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Araştırma Makalesi/Reserach Article

New Threat to Lemon Cypress (*Cupressus macrocarpa* cv. Goldcrest) in Turkey: *Seiridium cardinale*

Figen Mert[1]* Arjin Öndeş[1] Sibel Derviş[2]

[1]Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Plant Protection, Çanakkale 17100, Turkey
[2]Mardin Artuklu University, Vocational School of Kızıltepe, Department of Plant and Animal Production, Mardin 47000, Turkey
*Corresponding author: fmert@comu.edu.tr

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Abstract

Lemon cypress (*Cupressus macrocarpa* cv. Goldcrest) is a coniferous Cuppressaceae tree and is popular in urban landscapes of Turkey. In July 2021, dark-brown elongated bark lesions and resin exuding cracks with die-back symptoms were observed on the main stem of young *C. macrocarpa* cv. Goldcrest trees in a private house garden in the central district of Çanakkale Province of Turkey. Fungal isolations made from the margin of cankers of trees consistently yielded flat colonies with dense floccose aerial mycelium that were first off-white and grayish olive-green in some parts or in older cultures. Based on the morphological characteristics of obtained isolates, molecular analyses of the ITS rDNA region of a representative isolate, and pathogenicity tests on 4-year-old *C. macrocarpa* cv. Goldcrest plants, the causal agent was identified as *Seiridium cardinale* (Wagener) Sutton & Gibson. This is, to the best of our knowledge, the first report of *S. cardinale* causing canker on *C. macrocarpa* in Turkey.
Keywords: *Cupressus macrocarpa*, *Seiridium cardinale*, cypress canker, monterey cypress, lemon cypress

Türkiye'de Limoni Servilerde (*Cupressus macrocarpa* cv. Goldcrest) Yeni Bir Tehdit: *Seiridium cardinale*

Öz

Limoni servi (*Cupressus macrocarpa* cv. Goldcrest) Türkiye'de kentsel peyzaj alanlarında popüler olan Cuppressaceae familyasına ait iğne yapraklı bir ağaçdır. 2021 yılının temmuz ayında Çanakkale merkezinde özel bir mülke ait bir bahçede *C. macrocarpa* cv. Goldcrest bitkisinin ana gövdesinde geriye ölüm belirtileri ile kabukta uzunlamasına siyah-kahve renkli lezyonlar ve çatlaklardan sızan reçine gözlemlenmiştir. Kanserli dokuların kenarlarından yapılan izolasyonlar sürekli olarak; önce kirli beyaz, sonra bazı kısımlarda veya daha eski kültürlerde grimsi zeytin yeşili olan yoğun floccose havai miselyuma sahip düz koloniler sonuçlamıştır. Elde edilen izolatların morfolojik karakterlerine, temsilci bir izolatın ITS rDNA bölgesinin moleküler analizine ve 2 yaşındaki *C. macrocarpa* cv. Goldcrest bitilerinde yapılan patojenisite testlerine göre, hastalık etmeni *Seiridium cardinale* (Wagener) Sutton & Gibson olarak tanılanmıştır. Bu çalışma, şu anki bilgilerimize göre, Türkiye'de *C. macrocarpa*'da *S. cardinale*'nin sebep olduğu servi kanseri hastalığının ilk raporudur.
Anahtar Kelimeler: *Cupressus macrocarpa*, *Seiridium cardinale*, servi kanseri, monteri servi, limoni servi

Introduction

Cupressaceae, also known as the cypress family, is a type of conifer that grows all over the world. The Monterey cypress (*Cupressus macrocarpa* Hartw. ex Gordon: Syn: *Hesperocyparis macrocarpis*) is a species in this family that is native to California in the Western United States and is widely planted as an ornamental tree in many countries, including Western Europe, Turkey, Argentina, and New Zealand (Bean, 1981). GoldCrest (*C. macrocarpa* cv. Goldcrest), also known as lemon cypress, is a narrow columnar evergreen tree with bright yellow foliage with a strong scent of lemon verbena when crushed (Hanson, 1990). They are grown for ornamental purposes, hedges, shelter plants, and timber crops. Lemon cypress trees are popular ornamental plants in Turkey's parks, recreation areas, and home gardens.

Seiridium cardinale (W. W. Wagener) B. Sutton & I. A. S. Gibson is the most widespread and aggressive pathogen of *Cupressus* and several Cupressaceae, responsible for cypress canker (branch and trunk cankers) in many temperate regions and particularly in the Mediterranean area, and a cause

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of severe pandemic disease worldwide (Raddi and Panconesi, 1981; Graniti, 1986; and Graniti, 1998). Two other cypress canker fungi, *S. cupressi* and *S. unicorne*, also cause cankers on cypress to a lesser extent. *S. cardinale* was first reported on Monterey cypress in California in 1928 (Wagener, 1928), then spread worldwide, especially countries trading ornamental plants among continents. The disease spread gradually across the five continents, with New Zealand reporting the first case after California, followed by France and Italy (Birch, 1933; Barthelet and Vinot, 1944; Grasso, 1951). The disease was then spread to Argentina, Greece, Spain, the United Kingdom, Ireland, and Canada (Saravì Cisneros, R. 1953; Anastassiadis 1963; Torres, 1969; Strouts, 1970; Sutton 1980; Funk, 1974). California populations are significantly more genetically diverse than European populations, and a minimum spanning network confirms that California populations are most likely ancestral to those found in North Africa and Europe (Della Rocca et al. 2011). Sümer (1987) surveyed the costal parts of Turkey in order to assess the distribution of Mediterranean cypress (*C. sempervirens*) in Turkey as well as the current status of its pests and diseases, particularly cypress canker disease. The researcher reported the presence of cypress canker on *C. sempervirens* var. *horizontalis* at Gökova, south-west Aegean coast of Turkey, based on only visual observation of the symptoms on trees.

On susceptible hosts, the fungus causes both local and systemic symptoms (Graniti, 1998). The pathogen infects the host tissues and causes browning or reddening of the bark on branches or stems. Horizantal cracks and resin exudations of infected barks are followed by visible acervuli production. When cuttings are made through the inner bark of cambium, they can reveal a brownish red discoloration. A diffuse yellowing or reddening appears first on the foliage of the affected trees' twigs, branches, and apical parts, eventually turning brown or reddish-brown as the dieback progresses, especially on susceptible species such as *C. macrocarpa* and *C. sempervirens*. Depending on its age, susceptibility, and environment, the spread of one, several, or many infections on a single tree can kill the entire tree in a relatively short period of time.

In this study, canker symptoms were observed on the stems of young *C. macrocarpa* cv. Goldcrest trees, resulting in dieback and tree mortality of branches and trees in a private house garden in Çanakkale's central district. In order to diagnose the fungus, some diseased tissue surrounded by healthy barks was removed aseptically after symptomological observations. We describe the fungus's diagnosis on a symptomological, morphologic, and molecular level in this paper.

Materials and Methods

Observation of Symptoms and Fungal Isolations

On branches and/or the main stem of young *C. macrocarpa* cv. Goldcrest trees in the central district (40°04′07.6″N, 26°21′41.4″E) of Çanakkale, Turkey, dark-brown elongated bark lesions and resin exuding cracks were observed, leading to dieback and tree mortality. With a blade razor, the infected bark and wood tissues were removed and placed in polyethylene bags. The infected tissue parts were placed in a +4 refrigerator until the isolation procedures were completed.

Infected plant samples were cut into 0.5–1cm^2 pieces and surface-sterilized for 2 minutes with 2% NaClO solution before being rinsed with sterile distilled water. Before transferring the samples to potato dextrose agar, they were blotted dry on sterile filter papers (PDA; Merck, Darmstadt, Germany). A cork borer was used to remove the hyphal tip from actively growing margins, which was then transferred to fresh PDA for morphological and molecular analysis.

Pathogenicity Tests

To confirm Koch's postulates, stem inoculations were performed on seven 4-year-old *C. macrocarpa* cv. Goldcrest plants grown in pots in a growth chamber with a 16/8-h light/dark photoperiod at 23°C and 70% relative humidity. Colonies obtained from a single conidium of DRN1 isolate were used for pathogenicity tests. After removing a bark plug of the same size, a mycelial 3-mm-diameter PDA disk was applied to exposed xylem tissue in the midpoint of a healthy stem. Control trees (5) received sterile PDA disks. All wounds were wrapped with Parafilm. Inoculations were repeated once with a 2-week pause.

Isolation of Genomic DNA and Sequence-Based Identification of Isolate

For DNA isolation the Eur$_X$ GeneMATRIX Plant & Fungi DNA kit (Poland) was used. Spectrophotometric measurements were made by Thermo Scientific Nanodrop 2000 (USA) in order to identify the quantity and purity of the DNA. Universal ITS1 (5' TCCGTAGGTGAACCTGCGG 3') –

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ITS4 (5' TCCTCCGCTTATTGATATGC 3') primers were employed for fungal species diagnosis (White et al., 1990). The amplification conditions were initial denaturation at 95°C for 5-sec, then 40 cycles (95°C for 45-sec denaturation, 57°C for 45-sec annealing, 72°C for 60-sec extension) 72°C for 5-min final extension.

The PCR amplification product was run at 100 volts for 90 minutes on 1,5% agarose gel prepared with 1x TAE. The gel was dyed with ethidium bromide and visualized under a UV box. Following PCR amplification, a single band was obtained. The MAGBIO "HighPrepTM PCR Clean-up System" (AC-60005) was used for PCR product cleaning procedures. The sequencing was performed using a BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3730XL Sanger sequencer (Applied Biosystems, Foster City, CA). Readings from the ITS1 and ITS4 primers were assembled into a contig to form a consensus sequence. The BioEdit software's CAP contig assembly algorithm was used for this process. The results of the sequence analysis were evaluated using BLAST analysis and similarity scores obtained from the NCBI GenBank.

Results and Discussion

Symptomologic Diagnosis of the Disease

The disease's main symptom was die-back of trees on one or more top or lateral branches (Figure 1a). Close examination revealed dark-brown elongated bark lesions and resin-exuding cracks (Figure 1b). When the bark with canker was removed with a blade, the distinct dark dead tissue surrounded by healthy wood was visible. Pathogen acervuli were visible to the naked eye as small black dots scattered over the cankered areas of the bark, mostly within the resin exudate (Figure 1c). The symptoms observed during our survey were similar to those reported previously (Graniti, 1998). Aside from *Cupressus*, the fungus can infect and cause symptoms in other Cupressaceae genera such as *Chamaecyparis*, *Juniperus*, and *Thuja*. After observing the disease in the home garden, we discovered that the disease was more common than expected in lemon cypresses in the region, and adjacent trees no older than 10 years old died. A postgraduate study investigating the prevalence of the disease in lemon cypress trees in Çanakkale is continuing to this end by expanding our research.

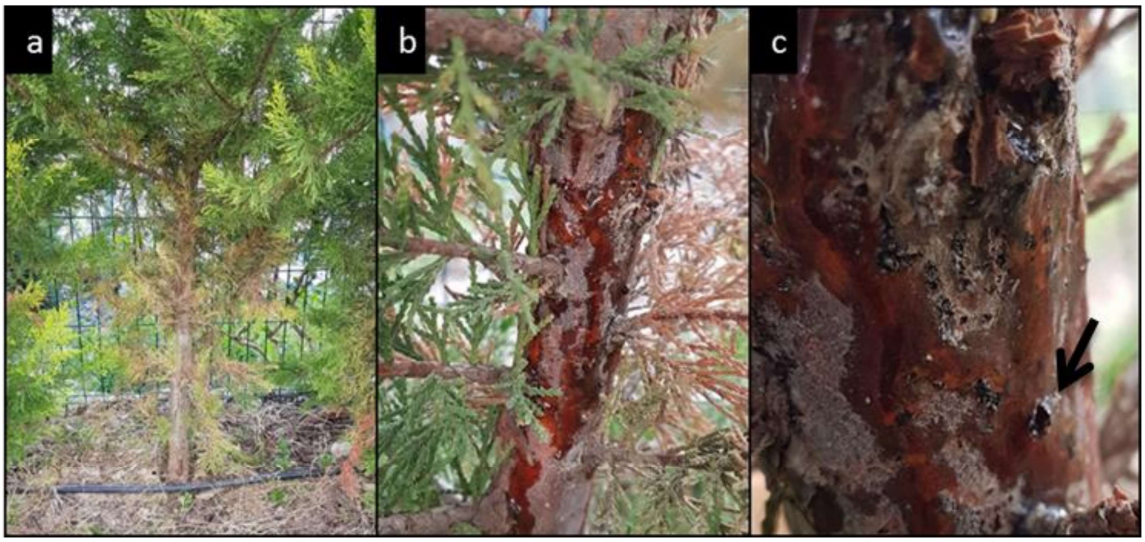

Figure 1. Symptoms of *Cupressus macrocarpa* cv. Goldcrest infected by the DRN1 isolate of *Seiridium cardinale*: (a) dieback of branches; (b) cracks and resin axudates on the infected bark; (c) acervulus in cankered areas (black arrow).

Morphological Diagnosis of the Fungus

Due to the formation of flat colonies with dense floccose aerial mycelium that was pale luteous and grayish olive-green in some parts or in older cultures, fungal isolates obtained from two trees were identified as the same fungus based on cultural features (Figure 2a). Cultures' reverse sides ranged from pinkish salmon to light orange (Figure 2b). Black acervuli were abundantly produced on the cypress seeds after three weeks of incubation on water agar amended with autoclaved *C.*

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sempervirens seeds at 18°C under a fluorescent and near UV light. Conidia were 6-celled, oblong-fusiform, straight to slightly curved, 22–28 × 8–10 µm long, with four brown median cells and two hyaline, short and conical apical cells with pores (Figure2c-d). The isolates' morphological characteristics were identical to those of *Seiridium cardinale* (Wagener) Sutton & Gibson (Bonthond et al 2018).

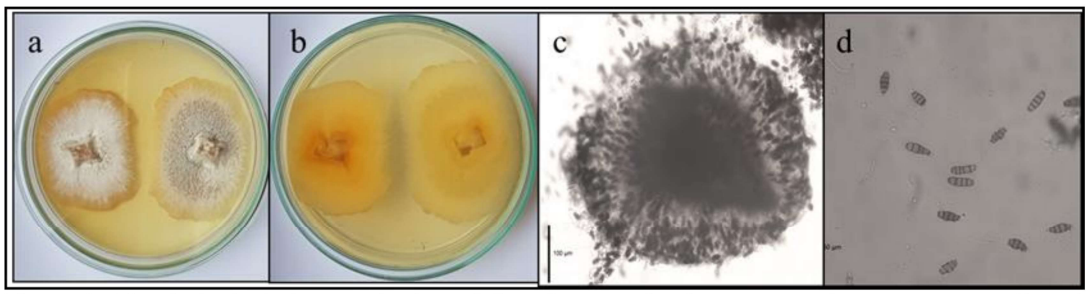

Figure 2. Morphological characterisation of *Seiridium cardinale* on potato dextrose agar medium 15 days after inoculation at 25°C: (a) dense floccose pale luteous aerial mycelium, (b) pinkish-salmon to light orange colony on reverse side, (c) acervulus, (d) curved 6-celled conidia.

Pathogenicity Tests

Three months after inoculations, elongated necrotic lesions (25.3 to 42.5-mm-length) with resin exudation were visible on all inoculated stems (Figure 3). There was no lesion development in the control plants. Three months after inoculation, the pathogen was successfully reisolated from the edges of lesions on the inoculated plants.

Figure 3. Resin exudation and canker development on stem of 4-year-old *Cupressus macrocarpa* cv. Goldcrest following inoculation with *Seiridium cardinale*.

Sequence-Based Molecular Diagnosis

The PCR product from the ITS1 and ITS4 primers yielded a single band of approximately 700 bp. The DNR1 isolate's sequence was uploaded to the NCBI GenBank and assigned the accession number OK287164. BLASTn analysis of the ITS sequence revealed 100% homology with the sequences of the *S. cardinale* USA isolate (GenBank accession no. AF346556) (Worapong, 2001) and 99.63% homology with the sequences of the South African isolate (GenBank accession no. LT853064.1) (Bonthond et al., 2018).

The fungus is found all over the world and has been reported in many countries across five continents. Sümer (1987) reported the presence of the fungus only symptomologically by visual observation of symptoms on *C. sempervirens* var. *horizontalis* in Gökova, Turkey, with no

morphological or molecular diagnosis. We searched the fungal databases, and this is the first report of the fungus on *Cupressus macrocarpa* cv. Goldcrest in Turkey, to the best of our knowledge (Farr and Rossman, 2021).

Conclusion

Following symptomologic, morphologic, and molecular diagnosis assays, we found that *S. cardinale* is the cause of canker in *Cupressus macrocarp*a cv. Goldcrest trees, which has caused significant economic damage worldwide. Presently, three *Seiridium* species (*S. cardinale*, *S. cupressi*, and *S. unicorne*) are thought to be responsible for a pandemic of cypress canker disease, which is affecting plantations for wood production and ornamental tree cultivation. (Boesewinkel, 1983; Graniti, 1986, 1998). Even though we only conducted a few surveys, we were able to identify seven isolates as *S. cardinale*. An MSc thesis is underway to survey a larger area for the disease and to determine whether other *Seiridium* species exist.

For species identification, we used universal ITS primers. The sequence of the fragment showed a high ratio of similarities to other *S. cardinale* isolates in GenBank.The next step in our research will focus on the phylogenetic analysis of the isolates using species-specific primers.

As reported previously and based on our observations, the infection of susceptible cypress trees is mostly fatal. Depending on the environmental conditions, the death of trees may occur in a few months to years. Lemon cypress is a popular recreational tree in Turkey, and it is obviously susceptible to the canker. Because the tree is propagated vegetatively and is planted as a hedge tree in many house gardens in Çanakkale, dieback symptoms spread quickly to nearby plants. The impact of exotic forest pathogens ranges from mortality to disruption of plantations of exotic and native trees, as *S. cardinale* did on planted Monterey cypress in California (Graniti, 1998). *Seiridium cardinale* is the largest pandemic threat to cupressaceous plants including *C. sempervirens*, the main cypress species distributed across Turkey, and urgent action is required to avoid further spread of this potentially high-risk disease. Sales of lemon trees may need to be symptom-checked immediately to slow the spread of this pathogen in new areas and species.

Authors' Contributions

Conception/Design of Study- F.M.; Data Acquisition- F.M., A.Ö.; Data Analysis/Interpretation- F.M., A.Ö. S.D.; Drafting Manuscript- F.M., S.D.; Critical Revision of Manuscript- F.M., S.D.; Final Approval and Accountability- F.M., A.Ö., S.D**.**

Conflicts of Interest Statement

Authors declared no conflict of interest

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