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Is *Macrophomina phaseolina* an emerging threat in Turkish forest nurseries?

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ABSTRACT **Research Article**

Background and Aims Macrophomina phaseolina is a globally significant soil-borne pathogen known for its wide host range and persistence under adverse conditions. This study reports a notable outbreak of *M. phaseolina* in a forest nursery in Osmaniye, southeastern Türkiye, with implications not only for seedling health and nursery management but also for field plantations, as the pathogen can be carried from nursery stock to planting sites.

Methods Healthy-looking, symptomatic and dead *Pinus brutia* and *Pinus pinea* seedlings were sampled from the nursery. Fungi were isolated from roots and stems of the seedlings. Isolates were identified morphologically and confirmed by ITS sequencing.

Results M. phaseolina was isolated from 50.4% of the seedlings, with higher frequencies in *P. pinea* (65.0%) compared to *P. brutia* (40.5%). Isolation rates were highest in dead seedlings (61.8%), followed by symptomatic ones (56.0%). Notably, the fungus was isolated from 62.9% of healthy-looking *P. pinea* seedlings, indicating widespread latent infections and a risk of undetected pathogen presence in asymptomatic nursery stock.

Conclusion The high isolation frequency and detection in asymptomatic seedlings signal *M. phaseolina* as an emerging threat to Turkish forest nurseries and raise concerns about nursery-to-field carryover and long-term impacts on plantation success. This aligns with recent global observations linking climate change to the rising prevalence of thermotolerant pathogens such as *M. phaseolina*. Considering its broad host range, persistence in soil and climate adaptability, *M. phaseolina* warrants increased attention in forest nurseries. To reduce potential losses in forest nurseries and mitigate nursery-to-field carryover risks, integrated disease monitoring and adaptive nursery practices are essential.

Key Words: Charcoal root rot, climate change, emerging forest pathogens, soilborne pathogens, forest nursery diseases

Macrophomina phaseolina Türkiye orman fidanlıklarında yükselen bir tehdit mi?

ÖZ

Giriş ve Amaçlar Macrophomina phaseolina, geniş konukçu yelpazesi ve olumsuz çevre koşullarına karşı dayanıklılığı ile bilinen, küresel ölçekte önemli bir toprak kökenli patojendir. Bu çalışma, Türkiye'nin güneydoğusunda yer alan Osmaniye orman fidanlığında *M. phaseolina*'nın neden olduğu bir salgını rapor etmekte ve bu patojenin yalnızca fidan sağlığı ve fidanlık yönetimi açısından değil, aynı zamanda fidanlıklardan plantasyon sahalarına taşınabilme riski nedeniyle sahadaki ağaçlandırmalar açısından da önemli sonuçlar doğurabileceğini vurgulamaktadır.

Yöntemler Sağlıklı görünümlü, simptomatik ve ölü *Pinus brutia* ve *Pinus pinea* fidanları örneklenmiş ve fidanların kök ve gövde dokularından fungal izolasyonlar yapılmıştır. Elde edilen izolatlar morfolojik olarak tanımlanmış ve ITS dizi analizleri ile teşhis doğrulanmıştır.

Bulgular M. phaseolina fidanların %50,4'ünden izole edilmiştir; bu oran *P. pinea*'da %65,0 ile *P. brutia*'ya kıyasla (%40,5) daha yüksektir. İzolasyon sıklığı ölü fidanlarda %61,8, simptomatik fidanlarda %56,0'dır. Fungusun, asimptomatik *P. pinea* fidanlarından izolasyon sıklığı %62,9 olup, bu durum latent enfeksiyonların yaygın olduğunu ve asimptomatik fidanlarda patojenin fark edilmeden taşınma riskinin yüksek olduğunu göstermektedir.

Sonuç M. phaseolina'nın yüksek izolasyon sıklığı ve asimptomatik fidanlardaki varlığı, bu patojenin Türkiye orman fidanlıklarında yükselen bir tehdit olabileceğine işaret etmekte ve fidanlıktan sahaya taşınma riski ve plantasyon başarısı üzerinde olası uzun vadeli etkileri konusunda ciddi kaygılar doğurmaktadır. Bu bulgular, *M. phaseolina* gibi termotolerant patojenlerin iklim değişikliğiyle birlikte artan yaygınlığına dair küresel gözlemlerle örtüşmektedir. Geniş konukçu yelpazesi, toprakta kalıcılığı ve iklim uyumu göz önüne alındığında, *M. phaseolina* orman fidanlıklarında daha fazla dikkat edilmesi gereken bir patojen olarak ön plana çıkmaktadır. Olası kayıpları azaltmak ve sahaya taşınma riskini en aza indirmek için entegre hastalık izleme sistemleri ve iklime uyarlanmış fidanlık uygulamaları büyük önem taşımaktadır.

Anahtar Kelimeler: Kömür çürüklüğü, iklim değişikliği, yeni ortaya çıkan orman patojenleri, toprak kökenli patojenler, orman fidanlığı hastalıkları

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1. Introduction

The warm-climate pathogen *Macrophomina phaseolina* (Tassi) Goid. (Botryosphaeriaceae) is a primarily soil-borne opportunistic plant pathogen present all over the world, affecting more than eight hundred herbaceous and woody plants in nurseries, field or in the wild (Khan and Javaid, 2023). It causes diseases such as stem and root rot, dry root rot, charcoal or crown root rot and seedling blight in a wide range of crops including seedlings of ornamental plants and forest trees in nurseries (Barnard, 1994; Farag et al., 2025; Gonthier and Nicolotti, 2013; Kumar and Dubey, 2023; Marquez et al., 2021; Reuveni and Madar, 1985). This necrotrophic fungus, a member of the Botryosphaeriaceae family, is widely distributed in tropical and subtropical regions and can infect over 800 plant species across more than 100 families (Farag et al., 2025; Kaur et al., 2012; Kumar and Dubey, 2023; Marquez et al., 2021). The fungus has a vast geographical distribution and is especially problematic in tropical and subtropical countries with arid to semiarid climates in Africa, Asia, Europe and North and South America (Kaur et al., 2012). *M. phaseolina* survives in the soil as microsclerotia for extended periods up to 15 years allowing it to persist under harsh environmental conditions and serve as a primary inoculum source. It is most aggressive under high temperatures and low soil moisture, conditions that are increasingly common in regions affected by drought and climate extremes (Farag et al., 2025; Kaur et al., 2012; Ranjan et al., 2024).

While *M. phaseolina* is well known as a root and collar rot pathogen in agricultural systems, in forestry it remains relatively less recognized. Nevertheless, it represents a serious threat, particularly in forest tree seedling production, where it causes charcoal root rot and seedling blight. Despite its global presence, reports of *M. phaseolina* in forest nurseries remain relatively scarce compared to other damping-off and root rot agents like *Phytophthora*, *Fusarium*, and *Rhizoctonia* species, particularly in European contexts (Gonthier and Nicolotti, 2013). Nevertheless, *M. phaseolina* can reduce seedling vigour and survival and lead to high mortality rates in nurseries and carryover effects after out planting (Barnard, 1994), undermining reforestation and afforestation efforts globally. The impact of the pathogen is particularly significant in southern forest nurseries in the United States, especially those that grow loblolly pine (*Pinus taeda* L.). In contrast, the occurrence of *M. phaseolina* in Europe is generally lower and more localized, with Mediterranean countries exhibiting greater susceptibility due to more favourable climatic conditions (Farag et al., 2025). Charcoal root rot caused by *M. phaseolina* was considered a serious problem in bare-root conifers in nurseries causing approximately 30% mortality in the southwestern United States (Fraedrich & Smith 1994). In Israel, the pathogen was identified as the primary cause of seedling mortality in several pine species, with disease outbreaks strongly associated with summer heatwaves and moisture stress (Reuveni and Madar, 1985). Similar findings have been reported in the United States, where *M. phaseolina* was shown to reduce survival of loblolly pine seedlings after out planting, having been carried over from contaminated nursery stock (Barnard, 1994). The pathogen has been also reported to cause significant loses to eucalypts, locusts, and other woody species in forest nurseries in various countries such as India, Italy, Chile and Iraq, underscoring its broad host range and ecological plasticity (Arias et al., 2013; Haleem et al., 2018; Prota et al., 1974; Reuveni and Madar, 1985; Soni et al., 1985).

In Türkiye, *M. phaseolina* has been recognized in numerous crops for decades; however, its impact on forest nurseries remains inadequately documented. Various studies have examined diseases affecting tree and woody ornamental plant seedlings in state forest nurseries in Türkiye (Aday Kaya, 2014; Aday Kaya et al., 2019; Aday Kaya and Beram, 2021; Akıllı et al., 2010; Aktaş and Şimşek, 2014; Doğmuş and Doğanoğlu, 2003; Gümüşdere and Ercan, 1984; Gürer, 2000; Kurt, 2011; Kurt et al., 2018, 2016; Kurt and Soylu, 2011; Lehtijärvi et al., 2017; Özdamar, 1999; Soylu et al., 2011; Sümer, 1987; Turhan and Özdamar, 1999; Uysal et al., 2021; Vural et al., 1984; Vural, 1989; Yeşilbaş and Özkazanç, 2014). Among the common damping off and root rot pathogens identified to date in these studies include *Fusarium* L. spp., *Rhizoctonia solani* J.G. Kühn, *Pythium* Pringsh spp., *Phytophthora* de Bary, spp., *Alternaria* Nees. spp. *Ilyonectria destructans* (Zinssm.) Rossman, L. Lombard & Crous *(Syn: Cylindrocarpon destructans* (Zinssm.) Scholten), *Verticillium dahlia* Kleb., and *Macrophomina phaseolina* (Tassi) Goid. According to these studies, *M. phaseolina* has been detected in Turkish forest nurseries in various forest tree and ornamental plant seedlings, including Turkish pine (*Pinus brutia* Ten), Anatolian black pine (*Pinus nigra* J. F. Arnold), stone pine (Pinus *pinea* L.), Taurus cedar (*Cedrus libani* A. Rich.) *Catalpa* Scop. (Özdamar, 1999; Turhan and Özdamar, 1999), *Buxus* L., *Salix* L., *Lavandula* L. *Nerium oleander* L., *Chamaecyparis* Spach, *Rosa* L., *Salvia rosmarinus* Spenn. and *Cryptomeria japonica* (Thunb. ex L.f.) D.Don (Uysal et al., 2021) (Table S1). Nevertheless, this fungus has not been detected in many other studies conducted in forest nurseries (Aday Kaya, 2014; Aday Kaya et al., 2019; Akıllı et al., 2010; Gürer, 2000; Vural et al., 1984) (Table S1).

Forest nurseries are essential for the regeneration of natural ecosystems and the success of large-scale afforestation and reforestation programs. In Türkiye, state nurseries annually produce hundreds of millions of seedlings to support national forestry goals. Among the most commonly cultivated species are conifers, such as the Turkish pine (*P. brutia*) and the stone pine (*P. pinea*) (General Directorate of Forestry, 2023). Forest nurseries are increasingly vulnerable to emerging soilborne pathogens, particularly under the pressures of climate change. Among these pathogens, *M. phaseolina* has drawn growing attention for its global prevalence and destructive potential. In Türkiye, *M. phaseolina* currently shows high habitat suitability and poses a significant risk to various crops, especially under the prevailing warm and dry conditions that favour its development. Climate models and distribution analyses indicate that Türkiye is among the Mediterranean and southern European regions with very high suitability for this pathogen, consistent with its known presence and impact on crops such as soybean, sorghum, and cereals (Farag et al., 2025). The climatic shifts create optimal conditions for *M. phaseolina*'s growth and infection cycles, potentially increasing disease incidence and severity in forest nurseries and plantations. Given Türkiye's extensive Mediterranean climate zones and its importance as a centre for pine forestry, understanding the current and future risks posed by *M. phaseolina* is critical for sustainable forest management.

This study reports the isolation of *Macrophomina phaseolina* from *P. brutia* and *P. pinea* seedlings in a Turkish forest nursery, where the pathogen was found at unexpectedly high frequencies. This finding is significant as it highlights a potentially emerging threat to pine seedling production in the region, which has been underrecognized relative to other root rot pathogens. The study aims to characterize the occurrence of *M. phaseolina* in this context, discuss its implications under ongoing climate change, and emphasize the need for increased surveillance and targeted management strategies in Mediterranean forest nurseries. Addressing this knowledge gap is essential to mitigate the pathogen's impact on pine regeneration and to safeguard forestry productivity in Türkiye and similar Mediterranean environments. The findings contribute to a growing body of international research that links *M. phaseolina* outbreaks with climate-induced stress in forest systems (Farag et al., 2025; Marquez et al., 2021) and call for integrated disease management strategies suited to Mediterranean nursery environments.

2. Materials and methods

2.1 Sampling

In early June 2021, bare-root seedlings of *P. pinea* and *P. brutia,* approximately six months old, were collected from the Osmaniye forest nursery in Osmaniye in South-eastern Türkiye (37°3'57.76"N, 36°14'8.81"E). A minimum of 100 seedlings per species was sampled from affected seedbeds, including healthy samples as well as those that were dead or showing symptoms of disease.

2.2 Morphological diagnosis

Sampled seedlings were visually grouped into four symptom severity classes based on crown symptoms: (a) healthy-looking (no crown symptoms), (b) symptomatic-low (chlorosis or top dieback ≤25% of crown), (c) symptomatic -high (chlorosis or top dieback >25% of crown), and (d) dead. After grouping, stem, root collar and roots were examined for the presence of signs such as pycnidia or microsclerotia of pathogenic fungi using a dissecting microscope (10x magnification) and compound microscope (40x).

2.3 Fungal isolations

From each symptom class, three to five seedlings per host were randomly selected for isolations. Prior to surface sterilization, the needles were removed, and the stems and roots of the seedling were washed under running tap water. The whole stem + root tissues were then rinsed in 4.28% sodium hypochlorite (NaOCl) solution for ten minutes, followed by two washes in sterile distilled water, and then dried on sterile filter papers. The tissues were aseptically cut into 1–2 cm fragments (five fragments/seedling), plated on 90 mm Ø Petri dishes containing potato-dextrose agar (PDA) amended with 250 mg/L streptomycin, and sealed with parafilm. The plates were incubated at 22°C in the dark for ten days. Emerging colonies were counted and subcultured onto fresh PDA, and then incubated at 22°C for ten days. The isolates were identified based on colony morphology, including colour, media pigmentation, and the presence of microsclerotia.

Macrophomina phaseolina was identified morphologically by characteristic black microsclerotia (50–200 µm) observed in culture (Barnard, 1994). Other fungi or oomycetes (i.e., *Fusarium* spp., *Pythium* sp., *Alternaria* spp.) were also identified morphologically. Isolation frequency was determined as the percentage of fragments yielding each fungus per host species and symptom class.

2.4 Molecular identification

Two isolates (TRAPB1 and TRAPB2) with morphological features of *M. phaseolina* were selected for molecular identification. Isolates were subcultured onto fresh PDA and incubated in dark at room temperature for 20 days. Approximately 50 mg mycelium from the colony edge was transferred to 2.0 ml micro centrifuge tubes, homogenized, and DNA was extracted using the GeneMATRIX Plant & Fungi DNA Purification Kit (EURX®, Poland), following the manufacturer's instructions.

The PCR assay was performed in a total volume of 35 µL containing 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.3 mM of each primer and 2 U DNA Polymerase (Solis Biodyne FIREPol® DNA Polymerase, Estonia) and 3 µl of genomic DNA using PCR thermocycler (Kyratec, SuperCycler Thermal Cycler, Australia). The thermal cycling conditions were as follows: an initial denaturation step at 95 °C for 5 minutes, 30 cycles of 95 °C for 45 seconds, 57 °C for 45 seconds, and 72 °C for 60 seconds, followed by the final extension step at 72 °C for 5 minutes. Amplicons were cleaned using the "HighPrep™ PCR Clean-up System (MagBio Genomics, Gaithersburg, MD, USA) according to manufacturer's instructions and sent to BM (Ankara, Turkey) for sequencing. Sequences were manually edited and aligned using BioEdit version 7.2.5.

2.5 Climate Data

Long-term climate data and climatic classification of the study site (Osmaniye) were retrieved from Turkish State Meteorological Service (Meteoroloji Genel Müdürlüğü, 2025). Köppen-Geiger Classification was obtained from free global dataset CHELSA (Climatologies at high resolution for the earth's land surface areas) (Karger et al., 2017). Meteorological Drought analysis for the year 2021 as presented in "2021 Climate evaluation report" (Meteoroloji Genel Müdürlüğü, 2022) were also examined. Long-term climate averages (1991-2020 and 1987–2021 periods) were compared to analyse 2020-2021 growing seasons trends.

3. Results

3.1 Morphological diagnosis

Observed disease symptoms included yellowing of the needles, followed by wilting and death of the seedlings. Swellings, blackening and cracking of cortical tissues at root collar extending to stem were observed. Abundant black microsclerotia were present within and beneath the cracked cortical tissues (Figure 1). On healthy-looking *P. brutia* seedlings, swelling or cracking of cortical tissues was not observed. No microsclerotia was observed on healthy-looking seedlings. Root rot symptoms included discoloration and loss of fine feeder roots. On *P. pinea* seedlings with green, healthy-looking crowns, root rot symptoms were also observed.

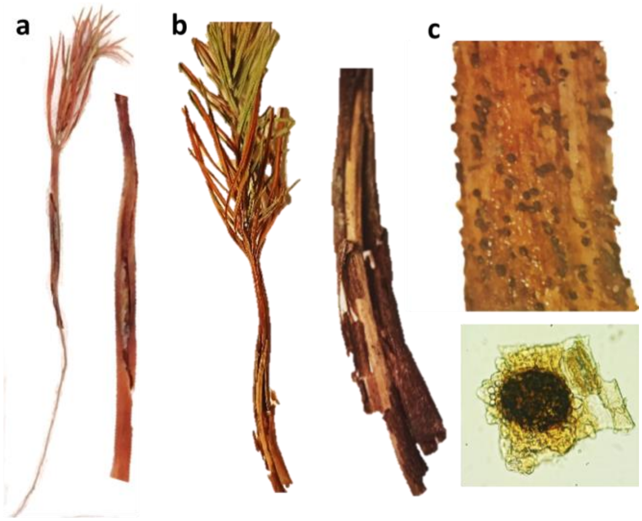

Figure 1. Swellings, blackening and cracking of cortical tissues at root collar extending to stem on dead (a) and symptomatic (b) seedlings and abundant black microsclerotia beneath the cracked cortical tissues (c)

3.2 Fungal isolates

From the root + stem fragments of *P. brutia* and *P. pinea* a total 248 fungal colonies were isolated. 50.4% of these were characterized by black fast-growing dark colonies with abundant microsclerotia production in media (Figure 2). Based on morphological characteristics, this continuously isolated fungus was identified as *M. phaseolina*.

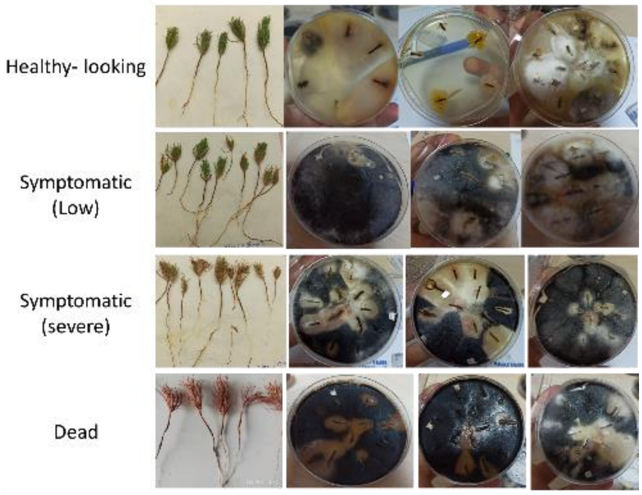

Figure 2. Fungal colonies growing from root and stem fragments of; healthy-looking, symptomatic, and dead *Pinus brutia* seedlings

The isolation frequency (%) of *M. phaseolina* from *P. brutia* and *P. pinea* seedlings with differing symptom severities (symptom classes) ranged from 0 to 69.4% (Table 1). The fungus was isolated from 49.3 and 61.5% of symptomatic and dead *P. brutia* seedlings respectively. The isolation frequency from symptomatic and dead *P. pinea* seedlings was higher than those of *P. brutia* (69.4, 62% respectively). The fungus was also isolated from the root and stems of healthy-looking *P. pinea* seedlings (63%), contrary to this, *M. phaseolina* was not isolated from healthy-looking *P. brutia* seedlings.

Table 1. Isolation frequency (%) of *Macrophomina phaseolina* from *Pinus brutia* and *Pinus pinea* seedlings across symptom classes

Symptom classes	*Pinus brutia*	*Pinus pinea*	Total
Healthy-looking seedlings	0.00	62.86	30.99
Symptomatic seedlings	49.32	69.44	55.96
Dead seedlings	61.54	62.07	61.76
Total	40.54	65.00	50.40

Other fungi isolated from the tissues, primarily from root fragments, included *Fusarium* spp. (5.2%, 13 colonies) and *Alternaria* spp. (2.8%, 7 colonies). Additionally, an oomycete identified as *Pythium* sp. was also isolated (3.6%, 9 colonies). Bacterial colonies were observed as well (8.1%, 20 colonies), predominantly from the roots, although further identification was not conducted.

3.3 Molecular Identification

To confirm the identification, the internal transcribed spacer (ITS) of rDNA of two representative isolates (TRAPB1 and TRAPB2) were amplified and sequenced. The resulting 537-bp sequence shared 99% similarity to those of *M. phaseolina* isolates CBS-231.33, CBS-457.70 and CBS-126630 (KF951631, KF951636 and MH864176, respectively) in the GenBank database.

3.4 Climate Conditions

The climatic classification of the study site in Osmaniye, derived from five systems (Aydeniz, Erinç, De Martonne, Trewartha, and Thornthwaite; see Table 2) sourced from the Turkish State Meteorological Service (2025), categorizes the region as semi-humid to semi-arid. It is characterized by hot summers marked by high summer evapotranspiration and limited rainfall during the growing season. According to the Köppen-Geiger classification, the climate is classified as a hot-summer Mediterranean climate (Csa). Climate data from 1991 to 2020 further validate this classification, confirming a semi-arid to semi-humid profile (Thornthwaite: C1) and highlighting very hot summers with an average temperature of 28.52°C in August (Trewartha).

Table 2. Climatic classification of the study site (Osmaniye) using five systems (Aydeniz, Erinç, De Martonne, Trewartha, Thornthwaite (1991-2020 climate periods were used in classifications Data source: (Turkish State Meteorological Service, 2025)

Classification System	Climate Type	Interpretation
Aydeniz	Semi-humid (Aridity coefficient: 0.55)	Transitional: slightly moist but vulnerable to drought stress
Erinç	semi-humid (Precipitation Effectiveness Index: 32.22)	Moderate rainfall effectiveness; not fully drought-resistant
De Martonne	Semi-arid – Semi-humid (Aridity Index:14.98)	Borderline dry; especially dry summers are expected
Trewartha	Winter climate type: Cool winters (8.80 °C) Summer climate type: Very hot summers (28.52 °C)	Warm temperate climate with very hot summers
Thornthwaite	C1, B'4, s2, b'4	C1: Semi-arid to slightly humid B'4: 4th degree mesothermal climate s2: Strong moisture surplus in winter b'4: High summer evapotranspiration (~51.4%)

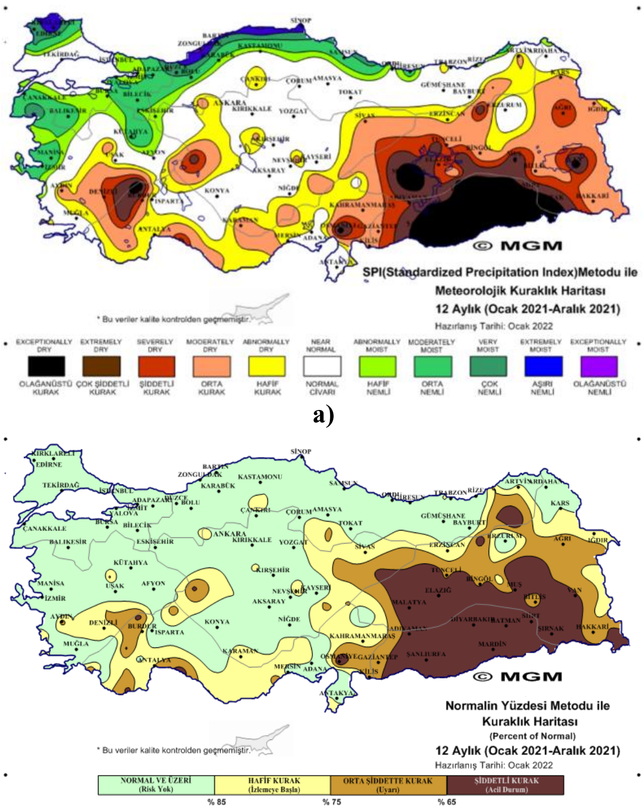

Figure 2. Meteorological drought maps based on "SPI (Standardized Precipitation)" (a) and "Percent of Normal" (b) methods for 2021 in Türkiye [Data source: (Meteoroloji Genel Müdürlüğü, 2022)]

Osmaniye experienced an exceptionally dry year in 2021 according to drought analysis estimated by Standardised Precipitation Index (SPI) and Percent of Normal Precipitation (PNP) method-based drought assessment by the Turkish State Meteorological Service (Meteoroloji Genel Müdürlüğü, 2022). The SPI-based map indicates that the region experienced exceptionally dry conditions throughout 2021, as represented by the black colour on the map (Figure 2a). This suggests a severe and prolonged drought, with precipitation levels far below the historical average. Osmaniye falls within the dark brown area, which corresponds to the "Şiddetli Kurak" (Severely Dry / Emergency) category in Percent of Normal Precipitation (PNP) method-based drought assessment for Türkiye during January–December 2021 (Figure 2b). This means that Osmaniye received significantly less precipitation than normal—less than 65% of the long-term average—throughout 2021.

Long-term climate normals for Osmaniye (1991–2021 period) indicates hot summers (June–August average highs >31°C) and very low summer precipitation, particularly in June (39.1–42.4 mm) and July (18.3–19.8 mm) (Meteoroloji Genel Müdürlüğü, 2025). Comparison with long-term averages (1987–2021) revealed a trend toward drier, sunnier conditions in 1991–2020, with June showing a 3.3 mm decrease in rainfall, 2.16 fewer rainy days, and 0.5 hours/day more sunshine.

4. Discussion

This study highlights the emergence of *M. phaseolina* as a significant pathogen associated with seedling mortality in *P. brutia* and *P. pinea* at the Osmaniye Forest Nursery in southeastern Türkiye. With an overall isolation frequency of 50.4%, *M. phaseolina* was the most frequently recovered pathogen, far surpassing other commonly encountered soilborne fungi or oomycetes such as *Fusarium* spp., *Alternaria* spp. and *Pythium* sp. (≤8.1%). While this level of infection is striking, earlier work by Özdamar (1999) reported similarly high frequencies up to 42.5% in *P. nigra* seedlings at the İzmir-Torbalı nursery, demonstrating that *M. phaseolina* has been present at substantial levels in certain Turkish nurseries for decades. However, the fungus was inconsistently detected across other sites and host species in that study, often with much lower rates or complete absence, suggesting that our findings reflect both a historically underrecognized presence and a potential resurgence in frequency and impact, likely driven by climatic and environmental changes. Interestingly, while our study is the first to report high frequencies of *M. phaseolina* in *P. brutia* and *P. pinea* seedlings from Osmaniye Forest Nursery, previous work had already identified the pathogen at the same nursery site. Uysal et al. (2021) detected *M. phaseolina* in ornamental and landscape plant species such as *Buxus*, *Salvia rosmarinus, Cryptomeria* spp., *Nerium oleander*, and *Cupressus* spp. during their 2018–2019 surveys. While, their study focused on ornamental and landscape plants, it remains unclear whether *M. phaseolina* was also affecting pine seedlings at that time. Nevertheless, the findings confirm that *M. phaseolina* was already present in the nursery's soil and production environment prior to the 2021 outbreak, potentially serving as a reservoir for later infection in pine seedling beds. Combined with the high isolation frequencies observed in this study, particularly in *P. pinea* (65.0%) and even in asymptomatic individuals (62.9%), our findings strongly suggest that the pathogen has expanded its host range or increased in aggressiveness under recent climatic stressors. This trend mirrors earlier observations by Özdamar (1999), who reported variable but occasionally high *M. phaseolina* isolation rates (up to 42.5%) from conifer and hardwood seedlings in other Turkish nurseries, including *P. nigra, C. libani*, and *Catalpa bignonioides*. Together, these results reinforce the emerging role of *M. phaseolina* as a persistent and adaptable threat in Turkish nursery systems, capable of persisting across multiple hosts and climatic regions.

The differential infection patterns observed between *P. brutia* and *P. pinea* further emphasize the potential for host-specific interactions. While both species showed high pathogen recovery from symptomatic and dead seedlings, *M. phaseolina* was detected in 62.9% of healthy-looking *P. pinea* seedlings, compared to none in *P. brutia*. This suggests that *P. pinea* may be more prone to latent infections, which could allow the pathogen to escape detection during nursery inspections and be transported to field. The presence of asymptomatic infection has important implications for post-planting disease emergence, echoing concerns raised in earlier studies of nursery-to-field pathogen transmission (Barnard, 1994; Slippers and Wingfield, 2007). A similar case was documented by Akıllı et al. (2010), where *Phytophthora cryptogea* isolated from *P. nigra* seedlings in the Kenbağ Forest nursery was later found in declining trees in afforestation areas established using those seedlings. These examples illustrate the need for stricter phytosanitary protocols, particularly for hosts like *P. pinea*, which may carry latent infections without visible symptoms.

The outbreak in Osmaniye coincided with severe drought conditions and elevated summer temperatures. During the 2021 growing season, monthly rainfall fell below 40 mm and maximum temperatures exceeded 31°C, conditions known to favour *M. phaseolina* infection. The fungus thrives under high temperatures (28–35°C) and low soil moisture, often outcompeting other soil microbes under such stress (Bashir, 2017; Reuveni & Madar, 1985). These conditions likely contributed to both disease development and the high rate of latent colonization observed in *P. pinea*. Drought-stressed seedlings may have had compromised defence responses, providing a favourable environment for the pathogen to establish and proliferate.

Despite regular treatment with a Thiram-based fungicide in the Osmaniye nursery, *M. phaseolina* was recovered at high frequencies. Thiram is commonly utilized in forest nurseries across Türkiye and has shown efficacy against several soilborne pathogens, including M. phaseolina, in controlled experiments (Turhan & Özdamar, 1999). However, its performance under field conditions may be reduced by environmental stressors such as drought and high temperature, which can limit fungicide persistence and efficacy. Such challenges highlight the need to re-evaluate reliance on chemical controls and consider more resilient, integrated management strategies under evolving climate conditions.

The scale of pine seedling production in Türkiye amplifies the relevance of our findings. In 2021, over 53 million *P. brutia* and nearly 3 million *P. pinea* seedlings were produced in Turkish nurseries (General Directorate of Forestry, 2023). Even localized outbreaks of *M. phaseolina* pose a significant risk, particularly when latent infections in asymptomatic seedlings go undetected. In Osmaniye, anecdotal records indicated that approximately one-third of all pine seedlings produced in 2021 were lost, a figure that aligns with the high isolation frequencies reported in this study and suggests that the pathogen's impact may be more widespread than officially documented.

While this study provides compelling evidence for the increasing significance of *M. phaseolina* in forest nurseries, it also has limitations. Pathogenicity tests under controlled conditions were not conducted, and molecular characterization was limited to selected isolates. Ongoing work to examine isolate aggressiveness, environmental fitness, and host responses will be critical to better understand the dynamics of this pathogen and guide effective disease management strategies.

5. Conclusions

This study documents the emergence of *M. phaseolina* as a significant pathogen affecting *P. brutia* and *P. pinea* seedlings in Osmaniye Forest Nursery, Türkiye. With an overall isolation frequency of 50.4%, *M. phaseolina* was the most frequently recovered fungus, surpassing traditionally dominant nursery pathogens such as *Fusarium*, *Pythium*, and *Alternaria* spp. The notably high infection rate in *P. pinea*, including latent infections in morphologically healthy seedlings, suggests differential host susceptibility and raises concerns about the potential for undetected carryover to plantation sites.

The outbreak coincided with extreme climatic conditions in 2021, characterized by record summer temperatures, severely reduced rainfall, and high evapotranspiration conditions that are highly favourable for *M. phaseolina* proliferation and infection. These findings support the hypothesis that climate change, through increased drought frequency and intensity, is accelerating the emergence and spread of opportunistic pathogens in forest nursery ecosystems.

Despite routine fungicidal treatments (e.g., Thiram), *M. phaseolina* incidence remained high, highlighting the limitations of conventional control methods under abiotic stress. As Türkiye continues to invest in large-scale afforestation, especially with climate-sensitive species like *P. pinea* and *P. brutia*, proactive strategies are urgently needed.

In conclusion, while earlier studies have reported the presence of *M. phaseolina* in Turkish nurseries, our findings emphasize its increasing relevance and pathogenic dominance under semi-arid Mediterranean conditions. This underscores the urgent need for systematic monitoring, improved diagnostic capabilities targeting latent infections, and the development of integrated nursery management strategies that account for local climate realities and host-specific vulnerabilities.

To address the rising threat of *M. phaseolina* in forest nurseries, we recommend a series of targeted actions. First, expanding systematic surveys across Turkish nurseries is essential not only to document pathogen presence but also to detect latent infections that may escape visual inspection.

Second, integrating climate-based disease forecasting models would help anticipate outbreak periods under projected warming and drought trends, enabling proactive nursery responses. Third, host resistance should be evaluated through coordinated, multi-location seedling trials to identify tolerant genotypes, especially for *P. brutia* and *P. pinea*. Finally, nursery management practices must be adapted to the realities of semi-arid environments, with particular focus on optimizing irrigation schedules, enhancing soil health, and minimizing stressors that predispose seedlings to disease. Together, these steps form a comprehensive strategy to reduce the impact of *M. phaseolina* and improve the resilience of forest nursery systems under changing climatic conditions. Ultimately, safeguarding the sustainability of Türkiye's pine seedling production under a changing climate will require a shift toward climate-adaptive, evidence-based forest nursery management and greater recognition of emerging threats such as *M. phaseolina*.

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Table S1. Summary of surveys on pathogens in Turkish forest nurseries with emphasis on detection and isolation frequencies and hosts of *Macrophomina phaseolina* (MP).

Studies*	Survey Period	Studied Nurseries	Main disease agents detected	Detection status of MP	Detection/isolation frequency of MP	MP Hosts
1	Na	FNs in Marmara, Aegean, Black Sea, Mediterranean regions	*Melampsora pinitorqua, Coleosporium* spp. *(C. inulae, C. euphrasiae, C. campuneale), Chrysoma* sp., *Cronartium flaccidum, Lophodermium pinastri*	No	-	-
2	Na	FNs in İzmir	*Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Cylindrocarpon* spp., *Alternaria spp., Phytopthora* spp. and *Macrophomina* spp.	Yes	na	*Pinus pinea*
3	1996	Denizli (Karahasanlı), İzmir (Torbalı), Manisa (Muradiye), Konya (Seydişehir), Burdur (Gölhisar), Isparta (Egirdir, Aşagı Gökdere) FNs	*Fusarium* spp., *Rhizoctonia solani, Pythium* spp., *Alternaria* spp. and *Macrophomina phaseolina*	Yes	6.0% (42.5% on *P. nigra* in Torbalı FN)	*Pinus brutia, Pinus nigra, Pinus pinea, Cedrus libani, Catalpa bignonioides*
4	Na	Kızılcahamam, Beypazarı, Çerkeş, Behiçbey FNs	*Alternaria* sp., *Mucor* spp., *Rhizoctonia* sp., *Fusarium acuminatum, F. oxysporum, Cladosporium* sp., *Botrytis cinerea, Sphaerotheca lanestris*	No	-	-
5	2008-2009	Artvin-Ardanuç, Bursa, Devrek-Gökçebey, Düzce-Akçakoca, Eskişehir, Kastamonu-Gölköy &Taşköprü, Ordu, Samsun, Zonguldak FNs	*F. solani, F. oxysporum, F. moniliforme, R. solani, Pythium* spp., *Phytophthora cryptogea, P. cinnamomi, Cylindrocarpon* sp., *Verticillium* sp.	No	-	-
6	2008-2010	Hatay, Serinyol FN	*Pythium* spp., *Fusarium oxysporum, Cylindrocarpon spp., Rhizoctonia solani, Phytophthora* spp., *F. solani, F. moniliforme, Macrophomina phaseolina, Sclerotinia sclerotiorum*	Yes	7.50%	na
7	2011-2013	İzmir-Torbalı, Denizli-Karahasanlı, Muğla-Gökova, Isparta-Eğirdir, Antalya-Elmalı, Eskişehir, Bursa, Adapazarı-Hendek FNs	*Fusarium oxypsorum, Fusarium solani, Fusarium verticillioides, Cylindrocarpon destructans, Rhizoctonia solani, Verticillium dahliae, Pestalatiopsis clavispora, Pythium aphanidermatum, Pythium intermedium, Pythium irregulare, Pythium ultimum, Phytophthora cactorum, Phytophthora citricola, Phytophthora megasperma, Phytophthora syringae*	No	-	-
8	2015-2018	FNs in Adapazarı, Kocaeli, Yalova, Bursa, İstanbul, Kırklareli, Edirne	*Fusarium solani, Fusarium oxysporum, Pythium aphanidermatum, Pythium irregulare, Pythium ultimum, Phytopythium vexans, Phytophthora syringae*	No	-	-
9	2018-2019	Gaziantep, Osmaniye, Hatay Serinyol, Hatay Mustafa Kemal University FNs	*Macrophomina phaseolina, Fusarium solani, Ceratobasidium* sp., *Rhizoctonia solani*	Yes	17.0%	*Buxus* sp., *Salix* sp, *Lavandula* sp., *Rosmarinus officinalis*, *Nerium oleander, Rosa* sp., *Chamaecyparis* sp., *Cryptomeria* sp., *Cupressus arizonica, Cupressus macrocarpa* 'Goldcrest'

FNs = Forest Nurseries; MP = *Macrophomina phaseolina*, "na" indicates data not available,
*References of the studies: 1=Vural et al., 1984; 2=Vural, 1989; 3=Özdamar, 1999; Turhan and Özdamar, 1999; 4=Gürer, 2000; 5=Akıllı et al., 2010; 6=Kurt and Soylu, 2011; 7=Aday Kaya, 2014; 8=Aday Kaya et al., 2019; 9=Uysal et al., 2021.