

Article

Identification and Pathogenicity of *Dothiorella sarmentorum* Causing Lavender Leaf Blight Disease in Xinjiang, China

Mengyao Li ^{1,†}, Chuli Liu ^{1,†}, Wanbin Shi ¹, Aifan Wang ², Rong Ma ^{1,*} and Xiujuan Su ^{2,*}

¹ College of Forestry and Landscape Architecture, Xinjiang Agricultural University, Urumqi 830052, China; limengyao0306@163.com (M.L.); 15199065502@163.com (C.L.); 18097723209@163.com (W.S.)

² Agricultural College, Xinjiang Agricultural University, Urumqi 830052, China; wangaifantop@163.com

* Correspondence: xjaumr@xjau.edu.cn (R.M.); suxiujuan@xjau.edu.cn (X.S.)

[†] These authors contributed equally to this work.

Abstract: Lavender is an oil-bearing plant, which has long been cultivated for oil, fresh flowers, dried products, and food. Leaf blight disease was observed on ‘Bandera Pink’, which belongs to *Lavandula stoechas* in Yining County, Xinjiang Uygur Autonomous Region, China. The causal agent of this disease was isolated, and Koch’s postulates were assessed to confirm its pathogenicity. The morphological characteristics of the pathogen were observed, and the LSU, ITS, *tef1*, and *tub2* loci were combined and analyzed. Based on morphological characterization and phylogenetic analyses, the causal agent was identified as a fungal species named *Dothiorella sarmentorum*. Pathogenicity tests revealed that *D. sarmentorum* can infect seven varieties of three lavender species. This is the first report of *D. sarmentorum* causing lavender leaf blight. This study provides a theoretical basis for the diagnosis of disease and the monitoring of disease occurrence and epidemics.

Keywords: Botryosphaeriaceae; leaf disease; morphology; phylogeny



Citation: Li, M.; Liu, C.; Shi, W.; Wang, A.; Ma, R.; Su, X. Identification and Pathogenicity of *Dothiorella sarmentorum* Causing Lavender Leaf Blight Disease in Xinjiang, China. *Diversity* **2024**, *16*, 148. <https://doi.org/10.3390/d16030148>

Academic Editor: Ipek Kurtboke

Received: 17 January 2024

Revised: 21 February 2024

Accepted: 22 February 2024

Published: 26 February 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Lavender (*Lavandula*, Lamiaceae) contains several commercial species that are cultivated extensively in temperate climates for the extraction of essential oils, as well as traditional medicine, culinary herbs, and ornamental plants [1–5]. Yili Prefecture of Xinjiang is the main cultivation area of lavender in China. At the present time, the lavender cultivation area in the Yili River Valley is 4900 hm², accounting for 98% of the national lavender cultivation area and more than 90% of the national essential oil production, making it the largest lavender cultivation base in China [6,7].

In field conditions, lavender can be affected by some potential disease factors like fungi and bacteria. The most recognizable root rot of lavender is caused by *Fusarium foetens*, *F. oxysporum*, and *Meloidogyne javanica* [8–10]. The Downy mildew disease of lavender is caused by *Peronospora belbahrii*, and net blotch is caused by *Rhizoctonia solani* [11,12]. Bacterial speck disease is caused by *Xanthomonas hortorum* in Korea [13]. In August 2023, lavender leaf blight occurred in Yining County, Xinjiang Uygur Autonomous Region, China.

Dothiorella species are known pathogens on a wide range of hosts, causing environmental or agricultural production losses and seriously affecting healthy tree growth. It caused severe decline of almond trees in orchards in Spain, with disease symptoms including branch collapse, chlorosis of leaves leading to sudden wilting, death, and shoot dieback [14,15]. It also caused the wilting and death of a large number of grapevines in Australia’s wine regions, severely affecting local wine production [16]. Severe stem and branch diseases of hazelnut trees have been observed in several groves in Italy, leading to crown dieback and total tree mortality, with a significant impact on crop production [17]. *Dothiorella* panicle and shoot blight has been identified as one of the major threats to the Californian pistachio industry [18]. It also causes ulceration and wilting shoot disease in elms in Europe, severely affecting healthy tree growth [19].

The identification of fungal pathogens on the basis of morphological, physiological, and biochemical tests is not precise enough as it is mostly dependent on subtle differences in nutrients, pH, humidity, and environmental acclimatization [20,21]. Thus, polyphasic identification involving morphological characteristics and more than one gene sequence phylogenetic analysis is recommended to remove this predicament [22]. Based on morphology and molecular data, Phillips revived *Dothiorella* for species with conidia that become brown and 1-septate while they were still attached to the conidiogenous cells; sexual morphs of *Dothiorella* have pigmented, 1-septate ascospores [23,24]. The research confirmed the presence of *D. sarmentorum* (Fr.). Spore size and shape are important taxonomic features and valuable criteria for distinguishing the species [25].

The objective of this study was to identify the pathogen causing blight disease on 'Bandera Pink' in Xinjiang Uygur Autonomous Region based on morphological characterization and molecular analyses and to compare the differences in the pathogenicity of pathogenic fungi to different varieties of lavender. This study is fundamental for subsequent research on lavender leaf diseases.

2. Materials and Methods

2.1. Sample Collection and Pathogen Isolation

The leaves of symptomatic lavender plants were collected in Yining County (43°94'35.49" N 81°54'88.12" E) Xinjiang Uygur Autonomous Region of China in August 2023. The symptomatic leaves were delivered to the laboratory and immersed in 75% ethanol for 30 s, then thoroughly washed in sterile distilled water. Small pieces (2 mm²) with necrotic tissue and healthy parts of the collected leaves were cut using a sterilized blade. Tissue pieces were disinfected by placing them in 3% sodium hypochlorite solution for 3 min, then they were rinsed three times in sterile distilled water and dried naturally on sterilized filter paper. These tissue pieces were incubated on potato dextrose agar (PDA) at 28 °C under 12 h/12 h photoperiod conditions. After 3–4 days, the growing fungal colonies were sub-cultured onto fresh PDA, and pure cultures were obtained by subculturing hyphal tips. After the colony to be cultured had produced conidia, the conidia were gently scraped off the colony using sterile inoculation needles and suspended in 1 mL of sterile distilled water. The suspension of conidia was coated on a PDA culture dish and incubated at 25 °C for 24 h, individual germinated conidia were transferred to fresh PDA plates under a stereomicroscope, and incubation was continued at 25 °C for 36 h for mycelial development to obtain a pure culture strain. Isolates were maintained as a spore suspension in 25% glycerol at −80 °C until ready for use in further studies. Living cultures were deposited in the Pathology Laboratory of the College of forestry and landscape architecture (XJAU).

2.2. DNA Extraction, PCR Amplification, and Sequencing

Total genomic DNA was extracted from 5-day-old mycelium grown on PDA using the CTAB method [26]. The water solution of obtained DNA was kept at −20 °C for further experiments. PCR was run using a BIO-RAD T100TM Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) to amplify the internal transcribed spacer (ITS) region using primers ITS1/ITS4. The nuclear ribosomal large subunit (LSU), the translation elongation factor 1-alpha (*tef1*), and the β -tubulin gene (*tub2*) were amplified using primers LROR/LR5, EF-AF/EF-BR, and BT2A/BT2B (Table 1). All PCR reactions were performed in a 30 μ L reaction volume containing 1.5 μ L of DNA template, 1.5 μ L of each forward and reverse primer, 15 μ L of Taq PCR master mix (2 \times , with blue dye), and 10.5 μ L of double-distilled water (dd H₂O). The cycling parameters were as follows: a first step of denaturation at 95 °C for 5 min followed by 35 cycles of (i) denaturation at 95 °C for 60 s, (ii) annealing at optimal temperature (55 °C for ITS, *tef1*, and LSU and 45 °C for *tub2*) for 80 s, (iii) elongation at 72 °C for 90 s, and a final elongation step of 5 min was applied. PCR products were detected using a 1% agarose gel under 120 V stable voltage, and the electrophoresis time was 25 min. PCR bands were observed in a gel imager to determine whether they were clear and to determine the size of the target bands. PCR products

were sent to Shanghai Sangon Bioengineering Co., Ltd. (Shanghai, China) for bidirectional sequencing.

Table 1. List of primer pair sets used for PCR and sequencing.

Gene Fragment	Primer	Primer Sequence	Reference
ITS	ITS1	TCCGTAGGTGAACCTGCGG	White et al., 1990 [27]
	ITS4	TCCTCCGCTTATTGATATGC	
LSU	LROR	GTACCCGCTGAACCTAAGC	Carbone & Kohn 1999 [28]
	LR5	ATCCTGAGGGAACTTC	
<i>tub2</i>	Bt-2a	GGTAACCAAATCGGTGCTTTTC	Glass & Donaldson 1995 [29]
	Bt-2b	ACCCTCAGTGTAAGTACCCTTGCC	
<i>tef1-α</i>	tef1-728F	CATCGAGAAGTTCGAGAAGG	Rehner et al. 2005 [30]
	tef1-986R	TACTTGAAGGAACCCTTACC	

2.3. Phylogenetic Analysis

The resulting sequences were edited and assembled by the SeqMan program within the DNASTAR software package (DNASTAR, Madison, WI, USA) and deposited in GenBank (Table 2). For molecular analysis, the ITS, LSU, *tef1*, and *tub2* sequences of the reference isolates of the *Dothiorella* species were retrieved from the NCBI database (Table 2) and aligned using MEGA 6 software. The phylogenetic analyses of the combined loci were performed using Maximum Likelihood (ML) implemented on the CIPRES Science Gateway portal using RAXML-HPG BlackBox 8.2.10, employing a GTRGAMMA substitution model with 1000 bootstrap replicates.

Table 2. Taxon names, strain or specimen numbers, and corresponding GenBank accession numbers of the taxa used for the phylogenetic studies. The newly generated sequences are indicated in bold.

Taxon Names	Strain/Specimen Numbers	GenBank Accession Numbers			
		ITS	LSU	<i>tef1-α</i>	<i>tub2</i>
<i>Diplodia mutila</i>	CBS 112553	AY259093	AY928049	AY573219	DQ458850
<i>Diplodia mutila</i>	CBS 112875	AY343484	–	AY343370	MT592509
<i>Diplodia mutila</i>	GZCC 23-0578	OR052057	OR020607	OR030454	OR030472
<i>Diplodia neojuniperi</i>	CBS 138652	KM006431	–	KM006462	MT592516
<i>Diplodia sapinea</i>	CBS 393.84	DQ458895	DQ377893	DQ458880	DQ458863
<i>Diplodia scrobiculata</i>	CBS 118110	AY253292	KF766326	AY624253	AY624258
<i>Diplodia seriata</i>	CBS 112555	AY259094	AY928050	AY573220	DQ458856
<i>Diplodia seriata</i>	CBS 112661	MT587378	–	MT592084	MT592541
<i>Diplodia seriata</i>	GZCC 23-0579	OR052058	OR052041	OR030455	OR030473
<i>Diplodia subglobosa</i>	CBS 124133	GQ923856	–	GQ923824	MT592576
<i>Dothiorella acacicola</i>	CBS 141295	KX228269	KX228320	KX228376	–
<i>Dothiorella acericola</i>	KUMCC 18-0137	MK359449	–	MK361182	–
<i>Dothiorella albisiae</i>	MFLUCC 22-0057	ON751762	ON751764	ON799588	ON799590
<i>Dothiorella alpina</i>	CGMCC 3.18001	KX499645	–	KX499651	–
<i>Dothiorella baihuashanensis</i>	CFCC 58549	–	–	OQ692933	OQ692927
<i>Dothiorella baihuashanensis</i>	CFCC 58788	–	–	OQ692934	OQ692928
<i>Dothiorella brevicollis</i>	CBS 130411	JQ239403	JQ239416	JQ239390	JQ239371
<i>Dothiorella camelliae</i>	CGMCC 3.24158	OQ190531	–	OQ241464	OQ275064
<i>Dothiorella capri-amissi</i>	CBS 121763	EU101323	KX464301	EU101368	KX464850
<i>Dothiorella casuarinae</i>	CBS 120688	DQ846773	MH874647	DQ875331	DQ875340
<i>Dothiorella citricola</i>	CBS 124728	EU673322	–	EU673289	KX464852
<i>Dothiorella citrimurcotticola</i>	CGMCC 3.20394	MW880661	–	MW884164	MW884193
<i>Dothiorella citrimurcotticola</i>	CGMCC 3.20395	MW880662	–	MW884165	MW884194
<i>Dothiorella diospyricola</i>	CBS 145972	MT587398	–	MT592110	MT592581
<i>Dothiorella dulcispinae</i>	CBS 130413	JQ239400	JQ239413	JQ239387	JQ239373
<i>Dothiorella dulcispinae</i>	CMW 36462	JQ239402	JQ239415	JQ239389	JQ239375
<i>Dothiorella eriobotryae</i>	CBS 140852	KT240287	–	KT240262	MT592582

Table 2. Cont.

Taxon Names	Strain/Specimen Numbers	GenBank Accession Numbers			
		ITS	LSU	<i>tef1-α</i>	<i>tub2</i>
<i>Dothiorella heterophyllae</i>	CMW46458	MN103794	–	MH548348	MH548324
<i>Dothiorella iranica</i>	CBS 124722	KC898231	–	KC898214	KX464856
<i>Dothiorella koae</i>	CMW 48017	MH447652	–	MH548338	MH548327
<i>Dothiorella lampangensis</i>	MFLUCC 18-0232	MK347758	–	MK340869	MK412874
<i>Dothiorella longicollis</i>	CBS 122068	EU144054	MH874718	EU144069	KF766130
<i>Dothiorella magnoliae</i>	CFCC 51563	KY111247	–	KY213686	–
<i>Dothiorella mangifericola</i>	IRAN 1584C	MT587407	–	MT592119	–
<i>Dothiorella moneti</i>	MUCC 505	EF591920	EF591937	EF591971	EF591954
<i>Dothiorella obovata</i>	MFLUCC 22-0058	ON751763	ON751765	ON799589	ON799591
<i>Dothiorella ovata</i>	MFLUCC 23-0035	OR052059	OR020691	OR030456	OR030474
<i>Dothiorella ovata</i>	MFLUCC 23-0036	OR052060	OR052042	OR030457	OR030475
<i>Dothiorella plurivora</i>	CBS 124724	KC898225	–	KC898208	KX464874
<i>Dothiorella pretoriensis</i>	CBS 130404	JQ239405	JQ239418	JQ239392	JQ239376
<i>Dothiorella prunicola</i>	CBS 124723	EU673313	EU673232	EU673280	EU673100
<i>Dothiorella rosacearum</i>	MFLUCC 23-0038	OR052061	OR052043	OR030458	OR030476
<i>Dothiorella rosacearum</i>	MFLUCC 23-0037	OR052062	OR052044	OR030459	OR030477
<i>Dothiorella santali</i>	WAC 13155	EF591924	EF591941	EF591975	EF591958
<i>Dothiorella sarmentorum</i>	CBS 115038	AY573206	DQ377860	AY573223	EU673101
<i>Dothiorella sarmentorum</i>	IMI 63581b	AY573212	AY928052	AY573235	–
<i>Dothiorella sarmentorum</i>	XJAU XYZ-1	OR947929	PP335474	PP335515	PP335512
<i>Dothiorella sarmentorum</i>	XJAU XYZ-2	OR947930	PP335475	PP335516	PP335513
<i>Dothiorella sarmentorum</i>	XJAU XYZ-3	OR947931	PP335476	PP335517	PP335514
<i>Dothiorella septata</i>	MFLUCC 23-0039	OR020942	OR020695	OR030462	OR030480
<i>Dothiorella septata</i>	GZCC 23-0583	OR019776	OR052047	OR030463	OR030481
<i>Dothiorella septata</i>	GZCC 23-0584	OR019803	OR052048	OR030464	OR030482
<i>Dothiorella striata</i>	CBS 124731	EU673321	–	EU673288	EU673143
<i>Dothiorella striata</i>	CBS 124730	EU673320	EU673240	EU673287	EU673142
<i>Dothiorella tectonae</i>	MFLUCC 18-0382	KM396899	–	KM409637	KM510357
<i>Dothiorella thailandica</i>	MFLUCC 11-0438	JX646796	JX646813	JX646861	JX646844
<i>Dothiorella thripsita</i>	CBS 125445	FJ824738	–	KJ5773639	KJ577550
<i>Dothiorella ulmacea</i>	CBS 138855	KR611881	KR611899	KR611910	KR611909
<i>Dothiorella ulmacea</i>	CBS 140005	KR611882	–	KR857697	MT592607
<i>Dothiorella uruguayensis</i>	CBS 124908	EU080923	MH874932	EU863180	KX464886
<i>Dothiorella vinea-gemmae</i>	DAR 81012	KJ573644	–	KJ573641	KJ577552
<i>Dothiorella viticola</i>	CBS 117009	AY905554	MH874565	AY905559	EU673104
<i>Dothiorella yunnana</i>	CGMCC 3.18000	KX499644	–	KX499650	–
<i>Dothiorella zanthoxyli</i>	CGMCC 3.24159	OQ190536	–	OQ241468	OQ275069

2.4. Morphology and Culture Characteristics

To study the morphological characteristics of pure isolates, 5 mm (diameter) mycelial plugs from the edge of 5-day-old cultures were transferred to fresh PDA. The plates were incubated at 25 °C in a 12 h/12 h photoperiod. Morphological observation was conducted mainly based on the conidiomata naturally formed on the host tissues, including size, shape, and color. Macromorphological photographs were obtained using a Leica stereomicroscope (M205, Leica, Wetzlar, Germany). Micromorphological observations including the size and shape of conidiophores and conidia were performed using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan). Twenty conidiomata were sectioned using a sterile scalpel, and then the lengths and widths of 50 randomly chosen conidia were measured. Colony morphology and growth rates were recorded [24].

2.5. Pathogenicity Test

The lavenders used in the experiment were all from the experimental base of the College of Agriculture, Xinjiang Agricultural University. The ‘Bandera Pink’ variety, belonging to *Lavandula stoechas*, was used to test Koch’s hypothesis; the ‘Blue Spear’, ‘Ellagance Sky’,

‘Avignon Early Blue’, ‘Taikonglan’, and ‘Faguolan’ varieties belonging to *L. angustifolia*; the Spanish Eyes variety belonging to *L. multifida*; and the ‘Bandera Pink’ variety belonging to *L. stoechas* were the different varieties used for the pathogenicity difference test.

Pathogenicity assays were performed on the leaves of lavender seedlings. The strain XJAU XYC-1 was prepared into a spore suspension with a concentration of 1×10^5 cfu/mL for use (the spore concentration was determined using a blood cell counting plate). The spore suspension was sprayed evenly on healthy lavender plants, spraying about 30 mL per plant. Then, a black plastic bag was used for moisturizing for 36 h, and sterile water treatment was sprayed as a control. These seedlings were raised in a growth chamber with a 12 h/12 h photoperiod at a 28 ± 2 °C temperature regime. After 36 h, the black silk plastic bag was removed and the disease incidence was monitored and recorded using the incidence area proportion counting method, which was used to calculate the ratio of the affected area to the total area every 2 days until the 15th day. Furthermore, to confirm Koch’s postulates, re-isolation was performed from lesions and identified based on morphological and molecular characteristics as described above.

2.6. Statistical Analysis

The means and standard errors of the data were calculated using Microsoft Excel formulas. Analysis of variance was used to detect differences among treatments using IBM SPSS Statistics 22.0 and Origin 9.5.

3. Results

3.1. Isolation of the Pathogen

A total of 19 strains of the fungal genus *Dothiorella* were obtained with similar colony morphology. Sequences of ITS, LSU, *tef1*, and *tub2* were compared and considered to be consistent among the 19 obtained strains. Three strains (XJAU XYC-1, XJAU XYC-2, and XJAU XYC-3) were then randomly selected for the following phylogenetic analysis for species identification.

3.2. Phylogenetic Analysis

The combined dataset of ITS, LSU, *tef1*, and *tub2* loci consisted of 45 strains, with *Diplodia subglobosa* (CBS 124133) as the outgroup taxon. The final alignment comprised 3152 characters including 512 characters in ITS, 801 characters in LSU, 242 characters in *tef1*, and 416 characters in *tub2*. The final ML optimization likelihood value of the best RAxML tree was −8740.98. The topology of our phylogenetic tree is nearly identical to previous publications. Three isolates from the present study formed a clade with CBS 115038 and IMI 63581b named *Dothiorella sarmentorum* (Figure 1).

3.3. Morphological Description of the Pathogen *Dothiorella sarmentorum*

On the PDA medium, the mycelium formed filamentous colonies. Initially, it was white (Figure 2A), subsequently forming small white colonies, within 4 days. Later, the mycelium turned leaden appressed and became smoke-grey to olivaceous grey at the surface, starting from the center (Figure 2B,C). The mycelium was immersed, consisting of septate, branched, brown, finely verruculose hyphae. Conidiomata that readily formed from the middle of colonies within 20 days were pycnidial, solitary, globose, dark brown to black, immersed in the medium, thick-walled, and up to 400 µm wide (Figure 2D). Conidiophores were hyaline, smooth, rarely branched, and aseptate (Figure 2G,H). The conidiogenous layer with developing conidia was thick and composed of dark brown 5–6-cell layers. Conidiogenous cells were cylindrical to fusiform, hyaline, thin-walled, smooth, and giving rise to periclinal thickenings (Figure 2E). Dark, oval to ovoid, often pigmented ellipsoid conidia were $18.9\text{--}24.9 \times 8.8\text{--}12.1$ µm ($x = 22.2 \times 8.7$ µm, $n = 60$), hyaline, later brown, thick-walled, 1-septate prior to release from conidiogenous cells, and occasionally slightly constricted at the septum with a broadly rounded apex and truncate base (Figure 2F,K).

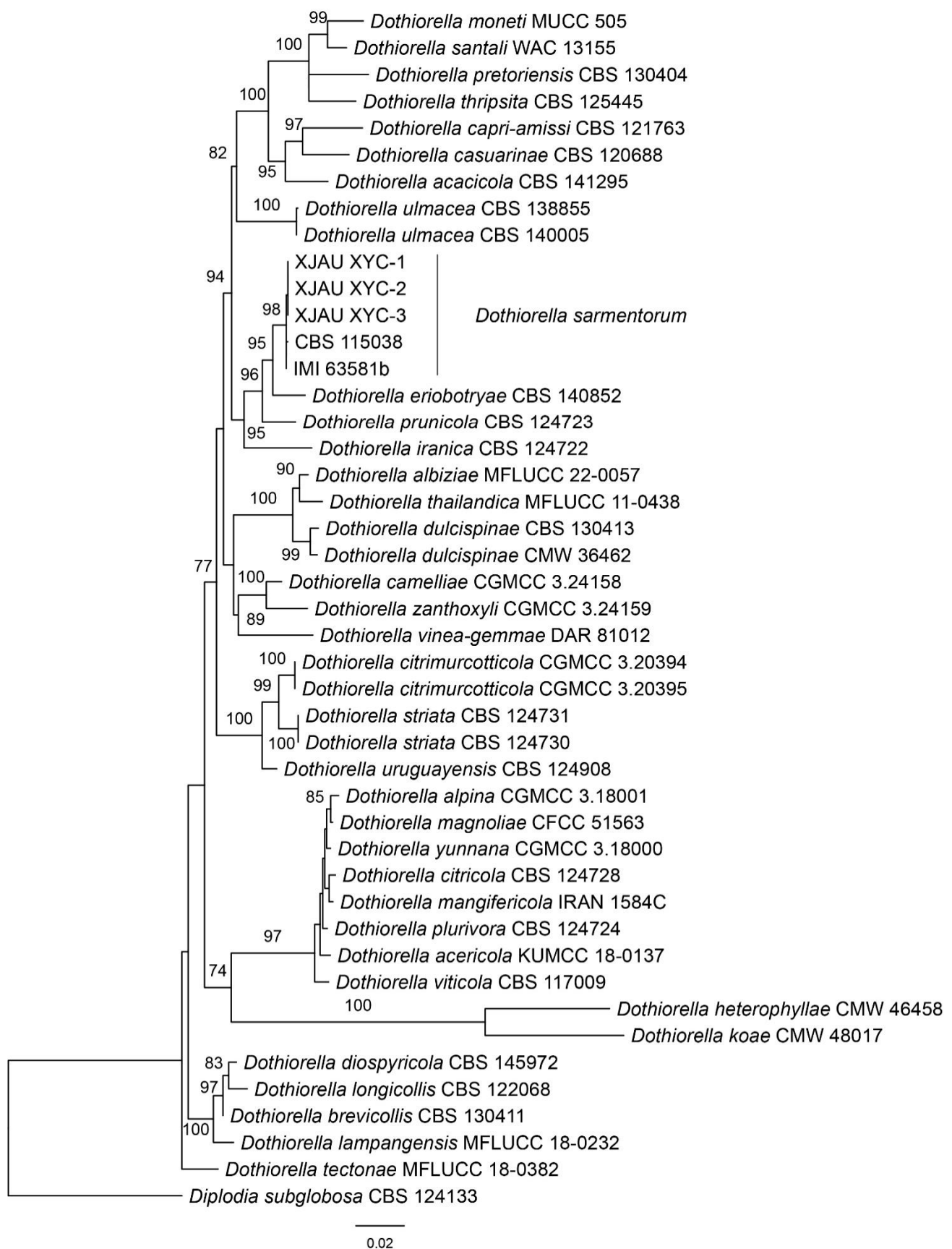


Figure 1. Phylogenetic tree of *Dothiorella* generated from the maximum likelihood (ML) analysis based on the combined loci of ITS, LSU, *tef1-α*, and *tub2* sequences.

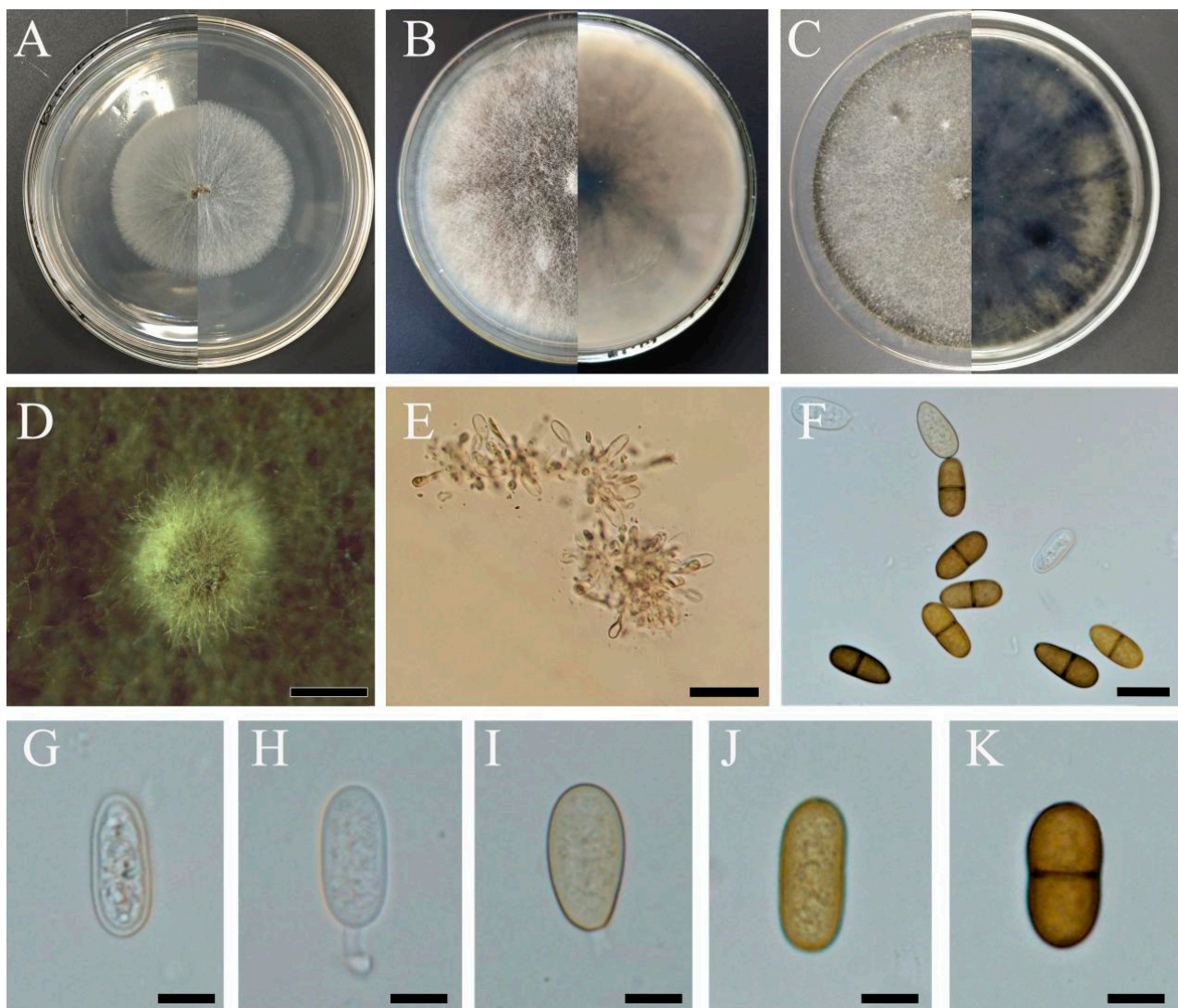


Figure 2. Morphological characteristics of *Dothiorella sarmentorum* (XIAU XYC-1). (A–C): colonies of white, later olivaceous grey mycelia after 2 (A), 6 (B), and 10 days (C) of incubation on PDA; (D): conidiomata; (E): conidiogenous cells; (F,K): dark, ovoid, diploid-like conidia. (G–J): immature conidia. Scale bars: (D) = 2 mm, (E) = 50 μ m, (F) = 20 μ m, (G–K) = 10 μ m.

3.4. Pathogenicity

Seedlings of ‘Bandera Pink’ inoculated with *Dothiorella sarmentorum* (XIAU XYC-1) developed symptoms that were the same as those observed in the field. The lavender leaves turned black and curled, and disease spots spread through the stems 4 d after inoculation (Figure 3D–F). The whole lavender plant was curled, withered, and necrotic, and the lavender roots were black 10 d after inoculation (Figure 3G–I,K,M). The negative control was sprayed with sterile water on healthy lavender plants and did not develop disease symptoms (Figure 3A–C,J,L). The fungus was reisolated from symptomatic leaves and identified as *D. sarmentorum* based on morphological characteristics and DNA sequence data, thus fulfilling Koch’s postulates.



Figure 3. Leaf disease symptoms observed in lavender seedlings after *Dothiorella sarmentorum* fungal inoculation. (A–C): negative control—leaves sprayed with sterile water. (D–F): *Dothiorella sarmentorum* conidial suspension was spray-inoculated onto seedlings of lavender; (G–I): symptoms of disease 4 d after inoculation; (G–I): symptoms of disease 10 d after inoculation. (J): negative control—the whole plant is healthy; (K): treatment—the whole plant has withered; (L): negative control—the roots of plants are brown; (M): treatment: the roots of plants are black.

Dothiorella sarmentorum showed different degrees of pathogenicity on ‘Blue Spear’, ‘Ellagance Sky’, ‘Spanish Eyes’, ‘Bandera Pink’, ‘Avignon Early Blue’, ‘Taikonglan’, and ‘Faguolan’ seedlings (Figures 4 and 5). The average disease incidence that developed on inoculated ‘Blue Spear’, ‘Ellagance Sky’, ‘Spanish Eyes’, ‘Bandera Pink’, ‘Avignon Early Blue’, ‘Taikonglan’, and ‘Faguolan’ seedlings were 38.96%, 41.46%, 16.38%, 31.78%, 11.62%, 7.99%, and 10.66%, respectively, 15 days after inoculation (Figure 4).

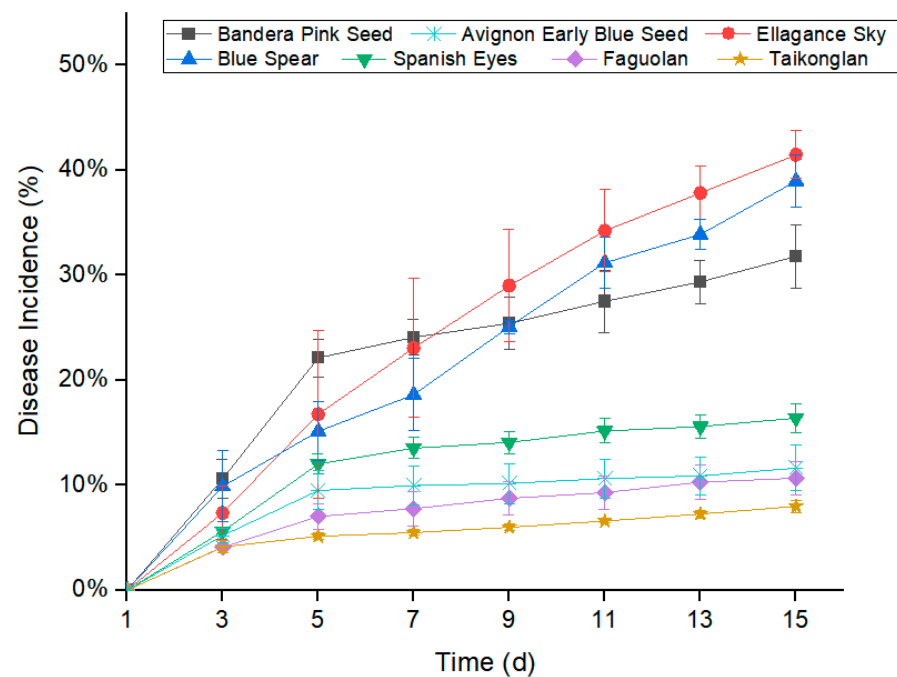


Figure 4. Disease incidence observed in different varieties of lavender after *Dothiorella sarmentorum* inoculation. Analysis of variance of disease incidence that developed on Blue Spear, Ellagance Sky, Spanish Eyes, Bandera Pink, Avignon Early Blue, Taikonglan, and Faguolan seedlings following inoculation with *Dothiorella sarmentorum*. Values shown are means \pm the standard errors.

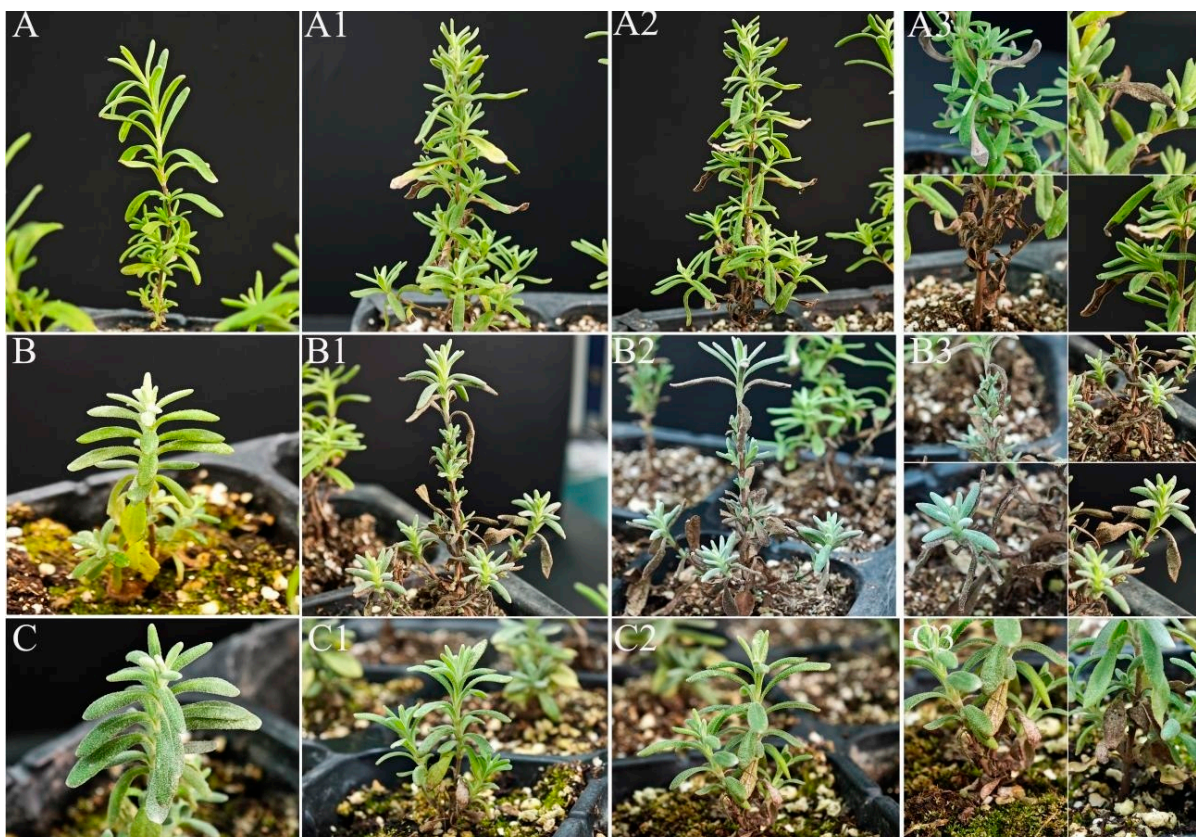


Figure 5. Cont.



Figure 5. Leaf disease symptoms observed in different varieties of lavender after *Dothiorella sarmentorum* inoculation. Leaf disease symptoms on (A–A3) Bandera Pink; (B–B3) Ellagance Sky; (C–C3) Blue Spear; (D–D3) Spanish Eyes; (E–E3) Faguolan; (F–F3) Taikonglan; and (G–G3) Avignon Early Blue lavender seedlings. (A–G) the negative control—leaves sprayed with sterile water (control—CK); the *Dothiorella sarmentorum* spore suspension was spray-inoculated onto seedlings of lavender. (A1–G1): 3 d after inoculation; (A2–G2): the overall image of 15 d after inoculation; (A3–G3): the partial image of 15 d after inoculation.

4. Discussion

According to morphological and molecular analyses as well as pathogenicity tests, we demonstrated that the dieback symptoms observed on lavender (*Lavandula stoechas*) plants in China are due to *D. sarmentorum*. To our knowledge, this is the first report of *D. sarmentorum* as a new pathogen of *L. stoechas*. The pathogenicity test confirmed that *D. sarmentorum* is a pathogenic fungus that can also infect *L. angustifolia* and *L. multifida*. There are variations in the pathogenicity of different varieties of lavender.

Dothiorella sarmentorum has been recorded in several countries around the world, and it can not only cause tree canker disease as a pathogenic bacterium but also parasitize 66 plant tissues such as Salicaceae and Rosaceae as endophytic fungi [24]. *D. sarmentorum* is a cosmopolitan species and has been isolated from 34 different host species including *Malus*, *Menispermum*, *Prunus*, *Pyrus*, and *Ulmus* genera. Molecular studies have described *D. sarmentorum* in 17 woody hosts [22,31]. However, there are relatively few reports on the diseases that *D. sarmentorum* causes in herbaceous plants. This study further confirms that lavender is a new host of *D. sarmentorum*, which is of great significance for understanding the source of this pathogen in nature. Considering that the lavender leaf spot disease caused by *D. sarmentorum* has the characteristics of a large lesion area, strong destructive power, and rapid development, it has the risk of widespread transmission and epidemics. Therefore, it is necessary to strengthen the knowledge about the ecology, epidemiology,

biogeography, and infection biology of this pathogen that represents a serious threat to the lavender industry.

5. Conclusions

Dothiorella sarmentorum is an emerging pathogen causing *L. angustifolia* leaf spot in Yining County, Xinjiang, China. The pathogenicity test confirmed that *D. sarmentorum* is a pathogenic fungus that can also infect *L. stoechas* and *L. pinnata*. The data obtained in this study should provide a theoretical basis for monitoring and preventing this disease. The potential impact of *D. sarmentorum* on lavender production in this area of China warrants further investigation to determine potential disease management strategies.

Author Contributions: Conceptualization, R.M.; methodology, R.M.; software, M.L. and C.L.; validation, M.L. and C.L.; formal analysis, W.S. and A.W.; investigation, A.W., and W.S.; resources, A.W., M.L. and X.S.; data curation, C.L., M.L. and R.M.; writing—original draft preparation, C.L.; writing—review and editing, R.M.; visualization, W.S.; supervision, R.M. and X.S.; project administration, R.M.; funding acquisition, R.M. and X.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 31960316, and the Key Research and development program of Xinjiang Province, grant number 2022B02036-1.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cavanagh, H.M.; Wilkinson, J.M. Lavender essential oil: A review. *Aust. Infect. Control.* **2005**, *10*, 35–37. [\[CrossRef\]](#)
2. Bradley, B.F.; Starkey, N.J.; Brown, S.L.; Lea, R.W. Anxiolytic effects of *Lavandula angustifolia* odour on the Mongolian gerbil elevated plus maze. *J. Ethnopharmacol.* **2007**, *111*, 517–525. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Prusinowska, R.; Smigielski, K.B. Composition, biological properties and therapeutic effects of Lavender (*Lavandula angustifolia*). A review. *Herba Pol.* **2014**, *60*, 56–66. [\[CrossRef\]](#)
4. Zhen, S.; Burnett, S.E. Effects of substrate volumetric water content on English lavender morphology and photosynthesis. *HortScience* **2015**, *50*, 909–915. [\[CrossRef\]](#)
5. Samuelson, R.; Lobl, M.; Higgins, S.; Clarey, D.; Wysong, A. The effects of lavender essential oil on wound healing: A review of the current evidence. *J. Altern. Complement. Med.* **2020**, *26*, 680–690. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Stierle, A.; Strobel, G.; Stierle, D. Taxol and Taxane Production by *Taxomyces andreanae*, an Endophytic Fungus of Pacific Yew. *Science* **1993**, *260*, 214–216. [\[CrossRef\]](#)
7. Tang, S.M.; Ran, B.; Zhu, L.; Dong, S.; Luo, W.; Zhang, X.; Huang, X. Evaluation of Cold Tolerance of Different Lavender Varieties. *Chin. Wild Plant Resour.* **2023**, *42*, 8–15. [\[CrossRef\]](#)
8. Jun, X.W.; Guo, B.J.; Xing, W. First report of *Fusarium foetens* causing root rot on lavender (*Lavandula angustifolia*) in China. *J. Plant Pathol.* **2023**, *105*, 1173–1174. [\[CrossRef\]](#)
9. Garibaldi, A.; Bertetti, D.; Pensa, P.; Ortu, G.; Gullino, M.L. First Report of *Fusarium oxysporum* Causing Wilt on Allard's Lavender (*Lavandula x allardii*) in Italy. *Plant Dis.* **2015**, *99*, 1868. [\[CrossRef\]](#)
10. Oliveira, S.A.; Dlugos, D.M.; Agudelo, P.; Jeffers, S.N. First report of *Meloidogyne javanica* pathogenic on hybrid lavender (*Lavandula × intermedia*) in the United States. *Plant Dis.* **2022**, *106*, 335. [\[CrossRef\]](#)
11. Aktaruzzaman, M.; Afroz, T.; Kim, S.B. First report of web blight on lavender caused by *Rhizoctonia solani* AG-1-IB in Korea. *Plant Dis.* **2020**, *104*, 2518. [\[CrossRef\]](#)
12. Marco, T.; Anthony, B.; Sebastian, P. Downy mildew of lavender caused by *Peronospora belbahrii* in Israel. *Mycol. Prog.* **2020**, *19*, 1537–1543. [\[CrossRef\]](#)
13. Rotondo, F.; Testen, A.L.; Horvat, M.M.; Roman-Reyna, V.; Klass, T.L.; Jacobs, J.M.; Miller, S.A. First report of *Xanthomonas hortorum* causing bacterial leaf spot of lavender (*Lavandula × intermedia*) in Ohio. *Plant Dis.* **2021**, *105*, 484. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Gramaje, D.; Agustí-Brisach, C.; Pérez-Sierra, A.; Moralejo, E.; Olmo, D.; Mostert, L.; Damm, U.; Armengol, J. Fungal trunk pathogens associated with wood decay of almond trees on Mallorca (Spain). *Persoonia* **2012**, *28*, 1–13. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Pitt, W.M.; Úrbez-Torres, J.R.; Trouillas, F.P. *Dothiorella* and *Spenceriartinsia*, new species and records from grapevines in Australia, *Australas. Plant Path.* **2015**, *44*, 43–56. [\[CrossRef\]](#)
16. Pitt, W.M.; Huang, R.; Steel, C.C.; Savocchia, S. Identification, distribution and current taxonomy of Botryosphaeriaceae species associated with grapevine decline in New South Wales and South Australia. *Aust. J. Grape Wine R.* **2010**, *16*, 258–271. [\[CrossRef\]](#)

17. Linaldeddu, B.T.; Deidda, A.; Scanu, B.; Franceschini, A.; Alves, A.; Abdollahzadeh, J.; Phillips, A.J.L. Phylogeny, morphology and pathogenicity of Botryosphaeriaceae, Diatrypaceae and Gnomoniaceae associated with branch diseases of hazelnut in Sardinia (Italy). *Eur. J. Plant Pathol.* **2016**, *146*, 259–279. [[CrossRef](#)]
18. Chen, S.F.; Morgan, D.P.; Michailides, T.J. Botryosphaeriaceae and Diaporthaceae associated with panicle and shoot blight of pistachio in California, USA. *Fungal Divers.* **2014**, *67*, 157–179. [[CrossRef](#)]
19. Jürisoo, L.; Adamson, K.; Padari, A.; Drenkhan, R. Health of elms and Dutch elm disease in Estonia. *Eur. J. Plant Pathol.* **2019**, *154*, 823–841. [[CrossRef](#)]
20. Liu, S.Y.; Wu, S.W. Application and progress in fungal taxonomy and nomenclature by molecular systematics. *Prog. Microbiol. Immunol.* **2015**, *43*, 48–53. [[CrossRef](#)]
21. Mukuma, C. Morphological and Molecular Identification and Characterization of Dry Bean Fungal Root Rot Pathogens in Zambia. M.Sc. Thesis, University of Nebraska, Lincoln, NE, USA, 2016.
22. Dar, G.J.; Nazir, R.; Wani, S.A.; Farooq, S. Isolation, molecular characterization and first report of *Dothiorella gregaria* associated with fruit rot of walnuts of Jammu and Kashmir, India. *Microb. Pathog.* **2023**, *175*, 105989. [[CrossRef](#)]
23. Phillips, A.J.L.; Alves, A.; Correia, A.; Lueg, J. Two new species of Botryosphaeria with brown, 1-septate ascospores and Dothiorella anamorphs. *Mycologia* **2005**, *97*, 513–529. [[CrossRef](#)]
24. Phillips, A.J.L.; Alves, A.; Abdollahzadeh, J.; Slippers, B.; Wingfield, M.J.; Groenewald, J.Z.; Crous, P.W. The Botryosphaeriaceae: Genera and species known from culture. *Stud. Mycol.* **2013**, *76*, 51–167. [[CrossRef](#)] [[PubMed](#)]
25. Ivanová, H. Identification and characterization of the fungus *Dothiorella sarmentorum* on necrotic shoots of declining ash in Slovakia. *Folia Oecologica* **2018**, *45*, 53–57. [[CrossRef](#)]
26. Fan, X.L.; Du, Z.; Liang, Y.M.; Tian, C.M. Melanconis (Melanconidaceae) associated with *Betula* spp. in China. *Mycol. Prog.* **2016**, *15*, 1–9. [[CrossRef](#)]
27. White, T.J.; Bruns, T.; Lee, S.; Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *Pcr Protoc. A Guide Methods Appl.* **1990**, *18*, 315–322. [[CrossRef](#)]
28. Carbone, I.; Kohn, L.M. A method for designing primer sets for speciation studies in filamentous ascomycete. *Mycologia* **1999**, *91*, 553–556. [[CrossRef](#)]
29. Glass, N.L.; Donaldson, G.C. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl. Environ. Microb.* **1995**, *61*, 1323–1330. [[CrossRef](#)]
30. Rehner, S.A.; Buckley, E. A Beauveria phylogeny inferred from nuclear ITS and EF1-alpha sequences: Evidence for cryptic diversification and links to *Cordyceps teleomorphs*. *Mycologia* **2005**, *97*, 84–98. [[CrossRef](#)]
31. Dissanayake, A.J.; Camporesi, E.; Hyde, K.D.; Phillips, A.J.L.; Fu, C.Y.; Yan, J.Y.; Li, X.H. Dothiorella species associated with woody hosts in Italy. *Mycosphere* **2016**, *7*, 51–63. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.