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Paecilomyces maximus as the causal agent of canker disease on *Eucalyptus camaldulensis* in Hormozgan Province, Iran

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Abstract

During a survey, a severe canker disease outbreak affecting *Eucalyptus camaldulensis* was found in various parts of Hormozgan Province, the south of Iran. The disease symptoms manifested through dark brown necrotic lesions, extensive cankers, longitudinal trunk cracks, wood discoloration, twig dieback, gummosis, and death. The disease incidence in the studied regions was 81%. This study aimed to characterize the disease, ascertain its etiological agent, and validate its pathogenicity using Koch's postulates. Fungal isolations from symptomatic tissues yielded colonies consistent with a *Paecilomyces* sp., subsequently identified as *P. maximus* based on morphological traits and molecular analysis of the internal transcribed spacer nrDNA region and β-tubulin (*TUB2*) gene. Pathogenicity tests demonstrated lesion development on healthy branches inoculated with mycelium plugs from *P. maximus*, confirming the association between the fungus and observed symptoms. Small brown lesions appeared on the inoculated branches during 15 to 18 days, proving that the fungus is the cause of the disease. *P. maximus* was only re-isolated from the inoculated symptomatic tissue. Notably, this study represents the first documentation of P. maximus causing canker disease on eucalyptus trees in Iran and globally.

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Introduction

The genus Eucalyptus belongs to the family *Myrtaceae* (*Myrtoideae*, *Eucalypteae*), containing more than 700 species of flowering plants. Species of this genus comprise trees and shrubs which are among the most important forest plants worldwide (Yu et al., 2009). *Eucalyptus* species are widely cultivated for their timber, pulp, and ornamental value; however, they are susceptible to various diseases that can significantly impact their health and productivity.

These species are known to be relatively tolerant to a range of undesirable environmental conditions, especially salinity and drought stresses. They are originally from Australia and grown as non-native plants in many tropical and subtropical countries throughout Africa, South America and Southeast Asia (Mugunga et al., 2015; Sadeghi et al., 2018). Some Eucalyptus species were imported to Iran more than 100 years ago and well adapted to the environmental and ecological conditions of the South of the country. They have been planted in semi-arid regions of Iran in the form of forestry projects and are considered one of the best options for wood cultivation in this area (Saadat et al., 2004; Asareh & Sardabi, 2007; Sadeghi et al., 2018). In Hormozgan Province, the southern province of Iran, *Eucalyptus* plantations consist of numerous species with *Eucalyptus camaldulensis* Dehn. as the most common species planted. Some of these species are industrially important, and others are grown in the urban green spaces, as ornamental trees for landscape design (Sadeghi et al., 2018). Canker is known as one of

the most common and important diseases of *Eucalyptus* in the world. The disease often involves most of the host species growing under unfavorable conditions and eventually results in the death of severely affected trees (Silva et al., 2015; Machua et al., 2016; Vitale et al., 2019). Eucalyptus canker was first reported by Wingfield et al. (1989) in South Africa, with *Cryphonectria cubensis* (Bruner) Hodges as the pathogen. Subsequently, several other species of fungi have been reported as the causal agents of Eucalyptus canker in other African countries as well as in South America and Southeast Asia, including *Botryosphaeria dothidea* (Moug.) Ces. & De Not. in South Africa and China (Smith et al., 1994; Yu et al., 2009), *Coniothyrium zuluense* M.J. Wingf., Crous & T.A. Cout. in South Africa, Ethiopia, Uganda, Hawaii, Mexico, Argentina, Vietnam and Thailand (Wingfield et al., 1996; Van Zyl et al., 1997; Roux et al., 2002; Gezahgne et al., 2003; Cortinas et al., 2004; Gezahgne et al., 2005), *Mycosphaerella aurantia* A. Maxwell, *M. heimii* Bouriquet ex Crous, *M. lateralis* Crous & M.J. Wingf., *M. scytalidii* Crous & M.J. Wingf., *Pseudocercospora norchiensis* Crous, *Teratosphaeria ohnowa* (Crous & M.J. Wingf.) Crous & U. Braun and *T. pluritubularis* (Crous & Mansilla) Crous & U. Braun in Uruguay (Perez et al., 2009) and *T. gauchensis* (M.-N. Cortinas, Crous & M.J. Wingf.) M.J. Wingf. & Crous in Italy, Portugal and Kenya (Silva et al., 2015; Machua et al., 2016; Vitale et al., 2019).

The genus *Paecilomyces* Bainier, belonging to the order *Eurotiales*, was first defined in 1907 as a genus closely related to *Penicillium* Link ex Fr., with only one species, *P. variotii* Bainier (Urquhart & Alexander, 2023). Currently, *Paecilomyces* comprises 145 species, both pathogens and saprobes, which have been isolated from a wide range of substrates, e.g. soils, composts, wood, marine litter, food, animals, insects and plants (Torabi et al., 2019, Moreno-Gavira et al., 2021). *Paecilomyces maximus* C. Ram has been described in 1968 based on its cultural characters and large-sized conidia (Ram, 1968). In 2009, sequencing of the ITS region and parts of the protein coding genes, β-tubulin (*TUB2*) and calmodulin (*CAL*), revealed that *P. formosus* (*nom. inval.*) may include three species, *P. formosus* Sakag., May. Inoue & Tada ex Houbraken & Samson, *P. lecythidis* C. Ram and *P. maximus*, which could not be distinguished by morphological characteristics (Samson et al., 2009), but subsequently, whole genome sequencing resolves the *P. formosus* clade into three distinct species (Urquhart & Idnurm, 2023). *Paecilomyces* species are important plant pathogens associated with canker and dieback diseases on a wide range of trees and shrubs with worldwide distribution. *Paecilomyces maximus* has been recently identified as a pathogen causing canker on apricot and pistachio in Turkiye (Ozan et al., 2022; Oren et al., 2023). In Iran, *Paecilomyces* species that have been associated with canker and dieback on trees include *P. variotii* on almond in Chaharmahal and Bakhtiari Province (Heidarian & Ershad, 1998) and pistachio in Khorasan Razavi Province (Alizadeh et al., 2000; Ghelichi et al., 2012) and *P. formosus* Urquhart on pistachio in Kerman, Yazd, Isfahan, Qom, Markazi, Qazvin, Tehran, Semnan, South Khorasan and Khorasan Razavi Provinces (Torabi et al., 2019), on apple in West Azarbaijan Province (Azizi et al., 2020) and on brook willow (*Salix acmophylla* Boss.), oak and Christ's thorn (*Paliurus spina-christi* Mill.) in Kermanshah Province (Rostami & Jamali, 2022; Rostami & Jamali, 2023; Ghaderi et al., 2024).

Recent surveys conducted in Hormozgan Province revealed an epidemic of a severe canker disease affecting *E. camaldulensis*. This paper aims to detail the disease characteristics, identify the fungal pathogen responsible, and establish its pathogenicity according to Koch's postulates.

Materials and Methods

Evaluation of the canker incidence

In June and July 2024, an epidemic of a serious canker on *Eucalyptus* trees was detected in five different regions of Hormozgan Province, including Bandar Abbas, Khamir Port, Lengeh Port, Minab and Roudan (Fig. 1, Table 1). The affected trees were estimated more than 10 years old. A total of 30 E. camaldulensis trees were randomly selected at five locations to calculate canker incidence, and 50 symptomatic trees were visually inspected to characterize disease symptoms.

Sample collection and fungal isolation

Samples were collected from bark and wood tissues of trunks, branches and twigs of *Eucalyptus* trees exhibiting disease symptoms. To isolate fungi, samples were washed under running tap water, cut into 5 × 5 mm pieces from the margin of necrotic and healthy tissues, surface-sterilized with 2% sodium hypochlorite solution for 5 min, rinsed again three times with sterile distilled water and aseptically dried on filter paper. The tissue pieces were plated onto Potato-Dextrose-Agar (PDA, Merck, Germany) medium supplemented with 150 µg ml−1 of streptomycin sulfate to inhibit bacterial growth. The plates were incubated at 27 °C in the dark for seven days and checked daily. Isolated fungal colonies were

purified based on the single spore method and used for further studies (Heidarian et al., 2018).

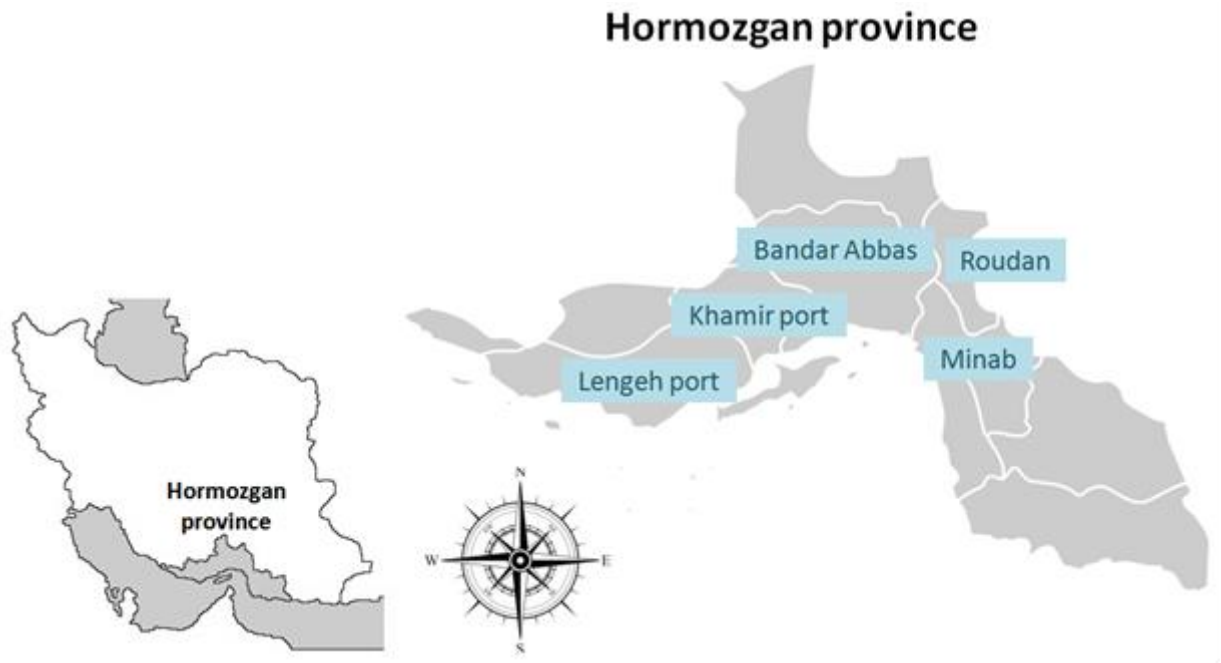

Fig. 1. Map of Hormozgan Province in the South of Iran showing the five surveyed regions.

Table 1. Geographic location, mean annual temperature (°C) and mean annual precipitation (mm) of the surveyed regions in this study

Region	Geographic location		Mean annual temperature (°C)	Mean annual precipitation (mm)
	N	*E*		
Khamir Port	26˚ 57’	55˚ 45’	33.4	211.1
Bandar Abbas	27˚ 11’	56˚ 17’	35.4	200
Minab	27˚ 07’	57˚ 06’	36.8	154.7
Lengeh Port	26˚ 33’	54˚ 54’	36.1	152.2
Roudan	27˚ 27’	57˚ 19’	37.7	124.2

Morphological identification

Pure isolates of *P. maximus* were examined for cultural and microscopic properties according to the description of Samson (1974) and Samson et al. (2009), with cultural properties including texture, color, and radial growth recorded after five days on PDA at 27 °C, colony color assessed using Rayner’s mycological color charts (Rayner, 1970), and microscopic characterization conducted on lactophenol slides made from 5-day-old colonies on WA at 27 °C, focusing on the shape and size of conidia, conidiophores / conidiogenous cells, and chlamydospores (n = 50).

Molecular phylogenetic analysis

Three *P. maximus* isolates were selected based on the morphological characteristics for sequencing. About 150 mg of mycelial mass was collected from 7-day-old colonies on Potato-Dextrose-Broth (PDB, Neogen, USA) medium and ground in liquid nitrogen. Genomic DNA was extracted based on CTAB protocol according to Zhong & Steffenson (2001). Partial sequence of the internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) and beta tubulin (*TUB2*) gene were amplified using the primer pairs ITS1/ITS4 and Bt2a/Bt2b, respectively (White et al., 1990; Glass & Donaldson, 1995). The PCR mixtures for all reactions included 10 ng/µL of genomic DNA, 0.4 µM of each primer, and 18 µL of reaction mix (Taq DNA polymerase 2× Master Mix Red, 2 mM MgCl2, Ampliqon, Denmark) in a total volume of 25 µL. Thermal conditions for PCR amplification of ITS and *β-tubulin* consisted of an initial denaturation step of 5 min at 95 °C, followed by 35 cycles of 40 s at 95 °C, 45 s at 56 °C (for ITS) and 57 °C (for *TUB2*), 60 s at 72 °C, and a final extension step of 7 min at 72 °C. The amplified products were purified and Sanger sequencing was carried out in forward and reverse directions with the same PCR primers at Microsynth AG (Switzerland). The obtained sequences

were trimmed manually in BioEdit v. 7.2.670 and consensus sequences were analyzed by BLASTN against previously deposited sequences in GenBank. Concatenated sequence dataset (ITS and *TUB2*) was produced in Mesquite v. 2.7472 and phylogenetic analyses were performed by using Bayesian Inference (BI) method with the species *Thermoascus crustaceus* CBS 181.67 as outgroup (Ronquist & Huelsenbeck, 2003). The combined sequences along with the sequences of closely related *Paecilomyces* species were aligned using the MAFFT v. 7 online service (Standley, 2013) and analyzed with MrModeltest v. 2.3 (Posada & Crandall, 1998) to select the best-fit base substitution model according to the Akaike Information Criterion (AIC). The Markov Chain Monte Carlo (MCMC) analysis was operated with two independent runs, four chains and 8 000 000 generations. Trees were saved each 1000 generations, 25% of the generated trees were discarded as the 'burn-in' phase and the remaining were used to determine posterior probabilities (PP). The GenbBank accession numbers of the isolates used in phylogenetic analysis are shown in Table 2.

Table 2. Isolates used in phylogenetic analysis. Taxa in bold were isolated and sequenced in the present study

Species	**Isolate**	**GenBank accession number**	
		ITS	*TUB2*
Paecilomyces maximus	**PM321**	**PQ305760**	**PQ308969**
Paecilomyces maximus	**PM752**	**PQ305761**	**PQ308970**
Paecilomyces maximus	**PM956**	**PQ305762**	**PQ308971**
Paecilomyces maximus	RU-QuBr-2	OR922491	PP025483
Paecilomyces maximus	DD54	OR501535	OR933703
Paecilomyces maximus	Pm-7	OR744819	OR757268
Paecilomyces maximus	WP2	OR574376	OR661290
Paecilomyces variotii	CBS 338.51	MH856886	FJ390007
Paecilomyces variotii	CBS 102.74	AY753328	EU037073
Paecilomyces variotii	CBS 110431	EU037054	EU037072
Paecilomyces lecythidis	CMW 18170	PP191151	PP197739
Paecilomyces lecythidis	CMW 18169	PP191150	PP197738
Paecilomyces lecythidis	CMW 18167	PP191149	PP197737
Paecilomyces formosus	DTO 49D6	GU968655	GU968691
Paecilomyces formosus	DTO 49D5	GU968654	GU968690
Paecilomyces formosus	DTO 63F4	GU968673	GU968688
Paecilomyces fulvus	CCF 3236	LR983928	LR983941
Paecilomyces niveus	SKI-4	OR488837	OR805346
Paecilomyces dactylethromorphus	CMW 18163	PP191146	PP197734
Paecilomyces dactylethromorphus	CMW 18162	PP191145	PP197733
Thermoascus crustaceus	CBS 181.67	MH858941	MN969423

Pathogenicity trials

The pathogenicity of three morphologically and molecularly identified *P. maximus* isolates was tested on detached branches from healthy *E. camaldulensis* trees, which were washed, surface-disinfested with 70% ethanol, and longitudinally cut to expose the wood; mycelial plugs (5 mm in diameter) were taken from the margins of 7-day-old *P. maximus* colonies on PDA and placed in the wounds, while control samples received sterile PDA plugs, all inoculation sites were sealed with Parafilm (American National Can, Greenwich, CT) and covered with sterile moist cotton towels, with each isolate inoculated on five branches and the experiment repeated twice, after which all branches were stored in a moist chamber at 27 °C and 80% relative humidity, monitored daily for disease symptoms, and wood discoloration length was measured after 30 days, with fungal re-isolation performed from all branches to confirm Koch's postulates and recovery of isolates identified as previously described (Goudarzi & Moslehi, 2020).

Results

Disease incidence and symptoms

During field surveys across different regions, canker symptoms were observed in an average of 81% of the

inspected *E. camaldulensis* trees, predominantly on mechanically damaged or weakened trees affected by unfavorable environmental factors (Fig. 2A); initial symptoms of infection presented as dark brown, measles-like necrotic lesions on branches and trunks that developed and coalesced into cankers (Fig. 2B, C), resulting in dark discoloration of wood and inner bark tissues (Fig. 2D), while trunks with large cankers and longitudinal cracks appeared malformed and burnt (Fig. 2E-G), with variable levels of dieback observed on twigs and branches (Fig. 2H), and in almost all infected trees, dark red to black exudates flowed down over the cankers like veins (Fig. 2I, J), leading to tree death in cases of extensive cankers that girdled the trunks (Fig. 2K, L).

Fig. 2. *Paecilomyces* canker disease on *Eucalyptus camaldulensis* in five different regions of Hormozgan Province. A) Canker symptoms on a mechanically damaged tree; B-C) Dark brown measles-like necrotic lesions on the trunks; D) Dark discoloration of wood tissue; E-G) Trunks with large cankers, longitudinal cracks and malformed and burnt appearance; H) Dieback of twigs and branches; I, J) Flow down of dark red to black exudates over the cankers; K, L) Girdling cankers on branch and trunk.

Fungal isolation and morphological identification

Totally, 1358 fungal isolates with identical cultural characteristics were recovered from the symptomatic samples of *E. camaldulensis*. The highest percentage of fungal isolation was obtained from the samples of Roudan (79.8%) followed by Minab (76.9%), Lengeh Port (76.6%) and Bandar Abbas (72.8%), and the lowest percentage of isolation (70.5%) were recorded for Khamir Port. The morphological features of the isolated fungi were in accordance with *Paecilomyces* (Samson, 1974; Samson et al., 2009). The isolates were characterized by fast growth on PDA, reaching 3.5 cm within three days at 27 °C. The colonies had a cottony texture, tufted or funiculose, initially white to yellowish-white, quickly turning yellow to pale olive green at the center as the conidia produced (Fig. 3A). The colony reverse was pale brown. Microscopical examination showed regularly branched, *Penicillium*-like conidiophores, bearing flask-shaped phialides with a subglobose to ellipsoidal basal portion, tapering to an elongated cylindrical neck (Fig. 3B, C). Occasionally, the phialides were solitarily arisen from hyphae. Conidia were one-celled, ellipsoidal to clavate, with truncate ends, hyaline to olive-brown, smooth-walled, 1.8-3.5 × 3-10 µm, produced in long divergent chains (Fig. 3D-F). Chlamydospores were present, singly, hyaline to pale olive green, subspherical to pyriform, smooth thick-walled, formed on short stalks (Fig. 3G). Ascomata were absent on PDA, though ascomatal initials could be observed as coils (Fig. 3H).

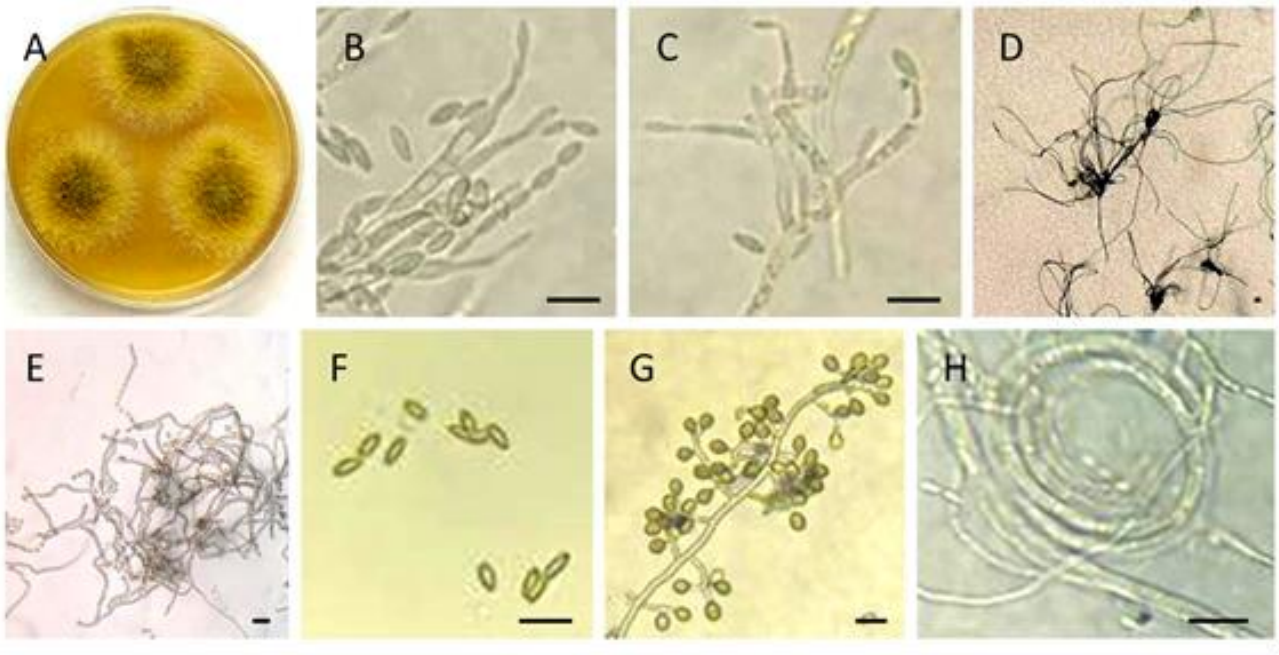

Fig. 3. *Paecilomyces maximus* PM321 isolated from *Eucalyptus camaldulensis* showing canker symptoms. A) Cultural morphology of a 3-day-old culture on PDA incubated at 27 °C; B, C) Phialides; D, E) Conidia in long divergent chains; F) Conidia; G) Chlamydospores; H) Ascoma initials; Scale bars = 10 µm

Molecular phylogenetic analysis

Identification of three *Paecilomyces* isolates obtained in this study was confirmed at the molecular level by partial amplification and sequencing of the ITS region and *β-tubulin* gene. Using the primers ITS1/ITS4 and Bt2a/Bt2b, DNA fragments, approximately 550 and 450 bp, were amplified, respectively. BLAST search of the sequences revealed 100% identity to several *P. maximus* strains in GenBank. The best-fit model of base substitution, K80+G, was selected, according to the Akaike criterion (AIC) and used for Bayesian inference analyses. Phylogenetic tree based on the combined sequences of ITS and *TUB2* of the isolates PM321, PM752 and PM956 as well as 17 selected isolates of *Paecilomyces* from GenBank (Table 2) revealed that our isolates are closely related to *P. maximus*. All three *Paecilomyces* isolates clustered together and constructed a distinct and well-supported clade with the *P. maximus* strains, separately from the other *Paecilomyces* species (Fig. 4). The sequences obtained in this study were deposited in GenBank with the accession numbers PQ305760 (ITS-PM321), PQ305761 (ITS-PM752), PQ305762 (ITS-PM956), PQ308969 (*TUB2*-PM321), PQ308970 (*TUB2*-PM752) and PQ308971 (*TUB2*-PM956).

Pathogenicity trials

The pathogenicity trials revealed that all three *P. maximus* isolates were pathogenic on the detached branches of *E. camaldulensis*. The inoculations resulted in the appearance of small brown lesions in the wood tissue, during 15 to 18 days. The lesions were developed into larger necrotic area upward and downward the inoculation sites which became dark in color, similar to those observed in the fields. The mean length of the necrotic tissues was 13.8 cm, 30 days after inoculation. No disease symptoms appeared on the control branches, up to the end of the trials (Fig. 5). Fungal isolates were reisolated only from the inoculated branches, though isolations from the controls failed to recover fungi, confirming Koch's postulates. The recovered fungi were morphologically and molecularly identified as *P. maximus*..

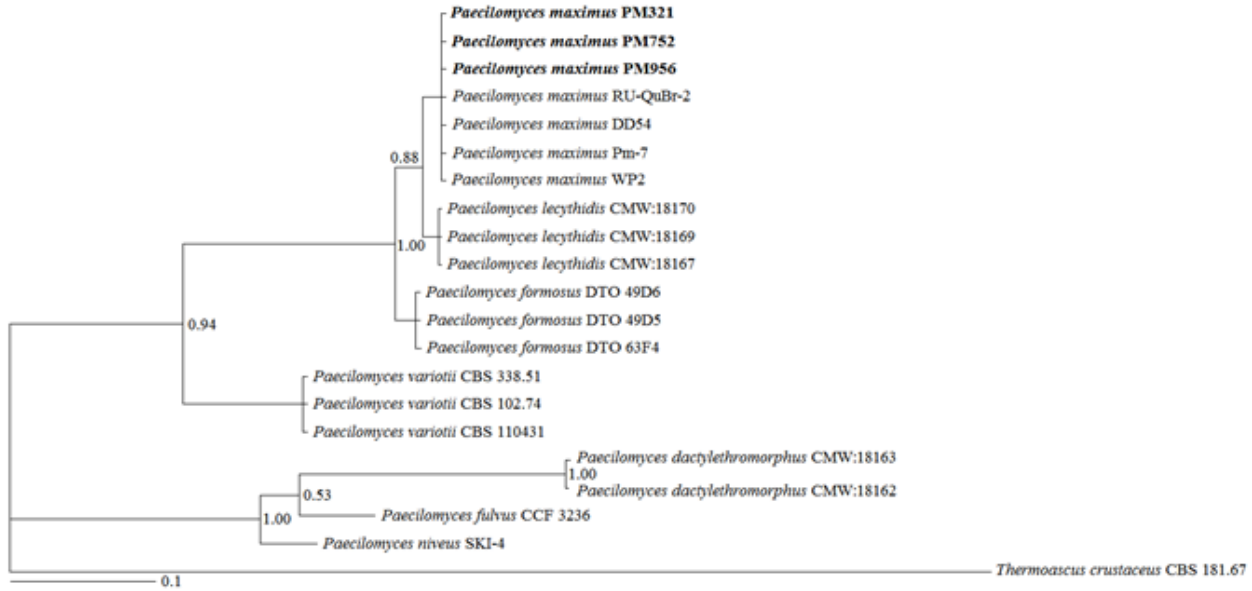

Fig. 4. Phylogeny of *Paecilomyces maximus* isolates inferred from Bayesian analysis using combined sequence data of the ITS region and *TUB2* gene. The isolates PM321, PM752 and PM956 that were obtained in this study are indicated in bold.

Fig. 5. Necrosis and dark discoloration of the wood tissue of *Eucalyptus camaldulensis* detached branches, 30 days after inoculation with *Paecilomyces maximus*, A) PM321 isolate; B) PM752 isolate; C) PM956 isolate; D) Control.

Discussion

The aim of this paper is to describe a significant canker on *E. camaldulensis* in Hormozgan Province, Southern Iran, and the fungus responsible for the disease. Based on the results, the fungal species *P. maximus* was consistently isolated from the symptomatic tissues of *E. camaldulensis* and confirmed as the pathogen for the epidemic. Although we did an extensive survey in all the studied regions, no other fungi were isolated from the diseased trees. Previous studies have shown that canker and dieback are serious diseases in several tree species in southern Iran (Goudarzi & Moslehi, 2020; Majidi et al., 2021). These diseases have increased with climate change during the past decades and threaten forests and commercial fruit plantations in this area (Goudarzi & Moslehi, 2020). Precipitation and temperature are considered as the most important climatic elements of any region. Hormozgan Province, a subtropical region of Iran, has seriously suffered from reduced rainfall and high temperatures over the last several years. In tropical and subtropical regions, environmental stresses are crucial issues and play important roles in the predisposition of plants to infection by pathogens (Goudarzi & Moslehi, 2020). In the same context, it has been demonstrated that *Paecilomyces* species are more virulent on drought-stressed trees (Heidarian et al. 2018). Investigations in five different regions of Hormozgan Province revealed that more than 80% of the inspected Eucalyptus trees were showing symptoms of a severe canker as well as twigs and branches dieback. The high incidence of the disease in all the surveyed regions may result from the

fact that the eucalyptus trees have been predisposed under the influence of some adverse factors of the environment. Based on our assessment, there were differences in the percentage of *P. maximus* isolation among the five sampling regions. In Roudan, the isolation percentage of the fungus was considerably higher than that of other regions. In this region, certain environmental factors including high mean annual temperature and low mean annual precipitation may increase colonization of the host tissues by the pathogen. On the other hand, *P. maximus* is known as a thermophilic species which grows fast at temperatures above 35 °C. In several studies, thermophilic members of *Paecilomyces* have been considered important threats to forest plantations where climate change is occurring (Sabernasab et al., 2019; Rostami & Jamali, 2022; Rostami & Jamali, 2023; Urquhart & Alexander, 2023).

Phylogenetic analyses based on DNA sequence data showed that the representatives of all 1358 isolates belong to *P. maximus*. It is well known that morphological observations or molecular analysis based on the ITS region alone are not sufficient for identification of *Paecilomyces* species and sequence comparison of the protein-coding genes such as β-tubulin and calmodulin is needed to distinguish these species (Samson et al., 2009; Sabernasab et al., 2019). ITS region and part of the *TUB2* gene have shown sufficient interspecific variations for reliable identification of *Paecilomyces* strains in clinical samples (Houbraken et al., 2010). Similarly, combined sequence data of ITS and *TUB2* distinctly placed our isolates in a separate clade with *P. maximus* strains from GenBank.

The results of the pathogenicity trials demonstrated that the *P. maximus* isolates obtained in this study were pathogenic on detached branches of *E. camaldulensis*. All three isolates caused wood discoloration and necrosis after 15 to 18 days which is extended upward and downward the inoculation sites. Different species of *Paecilomyces*, including *P. formosus*, *P. maximus* and *P. variotii*, have been identified as pathogens causing canker and dieback on a wide range of fruit and forest trees (Heidarian & Ershad, 1998; Alizadeh et al., 2000; Ghelichi et al., 2012; Torabi et al., 2019; Azizi et al., 2020; Ozan et al., 2022; Rostami & Jamali, 2022; Oren et al., 2023; Rostami & Jamali, 2023; Ghaderi et al., 2024). *Paecilomyces maximus* is only reported from apricot and pistachio as canker and dieback pathogen in Turkey (Ozan et al., 2022; Oren et al., 2023). In the previous studies conducted in different provinces of Iran, only *P. variotii* and *P. formosus* have been reported as causal agents of dieback, canker and decline diseases on fruit and forest tree species, including almond, apple, brook willow, Christ's thorn, oak and pistachio (Alizadeh et al., 2000; Azizi et al., 2020; Ghaderi et al., 2024; Ghelichi et al., 2012; Heidarian et al., 1998; Rostami and Jamali, 2022; Rostami and Jamali, 2023; Torabi et al., 2019). To our knowledge, this is the first report of *P. maximus* as the cause of canker disease on tree species in Iran and the first report of *Paecilomyces* eucalyptus canker worldwide. The occurrence of *E. camaldulensis* canker in southern Iran is of considerable concern given its known impact in other countries in Africa, South America and Southeast Asia. The high distribution of *P. maximus* in the studied regions shows that this fungus is well adapted to the environmental conditions of Southern Iran and may have very serious repercussions on other tree species in this area.

This research is the first report of *P. maximus* as the causative agent of canker disease on *E. camaldulensis* in Iran and the first global report of such a disease associated with *Paecilomyces*. Further studies are needed to understand the epidemiology and management of this emerging threat.

Conclusion

This study provides critical insights into the emergence of *P. maximus* as a significant pathogen of *E. camaldulensis* in Iran. The findings underline the importance of monitoring Eucalyptus diseases and implementing appropriate management strategies to mitigate future outbreaks.

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Conflict of interest

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

CRediT author statement

A. Goudarzi: Supervision, methodology, writing, reviewing & editing. **S. Kouchaki Hasankiadeh** and **H. Shabdar:** Field and laboratory works. **A. Bagheri:** reviewing**. S. S. Modarres Najafabadi:** reviewing.

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