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First report of *Neoscytalidium novaehollandiae* on common sage (*Salvia officinalis*)

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Abstract
In June 2020, many plants exhibited symptoms of root rot and foliar blight in the experimental field of common sage in Şanlıurfa province, Turkey. The pathogen was identified as *Neoscytalidium novaehollandiae* based on morphological characteristics and phylogenetic analysis of partial sequence of the transcription elongation factor 1-α gene and the internal transcribed spacer of rDNA. Koch's postulates were fulfilled by successful re-isolation of the pathogen from inoculated plants in the pathogenicity assay. To our best knowledge, this is the first report of *N. novaehollandiae* causing root rot and foliar blight of common sage worldwide.

Keywords *Salvia officinalis* · *Neoscytalidium novaehollandiae* · Foliar blight · Root rot

The genus *Salvia* L. belongs to the mint family Lamiaceae, and comprises about 900 species, spread throughout the world (Delamare et al. 2007). Some of the species have great economic value since they are used as spices and flavouring agents by cosmetic and perfumery industries. *Salvia* species are odorous small shrubs native to the Mediterranean region (Albania, Croatia, Greece, Italy, Turkey, etc.) and cultivated worldwide (Raal et al. 2007). In Turkey, the genus *Salvia* is represented by over 100 taxa, half of which are endemic, and 1,271 tons of sage production is conducted on a cultivation area of over 660 ha in 2020 (TURKSTAT 2021). Common sage (*Salvia officinalis* L.) is a perennial herb or sub-shrub and consumed for aromatic and medicinal species credited with a long list of medicinal use (Newall et al. 1996).

Several fungal and oomycetous species were reported on *S. officinalis* including *Golovinomyces biocellatus* (Radisek et al. 2012) and *G. neosalviae* (Venegas-Portilla et al. 2020) causing powdery mildew, *Sclerotinia sclerotiorum* causing white mold (Garibaldi et al. 2004) *Peronospora lamii* causing downy mildew (Humphreys-Jones et al. 2008), and *Phytophthora cryptogea* causing root rot (Garibaldi et al. 2015; Çakır et al. 2017). In June 2020, symptoms including yellowing and shedding of leaves, foliar blight, and root rot prior to plant death were observed on two-year-old *S. officinalis* cv. Elif plants in the sage fields of Koruklu village (36°42′ N; 38°58′ E, 410 m altitude), Akçatepe district of Şanlıurfa province (Fig. 1a, b). The incidence of diseased plants was approximately 25%.

To isolate the pathogen, 20 diseased plants were sampled from the field. Symptomatic roots of plants were cleaned thoroughly from soil residues under tap water. Portions (about 3–5 mm) of necrotic root tissues were surface disinfested in 1% sodium hypochlorite solution for 60 s and rinsed twice in sterile water, air dried, and placed onto potato dextrose agar (PDA) amended with 0.2 g/l streptomycin sulphate. After a five-day incubation period at 26 °C in the dark, fungal colonies with similar morphological characteristics were consistently isolated and transferred to new PDA plates using the hyphal tip method. The 7-day-old hyphae from the representative isolate (Nn-01Sf) were also transferred to 1.5% water agar containing sterile pine needles to promote the formation of conidiomata.

Colonies were initially powdery white with dense, hairy aerial mycelium, which becoming dark gray to black within ten days (Fig. 2a, b). Arthroconidia were dark brown, thick-walled, cylindrical to oblong, 0 to 1 septate, 6.7 × 4.2 µm

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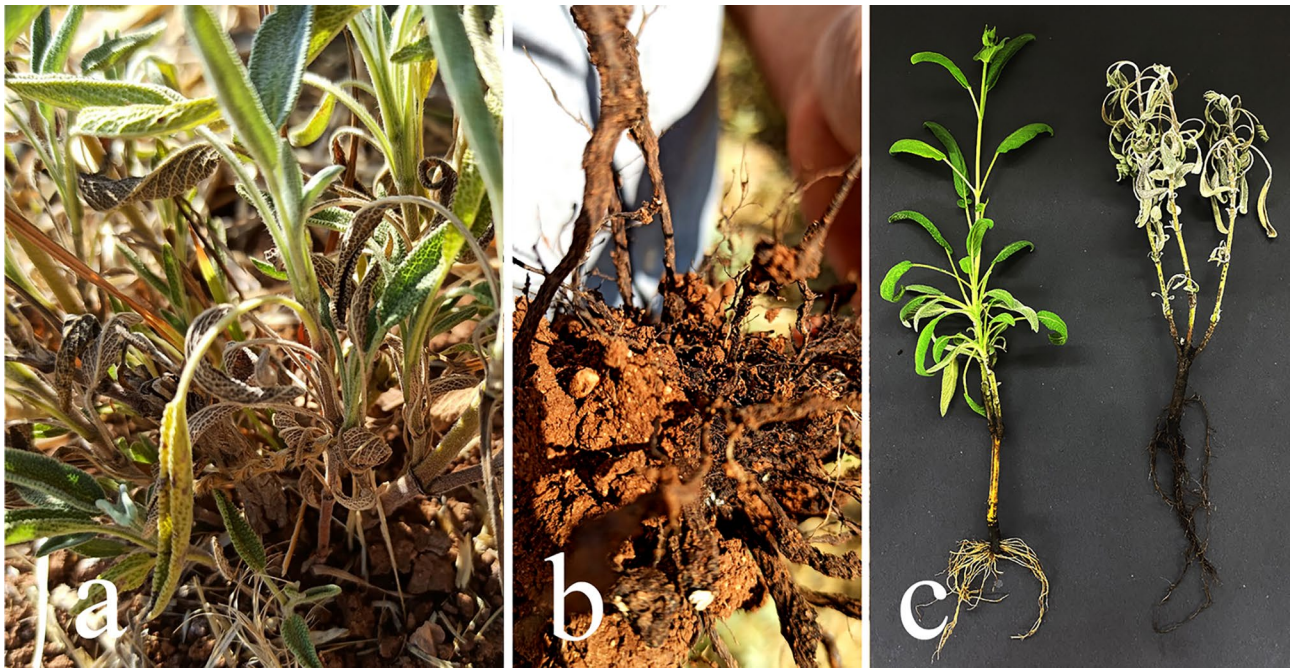

Fig.1 Symptoms on *Salvia officinalis* plants; **a** foliar blight in the field, **b** root rot in the field, **c** foliar blight and root rot symptoms on five healthy 2-year-old common sage cv. Elif plants after 14 days of inoculation

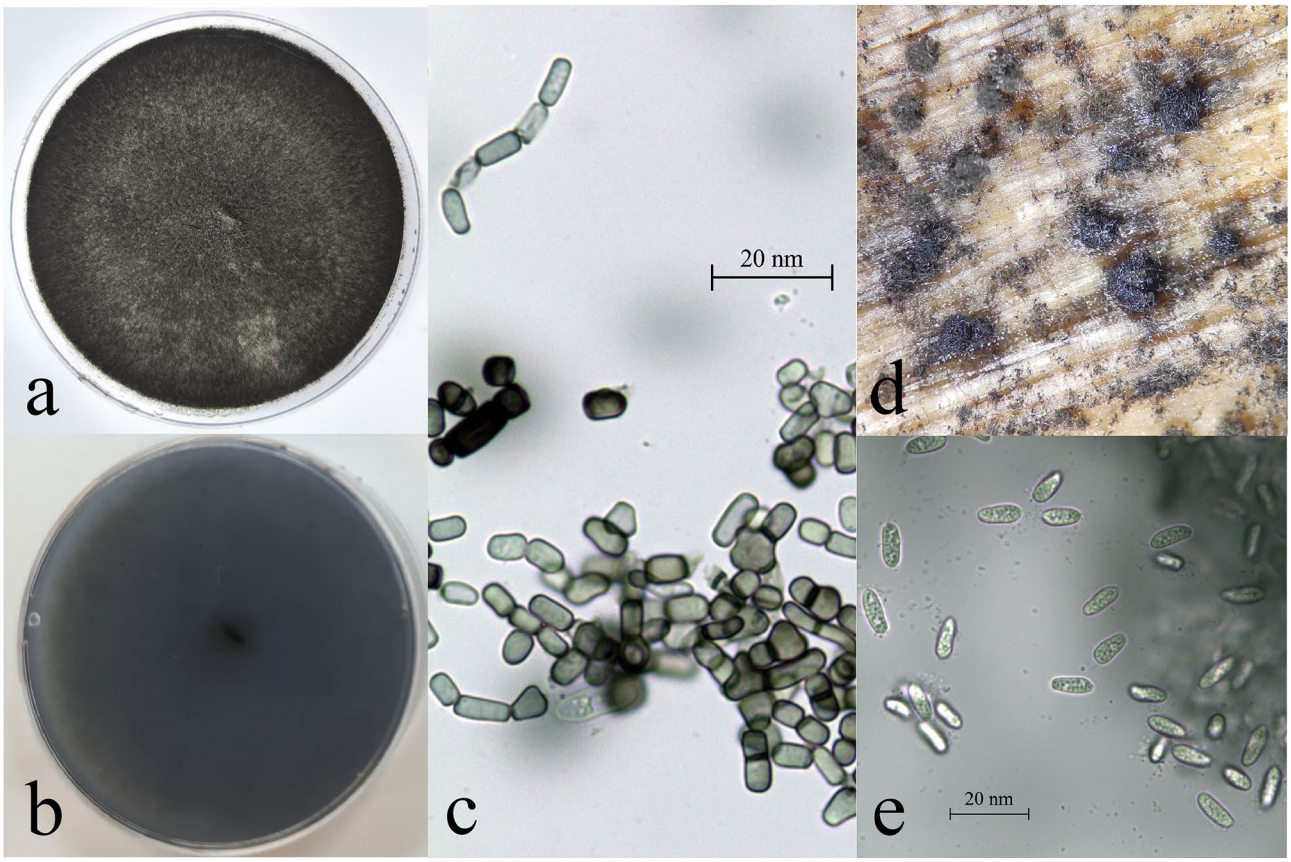

Fig. 2 Characteristics of *Neoscytalidium novaehollandiae* isolate Nn01Sf; **a, b** colony morphologies on PDA, **c** arthroconidia, **d** pycnidia, **e** conidia formed in pycnidia

($n=30$; Fig. 2c), formed both singly and in arthric chains by hyphal fragmentation. Pycnidia observed on pine needles on water agar were stromatic, semi-immersed, and black with a mean diameter of 310 µm (Fig. 2d). Conidia formed in pycnidia were hyaline, oblong to globose, apices rounded, 0- to 1-septate, and 11.2×4.6 µm in length ($n=30$; Fig. 2e). These morphological characteristics were consistent with the description of *Neoscytalidium* spp. by Phillips et al. (2013).

For further precise identification, genomic DNA of the representative isolate was extracted using DNeasy Blood and Tissue Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The sequences of the translation elongation factor 1-α gene (EF1-α) and the internal transcribed spacer region (ITS) of rDNA were amplified using primers EF1-728 F and EF1-986R (Carbone and Kohn 1999) and ITS1 and ITS4 (White et al. 1990), respectively, and both strands of the PCR products were sequenced at the Macrogen Inc. Sequencing Service (Seoul, Korea).

All sequences of the isolate were read and edited checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer directions using MEGA X software (Kumar et al. 2018). Multiple sequence alignment was constructed using the online sequence alignment tool, MAFFT (https://mafft.cbrc.jp/alignment/server/large.html, Katoh et al. 2019). Phylogenetic analysis on the combined dataset was done using PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b10 (Swofford 2003) for Maximum Likelihood analyses and visualized TREEFINDER version of March 2011 (Jobb 2011) and MEGA X. The isolate was clustered closely with the isolates of *N. novaehollandiae* in the phylogenetic tree constructed (Fig. 3). The resultant sequences were deposited in GenBank with Accessions Nos. MZ392224 and MZ389710 for the EF1-α and ITS loci, respectively, and compared with the sequences registered in GenBank based on nucleotide similarity. A BLASTn search of the resultant sequences showed 99.30 and 100.00% identity with the EF1-α (Accessions No. EF585581) and ITS (Accessions No. EF585535) sequences of *N. novaehollandiae* CBS122071 strain, respectively. Based on morphological and molecular identification, the isolates were identified as *Neoscytalidium novaehollandiae* Pavlic, T.I. Burgess & M.J. Wingf. The isolate Nn-01Sf was deposited in Erciyes University Culture Collection (EUCC—WDCM 1202) with the accession number EUCC- 21,068 M.

Pathogenicity tests performed were by spraying conidial suspension (1×10^6 conidia/mL) of *N. novaehollandiae* Nn-01Sf strain on leaves and stems of five healthy 2-year-old *S. officinalis* cv. Elif plants, till run off. Five control plants were treated with sterile water. After inoculation, the plants were maintained in a moist chamber constructed using plastic bags to maintain moisture for 24 h. These bags were removed, and all plants were transferred to a growth chamber at 25 °C with a 16-h light/dark photoperiod. Two

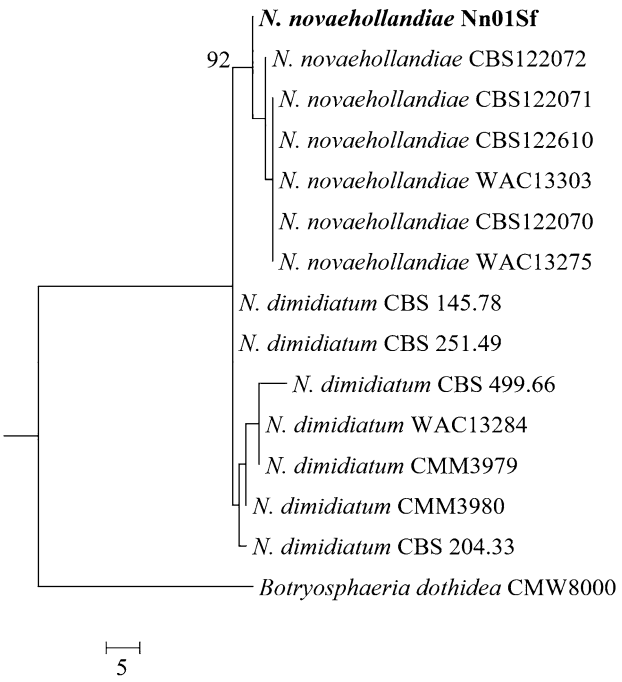

Fig. 3 Phylogenetic tree inferred by using the Maximum Likelihood method was reconstructed using the EF1-α and ITS sequences of *Neoscytalidium novaehollandiae* isolate Nn01Sf. The bootstrap value (percentage, based on 1,000 replications) is shown on the branch

weeks after inoculation, root rot and foliar blight symptoms (Fig. 1c) were observed on all plants inoculated, whereas all control plants remained symptomless. The pathogen was re-isolated from all inoculated plants and identified by conidial morphology, fulfilling Koch's postulates.

Neoscytalidium species cause canker, dieback, stem blight and root rot diseases on several hosts of agronomic importance. *N. novaehollandiae* has been reported as a destructive pathogen in Turkey causing stem canker and branch dieback of almond trees (Ören et al. 2020), stem blight on tomato (Derviş et al. 2020), and wood canker caused by on grapevine (Akgül et al. 2019). However, there is no available data on *N. novaehollandiae* associated with common sage. To our knowledge, this is the first report of *Neoscytalidium novaehollandiae* on common sage in Turkey and worldwide (Farr and Rossman 2021).

Declarations

Research involving human participants and/or animal The authors declare that no human participants and animals were involved in this study.

Informed consent This manuscript is new and not being considered elsewhere. All authors have approved the submission of this manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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