Oksal E (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 2, Article number 12716
DOI:10.15835/nbha50212716
Research Article

Prevalence, molecular characterization, and variety reactions of *Neoscytalidium novaehollandiae* on mulberries in Turkey

Erçin OKSAL

Malatya Turgut Özal University, Faculty of Agriculture, Department of Plant Protection, Malatya, Turkey; oksalercin@gmail.com

Abstract

Turkey is one of noteworthy countries for both fruit genetic resources and amount of fruit production in the world. Mulberry is cultivated throughout Turkey, most commonly in Central, Northeast and Southeast Anatolia. Mulberry has a great market potential thanks to its fresh consumption and usage of processed food products. In June 2019, a disease was observed causing deaths in shoots and branches on mulberry trees in Malatya province of Turkey. The causative agent of the disease was identified as *Neoscytalidium novaehollandiae* according to morphological characteristics and sequencing of TEF 1-α gene (Accession no. MT362602 and MT362603), ITS (Accession no. MT195554 and MT195555) and LSU (Accession no. MT195552 and MT195553). Based on the concatenated sequences of the ITS, TEF 1-α, and LSU, a phylogenetic tree was built using Bayesian analysis. Reactions of nine mulberry cultivars against the disease ('Ulukale', 'Ayaş', 'Ichinose', 'Poser', 'Kenmochi', 'Arapgir', 'Sarı aşı', 'Horum' and 'Istanbul') inoculated with Malatya isolate of *N. novaehollandiae* were evaluated under growth chamber conditions. All-mulberry cultivars artificially inoculated with *N. novaehollandiae* isolate exhibited severe necrosis symptoms on woody tissues of tested plants. It was confirmed that *N. novaehollandiae* is a fungal pathogen associated with dieback and canker on mulberry trees in Turkey for the first time. New mulberry plantations could be endangered by this emerging new disease.

Keywords: canker; dieback; mulberry; *Neoscytalidium novaehollandiae*; pathogenicity

Introduction

Mulberry is a fast-growing tree and widely distributed in many regions of the world. Mulberry trees are mostly cultivated for the silkworm, which feeds exclusively on their leaves (Tutin, 1996). However, in Turkey, mulberry is mostly cultivated for its delicious fruit, which is consumed dried or fresh (Sanchez, 2000). Three main mulberry species characterized by their fruit colour are predominant in Turkey; *Morus alba* L.: with white fruits, *M. rubra* L.: with red fruits, and *M. nigra* L.: with black fruits (Yaltirik, 1982). *Morus alba*, however, is the most important species (95%) in fruit production (Ercisli, 2004; Cocen *et al.*, 2020).

Many fungal diseases have been identified to damage mulberry trees in Turkey and across the world: *Neophloeospora maculans* causing leaf spot in Turkey, (Soylu *et al.*, 2003), *Pseudocercospora mori* causing grey spots on leaf in Australia (Grice *et al.*, 2006), *Diplodia seriata* causing twig dieback and canker in Iran (Arzanlou

Received: 29 Mar 2022. Received in revised form: 18 Apr 2022. Accepted: 20 Apr 2022. Published online: 23 May 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

and Dokhanchi, 2013), *Lasiodiplodia theobromae* causing root rot in China (Wei *et al*., 2014), *Curvularia lunata* causing leaf blight in Thailand (Bussaban *et al*., 2017), *Nigrospora sphaerica* causing shot hole disease in China and India (Chen *et al*., 2018; Arunakumar *et al*., 2019), *Botryosphaeria dothidea* causes shoot canker in China (Huang *et al*., 2019), and *Aplosporella javeedii* causes branch blight disease in China (Jia *et al*., 2019).

Several fungal species within the family Botryosphaeriaceae are considered serious plant pathogens related with cankers and dieback in a wide range of forest and fruit trees as well as ornamentals. The *Neoscytalidium* genus is distinguished by 0-2-septate, doliform, oblong-obtuse, dark brown, powdery conidia generated in arthric chains derived from aerial mycelium (Phillips *et al*., 2013). The members of the genus have stromatic and blackish-brown pycnidial conidiomata. The *Neocytalidium* genus is a phytopathogen that may be found in both plants and soil, which two of them are human pathogens (Machouart *et al*., 2013). Only three species have been described in plants and available in the MycoBank database: (1) *N. orchidacearum* was only identified associated with a leaf spot on orchid in Thailand (Huang *et al*., 2016; Suwannarach, 2018), (2) *N. dimidiatum* (Penz.) Crous & Slippers was described by Crous *et al*. (2006) as a type species of *Neoscytalidium* Crous & Slippers and accepted as the synonym of *N. hyalinum* by Phillips *et al*. (2013), (3) *Neoscytalidium novaehollandiae* was described by Pavlic *et al*. (2008) with similar morphological characteristics to *N. dimidiatum*, which can only be discriminated by sequence analyses from this species.

In June 2019, shoot blight symptoms were observed on white mulberry trees (*M. alba*) in orchards located in Malatya province, Turkey. The general disease incidences were 10% for orchards. Symptoms appeared as drying of branches, scorching of leaves and discolouring stems of the affected shoots, branch canker and dieback with internal vascular discoloration. The main objective of this study was to identify the causative agent of disease found on mulberries via molecular methods and morphological features, as well as to assess its pathogenicity.

Materials and Methods

Fungal isolates and morphological characteristics

The samples of shoots and branches showing symptoms were collected from each orchard in Malatya province for fungal isolation (Figure 1). Small pieces (5-10 mm^2) were sliced from symptomatic tissues and sterilized by immersing them in 1% sodium hypochlorite solution for 1 min, rinsed twice with sterile distilled water and then air-dried. The pieces were placed onto potato dextrose agar (PDA, Sigma-Aldrich, USA) including 0.05 g/L chloramphenicol (Sigma-Aldrich, USA) and 0.1 g/L streptomycin sulphate (Merch, Germany) to eliminate bacterial contamination. Twenty-six colonies showing similar cultural characteristics were obtained following an incubation period of five days at 25 ± 1 °C in climate chamber (Panasonic, Japan). One strain for each orchard was purified on PDA stock plates and stored at 4 °C in the microcentrifuge tubes (Eppendorf, USA) with filter papers (Whatman, USA) at -20 °C. The hyphae of 7-day-old of the strains were transferred to 1.5% water agar including sterile pine needles to induce conidiomata formation. The representative strains were deposited in a culture collection registered by the World Federation for Culture Collections. The cultural and conidial morphology were examined and measured at 400× magnification using Nikon E200 Eclips microscope and Nikon software (Nikon Microsystems, Japan).

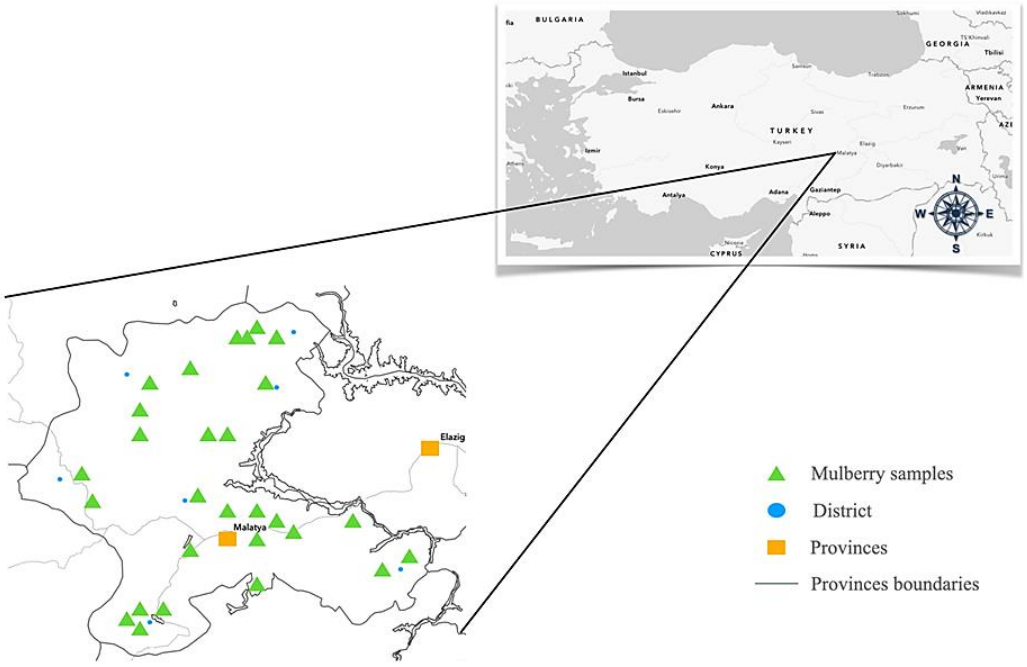

Figure 1. The surveyed area in Malatya province

Molecular identification

The DNA extraction of representative strains, which named as Mlty_Ma01 and Mlty_Ma02, was conducted with DNeasy Plant Mini Kit (Qiagen, Germany) in accordance with the commercial firm's protocol. To identify the species, the partial sequences of the translation elongation factor 1-α gene (TEF1-α), the internal transcribed spacer (ITS), and the large subunit (LSU) were amplified with primer pairs (Table 1). Twenty five microliters of total PCR reaction mix contained; 2.5 µl of diluted genomic DNA, 20 pmol of each primer (Macrogen Inc., Korea), 1.25 nmol of each deoxynucleotide (Thermo Scientific, USA), 0.5 U DreamTaq™ Green polymerase (Thermo Scientific, USA), 1.5 mM of MgCl2, 2.5 µl of 10x DreamTaq™ Green PCR buffer (Thermo Scientific, USA) and 14.5 µl H2O (Himedia, India). The cycling programme included an initial denaturation at 95 °C for 5 min, 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for ITS and 58 °C for LSU and TEF 1-α for 45 s, and elongation at 72 °C for 1 min in Arctik™ Thermal Cycler (Thermo scientific, USA). This was followed by a final extension for 5 min at 72 °C. PCR amplicons were electrophoresed on 1.5% agarose gel (Sigma-Aldrich, USA) and was stained with Pronasafe (Conda, Spain).

Table 1. Primer pairs used for PCR

Locus	Primer	Primer sequence 5' to 3'	Reference
Nuclear ITS rDNA	ITS1	TCCTCCGCTTATTGAAAGG	White *et al.*, (1990)
	ITS4	TCCTCCGCTTATTGATATGC	White *et al.*, (1990)
Nuclear LSU rDNA	LR0R	ACCCGCTGAACTTAAGC	Vilgalys and Hester (1990)
	LR5	TCCTGAGGGAAACTTCG	Vilgalys and Hester 1990)
Translation elongation factor 1-α	EF1-728F	CATCGAGAAGTTCGAGAAGG	Carbone and Kohn (1999)
	EF1-986R	TACTTGAAGGAACCCTTACC	Carbone and Kohn (1999)

The PCR products were sequenced bidirectionally via the same primers by a commercial company (Macrogen Inc., Seul, Korea).

TEF1-α, ITS, and LSU sequences of *Neoscytalidium* generated in this study, as well as sequences of closely related species downloaded (Table 2) after a BLAST search from the GenBank database (http://www.ncbi.nlm.nih.gov/), were included in separate datasets (one for each molecular marker) in the PhyDE program v.0.9971 (Müller *et al*., 2010). On the online Mafft service (Katoh *et al*., 2017), each dataset was individually aligned, and discrepancies were manually rectified. MEGA 7 was used to find the optimal evolutionary model for each dataset (Kumar *et al*., 2016). A concatenated dataset including previously aligned TEF 1-α + ITS + LSU sequences was used. For phylogenetic inference, Maximum Likelihood (ML) analysis with 1000 bootstrap repetitions in the same software for each dataset were used and concatenated multilocus dataset. For 1,000,000 repetitions, phylogenetic analyses were done with Mr- Bayes v 3.2.7a Bayesian Analysis (Ronquist *et al.,* 2012). Mega 7 and FigTree v1.4.4 (Rambaut, 2018) were used to depict the phylogenies generated by the ML and BI studies, respectively.

Table 2. List of strains used for phylogenetic tree construction

Species	Strain	Isolation source	Location	Collector and date of isolation	Sequences accession number		
					ITS	LSU	TEF1-α
Neoscytalidium novaehollandiae	Savur_SL02	*Solanum lycopersicum*, Tomato	Mardin, Turkey	Derviş, S. 2019	MT193667	MT192450	MT680928
Neoscytalidium novaehollandiae	Mlty_Ma01	*Morus alba*, Mulberry	Malatya, Turkey	Oksal, E. 2020	MT195552	MT195554	MT362602
Neoscytalidium novaehollandiae	Mlty_Ma02	*Morus alba*, Mulberry	Malatya, Turkey	Oksal, E. 2020	MT195553	MT195555	MT362603
Neoscytalidium dimidiatum	Arp2-D	*Vitis vinifera*, Gapevine	Malatya, Turkey	Oksal, E. 2018	MK813852	MK813853	MK816355
Neoscytalidium novaehollandiae	PTD-MA	*Pinus brutia*, Pinus	Iran	Alizadeh, M. 2018/2019	MW605153	MW605155	MW605154
Neoscytalidium dimidiatum	GCND1	*Hylocereus polyrhizus*, Dragon fruit	Taiwan	Wang, C. L. and Fung, J. A. 2020	MT323056	MT341860	MT358409
Neoscytalidium novaehollandiae	CBS:122071	*Mangifera indica*, Mango	Australia	Sakalidis, M., CBS Culture collection,	MH863173	MH874720	EF585580
Neoscytalidium novaehollandiae	NeNo3	*Quercus brantii*, Oak	Kermanshah, Iran	Sabernasab, M. and Jamali, S. 2013/2015	MH883623	MH899581	MH885094
Neoscytalidium dimidiatum	CPC:38666	*Aloidendron dichotomum*	South Africa	Crous, P.W., CBS Culture collection,	MW883431	MW883823	MW890096
Neoscytalidium novaehollandiae	Mlty_01	*Prunus armeniaca*, Apricot	Malatya, Turkey	Oksal, E. 2019	MT041243	MT038898	MT710710
Neoscytalidium dimidiatum	CBS:125695	*Homo sapiens*, Human toe tail	France	CBS Culture collection. 2016	KX464231	KX464051	KX464764

Pathogenicity and cultivar reaction tests

To confirm pathogenicity, six 2-years-old potted *M. alba* trees (cv. Ulukale) were used. For cultivar reaction tests, nine different mulberry cultivars, including Ulukale, Ayaş, Ichinose, Poser, Kenmochi, Arapgir, Sarı aşı, Horum and Istanbul were used (Table 3). Cultivars were inoculated with mycelial disks from the

margin of each 7-days-old fully-grown *N. novaehollandiae* strain. The inoculation zone was sterilized with 65% ethanol and injured up to bark depth with a cork borer, and 4 mm diameter mycelial disks were placed into the wounds and covered with parafilm (Isolab, Turkey). Sterile agar plugs were employed on the wounds as controls. One disease-free control and 3 inoculated plants were used for each cultivar. Inoculation sites were wrapped with parafilm to keep moisturized and the plants were incubated at 25 ± 1 °C with 85 to 90% relative humidity in a growth chamber with a 16-h photoperiod for a month. Four weeks later parafilms were removed, barks were peeled and necrosis lengths under the barks were measured. Results were subjected to analysis of variance (ANOVA) and significant differences between cultivars were detected using the Tukey test at $P < 0.01$ using SPSS statistical software V 17.0 (SPSS Inc., Chicago, IL, USA).

Table 3. Mulberry cultivars and their fruit characteristics

Cultivars	Fruit characteristics	Species
'Ulukale'	white fruit / seedless	*Morus alba* L.
'Ayaş'	white fruit / having seeds	*Morus alba* L.
'Ichinose'	black purple / seedless	*Morus alba* L.
'Poser'	white fruit / seedless	*Morus alba* L.
'Kenmochi'	black fruit / seedless	*Morus bombysis* Koidz.
'Arapgir'	white fruit / having seeds	*Morus alba* L.
'Sarı aşı'	Fruitless	*Morus alba* L.
'Horum'	black, dark red fruit / having seeds	*Morus nigra* L.
'Istanbul'	white fruit / seedless	*Morus alba* L.

Results

Disease symptoms and distribution

Mulberry samples showing symptoms like drying in shoots and brunches (Figure 2a) and xylem necrosis (Figure 2b) were collected from mulberry production areas in Malatya during June and July 2019. Branch cankers and trunk cankers related with dieback symptoms were observed in 28 orchards from different districts in Malatya. Cankers having longitude form caused dark discoloration of xylem tissue and were frequently related with extensive gumming.

Cultural and morphological characterization

In 48 hours, all strains formed fast expanding colonies that covered the surface of a 90-mm PDA plate. The mycelial growth began as white or hyaline colored colony in the middle, then progressed to an olive-green to greyish tone, and lastly to a dark-gray to black color after two weeks on PDA medium.

Blackish-brown pycnidia occurred on a woodchip placed on 1.5% water agar after three weeks incubation. Pycnidia were black in color, having a a mean diameter of 290 µm (n = 500), irregularly shaped to ovoid and superficial or semi-immersed with (Figure 2c). Pycnidial conidia were ellipsoidal, hyaline, becoming sepia 0-1-septate, 3.2 to 4.3 × 11.2 to 13.6 µm (n = 20) (Figure 2d). The isolates were developed as dark grey to black with plentiful arthroconidia in aerial mycelia was formed on PDA. Arthroconidia were rod-shaped, thick-walled, 5.4 to 9.7 × 2.7 to 4.6 µm (n = 50), formed in arthric chains or singly by hyphal fragmentation, and observed to have 0- to 1-septate (Figure 2e). The morphological characteristics of twenty six strains were similar to description of *Neoscytalidium* spp. reported by Phillips *et al.* (2013).

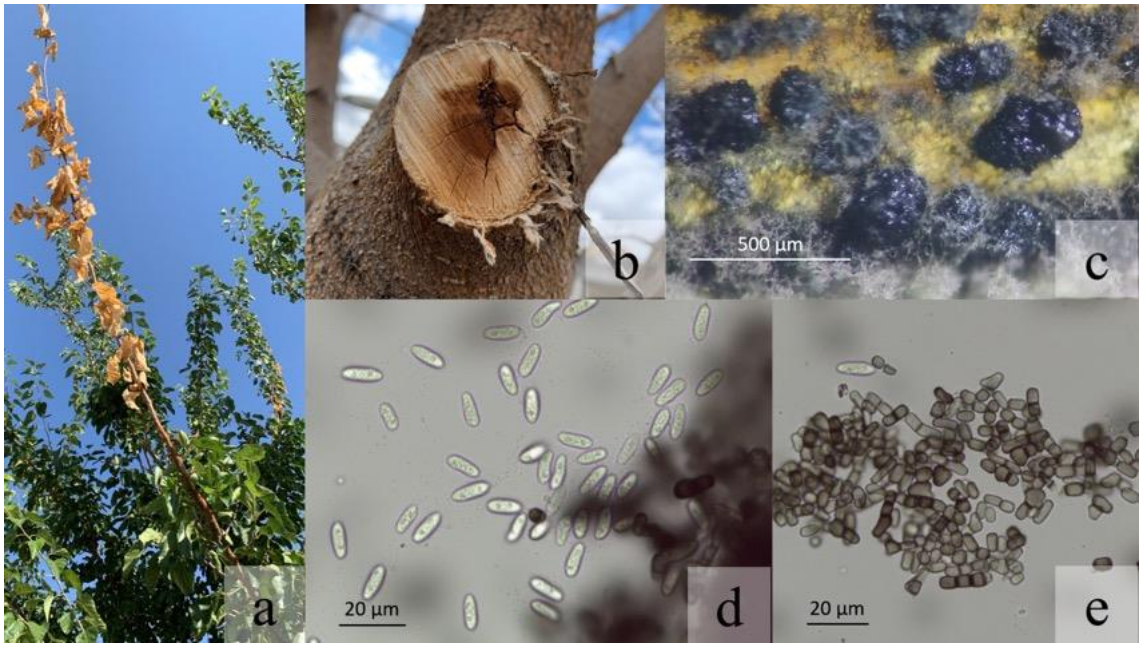

Figure 2. Disease symptoms on *Morus alba* in mulberry orchards caused by *Neoscytalidium novaehollandiae* (a); Branch of a mulberry tree with necrosis (b); Pycnidia formed on a woodchip 4 weeks after isolation and incubation in PDA at 25 ± 1 °C (c); Fusicoccum-like pycnidial conidia (d); Scytalidium-like anamorph showing various shapes and maturity stages of arthroconidia (e)

Molecular identification and phylogenetic analysis

The sequences of two representative strains for each primer were deposited in NCBI with the accession numbers MT362602-MT362603 (TEF1-α), MT195552-MT195553 (LSU), and MT195554-MT195555 (ITS), respectively. These strains showed 100% nucleotide identity with those of type strain CBS 122071 (TEF1-α EF585580; ITS MH863173; LSU MH874720) when assessed on its own. The combined or separate phylogenetic analyses based on TEF1-α, ITS, and LSU sequences of strain confirmed the species identification when compared with those of previously published sequences of ex-type and representative *Neoscytalidium* spp. strains available in GenBank. In Fig. 3, the phylogram displays the relationships between *Neoscytalidium* species identified in this study and additional *Neoscytalidium* species reported elsewhere. On the phylogenetic tree, three major phylogenetic clusters (Cluster I, II, and III) can be clearly recognized. The tree indicates that *N. novaehollandiae* mulberry isolates are genetically close to each other, whereas *N. dimidiatum* from human toe tail clustered in a separate branch (Cluster III) because of a high diversity of genetic relatedness. *N. novaehollandiae* isolates from mulberry formed a clear branch (cluster I) containing other *Neoscytalidium* species from tomato and grapevine from Turkey. Cluster II contained *Neoscytalidium* species from agronomically important crops such as dragon fruit, apricot and mango.

Specimens Mlty_Ma01 and Mlty_Ma02 were deposited with accession numbers EUCC- 20411 M and EUCC- 20412 M, respectively in Erciyes University Culture Collection (EUCC - WDCM 1202).

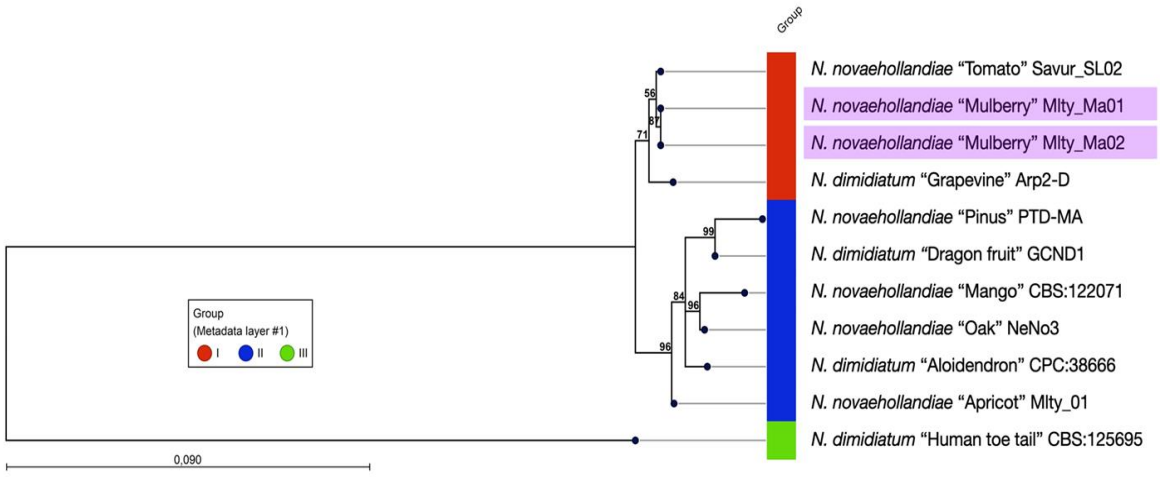

Figure 3. Concatenated three-locus (LSU, Tef1-α and ITS) Bayesian phylogenetic analysis by maximum likelihood of of *Neoscytalidium* species
The bootstrap values are shown on the branches

Pathogenicity test

Necrotic lesions above and below the point inoculation were observed on the bark of stems with a mean length of 2.74±0.07 cm, whereas control plants (cv. 'Ulukale') remained asymptomatic (Figure 4a). The fungus was re-isolated from symptomatic tissues of inoculated shoots, thus fulfilling Koch's postulates. Re-isolated fungal cultures were morphologically identical to *N. novaehollandiae*.

Necrosis symptoms were observed in all mulberry cultivars including cv. 'Ulukale' artificially infected with *N. novaehollandiae* Mlty_Ma01 isolate (Figure 4a-i). Healthy plant and dead seedlings as a result of *N. novaehollandiae* are shown (Figure 4j). No symptom was observed in control plants. Necrosis lengths of the 2 years old nine mulberry cultivars were measured (Figure 5).

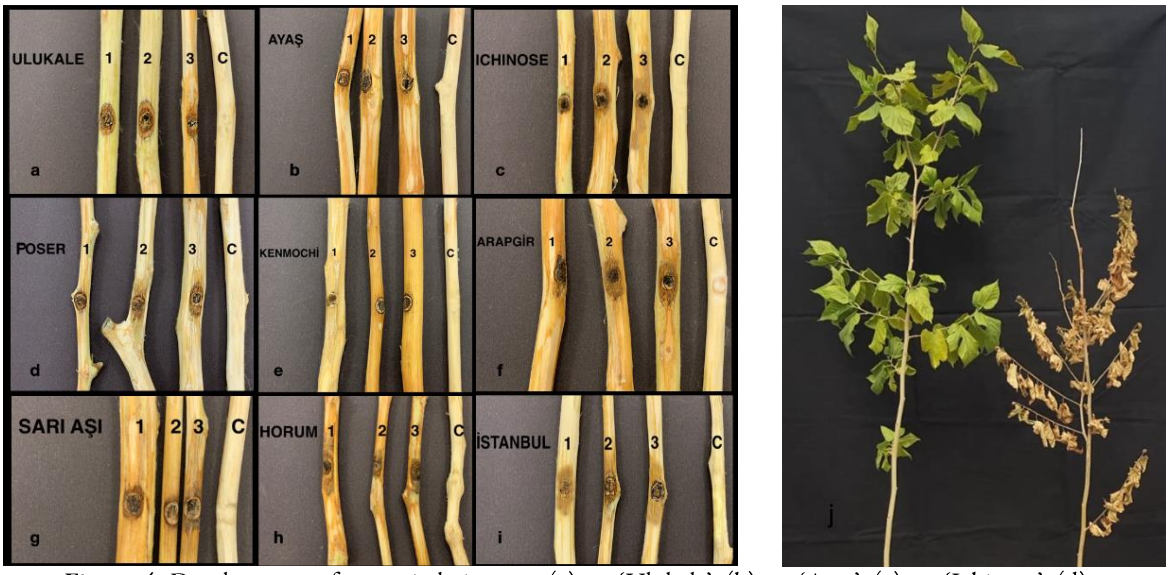

Figure 4. Development of necrotic lesions on (a) cv. 'Ulukale', (b) cv. 'Ayaş', (c) cv. 'Ichinose', (d) cv. 'Poser', (e) cv. 'Kenmochi', (f) cv. 'Arapgir', (g) cv. 'Sarı aşı', (h) cv. 'Horum' and (i) cv. 'Istanbul' artificially inoculated with *Neoscytalidium novaehollandiae*. Samples 1,2,3 are repetitions and C is for Control. (j) Healthy plant and dead plant caused by *Neoscytalidium novaehollandiae*

In cultivar reaction tests against *N. novaehollandiae*, statistical difference was observed among the mulberry cultivars in terms of necrosis lengths. *Morus nigra* cv. 'Horum' was the most affected mulberry cultivar followed by 'Sarı aşı', 'Arapgir', 'Ayaş', 'Kenmochi', 'Ichinose', 'Istanbul', 'Ulukale' and 'Poser' cultivars. The size of necrosis caused by the pathogen was found to be significant in both dark and white cultivars. the difference between the white cultivars was found to be significant, while there was no statistical difference in necrosis lengths of 'Ulukale' and 'Poser' cultivars which are white and seedless. The necrosis sizes in 'Ichinose' and 'Kenmochi' cultivars which are dark and seedless were found insignificant among themselves, whereas the difference between the dark 'Horum' cultivar was found to be significant.

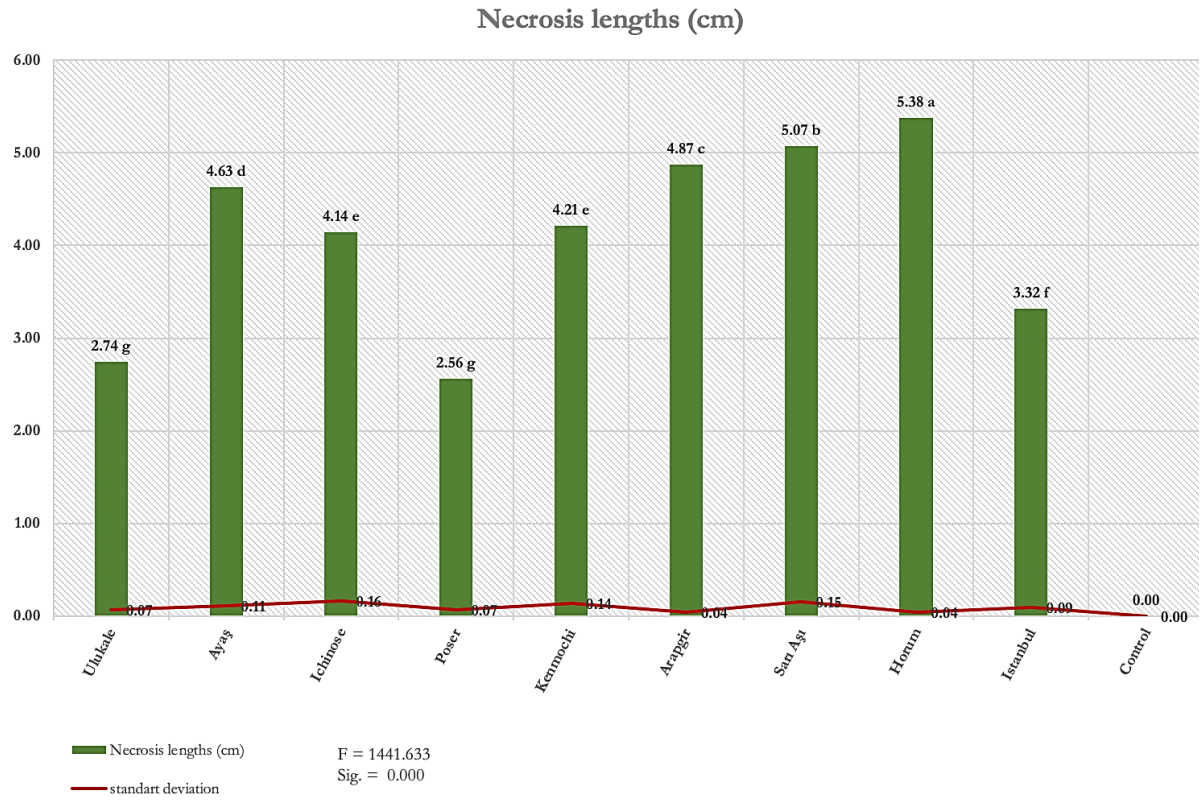

Figure 5. The lengths of necrosis caused by *Neoscytalidium novaehollandiae* on mulberry cultivars (cm)
The experimental results were analysed by one-way ANOVA and Tukey's HSD. Values (mean ± SD) with no letters in common are significantly different ($p < 0.05$) within a column.

Discussion

The present study recognizes *N. novaehollandiae* causing canker and shootblight of mulberry in Turkey. Symptoms include lengthwise growing branch and trunk cankers, as well as fruit-bearing spurs and shoot blight. Morphological observations of the genuine colony features and other anamorphic morphologies characteristic of the species validated the identification of *N. novaehollandiae* (Phillips *et al.*, 2013). Phylogenetic studies of ITS, LSU, and TEF1-a sequencing data were also used to validate identification. These investigations also demonstrated that strains collected from distinct mulberry symptoms (blighted shoots, trunk and branch cankers) and geographical areas constituted a single species. Phylogenetic investigations revealed that *N. novaehollandiae* strains from mulberry were genetically related to isolates from tomato, mango, apricot, and grapevine.

Since mulberry adapts easily to different soil and climate conditions, it is cultivated in different territories in the world. Anatolia is known to be the motherland of mulberry and grown in almost all provinces of Turkey. *Morus alba*, *M. rubra* and *M. nigra* cultivars are cultivated for high-quality mulberry production in some agro-ecological areas, such as Central Anatolia, Eastern Anatolia, Nort-East Anatolia and East Anatolia regions in Turkey (Ercisli and Orhan, 2007). Malatya is one of the most important provinces of mulberry production in the eastern Anatolia region.

Neoscytalidium spp. has been identified in a broad variety of ligneous plants worldwide (Punithalingam and Waterston, 1970; Sutton and Dyko, 1989). It has been linked to mango branch canker and dieback symptoms, as well as the formation of rot injury on mango fruit. (Marques *et al*., 2013; Ray *et al*., 2010). Pathogenicity studies revealed that *Neosytalidium* spp. was the most pathogenic species among various other Botryosphaeriaceae species. (Marques *et al*., 2013). The number of reports associated with *N. dimidiatum* and *N. novaehollandiae* causing plant diseases has rapidly increased worldwide as well as in Turkey (Akgul *et al*., 2019; Derviş *et al*., 2019, 2020; Oksal *et al*., 2019a; b; Ören *et al*., 2020; Oksal and Özer, 2021). Typical symptoms of *Neoscytalidium* sp. are leaf and branch drying, discoloration in xylem tissues, bark lesions, extensive gumming and longitudinal wood necrosis. Defoliation of leaves and death of the trees are also observed with a dieback in advanced stages. *Neoscytalidium* spp. has emerged as a new threat to agricultural output. *N. dimidiatum* has more hosts than *N. novaehollandiae* (Farr and Rossman, 2022).

This pathogen is known to be its vulnerability which primarily infects injured bark tissues by sunscald, hail, and mechanical wounds, but not healthy tissues (Calavan and Wallace, 1954). But latest studies have shown that it can also infect healthy plants (Polizzi *et al*., 2011; Agustí-Brisach *et al*., 2020). In our surveys we have also observed the plants that show brunch drying symptoms are young. But they had mechanical injuries which the causative agent can penetrate. Recently, *Neoscytalidium* spp. has been considered an emerging pathogen of pear, grapevine and apricot in Malatya (Oksal *et al*., 2019a; b; Oksal *et al*., 2019b; Oksal and Özer, 2021). These perennial plants are near or close to mulberry orchards across Malatya province, suggesting that cross infection between these crops is possible.

Mulberry cultivation has increased significantly in Malatya over the last decade, and intensive cultural practices such as clonal tree propagation, mechanical harvesting, and severe pruning, combined with long periods of drought causing trees increased water stress, may have tend the severity of this canker disease. Furthermore, it has been demonstrated that stressed trees are more vulnerable to Botryosphaeriaceae infestation (Ma *et al*., 2001).

Phylogenetic tree constructed with the sequence analysis data showed a difference between the *N. novaehollandiae* isolates. Sakalidis *et al*. (2011) and Al-Shuhaib *et al*., (2019) also determined the genetic difference in their studies. Only 4 spp have been identified *N. dimidiatum, N. oculus, N. orchidacearum*, and *N. novaehollandiae*. Florez-Muñoz *et al*. (2019) notified those 4 species can be cultured in vitro, but they are difficult to discriminate from each other morphologically, but they can be distinguished molecularly. In our study, strain CBS: 125695 reported as a human pathogen formed a separate cluster from the strains that were phytopathogens.

A statistically significant difference was observed between the cultivars in terms of host pathogen compatibility. In the cultivar reaction trials, it was observed that *N. novaehollandiae* isolate obtained from *M. alba* species caused serious necrosis not only in *M. alba* species but also in *M. bombysis* and *M. nigra* mulberry varieties, which are different varieties. The difference between cultivars has led to the understanding of genetically host-pathogen compatibility. Similar results were observed by Kazemzadeh *et al*. (2019) who studied host reaction of *N. novaehollandiae* on *Punica granatum*, *Alnus glutinosa*, *Pterocarya fraxinifolia*, *Mespilus germanica* and *Parrotia persica* in Iran.

The necessity to include more varieties and isolates in the pathogenicity study should be provided by further studies under different climatic conditions. Thus, it could be possible to use resistant/tolerant varieties

in infected orchards to manage the disease. *Neoscytalidium* is known to be aggressive on hosts having drought stress (Calavan and Wallace, 1954). Mulberry species are tolerant to unfavourable temperatures, but the emergence of *N. novaehollandiae* in *M. alba* orchards may be correlated with the changing in climate. The increase in temperature may have prolonged the growing seasons of the plants as well as the decrease of rainfall has probably caused of dehydration in plantation areas. Woody tissues dessicate because of drought stress and this causes mechanical strength of the barkwood bonds to lower, leading opportunistic fungal pathogens invade those injured plants easily (Bettucci *et al.*, 1999).

Results from the present study showed that *N. novaehollandiae* isolates produce cankers on *M. alba* seedlings. To our knowledge, this is the first report of occurrence, molecular characterization and pathogenicity confirmation for *N. novaehollandiae* causing mulberry in Turkey.

Conclusions

Neoscytalidium novaehollandiae should be considered as an extremely dangerous disease for mulberry production and other cultivated plant species. Further research should be performed to understand the epidemiology of the disease and to develop effective strategies to manage *N. novaehollandiae* and to decrease the impact of this new disease of mulberry in Turkey for the first time. It is thought that study about *N. novaehollandiae* will be helpful for further researches since there are limited studies on this subject.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

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

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