cap and on *Vitis vinifera* using excised shoot method.

Material and Methods

Fungal isolates

Phaeoacremonium fraxinopennsylvanicum (originated from *F. velutipes*; GenBank Acc. No. MT252927.1) and *Phaeoacremonium minimum* (originated from *V. vinifera*; GenBank Acc. No. KF179085.1) were obtained from CCTU (Culture collection of Tabriz University) housed at Mycology lab, Plant protection department.

Pathogenicity assay on *Agaricus bisporus*

Pathogenicity assay was performed following the protocol of Geo et al. (2016). Fresh and healthy *A. bisporus* mushroom (Sabalan mushroom for Ardabil Province) were purchased from hypermarket and surface-disinfected in 70% ethanol for 30s, rinsed three times in sterile distilled water and then dried on sterile filter papers under a laminar flow hood. For inoculation of *A. bisporus* with *P. fraxinopennsylvanicum* and *P. minimum*, 300 µl of conidial suspension (1×10^7 conidia ml^{-1}; containing Tween 20 (0.1% v v^{-1}) of each isolate was used to inoculate a total number of six *A. bisporus* caps (two caps in each container). Untreated control mushrooms were sprayed with sterile distilled water containing 0.1% Tween 20. The inoculated caps were kept at room conditions. Two weeks after inoculation, the shape and color of spots on the caps were used as an indicator of pathogenicity.

Pathogenicity assay on excised shoots of grapevine

For the inoculation of grapevine twigs with *P. fraxinopennsylvanicum* and *P. minimum*, 20 cm lengths of two- years- old twigs were cut from a healthy grapevine (Keshmeshi cultivar). The twigs were surface sterilized with 70% ethanol for 30s after removing of leaves and rinsing three times in sterile distilled water and then dried on sterile filter papers under a laminar flow hood. For inoculation, a wound (5 mm, reaching into the xylem) created by cutting to the pith with a sterile 5 mm diameter metal cork borer 10 cm above the first internode. A mycelial plug (5 mm diameter) obtained from the margin of the seven-day-old fungal colonies were placed in the wound with the mycelium facing the twig, and the wounds were wrapped with Parafilm. Control plants were inoculated with sterile PDA plugs. Pathogenicity tests were performed using three replicates for each fungal species. The results of the inoculation were recorded after one month. To check if the necrotic lesions appeared in the inoculation site or not the bark from the twigs was removed. As an indicator of pathogenicity, the length of necroses was measured. To observe of symptoms, inoculated twigs were incubated in lab condition at 25˚C under daylight condition. Following Koch's postulates, infected parts of inoculated mushrooms.

Results

Inoculation of *A. bisporus* with *P. fraxinopennsylvanicum* showed that this species is pathogenic on the inoculated mushroom. Tan spots with water-soaked looking lesions appeared at first on the mushrooms. Lesions diameters increased with the disease progress. These lesions were visible and completed within seven days after inoculation (Fig. 1). *Phaeoacremonium fraxinopennsylvanicum* was isolated from the inoculated mushrooms but the controls did not develop any disease symptoms. Inoculated mushrooms with *P. minimum* and grapevine twigs with *P. fraxinopennsylvanicum* did not show any disease symptoms after one week and one month, respectively (Fig. 2).

Discussion

Phaeoacremonium species are known to occur on a wide spectrum of substrates; although, cap fungi are not known as the natural host for the members of this genus. In this study, *P. fraxinopennsylvanicum* isolates were recovered from the hymenium of wild grown *F. velutipes* in Arasbaran forest (Torbati & Arzanlou, 2020). Most of the fungicolous fungi are reported to grow on surface of the fruiting bodies of other fungi instead of inner parts of them (Sun et al., 2019). However, in some fungal species, mycelia of different fungicolous fungi grow inside perennial carpophores (Gams et al., 2004). We did not record any disease symptoms on *F. velutipes*; which could be due to initial stages of pathogenicity on the host. *Phaeoacremonium fraxinopennsylvanicum* has been reported on *Actinidia chinensis* in Italy, *Juglans regia*, *Mespilus germanica*, *Parrotia persica* and *Syzygium cumini* in Iran (Kazemzadeh Chakusary et al., 2017; Panahandeh et al., 2019), *Fraxinus pennsylvanica*, *F. latifolia*, *Q. agrifolia* and *V. vinifera* from United States, *Pyrus communis*, *Malus domestica*, *Prunus salicina* from South Africa and *F. excelsior* from Sweden (Gramaje et al., 2015). Perithecia and conidia of *Phaeoacremonium* could co-occure in nature, and under condition, conidia may form on mycelium and near perithecia. It is reported that the sexual form of *P. fraxinopennsylvanicum* is formed on ash trees (*F. latifolia*) and *V. vinifera* in California. It shows that the trees near each other can transfer the pathogens to the neighboring trees. Therefore woody plants as hosts can be considered as sources of infective

inoculum of pathogens. In addition, insects also have an important role in pathogen spread. For example three *Phaeoacremonium* species, *P. scolyti*, *P. parasiticum* and *P. mortoniae* are reported from insect larvae as well as larval galleries inside tree bark (Mostert et al., 2006).

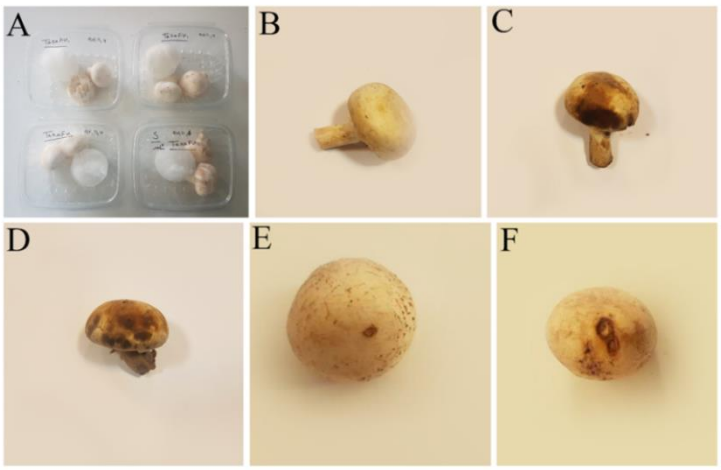

Fig. 1. Pathogenicity tests of *Phaeoacremonium fraxinopennsylvanicum* isolated from *Flammulina velutipes* on *Agaricus bisporus*, seven days after inoculation. (A): Inoculated and control treatments *in vitro* conditions, (B): Control, (uninoculated), (C-F): Tan spots with water-soaked looking lesions developed on inoculated caps.

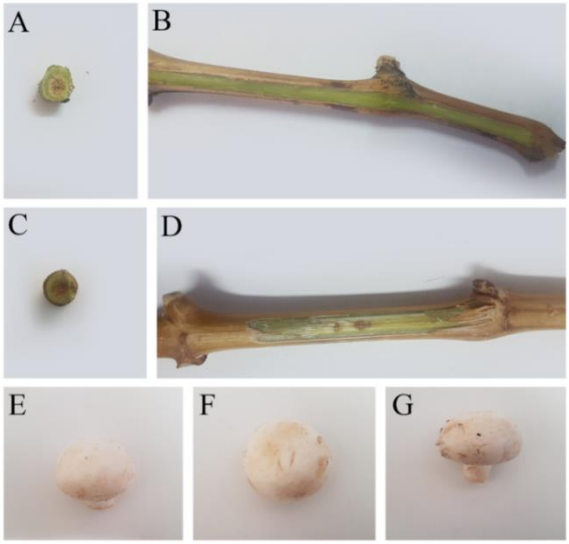

Fig. 2. Pathogenicity tests of *Phaeoacremonium fraxinopennsylvanicum* and *Phaeoacremonium minimum* on *Vitis vinifera* (A-D) and *Agaricus bisporus* (E-G): (C and D): Symptomless inoculated twigs of *V. vinifera* with *Phaeoacremonium fraxinopennsylvanicum*, (F-G): Symptomless inoculated *A. bisporus* with *Phaeoacremonium minimum* (A, B and E): Controls.

In the present study, *P. fraxinopennsylvanicum* was characterized from *F. velutipes.* Our study proves the pathogenicity of *P. fraxinopennsylvanicum* on *A. bisporus* but not on the *V. vinifera*. Aroca and Raposo (2009) reported that *P. fraxinopennsylvanicum* reduces root weight instead of vascular discoloration after five months and they showed that vascular discoloration symptom do not occur with all *Phaeoacremonium* species. The assay length may have influence on the symptoms occurrence (Halleen et al., 2007). There is no data available on species diversity and role of *Phaeoacremonium* spp. in dieback and decline of forest trees in Arasbaran forests. However, typical wood discoloration resembling *Phaeoacremonium* spp. have been observed on *Quercus* and *Carpinus* species in Arasbaran forests (Arzanlou unpublished data). *Phaeoacremonium fraxinopennsylvanicum* has been reported on *Alnus glutinosa*, *J. regia*, *M. domestica*, *M. germanica*, *P. persica*, *S.cumini*, and *V. vinifera* in Iran (Gramaje et al., 2015; Kazemzadeh Chakusary et al.,

2017; Spies et al., 2018; Panahandeh et al., 2019). Also recently, Mehrabioun et al., 2024 (unpublished) have reported this species from the galleries of bark beetles in the Marmisho region of West Azerbaijan of Iran. But this study reports the *P. fraxinopennsylvanicum* on *F. velutipes* for the first time from Iran. Before, *P. minimum*, *P. viticola* and *P. iranianum* were reported on *Vitis sylvestris* from Arasbaran forests (Ershad, 2022). Due to the diversity and density of woody plants in the Arasbaran forests, it is not far from the mind that different hosts for a pathogen may exist and the pathogen has adapted over time on their hosts. Also, the importance of *Phaeoacremonium* species as fungicolous fungi is demonstrated but it is not clear whether our isolates exerted any deleterious effect on their fungal hosts rather than acting as simple colonizers. *Phaeoacremonium* species are known from many woody hosts in Iran however, the occurrence of these species on mushrooms remains to be investigated. Although several fungal species have been isolated from mushrooms, little is known about the etiology of these fungicolous fungi of mushrooms in Iran.

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Conflict of interest

The authors declare that there are no conflicts of interest present.

CRediT author statement

M. Arzanlou: Supervision, Writing, Funding acquisition. **H. Hatef:** Laboratory work, technical assistance. **Z. Kalantri:** Data curation, Formal analysis, Visualization. **M. Torbati:** Field investigation, data collection.

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