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# Identification of *Curvularia spicifera* causing foliage blight on *Foeniculum vulgare*

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## Abstract

*Curvularia* is an important genus that contains plant pathogenic species that are found all over the world and have a broad host range. Fennel (*Foeniculum vulgare*) is a cultivated plant and has been commonly used as a traditional food and medicine. During August 2021, severe foliage blight symptoms were observed on fennel grown in Isparta province, Türkiye. The causal organism of this foliage blight was isolated from diseased tissues and identified as *Curvularia spicifera*, based on characteristics of morphology. The fungal isolate's morphological identification was confirmed through phylogenetic analysis of the internal transcribed spacer region (ITS) of the rDNA gene sequence. Pathogenicity to fennel was confirmed through inoculations of healthy plants. To our knowledge, this is the first report of *C. spicifera* causing foliage blight on fennel in Türkiye and worldwide.

***Keywords:*** *Curvularia spicifera*; fennel; foliage blight; ITS-rDNA; pathogenicity

## Introduction

Fennel (Rezene, local Turkish name), (*Foeniculum vulgare* Miller), belongs to Apiaceae family, is native to the Mediterranean region and widely used for both culinary and medicinal purposes. Fennel is an upright, branching perennial herb with soft, feathery, almost hair-like leaf that grows up to two meters tall (Badgujar *et al*., 2014). Fennel fruit and essential oil are used as constituents of cosmetic and pharmaceutical products and food industries such as bread, cheese, liqueurs, pickles, and pastries. Fennel has anti-inflammatory, anti-depressive, antispasmodic, antibacterial, antifungal, antioxidant, carminative, diuretic, and analgesic properties (Garg *et al*., 2010; Kooti *et al*., 2015).

Fennel production could be limited by several biotic stresses, with diseases being major limiting factors. Several diseases caused by fungi have been reported affecting fennel around the world, including *Aureobasidium*

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*pullulans, Alternaria* spp.*, Ascochyta phomoides, Cladosporium* spp.*, Botrytis cinerea, Diaporthe angelicae, Didymella glomerata*, *Didymospora* sp.*, Dendryphion nanum, Colletotrichum gloeosporioides, Epicoccum purpurascens, Erysiphe heracleid, Fusarium* spp.*, Fusoidiella anethi, Itersonilia pastinaceae, Gonatobotrys simplex, Macrophomina phaseolina, Melanconiales* sp.*, Ochraceocephala foeniculi*, *Leptosphaeria purpurea, Periconia* sp., *Pythium sulcatum, Phytophthora megasperma, Ramularia foeniculi, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia* spp., *Stemphylium botryosum, Subplenodomus apiicola*, and *Volucrispora* sp. (Odstrčilová *et al.,* 2002; Khare *et al.,* 2014; Erberk *et al.,* 2019; Köse, 2019; Aiello *et al.,* 2020). *Curvularia* species, on the other hand, have not previously been reported to be fennel pathogens.

The genus *Curvularia* Boedijn (1933) belongs to Pleosporaceae, Pleosporales, Pleosporomycetidae, Dothideomycetes, Pezizomycotina, Ascomycota (Marin-Felix *et al.,* 2017). Currently, there are 240 *Curvularia* species listed in Index Fungorum (accessed on 05th February 2024). *Curvularia* includes pathogens of many plants, particularly wild grasses and staple crops such as wheat, rice, maize, or sorghum, and could cause significant losses in production in agriculture (Manamgoda *et al.,* 2015; Marin-Felix *et al.,* 2017; Tan *et al.,* 2018; Bessadat *et al.,* 2023). Some species of this genera, such as *C. spicifera*, *C. lunata* and *C. hawaiiensis* can be found in association with both humans and plants (Manamgoda *et al.,* 2015).

Fungal populations are characterized and taxonomized using many kinds of polymerase chain reaction (PCR)-based methods. (Kılıçoglu and Özkoç, 2008). The rDNA complex's internal transcribed spacer (ITS) sections have been chosen as a barcode for fungal identification and are frequently utilized for this purpose (Brown *et al.,* 2014).

In 2021, a new disease was first observed on fennel in the experimental field of the Department of Field Crops, Faculty of Agriculture, Isparta University of Applied Sciences (Isparta, Türkiye). The disease symptoms were severe foliage blight observed on fennel plants. The current work aimed to identify the causal agent acquired from symptomatic fennel plants by analyzing morphological characteristics, sequencing the ITS region of rDNA, and testing for pathogenicity.

## Materials and Methods

*Sample collection and fungal isolation*

During August 2021, diseased fennel plants with symptoms of severe foliage blight (Figure 1A) were collected from the experimental field (37°50′18″ N 30°32′11″ E) of the Department of Field Crops, Faculty of Agriculture, Isparta University of Applied Sciences (Isparta, Türkiye).

Typically, symptomatic leaves were cut into tiny pieces (5 mm × 3 mm), and the tissue segments were surface-sterilized in 2% sodium hypochlorite for 2 min, and then washed twice with sterilized water (2 min each time) and excess liquid was removed by sterile filter papers. On potato dextrose agar (PDA) plates, the surface-disinfested tissues were placed, and they were then incubated at 25 °C (Ünal *et al.,* 2020). Using a sterile transfer needle, consistent fungal colonies that developed from diseased tissues were excised and cultivated on PDA. By transferring single hyphal tips to PDA, pure fungal cultures were produced. The cultures were then incubated at 25 °C for 5 to 7 days until the fungal colonies were large enough to be inspected. These were grown on PDA slants and kept at 4 °C until the identification processes were carried out. A representative isolate FV-11 which was deposited in the Department of Agricultural Biotechnology, Faculty of Agriculture, Isparta University of Applied Sciences (ISUBU-AB) was arbitrarily selected for morphological and molecular identification as well as pathogenicity assessment.

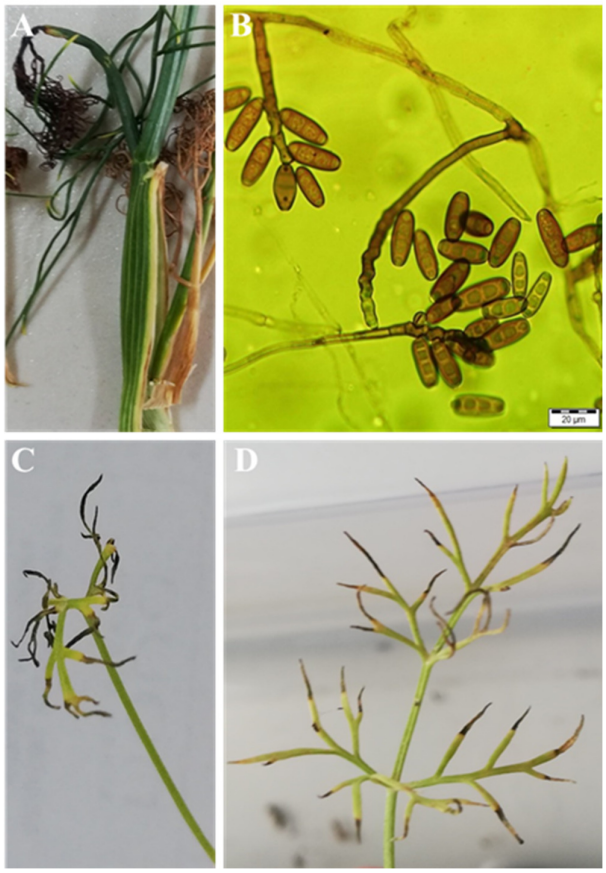


**Figure 1.** *Curvularia spicifera* causing foliage blight on fennel. (A) Field symptoms of fennel foliage blight; (B) Conidia and conidiophore of *Curvularia spicifera* isolate FV-11;(C-D) Symptomatic plant 7 days after inoculation with the *Curvularia spicifera* isolate FV-11

*Morphological and molecular identification*

Among the isolates obtained, isolate FV-11 was selected as the representative fungus for further studies. The morphological characteristics of the isolate were examined, photographed and measured with under a light microscope, including the size and shape of conidiophores and conidia as previously described (Jeon *et al*., 2015). Measurements were performed on 30 selected conidia.

One representative isolate (FV-11) was selected for molecular analysis, the isolate was grown on PDA for 7 days in the dark. Then, using a GeneMATRIX Plant & Fungi DNA Purification Kit (EURx Ltd., Poland), total DNA was extracted from fresh mycelium grown on PDA in accordance with the manufacturer's instructions. The universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify the rDNA's ITS region (White *et al*., 1990). For the sequencing and purification of the PCR products, they were sent for Sanger sequencing (BM Labosis, Ankara, Türkiye). After completing the sequence, the assembled isolate sequence was subjected to a BLAST search in the National Center for Biotechnology Information (NCBI) database, and the sequences were submitted to the NCBI GenBank for an accession number. The ITS sequence acquired in this work was put together using nucleotide sequences of additional isolates of *Curvularia* retrieved from GenBank (Table 1). The phylogenetic tree was generated by MEGA 11 program using the Neighbor-joining (NJ) technique with 1000 replications for each bootstrap value (Tamura *et al*., 2013). As an outgroup, *Alternaria alternata* (MN812275.1) sequence was used.

**Table 1.** Isolates and acession numbers of *Curvularia* spp. used in the analysis of ITS region

| Isolate no | ***Curvularia* species** | Country of origin | GenBank Accesssion No |
|---|---|---|---|
| FV-11 | *C. spicifera* | Türkiye | PP050235 |
| TN290D12 | *C. spicifera* | Tunisia | MH271090 |
| AY992b | *C. spicifera* | Namibia | MG250435 |
| LB-28 | *C. spicifera* | India | OQ845826 |
| DHFS1740 | *C. lunata* | China | OL790327 |
| Azerbaijan 46 | *C. inaequalis* | Azerbaijan | MH844811 |
| K1284 | *C. cactivora* | Malaysia | MH719232 |

*Pathogenicity tests*

The pathogenicity of the isolate FV-11 was determined using fennel seedlings according to Koch's postulates. Ten two-month-old fennel seedlings were sprayed with a conidial suspension of 1% Tween-20 solution of FV-11 calibrated to $1\times 10^6$ conidia/ml with a hemocytometer, while the other ten seedlings were treated with sterile water and utilized as the control group (Aslam *et al.,* 2020). To maintain high relative humidity, all seedlings were covered in plastic bags for 2 days. Then, the plants were transferred to a greenhouse and maintained at 25±2 °C until disease symptoms appeared. To verify Koch's postulates, the inoculated fungus was re-isolated and identified using the same process. The experiment was repeated twice.

## Results

The current study aimed to document a hitherto unreported foliage blight disease on fennel in Türkiye. The isolate FV-11 was identified as *Curvularia spicifera* based on its morphological characteristics. Colonies displayed velvety mycelium that turned dark green-grayish black and had a 5.5 cm colony diameter after one week of growth on PDA at 25 °C. Conidia of the isolate FV-11 were 16.9-26.2 × 6.9-10.8 µm, ellipsoidal or oblong, light brown, three-distoseptate, and narrower toward the ends (Figure 1 B). Conidiophores were light to medium brown, of variable length, septate, unbranched, erect or slightly curved. No chlamydospores or microconidia were observed.

The sequence of the FV-11 isolate's ITS region (568 bp) was successfully amplified, sequenced, and deposited in GenBank as PP050235. A BLAST search revealed a 100% sequence similarity between FV-11 and GenBank accession numbers MH271090, MG250435 and OQ845826. In the neighbor-joining method analysis, compared to sequences of other *Curvularia* species, the *C. spicifera* isolates formed a unique and well-supported clade (Figure 2) in the phylogenetic tree. The FV-11 was identified as *C. spicifera* based on morphological and phylogenetic analysis.

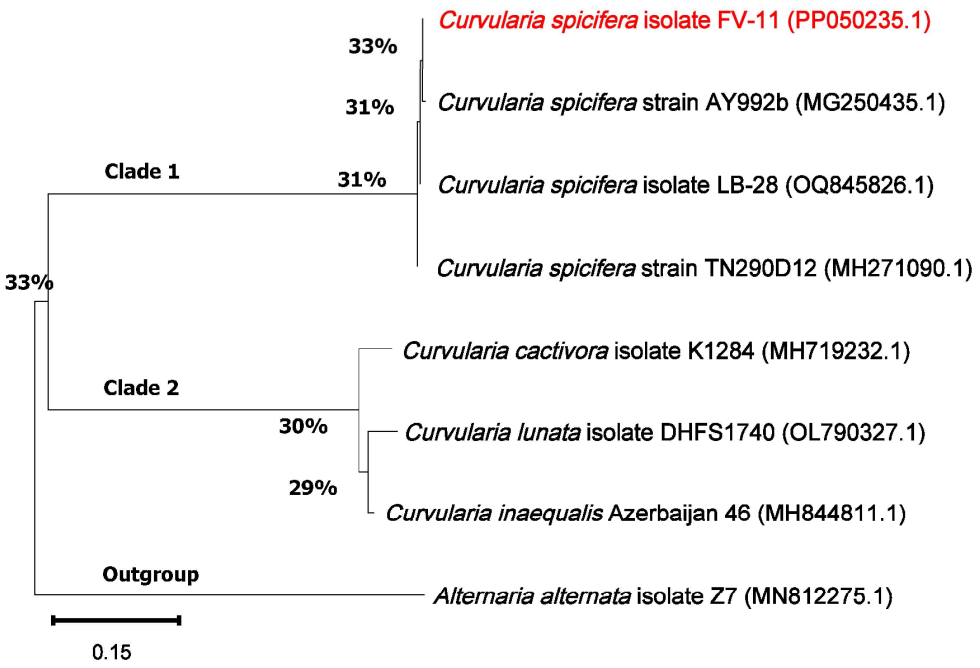


**Figure 2.** Phylogenetic tree for *Curvularia spicifera* and related species based on Neighbor-joining analysis of ITS sequences. The branch points display the bootstrap values obtained from 1000 replicates. The scale bar represents 0.10 substitutions per nucleotide. *Alternaria alternata* used as an outgroup. FV-11, isolated from *Foeniculum vulgare* is shown in red.

The pathogenicity test demonstrated that the representative isolate (FV-11) of *C. spicifera* is pathogenic to fennel plants. Symptoms of the disease was similar to those observed under field conditions and developed after inoculating the representative isolate. *Curvularia spicifera* induced similar symptoms on leaves within 7–10 days after inoculation (Figure 1 C-D). Control plants did not show any disease symptoms. In order to verify Koch's postulates, the inoculated fungus was recovered from the inoculated leaves and identified based on its morphology. As far as we know, this is the first report of *C. spicifera* causing fennel foliage blight in Türkiye and other countries.

## Discussion

The fungal genus *Curvularia* includes approximately 240 species belonging to the family Pleosporaceae (Jeon *et al.,* 2015). This genus's related species are plant pathogens that cause a wide range of diseases. For instance, *C. spicifera* has been reported to cause brown leaf spot in rice (*Oryza sativa*) (Ennaffah *et al.,* 1999; Aslam *et al.,* 2020), sugarcane (*Saccharum officinarum*) (Lin *et al.,* 2012), eggplant (*Solanum melongena*) (Pandey, 2010), tomato (*S. lycopersicum*) (Rao *et al.,* 2020), turfgrass species (Bessadat *et al.,* 2023), soybean (*Glycine max*) (González-Molotla *et al.,* 2021), *Aloe vera* (Ahmed *et al.,* 2023), watermelon (*Citrullus lanatus*) (El Mhadri *et al.,* 2009), pomagranate (*Punica granatum*) (Kadri *et al.,* 2011), switch grass (*Panicum virgatum*) (Vu *et al*., 2011), leaf blight in *Mentha arvensis* (Kumar *et al.,* 2013), strawberry (*Fragaria* × *ananassa*) (Ayoubi *et al.,* 2017), buffalograss (Amaradasa and Amundsen, 2014), Chinesee fir (*Cunninghamia lanceolata*) (Cui *et al.,* 2020), leaf blotch in cereals (Kochman, 2009), root rot in barley and wheat (Qostal *et al.,* 2019), and brown rot in mandarine (*Citrus reticulata*) (Garganese *et al.,* 2015). In Türkiye, *C. spicifera* was isolated from sorghum

(*Sorghum bicolor*) (Ünal *et al.,* 2010) and clover (*Trifolium repens*) (Ünal *et al.,* 2020). Also, many species of *Curvularia* are emerging opportunistic pathogens of animals and humans (Manamgoda *et al.,* 2015; Pham *et al.,* 2022). According to a review of the literature, *C. spicifera* has not been reported to cause fennel foliage blight. As a result, to our knowledge, this study is the first to report *C. spicifera* as a causal organism of fennel foliage blight in Türkiye and worldwide.

## Conclusions

For this investigation, we took samples of fennel plants from the experimental field of the Department of Field Crops, Faculty of Agriculture, Isparta University of Applied Sciences (Isparta, Türkiye), which display symptoms of the foliage blight disease. Following the causative fungus isolation from the infected plant tissues, the macro- and micromorphological characteristics of the fungal isolate, along with sequencing information from the ITS region of the rDNA gene, were used to characterize it morphologically and molecularly. *Curvularia spicifera* was identified as the causative fungus based on the research results. The pathogenic character of the recovered representative *C. spicifera* isolate FV-11 from fennel plants was confirmed by Koch's postulates. In Türkiye and around the world, this is the first report of *C. spicifera* leading to fennel foliage blight. Further studies will be necessary to determine the distribution and to assess the economic impact of this disease in Türkiye.

## Authors' Contributions

CE: Conceptualization; Investigation; Writing - original draft; MM: Investigation; Writing - review and editing; SÖ: Investigation; Visualization; Writing - review and editing; MT: Investigation; Writing - review and editing; SE: Investigation; Writing - review and editing.
All authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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