

Alternaria species associated with *Quercus brantii* in Zagros forests of Fars province, Iran

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INTRODUCTION

Persian oak (*Quercus brantii* Lindl.) is a significant tree throughout the forests of Zagros and has been in the area for approximately 5,000 years (Mollaei, 2019). Persian oak as the main tree species covers nearly five million hectares of Zagros Mountains, from the southwest to the northwest of Iran. Fars province has the first place in terms of forest and tree habitats (Eskandari et al. 2020; Ahmadi et al. 2014). Granted the role of oak trees in maintaining water supplies and in the mitigation of climate change (Mollaei 2019), any reduction and mortality of oak trees is considered an important threat to the ecosystem (Linaldeddu et al. 2011).

Endophytic fungi are diverse group of fungi that inhabit the internal tissues of living plants without causing visible symptoms and present in many kinds of plants (Hyde and Soyong 2008). Fungal endophytes are a taxonomically and ecologically heterogeneous groups and appear to make up a large fraction of the fungal biodiversity (Arnold et al. 2003). On the other hand, to achieve these benefits, it is important to explore the diversity of endophytic fungi in different ecosystems (DeMers 2022; Higgins et al. 2007).

Endophytic fungi can play significant ecological roles in plants and are known to help plant growth, improve plant's ability to tolerate abiotic and biotic stresses, and produce bioactive antimicrobial compounds, used in agriculture, commercial industry, and in medicine (Estrada et al. 2013). Nevertheless, some endophytic fungi have verified to be latent pathogens of the plant hosts. Additionally, the role of some endophytes in host plants is still unclear (Mirabolfathy 2013).

Many researches have been done to determine the diversity of endophytic fungi associated with oak trees worldwide (Gennaro et al. 2003; Ghobad-Nejhad et al. 2017). The diversity, abundance, and species composition of endophytic fungal species can

Abstract: Persian oak is the most important forest tree in Fars province (Iran). In order to identify the composition of *Alternaria* species associated with healthy and declined Persian oak trees in Fars Province, Iran, fungal isolates were recovered from twigs, trunks and leaves of healthy and declining Persian oak trees from early September to late March 2021-2022. Fungal species were identified based on both morphological and as well as molecular characteristics of the DNA sequence data of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and plasma membrane ATPase genes. In total, 29 *Alternaria* isolates were obtained, which were identified as *Alternaria alternata*, *Alternaria consortialis* and *Alternaria chlamydospora*. *Alternaria alternata* showed the highest frequency approximately 51.72% and were isolated from both healthy and declining oak trees. *Alternaria chlamydospora* and *A. consortialis* were obtained only from healthy oak trees without declining symptoms with 41.38% and 6.89% frequencies, respectively. Pathogenicity tests were conducted using all isolates in three replicates. Inoculation of different *Alternaria alternata*, *Alternaria consortialis* and *Alternaria chlamydospora* isolates on Persian oak seedlings did not show any symptoms after 60 days in greenhouse conditions. This is a new report of *Alternaria* species including *A. alternata*, *A. consortialis* and *A. chlamydospora* as endophytic fungi on Persian oak tree in Fars province of Iran.

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be affected by the locality in which a specific plant occurs (Higgins et al. 2007). It is possible that the diversity of endophytic fungi differs because of annual rainfall and latitude (Arnold et al. 2003). Up to now, several fungal species viz., *Cladosporium tenellum*, *Fusarium tricinctum*, *Deniquelata quercina*, *Cytospora ribis*, *Acremonium* sp., *Coniochaeta* sp., *Neoetophoma samarorum*, *Microsphaeriopsis olivacea*, *Diatrype* spp., *Leptosphaerulina* spp., *Comoclathris* sp., *Didymella glomerata*, *Tricothecium roseum*, *Fusarium solani*, *Thyrostroma* sp., *Paecilomyces formosus*, *Petriella guttulata*, *Preussia australis*, and *Sordaria sicutii* have been reported as endophytic fungi of Persian oak trees in Zagros forests (Alidadi et al. 2019a, 2019b; Ghobad-Nejhad et al. 2018; Hajizadeh et al. 2015).

In general, endophytic fungi survive in a latent phase in healthy trees for the whole lifetime or for an extended period of time, and may act as an opportunistic pathogen and attack weakened hosts under environmental stresses such as drought stress and high abnormal temperature (Vannini et al. 2009; Rodriguez et al. 2008). *Biscogniauxia mediterranea* (de Not.) Kuntze was introduced as an opportunistic pathogen responsible for charcoal canker in the inner bark of hardwood species under water stress conditions and high abnormal temperature (Linaldeddu et al. 2011; Mirabolfathy 2013; Safaei et al. 2016).

The objectives of the present study were to: a) isolate the fungal species associated with healthy tree and dead branches of Persian oak trees with decline symptoms in Zagros forests from Fars province b) identify fungal species based on morphological and molecular characteristics using phylogenetic analysis of the DNA sequence data of the glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and plasma membrane ATPase genes, and c) determine the pathogenicity of the recovered fungal species on Persian oak seedling in greenhouse conditions.

MATERIALS AND METHODS

Sampling, Isolation and Morphological identification

From early September to late March 2021-2022, fifty samples were collected from twigs, trunks and leaves from healthy trees and dead branches of Persian oak trees with decline symptoms in different forest areas of the Fars province, Iran. Samples were placed in separate paper bags, recorded their information, transferred to the laboratory, and kept at 4°C until diagnostic lab isolation were performed.

The samples were washed under running tap water in order to remove dusts or other surface contaminants, and subjected to air to be dried at room temperature for 2–3 h. With the intention of eliminate epiphytic contaminants, the samples disinfested by the 70% ethanol for 40 s, and by the 1% sodium hypochlorite for 50 s, and rinsed twice by sterile water. To isolate, the samples were cut into small segments (3–5 cm) with a sterile scalpel, sterilized by the 1% sodium

hypochlorite for 60 sec, rinsed in sterile deionized water, placed on 2% Water Agar (2% WA) as well as PDA (potato dextrose agar; 12 g/l potato extract, 10 g/l dextrose, 1.2% agar) with 50 µg of modified kanamycin to prevent bacterial contamination and then incubated at 25 °C for 5–7 days. Fungi purification was performed by transferring single spores and/or single hyphal tips grown on 2% Water Agar onto PDA medium. The purified isolates were stored on PDA slants at 4 °C for future studies.

To identify the fungal species, all recovered fungal isolates were morphologically evaluated and classified in accordance to genus and species descriptions in valid taxonomic references such as Woudenberg et al. (2013). Thus, fungal isolates were first cultured in a variety of culture media including Potato Carrot Agar (PCA), Oatmeal Agar (OA), PDA, Carnation Leaf Agar (CLA), Malt Extract Agar (MEA), and Synthetic Nutrient Agar (SNA). Macro and micro-morphological characteristics, such as colony morphology, cultural characteristics (growth rate, growth temperatures, and diameter of the hyphae), and teleomorph and anamorph states characteristics were recorded and compared with the literature (Woudenberg et al. 2013, 2015).

Molecular Identification

Isolates were obtained from one-week-old pure culture on PDA culture medium, and then a 4–6 mm mycelial plugs were transferred into PD broth on an orbital shaker at 20 °C for one week. Mycelia were harvested, washed with sterile distilled water and grinded in a sterile mortar using liquid nitrogen. Genomic DNA was extracted with the CTAB protocol according to the technique previously described by Murray and Thompson (1980). The quality and quantity of the extracted DNA were evaluated in 1.2% agarose gel, viewed by staining in ethidium bromide, and the DNA samples were stored at -20 °C.

For molecular characterization, 12 representative isolates were selected (Table 1) with the pair of primers *gpd1/gpd2*, and *ATPDF1/ATPDR1* (Lawrence et al. 2015) that amplified glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) and plasma membrane ATPase genes, respectively. The PCR reaction mixtures in 20 µl volume were prepared containing 0.04 µM of each primer (Microsynth, Switzerland), 0.4 µM dNTPs (MBI Fermentas, Germany), 1× Dream Taq Buffer (MBI Fermentas), and 0.4 U Dream Taq DNA polymerase (MBI Fermentas). PCRs were performed in a Biometra thermocycler based on a protocol that was described in detail by Ahmadi et al. (2022). The amplicons were analyzed in 1% agarose gel then stained with ethidium bromide, and viewed under UV light to check the amplification size and purity (GelDoc, Bio-Rad Laboratories, Hercules, CA, USA). The PCR products were purified by a PCR purification kit (Fermentas, UK), and DNA sequencing was performed in both directions by the DNA Sequencing Service of Macrogen Co. (South Korea).

GAPDH and ATPase sequences generated in this study, combined with sequences of representative taxa from GenBank (Table 1), to determine the taxonomic status of the Persian oak isolates. New nucleotide sequences were deposited in GenBank (<http://www.ncbi.nlm.nih.gov>), and the accession numbers of sequences are given in Table 1. The forward and reverse sequences of GAPDH and ATPase were aligned initially by program Sequencer ver. 5.1 (Gene Code). Final alignments were performed using Geneious version 7 (Biomatters, Auckland, New Zealand). Phylogenetic estimates were estimated using the maximum parsimony analyses (MP) in MEGA 6.0 (Tamura et al. 2013). Maximum parsimony (MP) phylogenies were performed using heuristic searches with 1000 random sequence additions and branch swapping with Tree-Bisection-Reconnection (TBR) algorithm, gaps treated as missing data and the reliability of resultant trees was determined with bootstrap analysis of 1000 replicates to test the support of the branches (Felsenstein 1985). The resulting trees were viewed and edited in FigTree v. 1.4.0., and sequences of *Stemphylium botryosum* (EGS08-069) were used as outgroup to root phylogenetic trees for *Alternaria* species.

Pathogenicity tests

To perform the pathogenicity tests, before sowing the seeds in soil packed plastic pots in the greenhouse, the seeds were surface- disinfested in 5% sodium hypochlorite for 2 min and were rinsed for 2 min three times in sterile distilled water. The seeds (one seed in each pot) were sown in free-draining plastic pots (14 × 16 cm) filled with a mixture of steam-sterilized soil (Loam and sand 2:1 v/v) (each pot as a replicate with five replications). Then, pathogenicity tests were done using two methods. The first method, after 24 months of growing under greenhouse conditions, the two-year-old cork Persian oak seedlings' stems were surface- disinfested in 10% sodium hypochlorite and artificially inoculated by creating a surface wound in the stem with a scalpel at a 10–25 cm height from the collar. A 6-mm mycelial plug from actively growing colony was placed on created wound. Lastly, the wounded stems were wrapped with parafilm (Ghaderi and Habibi 2021; Ahmadi et al. 2022). Control plants were treated similarly with a agar plug of sterile PDA medium after inoculation, the visual assessment was carried out after 60 days (Ghaderi and Habibi 2021; Ahmadi et al. 2022). In the second method, the isolates were grown for one week on PDA at 25°C, under a 12 h light/ darkness photoperiod, to favor fungal sporulation. For each isolate, the inoculum was prepared by flooding the agar plate surface with 10 ml of sterile distilled water and scraping with a spatula. Conidial suspensions were filtered through four layers of cheesecloth and adjusted at a final concentration of 10^6 conidia mL^{-1} . Leaves area of each seedling was sprayed with 4 ml of conidial suspension by using hand operated compressed air

sprayer. To favor fungal development, all seedlings were watered and enclosed in plastic bags for 3 days in a greenhouse condition at $25 \pm 2^\circ\text{C}$, 80% RH, 12 h light/darkness photoperiod. After inoculation, the first visual assessment was carried out after 10 days and then weekly for two months (Rabaoui et al. 2022). The control and inoculated seedlings were incubated in the greenhouse condition at an average temperature of 28–35°C for two months under natural daylight conditions.

RESULT

Sampling and Isolation

Fifty samples from branches of healthy/declining Persian oak trees in Zagros forests of Fars province were collected. In total, 29 pure isolates were obtained in this study which 15 out of 29 fungal isolates were obtained from both healthy and declining Persian oak trees and 14 out of 29 fungal isolates were obtained from healthy Persian oak trees without declining symptoms. All fungal isolates were efficiently recovered from September to the end of November. Nevertheless, no fungal isolate was obtained from tissues from December until the end of February.

Morphological and Molecular identification

In this study, 29 fungal isolates were obtained from twigs and branches of Persian oak trees. Finally, three fungal species including *Alternaria alternata*, *Alternaria consortialis* and *Alternaria chlamydospora*, were identified as endophytic fungi inhabiting twigs and branches of the Persian oak trees in Fars province, based on preliminary morphological characteristics as well as sequence data of *GAPDH* and *ATPase* genes.

The *A. alternata* species (15 isolates) showed the highest frequency approximately 51.72% and were isolated from both healthy and declining oak trees. *A. chlamydospora* (12 isolates) and *A. consortialis* (2 isolates) were obtained only from healthy oak trees without declining symptoms with 41.38% and 6.89% frequencies, respectively. Table 1 reports a summarized information about morphological characteristic of the identified species in the study (Fig.1).

In general, morphological identification could cause many errors in *Alternaria* species identification. Today, insufficiency of morphological features has been solved using molecular techniques (Somma et al. 2019). In Present study, different parts of the *Alternaria* genome are used in molecular phylogeny-based taxonomy such as *GAPDH* and *ATPase* genes. PCR amplification and sequencing were successful for 12 isolates. The obtained sequences of *Alternaria* isolates were submitted to GenBank under the following accession numbers: OR575023 to OR575034 for *GAPDH* and OR575011 to OR575022 for *ATPase* gene (Table 1).

Table 1. The ATPase and GAPDH sequences used in the phylogenetic analyses in this study. (Sequences were generated in this study are in bold letters).

Species name	strain	GenBank accession numbers	
		ATPase	GAPDH
<i>Alternaria alternata</i>	EGS 34-016	JQ671874	AY278808
<i>A. arbusti</i>	EGS 91-129	JQ693604	JQ693621
<i>A. californica</i>	EGS 52-082	JQ671813	JQ646285
<i>A. conjuncta</i>	EGS 37-139	JQ671824	AY562401
<i>A. daucicaulis</i>	EGS 36-1947	JQ671822	JQ646294
<i>A. ethzedia</i>	EGS 37-143	JQ671805	AY278795
<i>A. frumenti</i>	EGS 44-001	JQ671823	JQ646295
<i>A. consortialis</i>	CBS 104.31	JQ671839	KC584173
<i>A. graminicola</i>	EGS 41-139	JQ671819	JQ646291
<i>A. hordeiaustralica</i>	EGS 44-200	JQ671811	JQ646283
<i>A. hordeicola</i>	EGS 50-184	JQ671812	JQ646284
<i>A. humuli</i>	EGS 47-140	JQ671821	JQ646293
<i>A. incomplexa</i>	EGS 17-103	JQ671815	JQ646287
<i>A. infectoria</i>	EGS 27-193	JQ671804	AY278793
<i>A. intercepta</i>	EGS 49-137	JQ671826	JQ646297
<i>A. merytae</i>	EGS 46-153	JQ671820	JQ646292
<i>A. metachromatica</i>	EGS 38-132	JQ671809	AY562404
<i>A. novae-zelandiae</i>	EGS 48-092	JQ671825	JQ646296
<i>A. oregonensis</i>	EGS 29-194	JQ671827	FJ266491
<i>A. triticimaculans</i>	EGS 41-050	JQ671806	JQ646280
<i>A. triticina</i>	ITEM 630	JQ693607	JQ693623
<i>A. ventricosa</i>	EGS 52-075	JQ671818	JQ646290
<i>A. viburni</i>	EGS 49-147	JQ671816	JQ646288
<i>A. chlamydospora</i>	CBS 491.72	JQ671786	JQ646364
<i>A. limaciformis</i>	CBS 481.81	JQ671798	KC584123
<i>A. molesta</i>	CBS 548.81	JQ671789	KC584125
<i>A. brassicicola</i>	EEB 2232	JQ671843	AY278813
<i>A. selini</i>	EGS 25-198	JQ671853	AY278800
<i>A. panax</i>	EGS 29-180	JQ671846	JQ646299
<i>A. septospora</i>	CBS 109.38	JQ671829	FJ266500
<i>A. alternata</i>	Iran-Alt-1	OR575011	OR575023
<i>A. alternata</i>	Iran-Alt-2	OR575012	OR575024
<i>A. alternata</i>	Iran-Alt-3	OR575013	OR575025
<i>A. alternata</i>	Iran-Alt-4	OR575014	OR575026
<i>A. alternata</i>	Iran-Alt-5	OR575015	OR575027
<i>A. consortialis</i>	Iran-Alt-con1	OR575016	OR575028
<i>A. consortialis</i>	Iran-Alt-con2	OR575017	OR575029
<i>A. chlamydospora</i>	Iran-Alt-Chla-1	OR575018	OR575030
<i>A. chlamydospora</i>	Iran-Alt- Chla-2	OR575019	OR575031
<i>A. chlamydospora</i>	Iran-Alt- Chla-3	OR575020	OR575032
<i>A. chlamydospora</i>	Iran-Alt- Chla-4	OR575021	OR575033
<i>A. chlamydospora</i>	Iran-Alt- Chla-5	OR575022	OR575034
<i>Stemphylium botryosum</i>	EGS08-069	AY329271	AY316968

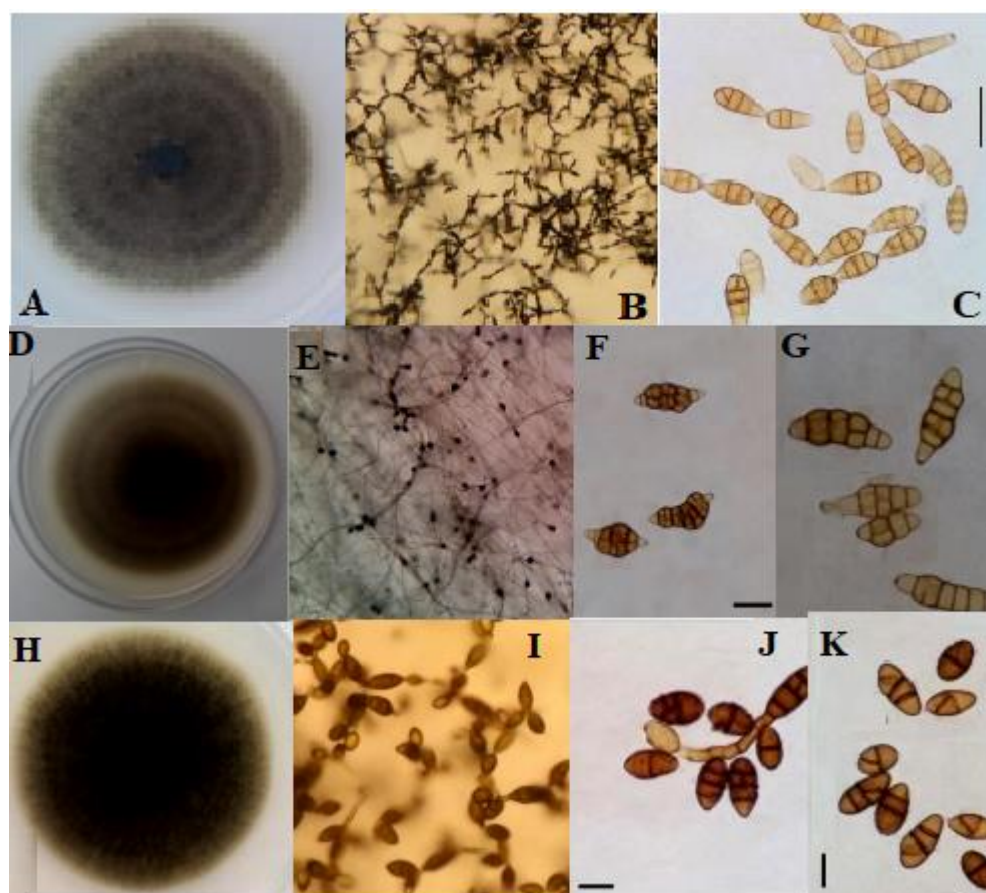


Fig. 1. A-C) *Alternaria alternata*: A) 7-d-old colony on PCA, B) Sporulation pattern, C) Conidia. D-G) *Alternaria chlamydospora*: D) 7-d-old colony on PCA, E) Sporulation pattern, F) Chlamydospore, G) Conidia. H-K) *Alternaria consortialis*: H) 7-d-old colony on PCA, I) Sporulation pattern, j-k) Conidiophores and Conidia. Scale bar = 10 µm

The aligned datasets for GAPDH and ATPase consisted of 590 and 1260 characters, respectively. The aligned multigene dataset contained 1836 characters. The topology and branch lengths of the phylogenetic inferences are shown in Figures 2. The multigene phylogenies of combined dataset of GAPDH and ATPase sequences revealed that Five isolates Iran-Alt-1 to Iran-Alt-5 are placed in a monophyletic group with *A. alternata* (strain: EGS 34-016) in a well-supported clade (posterior probability = 100%). Five isolates Iran-Alt-Chla-1 to Iran-Alt-Chla-5 formed a monophyletic group with *A. chlamydospora* (strain: CBS 491.72) in a well-supported clade (posterior probability = 100%). Finally, two isolates Iran-Alt-con1 and Iran-Alt-con2 formed a monophyletic group with *A. consortialis* (strain: CBS 104.31) in a well-supported clade (posterior probability = 99%) (Fig. 2).

Pathogenicity tests

Pathogenicity tests were conducted using all the isolates of *Alternaria* species in three replicates. Inoculation of different *Alternaria alternata*, *Alternaria consortialis* and *Alternaria chlamydospora*

isolates on Persian oak seedlings did not show any symptoms after 60 days in greenhouse conditions.

DISCUSSION

Species of genus *Alternaria* are significant and comprise a wide variety of saprophytic, pathogenic, and endophytic fungus extensively distributed on worldwide growing on a great variety of substrates (Lawrence et al. 2015; Alidadi et al. 2019a, 2019b). About 300 species have been defined and their taxonomic position is being the key to relevant changes on the basis of nucleotide sequence data (Woudenberg et al. 2013). The present study focused on isolation, Morphological and molecular characterization of *Alternaria* species from healthy/declining Persian oak trees.

In the present study, *A. alternata* is the most important *Alternaria* species detected from *Quercus brantii* from Fars province for the reason that this species was obtained from both branches of healthy and declining trees with 31.25% and 15.62% frequencies, respectively.

Table 2. Morphological characteristics of the identified species in the present study

Species	No. of isolates	Colony characteristics	morphological characteristics
<i>Alternaria alternata</i>	15	Dark colors ranging from gray to olive or olive brown on PDA	The formation of conidial chains 6 to 14 conidia in length. Chain branching occurred in a sympodial manner through the elongation of secondary conidiophores from distal terminal conidial cells and subsequent conidia formation or through the lateral growth of secondary conidiophores from median or basal conidial cells and subsequent conidia formation. Conidia were typically ovate in shape.
<i>A. chlamydospora</i>	12	Colonies were gray on PDA	Conidia were light brown in color, solitary, straight, ovoid to subcylindrical in shape, dark transverse septa. Conidiophores were simple, septate, straight, or with geniculate sympodial proliferation. Chlamydospores were abundant and formed in single chains.
<i>A. consortialis</i>	5	The colony olive green to brownish with a velvety texture	Conidia brown, spherical, elliptic to cylindrical, with a flat, sometimes dotted, the conidia often are produced singly or in short chains (2–3 spores), usually with smooth and rarely verrucous wall surface, 1–5 transverse septa and 1–3 longitudinal septa, conidiophores straight and short, simple or branched, and light brown.

This species was non-pathogenic on Persian oak seedlings and no symptoms was observed based in pathogenicity tests. The present study reported the *brantii* in Dena region of Kohgiluyeh & Boyer-Ahmad province (Ghobad-Nejhad et al. 2017). *Q. serrata* in Korea (Nguyen et al. 2020) and *Q. robur* L. in Poland (Jankowiak et al. 2022). The *A. alternata* species is presently considered as a cosmopolitan species and has a wide host range (Sánchez-García et al. 2020).

This species is pathogenic on many important crop plants (El Gobashy et al. 2014) but also lives in asymptomatic symbiosis as an endophyte of various plants (Lawrence et al. 2015), it is essential for plant pathologists to recognize whether a given strain of *A. alternata* indicates a danger to food safety or simply the presence of an endophyte (Patriarca et al. 2019). Given the prevalence of endophytic *A. alternata* (Lawrence et al. 2015), it is also essential for conservationists to understand if modifications to the abiotic environment might cause asymptomatic infections of *A. alternata* to move into parasitism,

fungus *A. alternata* is not a pathogen of Persian oak. This species has been previously reported from different species of the *Quercus* genus, including *Q.* leading to further stress to susceptible plant populations (Patriarca et al. 2019). These questions are difficult to address due to a history of unsteady taxonomy in the genus *Alternaria* (Andrew et al. 2009; Woudenberg et al. 2015; Patriarca et al. 2019). Other *Alternaria* species were *A. chlamydospora* and *A. consortialis*, which were isolated as endophyte fungi only from healthy trees without symptoms with 41.38% and 6.89% frequencies frequency, respectively, did not cause any symptoms on oak seedlings after 60 days in greenhouse conditions. *A. consortialis* was previously placed in genus *Ulocladium* as *U. consortiale* (Woudenberg et al. 2013). It has shifted to the genus *Alternaria* by the mutli-gene phylogeny, and currently known as *A. consortialis* (Woudenberg et al. 2013).

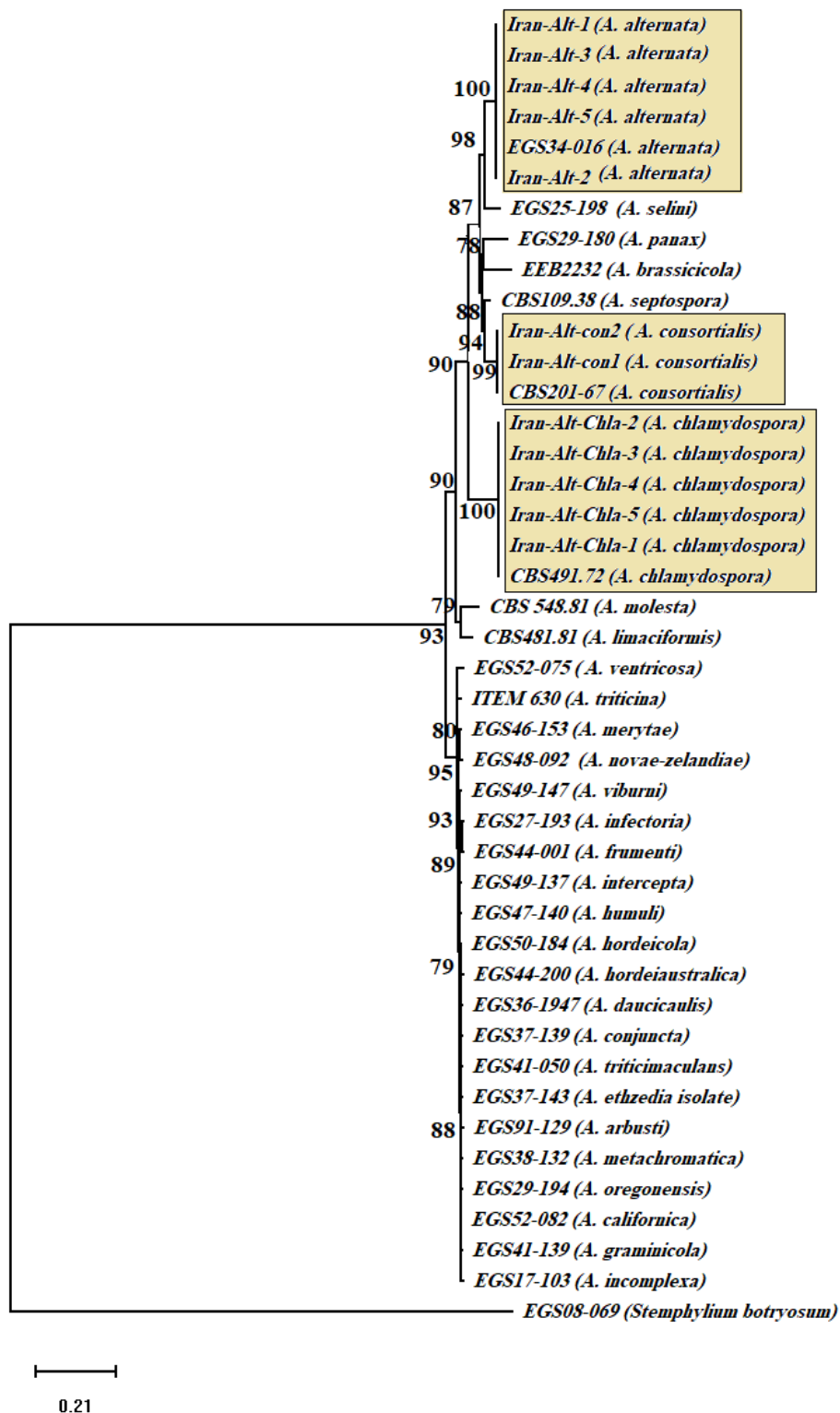


Fig. 2. Maximum likelihood phylogenetic tree inferred from of GAPDH and ATPase data set of 30 *Alternaria* species, nucleotide sequences of the current study were enclosed in a brown box. Bootstrap values (in %) are indicated at the nodes. *Stemphylium botryosum* is used as outgroup taxon. Scale bars indicate 0.21 changes per site per branch.

This species was formerly reported as an endophytic fungus of peach and apricot trees in Iran (Hashemlou et al. 2015). The *A. chlamydospora* species is a worldwide distributed soil fungus, which has been isolated from various kinds of soil (Ellis 1976), also as a cause of fungal skin and nail disease (Singh et al. 1990). This species has also been identified as one of the causes of mold in cereal grains (Skrmjar et al. 1999).

This is a new report of *Alternaria* species including *A. alternata*, *A. consortialis* and *A. chlamydospora* as endophytic fungi and are not pathogen on Persian oak trees in Zagros forests of Fars province, Iran.

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گونه‌های *Alternaria spp.* در ارتباط با بلوط ایرانی (*Quercus brantii*) در جنگل‌های زاگرس از استان فارس، ایران

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چکیده: بلوط از درختان جنگلی مهم در استان فارس از ایران می‌باشد. هدف از مطالعه حاضر، شناسایی قارچ‌های مرتبط با درختان بلوط سالم و در حال زوال می‌باشد. از اوایل شهریورماه تا اواخر اسفند ماه ۲۰۲۱ تا ۲۰۲۲، جدایه‌های قارچی از شاخه‌ها، تنه‌ها و برگ‌های درختان بلوط سالم و بیمار در استان فارس جمع‌آوری شدند. گونه‌های قارچی براساس ویژگی‌های ریخت‌شناختی و مولکولی (ژن‌های *GAPDH* و *ATPase*) شناسایی شدند. مطابق واکاوی‌های فیلوژنتیکی و ریخت‌شناختی، سه گونه، *Alternaria alternata*، *Alternaria consortialis* و *Alternaria chlamydospora* تشخیص داده شد. گونه *A. alternata* از بالاترین فراوانی نسبی برخوردار بود و از هر دو درختان بلوط سالم و در حال زوال جداسازی گردید. اما دو گونه *A. consortialis* و *A. chlamydospora*. فقط از درختان بلوط سالم جداسازی شدند. آزمون بیماری‌زایی نشان داد که هیچکدام از جدایه‌های قارچی قادر به آلوده کردن نهال‌های بلوط بعد از ۶۰ روز در شرایط گلخانه نمی‌باشند. این یک گزارش جدیدی از گونه‌های *A. alternata*، *A. consortialis* و *A. chlamydospora* به عنوان قارچ‌های درون‌رُست در استان فارس می‌باشد.

کلمات کلیدی: بلوط، قارچ‌های درون‌رُست، *Alternaria chlamydospora*، *Alternaria consortialis*