


Armillaria root rot on some plant species in Pasargad County, Fars Provinces (Iran)

Hamid Mohammadi , Hadi Panahi , Mahboobeh Sohrabi 

Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

✉ Corresponding authors: hmohammadi@uk.ac.ir

Article Info.	Abstract
<p>Article type: Original article</p> <p>Article history: Received 7 Jun. 2025 Received in revised form 22 Jun. 2025 Accepted 23 Jun. 2025 Available Online 23 Jun. 2025</p> <p>Keywords: Basidiomycota, Crown and root rot, Molecular studies, <i>Tef-1α</i>.</p>	<p><i>Armillaria</i> species (Basidiomycota, Agaricales, Physalacriaceae) cause root and crown rot diseases on a wide range of plant species including fruit, forest, and ornamental trees throughout the world. During the fall and spring of 2023 and 2024, signs and disease symptoms similar to those of <i>Armillaria</i> root and crown rot were observed on Atlantic pistachio (<i>Pistacia atlantica</i>), tree of heaven (<i>Ailanthus altissima</i>), ash (<i>Fraxinus excelsior</i>), oriental plane (<i>Platanus orientalis</i>), elm (<i>Ulmus minor</i> and <i>Ulmus</i> sp.) as well as Iranian rose (<i>Rosa damascena</i>) in Pasargad County, Fars Province, Iran. This study aimed to isolate and identify suspected basidiomycetes associated with the decline of these plants. Root and crown samples from the affected trees as well as the basidiocarps were collected. Rotted tissues were surface sterilized with 0.5% sodium hypochlorite, rinsed with sterile water, and plated on potato dextrose agar (PDA). Monosporic cultures were also obtained from each collected basidiocarp. Cultural identifications of 10 representative isolates were confirmed by sequence analysis of a partial sequence of the translation elongation factor 1-alpha (<i>tef-1α</i>) gene using EF595F and EF1160R primers. According to the results, all fungal isolates were identified as <i>Armillaria mellea</i>. Literature review indicates this is the first report of <i>A. mellea</i> on <i>P. atlantica</i>, <i>A. altissima</i>, and <i>R. damascene</i> and first record of this fungus on <i>P. orientalis</i>, <i>F. excelsior</i> and <i>Ulmus</i> spp. in Fars Province.</p>
<p>Cite this article: Mohammadi, H., Panahi, H., & Sohrani, M. (2025). <i>Armillaria</i> root rot on some plant species in Pasargad County, Fars Provinces (Iran). <i>Journal of Advances in Plant Protection</i>, 2(1), 57–70.</p>	
<div>  <p>© The Author(s).</p> <p>Publisher: Shahid Bahonar University of Kerman</p> </div>	

Introduction

The genus *Armillaria* as a known cosmopolitan member in phylum Basidiomycota (family Physalacriaceae) includes over 50 described species (Coetzee et al., 2011; Heinzelmann et al., 2019; Kim et al., 2022). The species of the genus are considered saprophytes (Heinzelmann et al. 2019), symbionts (Kikuchi & Yamaji, 2010; Guo et al., 2016) or pathogens on different plant species (Dai et al., 2007). Most of the *Armillaria* species cause crown and root rot diseases on a wide range of tree species worldwide (Hood, 1991; Coetzee et al., 2011). The abundance and importance of *Armillaria* species may vary depending on the regions. In Asia, two species, *A. ostoyae*, and *A. mellea*, have been reported as important tree pathogens. In the USA, *A. mellea* has been reported a prominent fungal pathogen of broad-leaved and other woody trees while in Australia, *A.*

luteobubalina has been considered as a dominant *Armillaria* species on natural and planted eucalypt forests (Shearer et al., 1997). In Europe, two *Armillaria* species, *A. ostoyae* and *A. mellea* are considered the most important *Armillaria* root disease pathogens of coniferous forests and fruit-tree plantations, respectively (Guillaumin et al., 1991).

In recent years, molecular studies have played an important role in identifying and distinguishing *Armillaria* species and various protein-coding genes have been used to distinguish and phylogeny species of this genus (Hasegawa et al., 2010; Guo et al., 2016; Koch al., 2017). Based on available references, the use of translation elongation factor 1-alpha (*tef1-a*) gene has shown more efficiency than the other loci such as actin (*act*), glyceraldehyde 3-phosphatedehydrogenase (*gpd*), RNA polymerase subunitII gene (*rpb2*), and beta-tubulin (*tub2*) genes (Maphosa et al., 2006;

Baumgartner *et al.*, 2011; Ross-Davis *et al.*, 2012; Tsykun *et al.*, 2013; Klopfenstein *et al.*, 2017). Many *Armillaria* species cause Armillaria root and crown rot (ARCR) in a wide range of plant species, nevertheless, *A. mellea* is remarkable for its wide host range and greatest distribution. This species as oak root fungus, honey fungus, mushroom root rot, and shoestring fungus can infect various plant species including deciduous and coniferous trees as well as herbaceous plants at any age of growth (Mańka, 1953; Coetzee *et al.*, 2000; Morrison *et al.*, 2000; Robinson & Fox, 2002; Cleary *et al.*, 2008; Baumgartner, 2004; Baumgartner *et al.*, 2011; Denman *et al.*, 2016; Ford *et al.*, 2017). This fungus as a white-rot basidiomycetous fungal species is capable to degrade cellulose and lignin in wooden tissues of hosts (Kile *et al.*, 1991; Guillaumin & Legrand, 2013). ARCR is currently the serious threat for horticulture and forestry industries in various countries and causes extensive economical losses (Hood *et al.*, 1991; Kile *et al.*, 1991; Pegler, 2000; Cox *et al.*, 2006). Armillaria root disease was first reported on *Castanea crenata* in Japan (Nomura, 1903). In Iran, root and crown rot disease caused by *Armillaria* species was first reported on apple trees (Saber, 1974) and currently is widely distributed in the country (Hood *et al.*, 1991). The fungus *A. mellea* (as *A. mellea sensu lato*) has been reported from a diverse fruit, ornamental and forest tree species in Iran (Ershad, 1995; Asef *et al.*, 2003; Dalili *et al.*, 2008, 2010). Based on literature reviews, at least four *Armillaria* species, *A. ellea*, *A. cepistipes*, *A. gallica* and *A. borealis* have been reported from forest and fruit trees in Iran (Asef *et al.*, 2003). This study aimed to isolate and identify suspected basidiomycetes associated with the decline of some plant species in Pasargad County.

Materials and Methods

Sites, symptoms and sampling

During the fall and spring of 2023 and 2024, signs and disease symptoms similar to those of Armillaria root and crown rot were observed on Atlantic pistachio (*Pistacia atlantica*), tree of heaven (*Ailanthus altissima*), ash (*Fraxinus excelsior*), oriental plane (*Platanus orientalis*), elm (*Ulmus minor* and *Ulmus* sp.) trees and Iranian rose (*Rosa damascena*) in Pasargad County (Latitude: 30° 04' 18.98" N Longitude: 53° 03' 7.99" E), Fars Province. Affected trees showed leaf yellowing, growth reduction, dwarfing of foliage, and branch dieback as external disease symptoms. Some trees showed extensive wood rot symptoms and fruiting bodies (basidiocarps) of a basidiomycetous fungus were

also commonly observed on decayed stumps and roots. Based on disease symptoms, samples were collected from fruiting bodies, as well as from root and crown tissues of plants showing decay symptom. Plant and fungal samples were transferred to the laboratory for more studies and fungal isolations.

Fungal isolation and morphological studies

The infected wood tissues showing wood decay symptoms and collected basidiocarps were cut into 5–7 mm segments. All segments were surface-disinfected with 1.5% sodium hypochlorite solution for 1.5 min, rinsed two times with sterile distilled water (SDW), dried on a sterile filter paper and then plated on potato dextrose agar (PDA, Merck, Germany). Cultures were incubated at 25°C for 7 to 10 days and colonies grown from the segments were transferred to fresh PDA plates. Each colony was purified based on the hyphal tip method. A suspension of basidiospores was also prepared from the collected basidiocarps, spread on the surface of PDA with a sterile glass rod and incubated at 25°C for three to six days. Germinated basidiospores were transferred to fresh PDA plates. In some cases, mycelial fans were also observed and collected. All pure cultures obtained from the infected tissues, basidiocarps and mycelial fans were preserved on filter paper and maintained at -17 to -20 °C in the personal culture collection of the Department of Plant Protection at the Shahid Bahonar University of Kerman, Kerman.

Molecular studies and phylogenetic analyses

Ten representative isolates were selected for molecular identification: One isolate from each of *P. atlantica*, *F. excelsior*, *A. altissimam*, *R. damascene* and *Ulmus* species, two isolates from *U. minor* and three isolates from *P. orientalis*. Isolates were grown on PDA and incubated on 25 °C until sufficient mycelial growth was obtained. Total genomic DNA was extracted from mycelium of the isolates using CTAB method (Doyle & Doyle 1990). All DNA samples were incubated at -15 to -17 °C until use for PCR amplification. Two oligonucleotide primers EF595F and EF1160R (Kausrud & Schumacher, 2001) were used to amplify a part of the translation elongation factor 1-alpha (*tef* 1- α) gene. All DNA samples and PCR amplicons were visualized under UV light on a 1.0 % agarose gel stained with ethidium bromide and a 100-bp ladder (GeneRuler 100 bp DNA Ladder, Thermo Scientific, Vilnius, Lithuania) was used to evaluate the bands. PCR reaction mixtures were set up in a 25µl with 1 × PCR buffer, 200 µM of each dNTP, 1.5 mM MgCl₂, 1.25

unit of DNA Taq polymerase (Cinnagen, Tehran, Iran) and 1 µl DNA of template from each isolate and 0.5 µM of each primer of each used primer. This mixture was adjusted to final volume with water [Chromasolv Plus (Sigma-Aldrich, Steinheim, Germany)]. PCR amplifications were performed on a Techne TC-312 Thermal Cycler (Techne, Cambridge, UK) with an initial denaturation step at 94°C for 5 min and 40 cycles consisting of denaturation at 94°C for 30 s, primer annealing for 30 s at 52°C, and extension for 50 s at 72°C. A final extension step for five min at 72°C was also used to complete the reaction. The PCR products were purified and directly sequenced by Bioneer Corporation (Daejeon, South Korea). Preliminary molecular identification of the selected isolates was carried out by using the BLASTn search tool and comparing the *tefl-α* gene sequences of the Iranian isolates with the *tefl-α* gene sequences of *Armillaria* species deposited in GenBank at NCBI (the National Center for Biotechnology Information, [http:// www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov), accessed on 20 May 2025). A phylogenetic analysis was performed for 10 *Armillaria* isolates recovered from related plant hosts. For this group of the isolates, individual loci sequences obtained in this study and those references retrieved from Genbank (Table 1) were aligned using default settings of Clustal W algorithm (Thompson et al., 1994) included within MEGA X software package (Kumar et al., 2018). The alignments were manually checked and improved. Phylogenetic analyses were based on Maximum Parsimony (MP). Maximum-parsimony analysis was performed in MEGA X (Kumar et al., 2018) with the Tree-Bisection-Reconnection (TBR) algorithm, where gaps were treated as missing data. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC). The robustness of the topology was evaluated by 1000 bootstrap replications (Felsenstein, 1985). Trees were visualized using MEGA X. All sequences were deposited in GenBank (Table 1).

Table1. Sequences used in the phylogenetic analyses; Iranian isolates are indicated in bold face.

Species	Isolate code	GenBank no. (<i>tefl-α</i>)	Country	Host	Reference
<i>Armillaria</i> (<i>A.</i>) <i>mellea</i>	HKAS85599	KT822342	China	<i>Gastrodia elata</i>	Guo et al., 2016
	HKAS86591_G_01010_1	KT822343	China	-	Guo et al., 2016
	HKAS86593_G_04054_14	KT822345	China	-	Guo et al., 2016
	IRNHM-ARM1	PV699544	Iran	<i>Pistacia atlantica</i> (root)	This study
	IRNHM-ARM18	PV699545	Iran	<i>Fraxinus excelsior</i> (root)	This study
	IRNHM-ARM22	PV699546	Iran	<i>Ailanthus altissimam</i> (root)	This study
	IRNHM-ARM4	PV699547	Iran	<i>Platanus orientalis</i> (basidiocarp)	This study
	IRNHM-ARM20	PV699548	Iran	<i>Platanus orientalis</i> (stump)	This study
	IRNHM-ARM6	PV699549	Iran	<i>Platanus orientalis</i> (root)	This study
	IRNHM-ARM27	PV699550	Iran	<i>Ulmus minor</i> (root)	This study
	IRNHM-ARM8	PV699551	Iran	<i>Rosa damascene</i> (root)	This study
	IRNHM-ARM9	PV699552	Iran	<i>U. minor</i> (basidiocarp)	This study
	IRNHM-ARM11	PV699553	Iran	<i>Ulmus</i> sp. (basidiocarp)	This study
<i>A. mexicana</i>	MEX 87	KR061314	Mexico	<i>Prunus persica</i>	Elías-Román al. 2018
	MEX 85	KR061313	Mexico	<i>Prunus persica</i>	Elías-Román al. 2018
<i>A. aotearoa</i>	NZFRIM 5283	KU295542	Canada	<i>Nothofagus</i> sp.	Hood & Ramsfield 2016
<i>A. pallidula</i>	3626	FJ618665	Australia	-	Elías-Román et al., 2018
	CMW 4971	DQ435647	Australia	-	Maphosa et al. 2006
<i>A. hinnulea</i>	CMW 4980	DQ435648	Australia	-	Maphosa et al. 2006
<i>A. limonea</i>	CMW 4991	DQ435656	New Zealand	-	Maphosa et al. 2006
	CMW 4680	DQ435655	New Zealand	-	Maphosa et al. 2006
<i>A. luteobubalina</i>	CMW 4977	DQ435657	Australia	-	Maphosa et al. 2006
<i>A. puiggarii</i>	MCA 3111	KU289104	Guyana	-	Koch et al., 2017
<i>A. novae-zelandiae</i>	CMW 5448	DQ435653	Australia	-	Maphosa et al. 2006
	CMW 4722	DQ435652	New Zealand	-	Maphosa et al. 2006
<i>A. borealis</i>	99025	KM878688	Finland	-	Maphosa et al. 2006
<i>A. altimontana</i>	POR100	JN944606	USA	-	Brazee et al., 2012
	D82	JN944611	USA	-	Brazee et al., 2012
<i>A. calvescens</i>	ST 18	JF895837	USA	-	Brazee et al., 2011
	ST 17	JF895836	USA	-	Brazee et al., 2011
<i>A. gallica</i>	ST22	JF313126	USA	-	Ross-Davis et al., 2012
<i>A. nabsnona</i>	C21	JF313119	USA	-	Ross-Davis et al., 2012
<i>A. algida</i>	Dai26847_2	PP443423	China	-	Qin et al., 2024
<i>A. cepistipes</i>	S20	JF313116	Canada	-	Ross-Davis et al., 2012
	HKAS 86586	KT822416	China	-	Guo et al., 2016
<i>Armillaria gemina</i>	ST11A	PP481738	USA	-	Ross-Davis et al., 2012
	ST8	JF313136	USA	-	Ross-Davis et al., 2012
<i>Oudemansiella cubensis</i>	MCA 5434	KU289105	Guyana	-	Koch et al., 2017

Results

Disease symptoms

In the current study, signs and disease symptoms similar to *Armillaria* root and crown rot were observed on seven plant species including *P. atlantica*, *A. altissima*, *F. excelsior*, *P. orientalis*, *Ulmus minor*, *Ulmus* sp. and *R. damascena* in Pasargad County. Above-ground or external disease symptoms included leaf yellowing, growth reduction, branch dieback and defoliation. Some affected species such as *P. atlantica*, *A. altissima*, *P. orientalis* and *R. damascena* showed sever dieback, root

and crown rot and extensive wood decay symptoms. *Ulmus* spp., *F. excelsior* and *P. orientalis* trees that were damaged by this fungus often were died (Fig. 1). About 20 to 30 percent of these trees had also been cut down just above the soil surface or their infected stumps and roots had been almost completely removed from the soil. Basidiocarps of an *Armillaria* like fungus were also commonly observed on the decayed stumps and roots of *Ulmus* spp. and *P. orientalis*. White mycelial fans were observed underneath the bark of the affected stumps of *P. orientalis*, *Ulmus* sp. and *U. minor* (Fig. 2).



Fig. 1. Disease symptoms associated with *Armillaria mellea* on plants in Pasargad County, Fars Province in Iran. A: Tree death of *Ulmus minor* and *Platanus orientalis*. B and C: Typical dieback symptoms on *P. orientalis* (B) and *Fraxinus excelsior* (C); D and E: Tree death of *U. minor* (D) and *P. orientalis* (E).

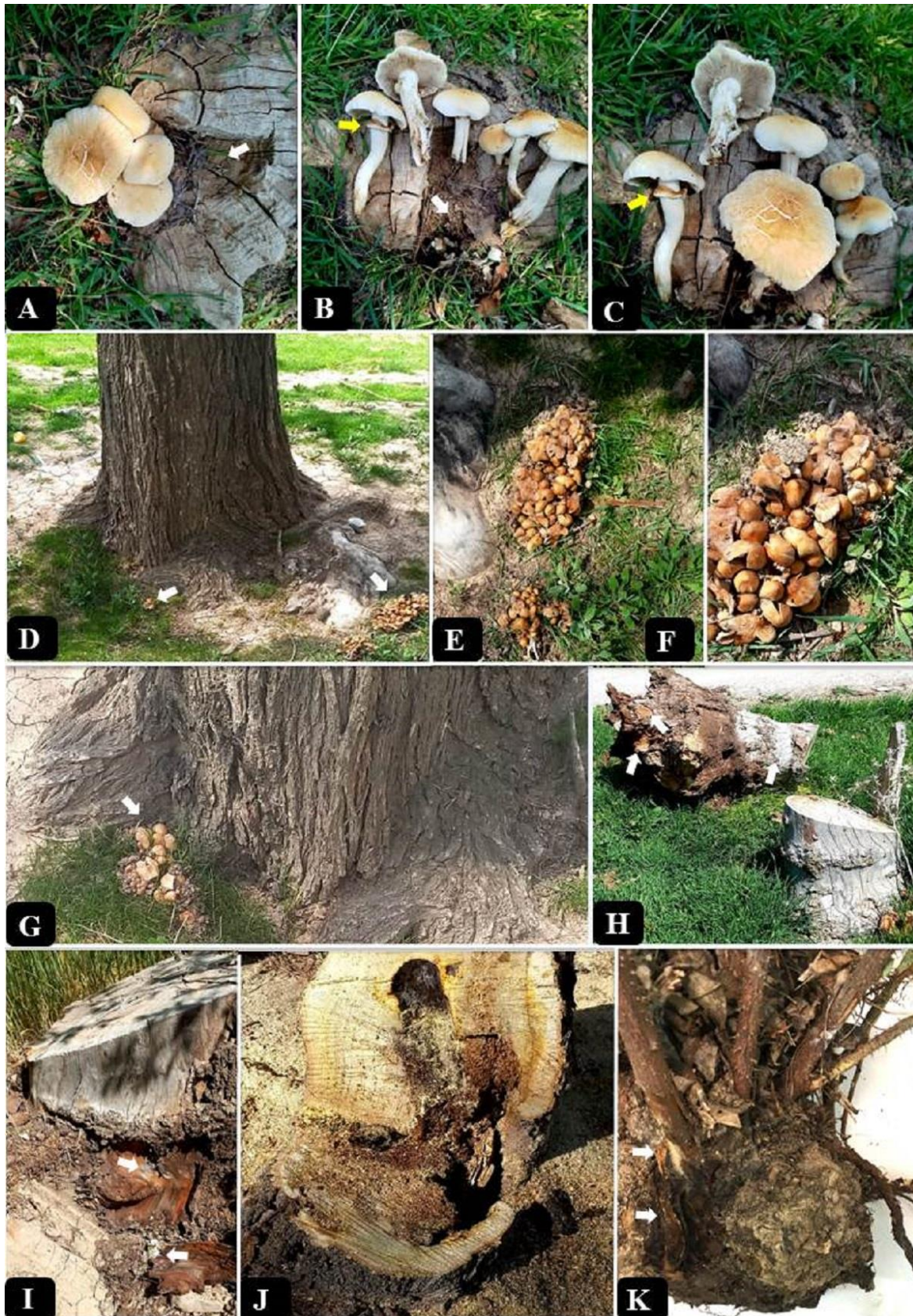


Fig. 2. Common signs and disease symptoms associated with *Armillaria mellea* on trees in Pasargad County, Fars Province in Iran. A-C: Clusters of yellowish brown basidiocarps of *A. mellea* on *Platanus orientalis* (A) and *Ulmus minor* (B and C), rings near the caps and the decayed areas of the wood tissues are indicated by yellow and white arrows, respectively; D-G: Clusters of basidiocarps around the base of *U. minor* trunk, H and I: Wood decay and white mycelial fans of *A. mellea* formed between the bark and wood of *P. orientalis* stumps, J: Typical wood decay symptoms with zone lines on *Ailanthus altissima* stump, K: Root and crown rot symptoms on *Rosa damascene*.

Sampling and fungal isolation

In this study, 44 samples were collected from seven plant species, *P. atlantica*, *A. altissima*, *F. excelsior*, *P. orientalis*, *U. minor*, *Ulmus* sp. and *R. damascene*. Of these 16 samples (36.4%) were related to Basidiocarps (10 samples from *P. orientalis*, four samples from *U. minor* and two sample from *Ulmus* sp.), two samples (4.5%) were collected from mycelial fans formed on *P. orientalis* and the remaining samples (26 samples: 59.1%) were collected from root and crown of affected trees including *P. atlantica* (one sample) *A. altissima* (two samples), *F. excelsior* (three samples), *P. orientalis* (six samples), *U. minor* (six samples), *Ulmus* sp. (five samples) and *R. damascene* (three samples). The highest and lowest percent of samples were collected from *P. orientalis* (18 samples: 40.9%) and *P. atlantica* (one sample: 2.3%), respectively.

Fungal isolation and morphological identification

Totally, 46 fungal isolates were obtained in this research. Of these 16 isolates were recovered from the collected Basidiocarps (12 isolates from *P. orientalis* and two isolates from *U. minor* and *Ulmus* sp.), two isolates were obtained from mycelial fans formed on *P. orientalis* and 28 isolates were also isolated from root and crown tissues showing wood decay symptoms. The later isolates were obtained from *P. atlantica* (one isolates), *A. altissima* (two isolates), *F. excelsior* (three isolates), *P. orientalis* (11 isolates), *U. minor* (seven isolates), *Ulmus* sp. (two isolates) and *R. damascene* (three isolates). Therefore, the highest and lowest percent of the isolated colonies were recovered from *P. orientalis* (25 isolate: 54.3%) and *P. atlantica* (one isolate: 2.2%), respectively.

In the current study, clusters of a yellow mushroom were observed at the base of *P. orientalis*, *U. minor* and *Ulmus* sp. trunks and stumps. They had honey-yellow to tan-brown caps (with a darker area near the centre (Fig. 2 A-G), light-colored gills, and a ring near the cap base (Fig. 2 B and C) and ovoid and hyaline basidiospores (mean=7-9 × 3-5 µm). The colonies were pale whitish to yellowish-brown, without spore structures, and clamp connections.

Molecular studies and phylogenetic analyses

To confirm the morphological identification of the isolates, BLASTn searches in GenBank showed that *tef-1a* sequences of the Iranian isolates had 99–100% identity with those of *A. mellea* isolates deposited in GenBank (accession numbers: KT822342, KT822343 and KT822345). The *tef-1a* sequences were obtained for 10 representative Iranian isolates and aligned with 27 reference sequences and *Oudemansiella cubensis* as the outgroup taxon. The alignment consisted of 580 characters including gaps. Of these, 388 were constant and 138 parsimony informative. The heuristic search resulted in 2 equally most parsimonious trees with TL=333, CI=0.640, RI=0.817 and RC=0.523. The showed MP tree revealed well-supported clade corresponding to established species (Fig. 3). Based on results, Iranian isolates belonged to the previous described species, *A. mellea* (Guo *et al.*, 2016).

Discussion

The current work reports the results of a study to characterize a basidiomycetous fungus associated with crown and root rot diseases of some plant species in Pasargad County in Fars Province. Based on the morphology of the basidiomata and analysis of DNA sequences, the *Armillaria* species associated with root and crown rot diseases on seven plant species, *P. atlantica*, *A. altissima*, *F. excelsior*, *P. orientalis*, *U. minor*, *Ulmus* sp. and *R. damascene* was identified as *A. mellea*. *Armillaria* species are responsible for crown and root rot diseases in various plantations worldwide. *Armillaria mellea* is considered an important plant pathogenic fungus on fruit and ornamental plants in Iran (Ershad, 2022) as well as in other countries of the world (Baumgartner *et al.*, 2011). This fungus can infect over than 500 plant species including trees, shrubs and herbaceous plants (Raabe, 1962; Ford *et al.*, 2017; Cromey *et al.*, 2019). Based on our field observations, crown and root rot, leaf yellowing, growth reduction, dwarfing of foliage, and branch dieback were the main disease symptoms on the studied plant species in Pasargad County. Dieback and death of trees on *F. excelsior*, *P. orientalis* and *U. minor*, *Ulmus* sp. were almost common disease symptoms. Similar disease symptoms caused by this fungus had been previously reported by various authors on fruit and forest trees (Cleary *et al.*, 2008; Baumgartner *et al.*, 2011; Anselmi *et al.*, 2021). However, disease symptoms caused by

Armillaria species including *A. mellea* depend on the host and associated environmental conditions.

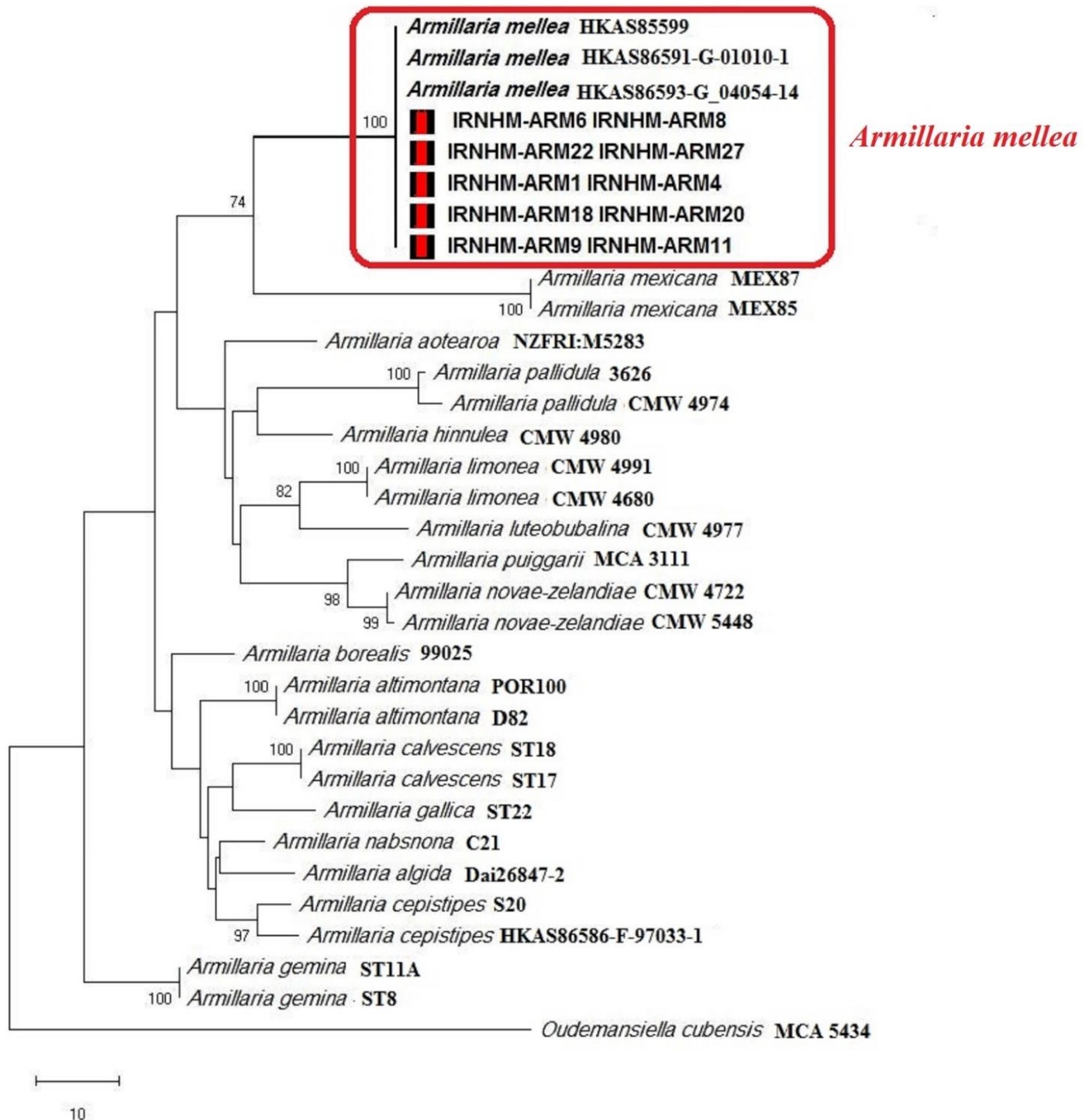


Fig 3. One of the most parsimonious trees for *Armillaria* isolates obtained from *tef-1α* sequence data. MP bootstrap supports (1000 replicates) above 70% are shown at the nodes. *Oudemansiella cubensis* (MCA 5434; accession number: KU289105) was used as outgroup and the Iranian isolates obtained in this study shown in bold face and red and black squares. Bar represents 10 changes.

Armillaria species can be present in three morphological forms including basidiocarps, mycelial fans and rhizomorphs (Devkota & Hammerschmidt, 2020). In the

current study, two clear signs of *Armillaria* infections including basidiocarps and mycelial fans were observed on *P. orientalis*, *Ulmus* sp. and *U. minor* planted close

to each other. Basidiocarps commonly were observed in clusters on infected stumps and at the base of infected trees. These fruiting bodies produce and release basidiospores through the gills, on the underside of their caps. Mycelial fans were also detected under the bark of the rotted roots and crowns of severely weakened and drying trees during this study. This form is known as the typical signs of *Armillaria* root and crown rot disease (Fox, 2000), which can infect phloem and cambium of the host and eventually leading to tree death (Ross, 1970). Mycelial fans can survive within the colonised tree stumps and decaying wood for decades and can be used as an important inoculum source to cause disease in surrounding trees. Therefore, basidiospores and mycelial fans can infect new hosts that are close to each other. However, spore dispersal is less important in disease spread than the other structures (Garbelotto, 2004).

In this study an extensive wood decay were recorded on *P. atlantica*, *A. altissima*, *P. orientalis* and *R. damascene*. Previous studies have reported that *Armillaria* species are capable of degrading woody tissues, causing wood decay and white rot in their hosts (Baumgartner *et al.*, 2011; Chandelier *et al.*, 2016; Kim *et al.*, 2022).

In Iran, *Armillaria* root rot was first recorded on apple trees in 1965 and since that time, the disease was reported from various forest and horticultural plants in Mazandaran, Kerman, Semnan, Tehran and Esfahan Provinces (Asef *et al.*, 2003; Amirahmadi *et al.*, 2006; Mohammadi & Haghdel, 2006; Honarjoo *et al.*, 2018; Ershad, 2022). However, based on pairing tests, it has been reported that three other *Armillaria* species, *A. cepistipes*, *A. gallica*, *A. borealis* are also associated with some forest trees in the Iran (Asef *et al.*, 2003; Ershad, 2022).

This species has previously been reported affecting *Rosa* sp. in Iran (Saber 1973; Asef *et al.*, 2003). Therefore our study is the first report of *A. mellea* on *R. damascene*. According to our results *A. mellea* was isolated for the first time from *P. orientalis* in Fars Province. This fungus was previously reported from *P. orientalis* in Esfahan (Saber, 1973; Behboudi & Saber, 1983), Tehran, Alborz and Mazandaran Provinces (Asef *et al.*, 2003). The results of the isolations made during the current study showed that *A. mellea* can infect *P. atlantica* in Pasargad County. This species has previously been reported affecting *Pistacia vera* in Iran

(Ershad, 2022). This study also represents the first record of *A. mellea* on *P. atlantica* in the world.

In a study conducted by Asef *et al.* (2003) in Iran, *A. mellea* was isolated from *U. minor* in Azarbaijan Province. Our work shows for the first time that *U. minor* and *Ulmus* sp. can also be infected by this basidiomycetous fungus in Fars Province. Based on literature reviews, *F. excelsior* can also be infected by *Armillaria* spp. (Madsen *et al.*, 2021; Peters *et al.*, 2023). However, in some countries such as Lithuania, *Armillaria cepistipes* has been reported as the most common and a dominant *Armillaria* species associated with ash trees showing crowns and root rot as well as decline symptoms (Lygis *et al.*, 2005; Bakys *et al.*, 2011).

In Ireland, *A. mellea* is known the most abundant *Armillaria* species affecting ash trees (FRDBI database, 2021). According to studies conducted in Belgian forests, *A. gallica*, *A. cepistipes* and *A. mellea* have been reported from ash trees with dieback symptoms (Chandelier *et al.*, 2016). To the best of our knowledge, the current study is the first report of *A. mellea* on *F. excelsior* in Iran. *Armillaria mellea* was also isolated from *U. minor* and *Ulmus* sp. in this study. This species has previously been isolated and reported from wych elm (*U. glabra*) trees showing root rot in Montenegro (Vemić, 2022). Other species of the genus *Armillaria*, such as *A. sinensis* and *A. luteopileata*, have also been isolated and reported from stump of *Ulmus* sp. and fallen trunk of *U. pumila*, respectively (Qin *et al.*, 2024). In Iran, *U. minor* has also been reported as a plant host for *A. mellea* in Azarbaijan Province (Asef *et al.*, 2003). Therefore, our research represents the first report of this species on *U. minor* and *Ulmus* sp. in Fars Province. *Ailanthus altissima* was another species identified as a host plant for *A. mellea* in this study. *Armillaria mellea* was reported on both trees and stumps of declining *A. altissima* in Italy (Sciré *et al.*, 2011). Therefore, *A. altissima* is reported here as new woody host for this pathogen in Iran. This study has revealed new insights into distribution and host range of *A. mellea* in Iran. *Armillaria mellea* is known to attack a various plants species in landscapes, parks, boulevards, sidewalks, along streets and roads as well as and orchards. In recent years, ornamental plants, especially *F. excelsior*, *P. orientalis* and *Ulmus* spp. in Pasargad County have damaged in large numbers due to the interactions of drought stress and bark beetles attack. These factors can predispose all trees to *Armillaria* root

rot disease (Sturrock *et al.*, 2012; Kolb *et al.*, 2016; Dempster, 2017). It seems that Armillaria root rot disease will continue to increase in this area in the future. Therefore all of these factors should be considered to control of Armillaria root rot disease on ornamental or forest trees. We did not study the pathogenicity of *A. mellea* isolates in the current work; therefore, more sampling of diverse plant species in different regions as well as pathogenicity testing can indicate the importance of this fungal species in Iran.

Acknowledgments

We would like to express our gratitude to the Shahid Bahonar University of Kerman, Iran, for their support.

Conflict of interest

The authors declare that they have no conflict of interest.

CRediT author statement

H. Mohammadi: Supervision, methodology, sampling, writing & reviewing. **H. Panahi:** Laboratory works. **M. Sohrabi:** Methodology & molecular analysing.

References

- Amirahmadi, H., Khabbaz Jolfaei, H., & Asef, M. R. (2006). First report of *Armillaria mellea* on pistachio, apricot, pomegranate and fig from Iran, *Proceedings of 17th Iranian Plant Protection Congress*, Karaj, Iran, pp. 410. (In Persian with English Abstract).
- Anselmi, N., Saraceni, A., & Anselmi, A. (2021). Incidence of *Armillaria* species in agrarian, forest and ornamental ecosystems of the Lazio region. *Agriculture and Forestry*, 67 (1), 7–25.
- Asef, M. R., Mohammadi Goltahpeh, E., & Alizadeh, A. (2003). Identification of *Armillaria* biological species in Iran. *Fungal Diversity*, 14, 51–60.
- Bakys, R., Vasiliauskas, A., Ihrmark, K., Stenlid, J., Menkis, A., & Vasaitis, R. (2011). Root rot, associated fungi and their impact on health condition of declining *Fraxinus excelsior* stands in Lithuania. *Scandinavian Journal of Forest Research*, 26(2), 128–135.
<https://doi.org/10.1080/02827581.2010.536569>.
- Baumgartner, K. (2004). Root collar excavation for postinfection control of Armillaria root disease of grapevine. *Plant Disease*, 88, 1235–1240.
- Baumgartner, K., Coetzee, M. P. A., & Hoffmeister, D. (2011). Secrets of the subterranean pathosystem of *Armillaria*. *Molecular Plant Pathology*, 12(6), 515–534.
<https://doi.org/10.1111/j.1364-3703.2010.00693.x>.
- Behboudi, B., & Saber, A. (1983). Study of honey mushroom in Esfahan, identification of damaged regions and factors affecting the intensity of pathogenecity. *Proceedings of the 7th Iranian Plant Protection Congress*, 3-7 Sept. Karaj, Iran. 106–107.
- Chandelier, A., Gerarts, F., San Martin, G., Herman, M., & Delahaye, L. (2016). Temporal evolution of collar lesions associated with ash dieback and the occurrence of *Armillaria* in Belgian forests. *Forest Pathology*, 46(4), 289–297.
<https://doi.org/10.1111/efp.12258>.
- Cleary, M., Van der Kamp, B., & Morrison, D. (2008). British Columbia's Southern Interior forests Armillaria root disease stand establishment decision aid. *Journal of Ecosystems and Management*, 9(2), 60–65. <https://doi.org/10.22230/jem.2008v9n2a397>.

- Coetzee, M. P. A., Bloomer, P., Wingfield, M. J., & Wingfield, B. D. (2011). Paleogene radiation of a plant pathogenic mushroom. *PloS One*, 6(12), e28545. <https://doi.org/10.1371/journal.pone.0028545>.
- Coetzee, M. P. A., Wingfield, B. D., Harrington, T. C., Dalevi, D., Coutinho, T. A., & Wingfield, M. J. (2000). Geographical diversity of *Armillaria mellea* based on phylogenetic analysis. *Mycologia*, 92(1), 105–113. <https://doi.org/10.1080/00275514.2000.12061134>.
- Cox, K. D., Scherm, H., & Riley, M. B. (2006). Characterization of *Armillaria* spp. from peach orchards in the southeastern United States using fatty acid methyl ester profiling. *Mycological Research*, 110, 414–422. <https://doi.org/10.1016/j.mycres.2005.12.004>.
- Cromey, M. G., Drakulic, J., Beal, E. J., Waghorn, I. A. G., Perry, J. N., & Clover, G. R. G. (2020). Susceptibility of garden trees and shrubs to *Armillaria* root rot. *Plant Disease*, 104(2), 483–492. <https://doi.org/10.1094/PDIS-06-19-1147-RE>.
- Dai, Y. C., Cui, B. K., Yuan, H. S., & Li, B. D. (2007). Pathogenic wood-decaying fungi in China. *Forest Pathology*, 37(2), 105–120. <https://doi.org/10.1111/j.1439-0329.2007.00485.x>
- Dalili, S. A. R., Nanagulyan, S. G., & Alavi, S. V. (2008). Identification of *Armillaria* species on different hosts from Iran. *Journal Mycologia Balcanica*, 5, 119–122.
- Dalili, S. A. R., Nanagulyan, S. G., Alavi, S. V., & Razavi, M. (2010). Investigation of the wood destroying activity of *Armillaria mellea* on horticultural and forest plants species. *Australian Journal of Crop Science*, 4(4), 209–215.
- Denman, S., Barrett, G., Kirk, S. A., McDonald, J. E., & Coetzee, M. P. A. (2017). Identification of *Armillaria* species on declined oak in Britain: implications for oak health. *Forestry (London, England)*, 90(1), 148–161. <https://doi.org/10.1093/forestry/cpw054>.
- Dempster, W. R. (2017). Impact of climate on juvenile mortality and *Armillaria* root disease in lodgepole pine. *Forestry Chronicle*, 93, 148–160. <https://doi.org/10.5558/tfc2017-021>.
- Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15.
- Elías-Román, R. D., Medel-Ortiz, R., Alvarado-Rosales, D., Hanna, J. W., Ross-Davis, A. L., Kim, M. S., & Klopfenstein, N. B. (2018). *Armillaria mexicana*, a newly described species from Mexico New *Armillaria* species from Mexico. *Mycologia*, 110, 347–360. <https://doi.org/10.1080/00275514.2017.1419031>.
- Ershad, D. (1995). Fungi of Iran. 2nd edition, Agricultural Research, Education and Extension Organization, Iran.
- Ershad, J. (2022). *Fungi and fungal analogues of Iran* (4nd ed.). Ministry of Agriculture, Agricultural Research, Education and Extension Organization, Iranian Research Institute of Plant Protection, Iran, 695 p.

- Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791.
- Ford, K. L., Henricot, B., Baumgartner, K., Bailey, A. M., & Foster, G. D. (2017). A faster inoculation assay for *Armillaria* using herbaceous plants. *The Journal of Horticultural Science & Biotechnology*, 92(1), 39–47. <https://doi.org/10.1080/14620316.2016.1223528>.
- Fox, R. T. V. (ed.). (2000). *Armillaria root rot: biology and control of honey fungus*. Intercept, Andover, UK.
- Chandelier, A., Gerarts, F., San Martin, G., Herman, M., & Delahaye, L. (2016). Temporal evolution of collar lesions associated with ash dieback and the occurrence of *Armillaria* in Belgian forests. *Forest Pathology*, 46, 289–297.
- Guillaumin, J. J., & Legrand, P. (2013). *Armillaria* root rots. In P. Gonthier & G. Nicolotti (eds.), *Infectious forest diseases* (pp. 159–177). Wallingford: CABI Publishing.
- Guillaumin, J. J., Pierson, J., & Grassely, C. (1991). The susceptibility to *Armillaria mellea* of different *Prunus* species used as stone fruit rootstocks. *Scientia Horticulturae*, 46(1–2), 43–54. [https://doi.org/10.1016/0304-4238\(91\)90091-c](https://doi.org/10.1016/0304-4238(91)90091-c).
- Guo, T., Wang, H. C., Xue, W. Q., Zhao, J., & Yang, Z. L. (2016). Phylogenetic analyses of *Armillaria* reveal at least 15 phylogenetic lineages in China, seven of which are associated with cultivated *Gastrodia elata*. *PloS One*, 11(5), e0154794. <https://doi.org/10.1371/journal.pone.0154794>.
- Hasegawa, E., Ota, Y., Hattori, T., & Kikuchi, T. (2010). Sequence-based identification of Japanese *Armillaria* species using the elongation factor-1 alpha gene. *Mycologia*, 102(4), 898–910. doi:10.3852/09-238.
- Heinzelmann, R., Dutech, C., Tsykun, T., Labbé, F., Soularue, J.-P., & Prospero, S. (2019). Latest advances and future perspectives in *Armillaria* research. *Canadian Journal of Plant Pathology. Revue Canadienne de Phytopathologie*, 41(1), 1–23. <https://doi.org/10.1080/07060661.2018.1558284>.
- Honarjoo, M., Alaei, H., Mohammadi, A. H., & Sedaghati, E. (2018). PCR based detection of *Armillaria mellea* the causal agent of pistachio root and crown rot in soil. *Proceedings of 2nd National conference on Iran Pistachio*, Rafsanjan, Iran.
- Hood, I. A., Redfern, D. B., & Kile, G. A. (1991). *Armillaria* in planted hosts. In C. G. Shaw, III & G. A. Kile (eds.), *Armillaria root disease* (pp. 122–149). Agricultural Handbook No. 691. USDA Forest Service, Washington, District of Columbia.
- Kausarud, H., & Schumacher, T. (2001). Outcrossing or inbreeding: DNA markers provide evidence for type of reproductive mode in *Phellinus nigrolimitatus* (Basidiomycota). *Mycological Research*, 105(6), 676–683. <https://doi.org/10.1017/s0953756201004191>.
- Kikuchi, G., & Yamaji, H. (2010). Identification of *Armillaria* species associated with *Polyporus umbellatus* using ITS sequences of nuclear

- ribosomal DNA. *Mycoscience*, 51(5), 366–372. <https://doi.org/10.1007/s10267-010-0053-8>.
- Kile, G. A., McDonald, G. I., & Byler, J. W. (1991). Ecology and disease in natural forests. In C. G. Shaw & G. A. Kile (eds.), *Armillaria root disease* (pp. 102–121). Agricultural Handbook No. 691. USDA Forest Service, Washington, District of Columbia.
- Kim, M. S., Heinzlmann, R., Labbé, F., Ota, Y., Elías-Román, R. D., Pildain, M. B., Stewart, J. E., Woodward, S., & Klopfenstein, N. B. (2022). In: F. O. Asiegbu, & A. Kovalchuk (eds.), *Armillaria root diseases of diverse trees in wide-spread global regions* (pp. 361–378), *Forest microbiology forest tree health*. Academic Press (Elsevier); London, UK.
- Klopfenstein, N. B., Stewart, J. E., Ota, Y., Hanna, J. W., Richardson, B. A., Ross-Davis, A. L., Elías-Román, R. D., Korhonen, K., Keča, N., Iturrirxa, E., Alvarado-Rosales, D., Solheim, H., Brazee, N. J., Łakomy, P., Cleary, M. R., Hasegawa, E., Kikuchi, T., Garza-Ocañas, F., Tsopelas, P., Rigling, D., Prospero, S., Tsykun, T., Bérubé, J. A., Stefani, F. O., Jafarpour, S., Antonín, V., Tomšovský, M., McDonald, G. I., Woodward, S., & Kim, M. S. (2017). Insights into the phylogeny of Northern Hemisphere *Armillaria*: Neighbor-net and Bayesian analyses of translation elongation factor 1- α gene sequences. *Mycologia*, 109(1), 75–91. <https://doi.org/10.1080/00275514.2017.1286572>.
- Koch, R. A., Wilson, A. W., Séné, O., Henkel, T. W., & Aime, M. C. (2017). Resolved phylogeny and biogeography of the root pathogen *Armillaria* and its gasteroid relative, *Guyanagaster*. *BMC Evolutionary Biology*, 17(1). <https://doi.org/10.1186/s12862-017-0877-3>.
- Kolb, T. E., Fettig, C. J., Ayres, M. P., Bentz, B. J., Hicke, J. A., Mathiasen, R., Stewart, J. E., & Weed, A. S. (2016). Observed and anticipated impacts of drought on forest insects and diseases in the United States. *Forest Ecology and Management*, 380, 321–334. <https://doi.org/10.1016/j.foreco.2016.04.051>.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>.
- Lygis, V., Vasiliauskas, R., Larsson, K. H., & Stenlid, J. (2005). Wood-inhabiting fungi in stems of *Fraxinus excelsior* in declining ash stands of northern Lithuania, with particular reference to *Armillaria cepistipes*. *Scandinavian Journal of Forest Research*, 20(4), 337–346. <https://doi.org/10.1080/02827580510036238>.
- Madsen, C. L., Kosawang, C., Thomsen, I. M., Hansen, L. N., Nielsen, L. R., & Kjær, E. D. (2021). Combined progress in symptoms caused by *Hymenoscyphus fraxineus* and *Armillaria* species, and corresponding mortality in young and old ash trees. *Forest Ecology and Management*, 491(119177), 119177. <https://doi.org/10.1016/j.foreco.2021.119177>.
- Mańka, K. (1953). Badania terenowe i laboratoryjne nad opieńką miodową- *Armillaria mellea* (Vahl) Quel. *Prace Instytutu Badawczego Leśnictwa*, 94, 1–96.

- Maphosa, L., Wingfield, B. D., Coetzee, M. P. A., Mwenje, E., & Wingfield, M. J. (2006). Phylogenetic relationships among *Armillaria* species inferred from partial elongation factor 1-alpha DNA sequence data. *Australasian Plant Pathology: APP*, 35(5), 513. <https://doi.org/10.1071/ap06056>.
- Mohammadi, A. H., & Haghdel, M. (2020). Technical report of contamination of Aran and Bidgol pistachio orchards in Kashan to *Armillaria* rot and providing necessary solutions for disease management. Pistachio Research Center, 12p. (In Persian).
- Morrison, D. J., Pellow, K. W., Norris, D. J., & Nemec, A. F. L. (2000). Visible versus actual incidence of *Armillaria* root disease in juvenile coniferous stands in the southern interior of British Columbia. *Canadian Journal of Forest Research*, 30(3), 405–414. <https://doi.org/10.1139/x99-222>.
- Nomura, H. (1903). Root rot disease of mulberry trees in Kanagawa prefecture. *Sanjihokoku*, 19, 443–461.
- Peters, S., Fuchs, S., Bien, S., Bußkamp, J., Langer, G. J., & Langer, E. J. (2023). Fungi associated with stem collar necroses of *Fraxinus excelsior* affected by ash dieback. *Mycological Progress*, 22, 52. <https://doi.org/10.21203/rs.3.rs-2484538/v1>.
- Qin, G.-F., Qin, W.-M., Wang, H.-C., Zhao, J., Korhonen, K., Chen, J., Zhao, J., Korhonen, K., Chen, J., Dai, Y. C., & Yuan, Y. (2025). Phylogeny and species diversity of *Armillaria* in China based on morphological, mating test, and GCPSR criteria. *Mycology*, 16(2), 777–811. <https://doi.org/10.1080/21501203.2024.2404121>.
- Raabe, R. D. (1962). Host list of the root rot fungus *Armillaria mellea*. *Hilgardia*, 33, 25–88.
- Robinson-Bax, C., & Fox, R. T. V. (200). Root rots of herbaceous plants caused by *Armillaria mellea*, *Mycologist*, 16(1), 21–22.
- Ross, E. W. (1970). Sand pine root rot pathogen: *Clitocybe tabescens* *Journal of Forestry*, 68, 156–158.
- Ross-Davis, A. L., Hanna, J. W., Klopfenstein, N. B., & Kim, M.-S. (2012). Advances toward DNA-based identification and phylogeny of North American *Armillaria* species using elongation factor-1 alpha gene. *Mycoscience*, 53(2), 161–165. <https://doi.org/10.1007/s10267-011-0148-x>.
- Saber, M. (1973). *Armillaria* root rot. *Iranian Journal of Plant Pathology*, 9, 45–62 (In Persian with English abstract).
- Sciré, M., D'Amico, L., Gaglioppa, P., Puddu, G., Tizzani, L., & Motta, E. (2011). First record of *Armillaria mellea* on *Ailanthus altissima* in Italy. (Prima segnalazione di *Armillaria mellea* su *Ailanthus altissima* in Italia). *Micologia italiana*, 3, 17–22.
- Shearer, B. L., Crane, C. E., Fairman, R. G., & Grant, M. J. (1997). Occurrence of *Armillaria luteobubalina* and pathogen-mediated changes in coastal dune vegetation of south-western Australia. *Australian Journal of Botany*, 45(5), 905. <https://doi.org/10.1071/bt96084>.

- Sturrock, R. N., Frankel, S. J., Brown, A. V., Hennon, P. E., Kliejunas, J. T., Lewis, K. J., Worrall, J. J., & Woods, A. J. (2012). Climate change and forest diseases. *Plant Pathology*, 60, 133–149. <https://doi.org/10.1111/j.1365-3059.2010.02406.x>.
- The Fungal Records Database of Britain and Ireland (FRDBI). Available at <http://frdbi.info/>. Accessed 7 Jun 2025.
- Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22), 4673–4680. <https://doi.org/10.1093/nar/22.22.4673>.
- Tsykun, T., Rigling, D., & Prospero, S. (2013). A new multilocus approach for a reliable DNA-based identification of *Armillaria* species. *Mycologia*, 105(4), 1059–1076. <https://doi.org/10.3852/12-209>.
- Vemic, A. (2022). The most important fungi on wych elm (*Ulmus glabra*) trees in Montenegro. *The Journal Agriculture and Forestry*, 68(3). <https://doi.org/10.17707/agricultforest.68.3.05>.