horticulturae

MDPI

Article

First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.)

Elena Petrović [1], Karolina Vrandečić [2], Jasenka Ćosić [2], Edyta Đermić [3] and Sara Godena [1,*]

[1] Institute of Agriculture and Tourism, Karla Huguesa 8, 52440 Poreč, Croatia; elena@iptpo.hr
[2] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Vladimira Preloga 1, 31000 Osijek, Croatia; kvrandecic@fazos.hr (K.V.); jcosic@fazos.hr (J.Ć.)
[3] Faculty of Agriculture, University of Zagreb, Svetošimunska cesta 25, 10000 Zagreb, Croatia; edermic@agr.hr
* Correspondence: sara@iptpo.hr

Abstract: Leaf spot symptoms were spotted in two olive orchards in Istria and in Kvarner Gulf, Croatia. Fungal species from three representative isolates (P13 LECIII, R18 BI, JA20 NP) have been morphologically characterized based on the colony and conidial characteristics. Several techniques were performed for inducing the sporulation of the JA20 NP isolate. Only PDA + banana medium was successful. PCR was conducted for ITS, TUB, and EF1α gene regions. Phylogenetic analyses were performed using internal transcribed spacer, beta-tubulin, and translation elongation factor 1-alpha sequence data. Three types of tests were conducted: a pathogenicity test on detached leaves, on detached and scratched leaves, and on olive seedlings. Ultimately, from the morphological characterizations, DNA sequence analysis of ITS, TUB, and EF1α gene regions, and phylogenetic analysis, these species were identified as *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai, and *Nigrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai. This is the first report of *Nigrospora* species causing leaf spot on olive trees and the first report of *Nigrospora philosophiae-doctoris* as a plant pathogen. Fungal leaf diseases in conditions that are favorable for infection and disease development can lead to a decrease in the yield and olive oil quality. Therefore, it is necessary to conduct further research and the monitoring of fungal leaf diseases.

Keywords: fungal disease; isolate; olive tree; pathogenicity

Citation: Petrović, E.; Vrandečić, K.; Ćosić, J.; Đermić, E.; Godena, S. First Report of *Nigrospora* Species Causing Leaf Spot on Olive (*Olea europaea* L.). *Horticulturae* **2023**, *9*, 1067. https://doi.org/10.3390/horticulturae9101067

Academic Editor: Zhi Li

Received: 23 August 2023
Revised: 18 September 2023
Accepted: 19 September 2023
Published: 22 September 2023

1. Introduction

The olive tree, *Olea europaea* L., is among the world's most important crops. At present, the approximate production levels per year are 23.0 million tons of olives and 3.0 million tons of oil [1]. The crop is indigenous to the Mediterranean region with a mild, rainy winter and a hot, dry summer [2]. In the Republic of Croatia, the production area includes from the north of the Istrian peninsula, the Kvarner Gulf, and the Dalmatia coastal belt with the islands to the south. In recent years, interest in olive oil production has been rising due to certain socio-economic factors, such as a higher demand for olive oil, the possibility of achieving higher prices, and tourism development.

Among all fungal pathogens affecting olives, *Venturia oleaginea* (Castagne) Rossman & Crous and *Pseudocercospora cladosporioides* (Sacc.) U. Braun are two of the most important pathogens causing leaf spots: peacock spot disease (syn. bird's-eye spot, olive leaf scab, olive leaf spot) and cercosporiosis (syn. cercospora leaf spot) [3]. Olive leaf spots caused by *V. oleaginea* and *P. cladosporioides* result in the defoliation of leaves and weakness or death of branches, a reduced fruit set, and a decrease in the oil yield in the following years [4–8].

Other fungal causal agents of leaf spot symptoms on olives are *Alternaria alternata* (Fr.) Keissl. [9], *Colletotrichum acutatum* J.H. Simmonds and *C. gloeosporioides* (Penz.) Penz. & Sacc. [10], *Neofabraea kienholzii* (Seifert, Spotts & Levesque) Spotts, Levesque & Seifert, and *Phylctema vagabunda* Desmazières [11].

Leaf spot usually manifests as small, circular-to-elliptical spots on the leaves. Spots are initially green or yellow-green but gradually turn brown or black as the disease progresses. They often have a dark border and may have a yellow halo around them. In severe cases, the spots can merge, leading to significant defoliation. Leaf spot fungi usually thrive in warm and humid conditions. Rain or overhead irrigation can facilitate the spread of the disease by splashing fungal spores onto healthy leaves. The disease exhibits its highest activity during the wetter months of the year. While leaf spot primarily affects the leaves, a severe infection can weaken the tree and reduce its overall vitality. In severe cases, defoliation can expose fruit to direct sunlight, leading to sunburn and reduced fruit quality. Leaf spot diseases also weaken trees by interrupting the process of photosynthesis. This means the tree produces less energy, which can lead to stunted growth and smaller and less flavorful olives. This can be problematic for olive growers who rely on high-quality fruit for oil production or table olives. Leaf spot diseases can also affect the appearance of olive trees, which may be a concern for growers who want their orchard to be visually appealing. Management practices, such as maintaining good tree spacing and air circulation, pruning, avoiding excessive moisture on the leaves, proper disposal of infected leaves and pruning debris, planting resistant cultivars, etc., can help to maintain the health and productivity of olive orchards.

This study focuses on fungal species from the *Nigrospora* genus, new causal agents of a leaf spot symptom on olives. The *Nigrospora* genus has been introduced in 1902. for *N. panici*, which was isolated as an endophyte from leaves of *Panicum amphibium* in Java, Indonesia [12]. Based on its conidial characteristics, *Nigrospora* was placed in *Dermateaceae* (*Moniliales*) by Barnett and Hunter [13]. Kirk et al. [14] assigned *Nigrospora* and its *Khuskia* sexual morph to *Trichosphaeriaceae* (*Trichosphaeriales*). Wang et al. [15] placed the *Nigrospora* species in the family *Apiosporaceae* based on the phylogenetic analyses of combined ITS, TUB, and EF1-α sequence data of 165 strains from China and Europe [16].

Currently, there are 45 records of *Nigrospora* species in the MycoBank database, namely *Nigrospora aerophila*, *N. arundinacea*, *N. aurantiaca*, *N. bambusae*, *N. brasiliensis*, *N. camelliae-sinensis*, *N. canescens*, *N. chinensis*, *N. cooperae*, *N. covidalis*, *N. endophytica*, *N. falsivesicularis*, *N. gallarum*, *N. globosa*, *N. globospora*, *N. gorlenkoana*, *N. gorlenkoanum*, *N. gossypii*, *N. guangdongensis*, *N. guilinensis*, *N. hainanensis*, *N. javanica*, *N. lacticolonia*, *N. macarangae*, *N. magnoliae*, *N. manihoticola*, *N. maydis*, *N. musae*, *N. oryzae*, *N. osmanthi*, *N. padwickii*, *N. panici*, *N. pernambucoensis*, *N. philosophiae-doctoris*, *N. pyriformis*, *N. rubi*, *N. sacchari*, *N. sacchari-officinarum*, *N. saccharicola*, *N. singularis*, *N. sphaerica*, *N. vesicularifera*, *N. vesicularis*, *N. vietnamensis*, and *N. zimmermanii* [17].

Until now, only *Nigrospora oryzae* was isolated from olive trees [18,19], but it was not described as a pathogen on olives.

The aims of this research were to determine the causal agent of leaf spot symptoms on olive trees in Croatia, to morphologically characterize the fungal species from representative isolates, to molecularly identify the isolates of phytopathogenic fungal species using PCR and DNA sequence analysis of ITS, TUB, and EF1α gene regions, and to determine isolate pathogenicity in pathogenicity tests conducted in the laboratory and in a greenhouse experiment.

2. Materials and Methods

2.1. Collection of Plant Materials and Fungal Isolations

During the summer and autumn of 2021, leaf spot symptoms were spotted on olive trees on the coastal belt in Jadranovo, Kvarner Gulf (45°13′46″ N, 14°36′40″ E), and in olive orchards in Vabriga (45°17′14″ N, 13°36′41″ E) and Španidiga (45°03′02.2″ N, 13°42′43.9″ E) in Istria, Croatia (Figure 1).

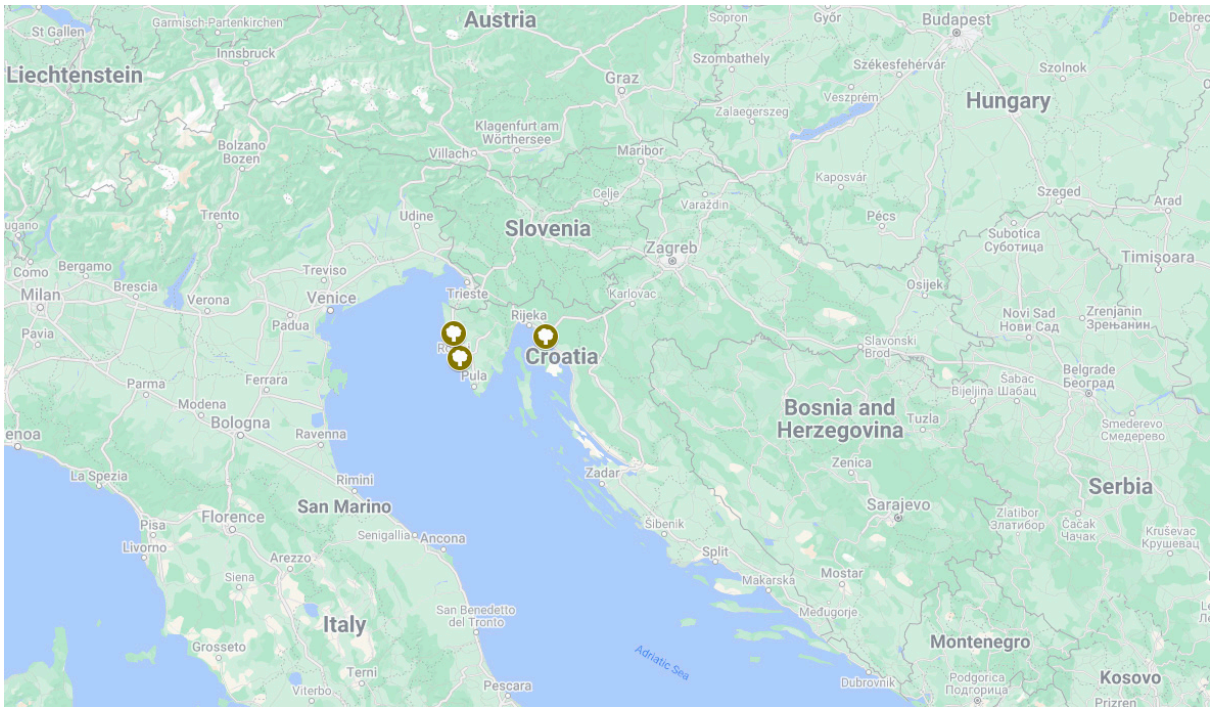

Figure 1. Locations of collected samples: Vabriga, and Špainidiga in Istria, and Jadranovo in Kvarner Gulf.

The symptoms were yellow and brown spots on leaves and defoliation. The samples from symptomatic trees were collected (30 leaves from each tree) in a sterile plastic bag, placed in a portable refrigerator at +4 °C, and immediately brought to the Laboratory for Plant Protection at the Institute of Agriculture and Tourism in Poreč (Croatia) for analysis. Olive varieties on which samples were collected in Istria were 'Buža' and 'Leccino'. The olive variety on which samples were collected in Kvarner Gulfs remains unknown.

Fresh olive leaves were used to isolate the causal agent of yellow and brown spots on leaves. For surface sterilization, leaves were rinsed under tap water for 1 min and transferred to aseptic conditions. Leaves were submerged in 70% ethanol for two minutes, rinsed in sterile distilled water, and placed on a sterile paper sheet in a laminar flow cabinet to surface-dry. Entire leaves were plated on potato dextrose agar (PDA) supplemented with 35 mg/L of penicillin and incubated at 25 °C under dark conditions. After seven days of incubation, the isolates were transferred into the fresh PDA medium for pure culture.

2.2. Morphological Characterization

After 7 and 30 days of incubation at 28 °C in dark conditions, pure fungal cultures were taken for examination. Fungal species, from three representative isolates (P13 LECIII, R18 BI, JA20 NP), have been characterized based on the colony characteristics (color, form, elevation, margin, surface, and opacity) and conidial characteristics (color, shape, presence or absence of septum, dimensions). A Boeco BM-2000 microscope, Boeco BCAM10 camera, and B-View software (Boeckel + Co (GmbH + Co), Hamburg, Germany) were used to capture conidia and hyphae. Isolate JA20 NP did not sporulate on PDA. Several techniques were performed for inducing the sporulation of the JA20 NP isolate. Pine needle medium was prepared according to Su et al. [20]. The banana peel technique was performed based on Kindo et al. [21]. The PDA + banana medium was prepared based on a technique for the pine needle medium described in Su et al. [20], by putting 100 g of fresh banana peel (instead of pine needle) and 20 g of potatoes into 1 L of distilled water, boiling for 30 min, filtrating, and keeping the volume at 1 L by adding distilled water. After filtration, it was amended with 20 g of agar and autoclaved for 20 min at 121 °C.

2.3. DNA Extraction, Amplification, and Sequencing

Fresh fungal mycelia of fungal isolates grown on PDA for 5 days, at 28 °C, under dark conditions, were scraped with a sterile laboratory needle from the colony margins and used for genomic DNA (deoxyribonucleic acid) extraction. Total DNA from the isolate was extracted using the Extract-N-Amp™ Plant PCR kit (Sigma-Aldrich, Merck, Saint Louis, MO, USA) according to manufacturer's protocol. The PCR (polymerase chain reaction) amplification process was performed using ITS1/ITS4 [22], ITS5/ITS4 [23], Btub2Fd/Btub4Rd [24], and EF1-728F/EF1-986R [25] pairs of primers (Table 1). The PCR reaction mixture was composed of 12.5 µL of EmeraldAmp® GT PCR Master Mix, 0.5 µL (10 µM) of each primer, 6.5 µL of nuclease-free water, and 5 µL (4.5 ng/µL) of genomic DNA. The PCR was conducted in a SureCycler 8800 Thermal Cycler (Agilent Technologies, Santa Clara, CA, USA) using different PCR conditions for ITS, TUB, and EF1α gene regions (Table 2). In the case of the R18 BI isolate, amplification of the EF1-alpha region was unsuccessful. Therefore, a second PCR was conducted using 1 µL of the initial PCR amplification as the template. Electrophoresis was performed using 1% agarose gel amended with two drops of GelRed® Nucleic Acid Gel Stain (Biotium, Fremont, CA, USA) at 110 V for 30 min in 1x TAE buffer with a BIO-RAD Power Pac 300 electrophoresis power supply (Agilent, Santa Clara, CA, USA). After electrophoresis, the PCR products were visualized using an iBright CL1000 Imaging System (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Purification of PCR products was performed with the GenElute™ PCR Clean-Up Kit (Sigma-Aldrich®, Burlington, MA, USA).

Table 1. List of the primers used for PCR and sequencing.

Locus	Primer	Sequence (5′-3′)
Internal transcribed spacer (ITS)	ITS1	5′ TCCGTAGGTGAACCTGCGG 3′
	ITS4	5′ TCCTCCGCTTATTGATATGC 3′
	ITS5	5′ GGAAGTAAAAGTCGTAACAAGG 3′
Beta-tubulin	Btub2Fd	5′ AACATGCGTGAGATTGTAAGT 3′
	Btub4Rd	5′ TAGTGACCCTTGGCCCAGTTG 3′
Translation elongation factor 1-alpha	EF1-728F	5′ CATCGAGAAGTTCGAGAAGG 3′
	EF1-986R	5′ TACTTGAAGGAACCCTTACC 3′

Table 2. PCR amplification program for ITS and EF1α region sets, according to White et al. [22], and for ITS and TUB region sets, according to Hao et al. [26].

ITS1/ITS4 and EF-728F/EF-986R						
HOT START 95 °C	Start Cycle 34 times	Denaturation 95 °C	Annealing 58 °C	Elongation 72 °C	End Cycle	Elongation 72 °C
3 min		30 s	30 s	1 min		10 min
ITS5/ITS4, and Btub2Fd/Btub4Rd						
HOT START 95 °C	Start Cycle 34 times	Denaturation 95 °C	Annealing 58 °C	Elongation 72 °C	End Cycle	Elongation 72 °C
3 min		30 s	30 s	1 min		10 min

2.4. DNA Sequence Assembly and Phylogenetic Analysis

Sequencing of the PCR products was performed by Macrogen Europe (Amsterdam, The Netherlands). Sequences were edited in Sequencher® (Gene Codes Corporation, Ann Arbor, MI, USA) and compared with sequences from GenBank®.

The phylogenetic trees were constructed and the evolutionary history of the isolated fungi was concluded based on the Neighbor-joining method [27]. Phylogenetic analysis was

performed using ITS, TUB, and EF1α sequence data from isolates and relevant sequence data of *Nigrospora* and *Botryosphaeria dothidea* (outgroup) isolates from GenBank® (a list of isolates used is presented in Table 3). The sequences were aligned using ClustalX2 (UCD Dublin, Dublin, Ireland) software, and a phylogenetic tree was made using MEGA11 software (Pennsylvania State University, State College, PA, USA).

Table 3. Genbank accession numbers of isolates used for phylogenetic analysis based on research carried out by Chen et al. [28].

Species	GenBank Accession Number			References
	ITS	TUB	TEF1α	
Botryosphaeria dothidea	AY236949	AY236927	AY236898	[29]
Nigrospora aurantiaca	KX986064	KY019465	KY019295	[15]
	MN215771	MN329935	MN264010	[30]
N. bambusae	KY385307	KY385319	KY385313	[15]
	KY385306	KY385320	KY385314	[15]
N. brasiliensis	KY569629	MK720816	MK753271	[31]
	KY569630	MK720817	MK753272	[31]
N. cameliae-sinensis	KX985986	KY019460	KY019293	[15]
	MN215775	MN329939	MN264014	[30]
N. chinensis	KX986023	KY019462	KY019422	[15]
	KX986026	KY019548	KY019445	[15]
N. covidalis	OK335209	OK431479	OK431485	[28]
	OK335210	OK431480	OK431486	[28]
N. falsivesicularis	MN215778	MN329942	MN264017	[30]
	MN215779	MN329943	MN264018	[30]
N. globospora	OK335211	OK431481	OK431487	[28]
	OK335212	OK431482	OK431488	[28]
N. gorlenkoana	KX986048	KY019456	KY019420	[15]
N. guilinensis	KX985983	KY019459	KY019292	[15]
	KX986063	KY019608	KY019404	[15]
N. hainanensis	KX986091	/	KY019415	[15]
	MN215780	MN329944	MN264019	[30]
N. lacticolonia	KX985978	KY019458	KY019291	[15]
	/	MN329948	MN264023	[30]
N. musae	KX986076	KY019455	KY019419	[15]
	KX986042	KY019567	KY019371	[15]
N. oryzae	KX985931	KY019601	KY019396	[15]
	KX985954	KY019481	KY019307	[15]
N. osmanthi	KX986010	KY019461	KY019421	[15]
	KX986017	KY019540	KY019438	[15]
N. philosophiae-doctoris	OK335213	OK431483	OK431489	[28]
	OK335214	OK431484	OK431490	[28]

Table 3. *Cont.*

Species	GenBank Accession Number			References
	ITS	TUB	TEF1α	
N. pyriformis	KX985940	KY019457	KY019290	[15]
	MN215787	MN329988	MN264026	[30]
N. rubi	KX985948	KY019475	KY019302	[15]
N. sacchari-officinarum	MN215791	MN329954	MN264030	[30]
	MN215792	MN329955	MN264031	[30]
N. saccharicola	MN21578	/	MN264027	[30]
	MN215789	MN329952	MN264028	[30]
N. singularis	MN215793	MN329956	MN264032	[30]
	MN215794	MN329957	MN264033	[30]
N. sphaerica	KX985965	KY019492	KY019318	[15]
	MN215811	MN329974	MN264050	[30]
N. vesicularifera	MN215812	MN329975	MN264051	[30]
	MN215814	MN329977	MN264053	[30]
N. vesicularis	KX986088	KY019463	KY019294	[15]
	KX985939	KY019467	/	[15]
N. zimmermanii	KY385309	KY385317	KY385311	[15]
	MN215824	MN329987	MN264063	[30]

2.5. Pathogenicity Test

Three pathogenicity tests were conducted to determine the isolates' pathogenicity: pathogenicity test on detached leaves, pathogenicity test on detached and scratched leaves, and pathogenicity test on olive seedlings.

2.5.1. Pathogenicity Test on Detached Leaves and Scratched Detached Leaves

In May 2022, healthy olive leaves of the cultivars Leccino and Buža were collected from a collection orchard at the Institute of Agriculture and Tourism in Poreč (Istria, Croatia). Leaves were washed with tap water, surface-sterilized in 1% sodium hypochlorite solution for three minutes, rinsed with sterilized distilled water for one minute, and placed in a laminar flow cabinet, on sterile paper, until dry. After air-drying, ten leaves of each cultivar (per isolate), scratched with a needle, and ten unscratched leaves of each cultivar (per isolate), were inoculated by placing a 5 mm-diameter mycelium plug, taken from the margins of a five-day-old PDA culture of the isolates. The same numbers of scratched and unscratched leaves inoculated using pure PDA agar plugs were used as controls. Leaves were placed on a sterile filter paper, sprayed with sterile distilled water, in a Petri dish, and protected with parafilm. Two replicates were performed. Leaves were incubated at 28 °C, in dark conditions. After nine days, samples were analyzed.

2.5.2. Pathogenicity Test on Olive Seedlings

For the whole plant assay, the fungus was grown on PDA at 28 °C for five days, and mycelia were collected. Approximately one gram of mycelia was grinded in 10 mL of sterilized distilled water. The grinded mycelia were homogenized by mixing with a vortex mixer. In the greenhouse of the Institute of Agriculture and Tourism, the grinded mycelia were injected into the petiole of the leaves of three-year-old olive seedlings of cultivar Rosinjola. Thirty leaves (ten per isolate) were inoculated with a grinded mycelium. An equal number of control leaves were inoculated with sterile distilled water to serve as a

negative control. Two replicates were performed. The inoculated plants were kept in a greenhouse for two weeks. After the incubation period, samples from all tests were collected and, in an effort to adhere to Koch's postulate, small sections of necrotic tissue from the periphery of lesions were excised and placed on PDA to isolate the inoculated fungus.

3. Results

3.1. Field Survey and Disease Symptoms

Symptoms of leaf spot were observed on mature 10- to 38-year-old trees in Istria, Croatia. Leaves were dry and yellowish to chocolate-brown in color (Figure 2). Some of them had brown spots. Defoliation was observed on leaves with expanded spots, and symptoms affected only part of the trees.

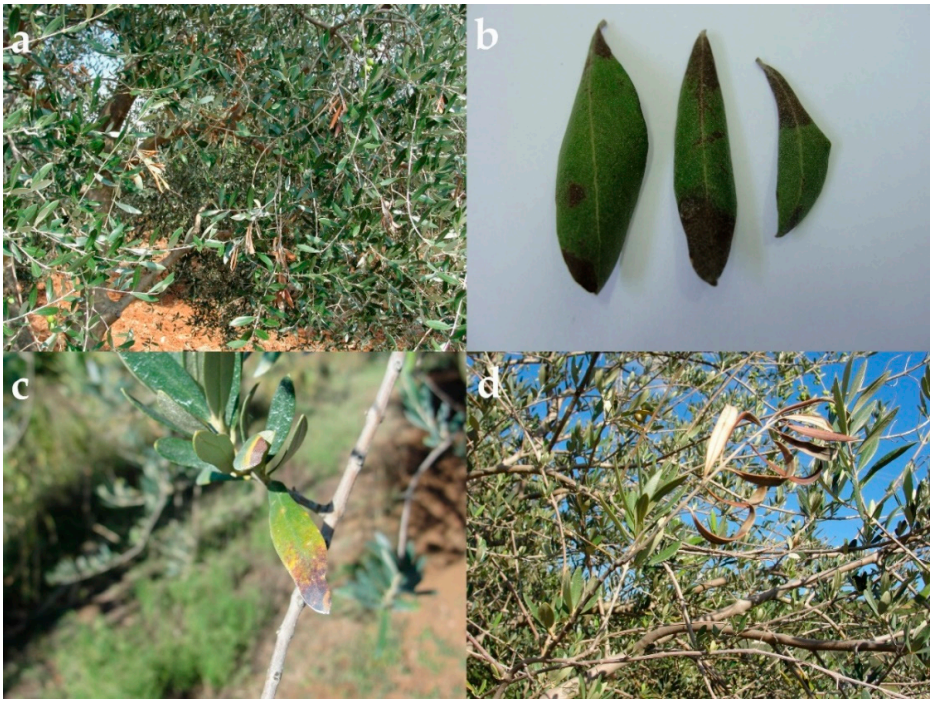

Figure 2. Symptoms on olive leaves, (**a**) *Nigrospora gorlenkoana*, (**b**) *Nigrospora osmanthi*, (**c**,**d**) *Nigrospora philosophiae-doctoris*.

3.2. Molecular Phylogenetic Identification

For molecular identification, consensus sequences of isolates were produced and registered in GenBank®. GenBank® accession numbers for each isolate and its genome region are represent in Table 4.

Table 4. GenBank accession numbers of the sequences.

SPECIES	ISOLATE	COLLECTION DATE	Varieties	Genbank Accession Number		
				ITS	TUB	Ef1α
Nigrospora gorlenkoana	P13 LECIII	24 September 2021	Leccino	OP999642	OQ286068	OQ286069
Nigrospora osmanthi	JA20 NP	31 October 2021	Unknown	OP999639	OQ275027	OQ275028
Nigrospora philosophiae-doctoris	R18 BI	14 October 2021	Buža	OP999644	OQ286067	OQ286066

BLAST analysis of the sequences from the P13 LECIII isolate showed 100% similarity for ITS and TUB and 99.27% similarity for the EF1α gene region to *N. gorlenkoana*. BLAST

analysis of the sequences from the JA20 NP isolate showed 100% similarity for ITS, TUB, and EF1α gene regions to *N. osmanthi*. BLAST analysis of the sequences from the R18 BI isolate showed 100% similarity for ITS and TUB and 98.64% similarity for the EF1α gene region to *N. philosophiae-doctoris*. Phylogenetic trees were constructed by aligning ITS, TUB, and EF1α sequences, and the evolutionary history was inferred using the Neighbor-Joining method [27]. Finally, a multilocus tree was created from a combination of ITS, TUB, and EF1α sequence alignments. The optimal trees are displayed in Figures 3–6. Beneath the branches are the percentages of replicate trees where related taxa clustered together in the bootstrap test based on 1000 replicates [32]. The Maximum Composite Likelihood method [33] was used to calculate evolutionary distances, expressed in the units of base substitutions per site. The *Botryosphaeria dothidea* isolate CMW8000 was used as the outgroup. Ambiguous positions were excluded via pairwise detection using MEGA11 software [34].

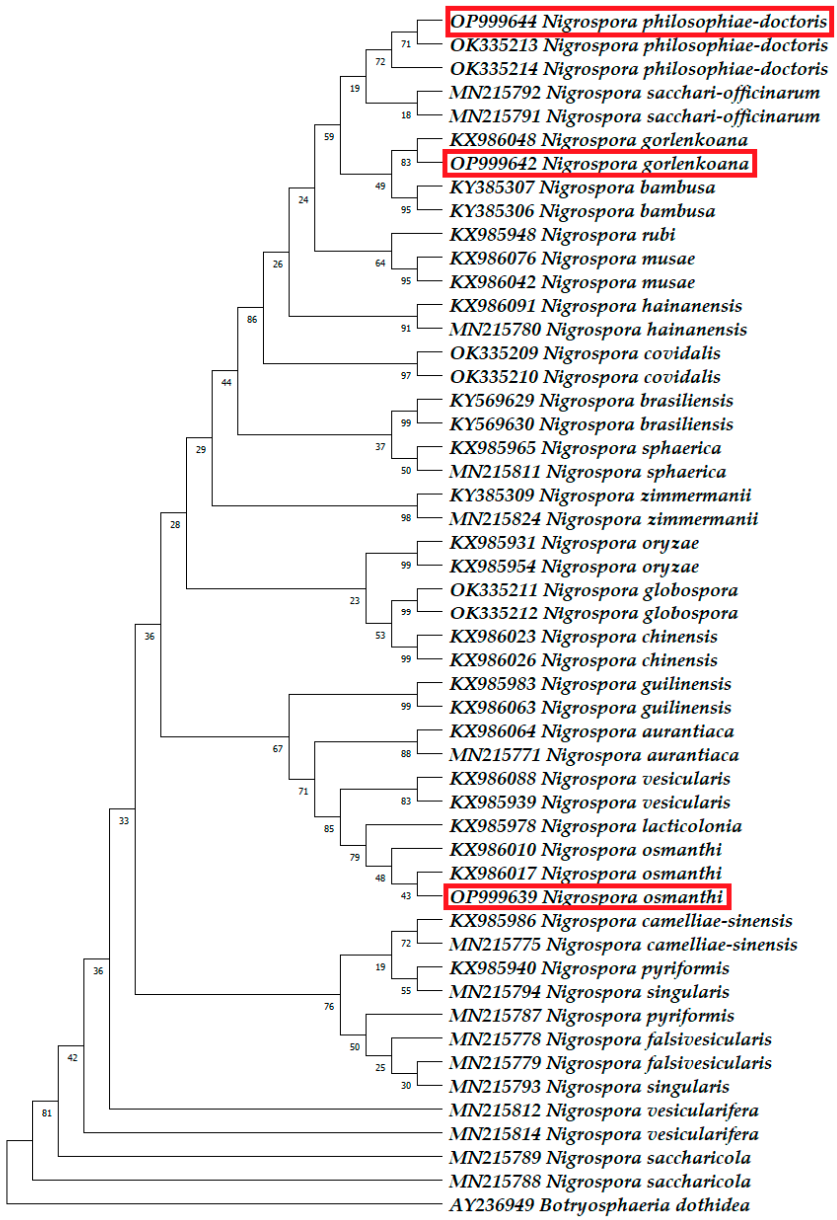

Figure 3. Phylogenetic tree based on internal transcribed spacer sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 51 nucleotide sequences, resulting in a final dataset comprising 595 positions.

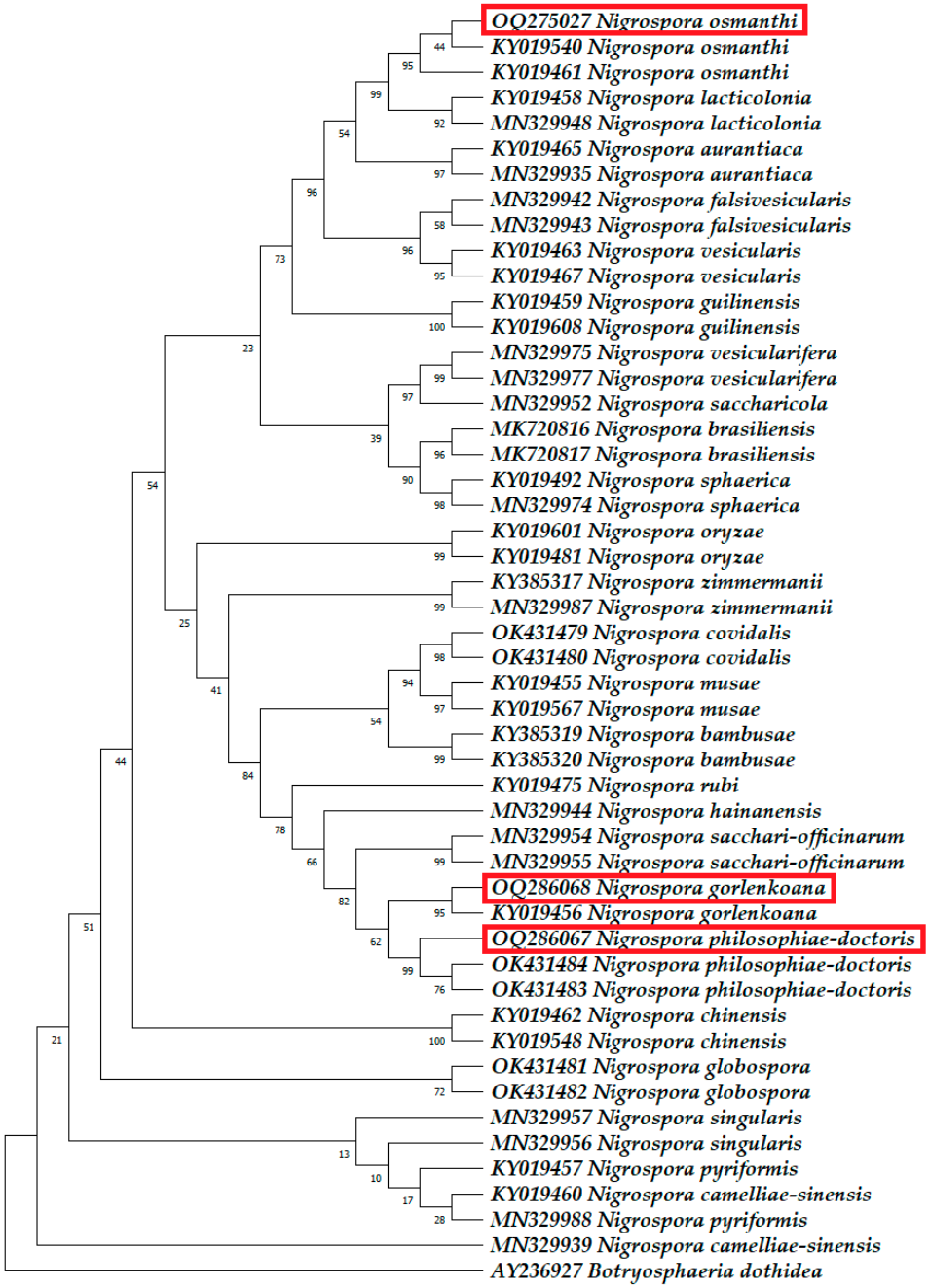

Figure 4. Phylogenetic tree based on beta-tubulin sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 50 nucleotide sequences, resulting in a final dataset comprising 808 positions.

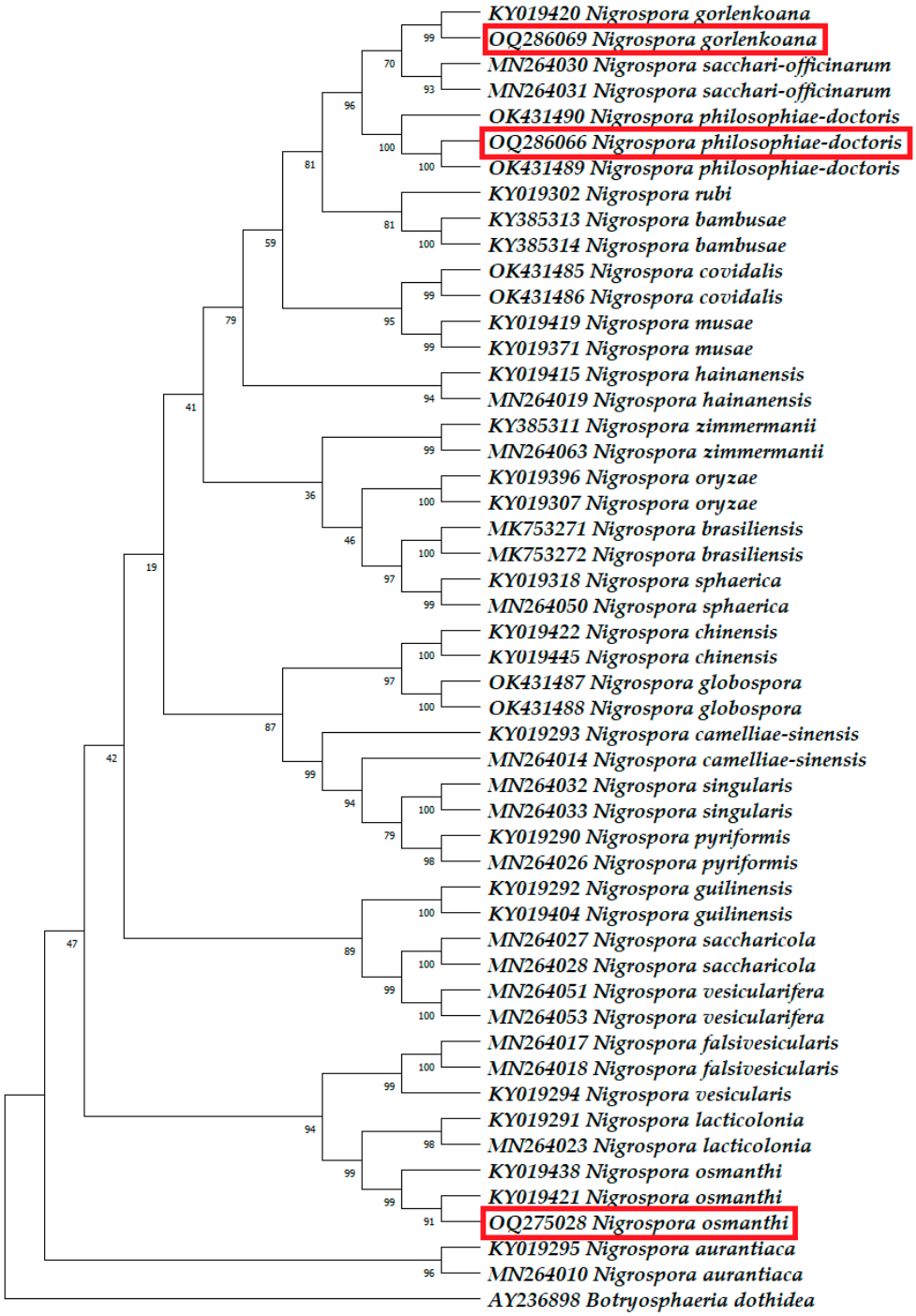

Figure 5. Phylogenetic tree based on translation elongation factor 1-alpha sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 51 nucleotide sequences, resulting in a final dataset comprising 567 positions.

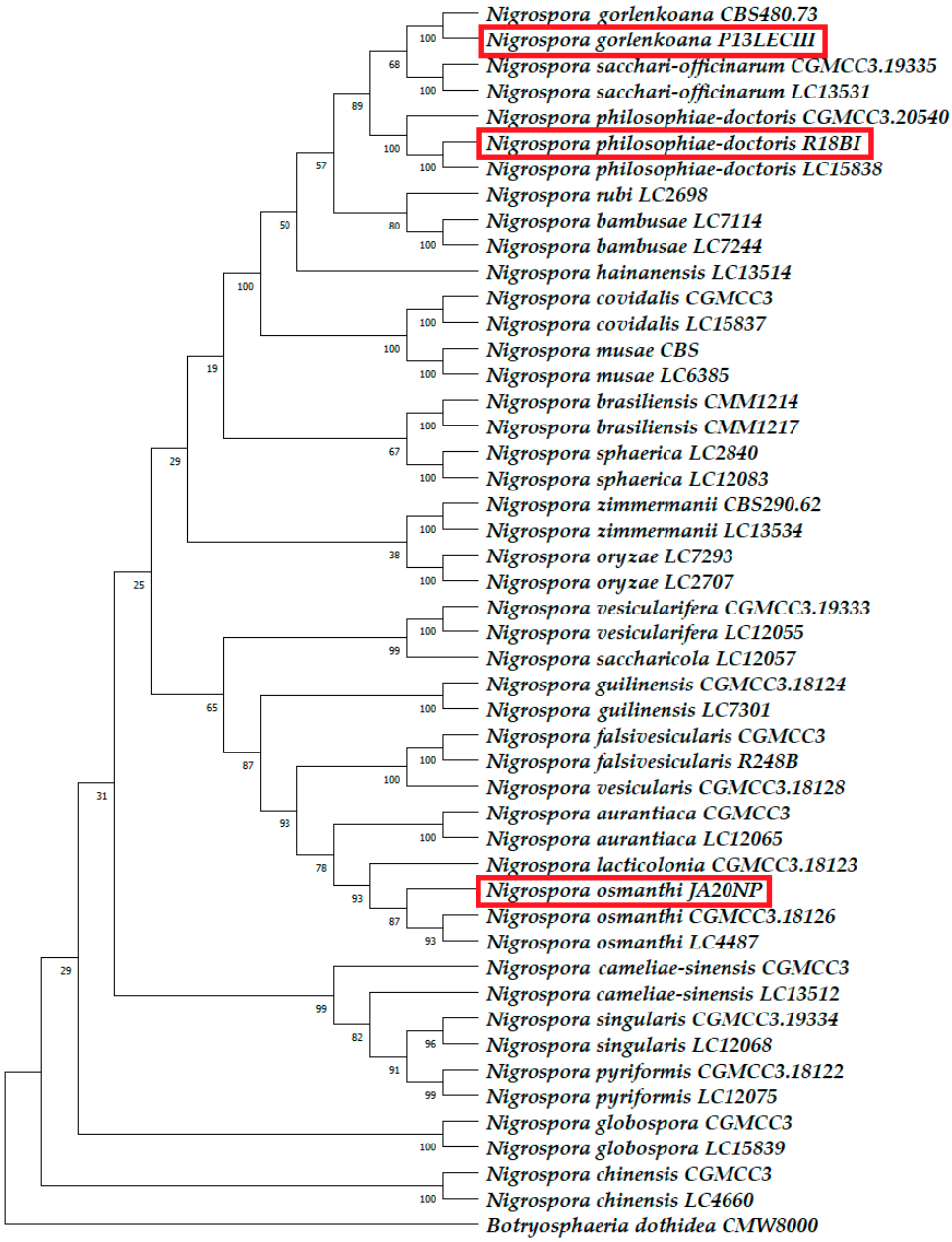

Figure 6. Multilocus tree based on internal transcribed spacer, beta-tubulin, and translation elongation factor 1-alpha sequence alignment. Sequences identified in this research are highlighted with red rectangles. This analysis encompassed 48 nucleotide sequences, resulting in a final dataset comprising 1581 positions.

Ultimately, from the DNA sequence analysis of ITS, TUB, and EF1α gene regions and the phylogenetic analysis, these species were identified as *Nigrospora gorlenkoana* Novobr., *Nigrospora osmanthi* Mei Wang & L. Cai, and ***N****igrospora philosophiae-doctoris* M. Raza, Qian Chen & L. Cai.

3.3. Morphological Characterization and Fungal Incidence

Regarding sporulation, successful sporulation of the JA20 NP isolate was observed exclusively when it was cultured on a PDA + banana medium, as shown in Table 5.

Table 5. List of techniques used for inducing sporulation of the JA20 NP isolate and results.

TECHNIQUES									
PDA Temperatures: 22 °C, 25 °C, 28 °C, 30 °C	1/2 strength PDA medium	WA Temperatures: 22 °C, 25 °C, 28 °C, 30 °C	Pine needle extracts + WA	MEA Temperatures: 22 °C, 25 °C, 28 °C, 30 °C	Host tissue	Slide culture	Exposure to near-ultraviolet light (12 h day/12 h night)	Banana peel	PDA + banana medium
x	x	x	x	x	x	x	x	x	✓

x—sporulation not determined, ✓—sporulation recorded, WA—water agar, MEA—malt extract agar.

3.3.1. *Nigrospora gorlenkoana*

Colonies on PDA had reached a nine-centimeter diagram after two days at 28 °C and on WA after five days and sporulated after three days of incubation on PDA medium. Colonies of *N. gorlenkoana* developing on PDA (Figure 7a,b) were circular-shaped with aerial, woolly mycelium, entire-margined, opaque, floccose, raised with fuzzy edges, and growing rapidly; at first, they were white, becoming light grey when they matured and the reverse initially white, becoming darker grey when they matured. Conidia of *N. gorlenkoana* were round-shaped, light-brown-to-black-colored and aseptated, solitary, smooth, and 10.5–13.8 × 13.5–17.3 µm in diameter ($\bar{x}$= 11.9 × 15.1 µm, n = 30). Hyphae were smooth, septate, hyaline, and yellowish. On WA, colonies were white and growing poorly.

3.3.2. *Nigrospora osmanthi*

Colonies on PDA had reached a nine-centimeter diagram after three days at 28 °C and on WA after seven days and sporulated after five days of incubation on PDA + banana medium. Colonies of *N. osmanthi* developing on PDA (Figure 7c,d) were circular-shaped with an aerial, slightly woolly mycelium, entire-margined, opaque, raised a little, filiform, and growing rapidly; they were creamy-white-colored up and the reverse becoming greyish when mature. Conidia of *N. osmanthi* were round-shaped, black-colored, and aseptated, solitary, smooth, and 11.2–12.8 × 12.7–15.4 in diameter ($\bar{x}$ = 12.5 × 15.3 µm, n = 30). Hyphae were smooth, septate, hyaline, and yellowish. On WA, colonies were white and growing poorly.

3.3.3. *Nigrospora philosophiae-doctoris*

Colonies on PDA had reached a nine-centimeter diagram after three days at 28 °C and on WA after nine days and sporulated after four days of incubation on PDA medium. Colonies of *N. philosophiae-doctoris* developing on PDA (Figure 7e,f) were circular-shaped with aerial, woolly mycelium, entire-margined, opaque, floccose, raised with fuzzy edges, and growing rapidly; they were white-to-greyish-colored and reverse initially white, becoming creamy white when mature. Conidia of *N. philosophiae-doctoris* were round-shaped, brown-to-black-colored, aseptated, solitary, smooth, and 10.8–15.6 × 8.4–14.4 in diameter ($\bar{x}$ = 11.6 × 13.2, n = 30). Hyphae were smooth, septate, and hyaline. On WA, colonies were white to greyish and growing poorly.

3.4. Pathogenicity Tests

3.4.1. Pathogenicity Test on Detached Leaves

The symptoms of the disease on olive leaves tested in the laboratory showed similar symptoms as the leaf samples collected from the field survey. All inoculated leaves had yellowish-to-chocolate-brown spots (Figure 8). No symptoms were spotted on the control leaves. The re-isolated fungus from the diseased leaves was identical to the *Nigrospora* species.

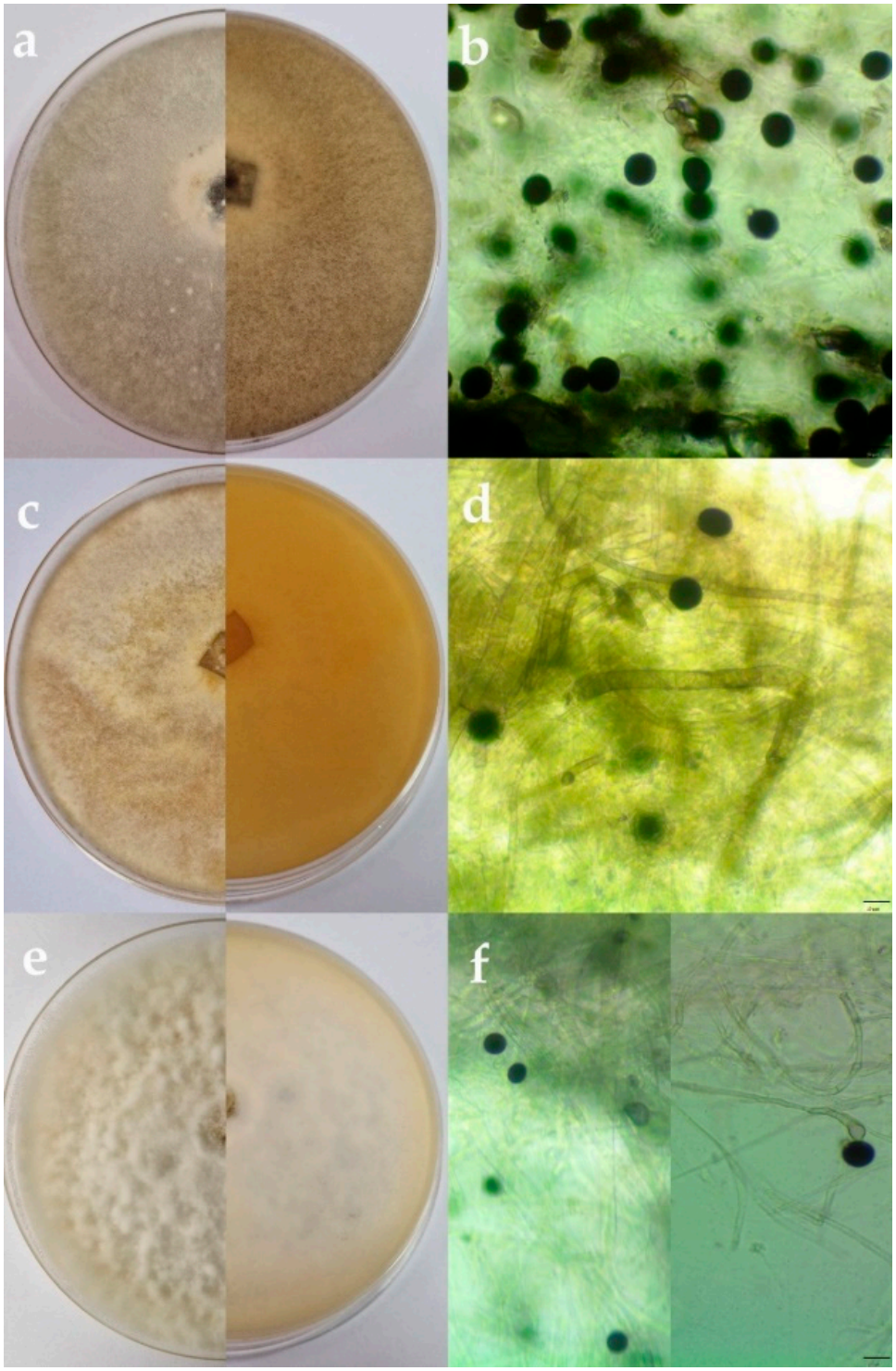

Figure 7. Left: Upper surface and reverse overview of cultures five days after incubation at 28 °C on PDA medium. Right: micrographs of isolates under the microscope with conidia. Scale bar = 10 µm, (**a**,**b**) *Nigrospora gorlenkoana*, (**c**,**d**) *N. osmanthi*, (**e**,**f**) *N. philosophiae-doctoris*.

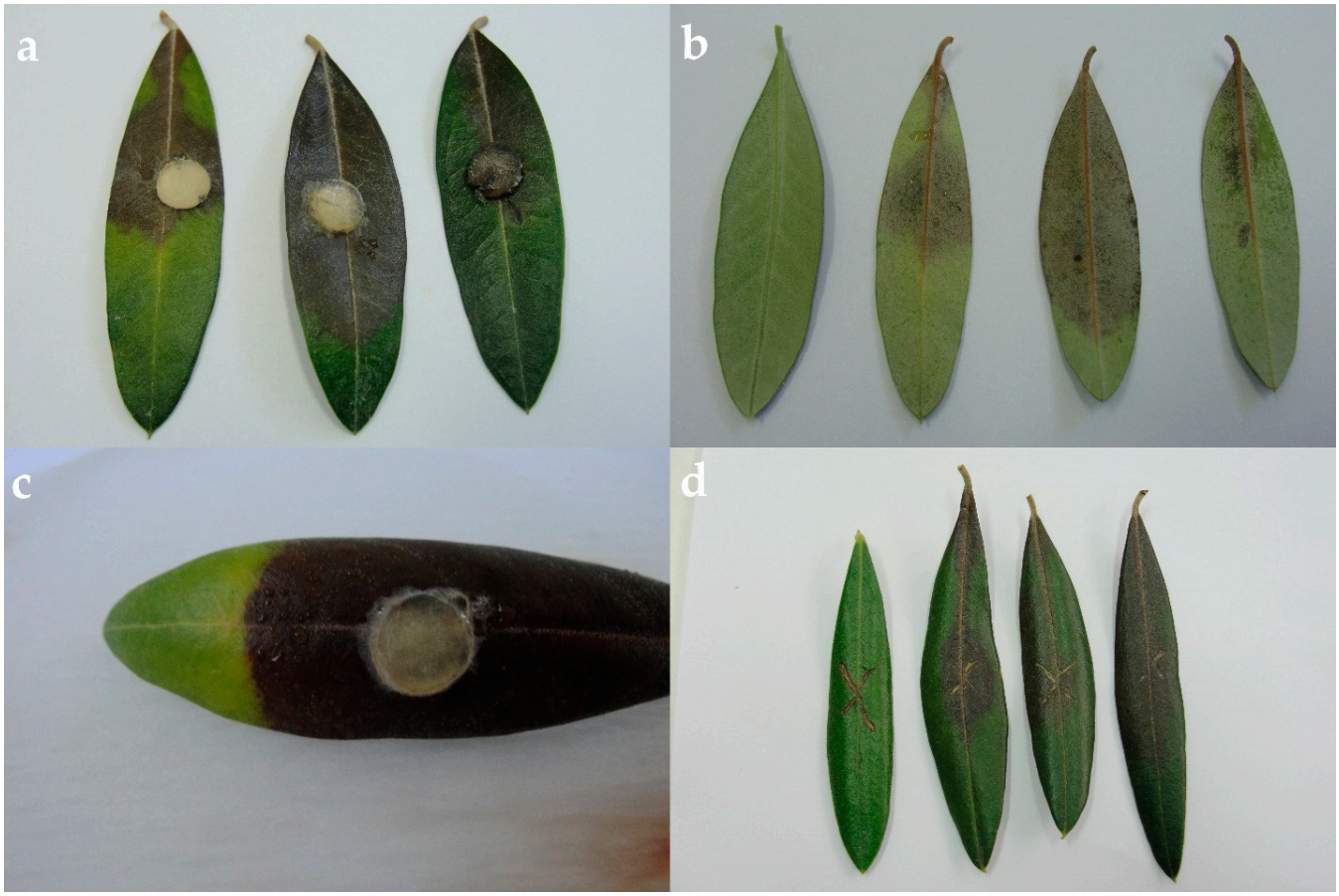

Figure 8. (**a**) Symptoms on unwounded leaves, from left to right: *N. philosophiae-doctoris*, *N. gorlenkoana*, *N. osmanthi*. (**b**) Symptoms on the bottoms of unwounded leaves, top left: control, from control to right: *N. philosophiae-doctoris*, *N. gorlenkoana*, *N. osmanthi*. (**c**) Symptoms on unwounded leaf: *N. philosophiae-doctoris*. (**d**) Symptoms on wounded leaves, from left to right: control, *N. philosophiae-doctoris*, *N. gorlenkoana*, *N. osmanthi*.

3.4.2. *Pathogenicity Test on Olive Seedlings*

The first symptoms were observed three days after inoculation. All inoculated leaves had chocolate-brown spots, similar to those observed in the field. The symptoms progressed until the entire leaf surface was covered (Figure 9). No symptoms were observed on the control leaves. The re-isolated fungus from the diseased leaves was identical to the *Nigrospora* species.

Figure 9. Symptoms on olive seedling leaves (cv. Rosinjola) in the pathogenicity test in the greenhouse, (**a**) *Nigrospora gorlenkoana*, (**b**) *N. osmanthi*, (**c**) *N. philosophiae-doctoris* after 9 days, (**d**) *N. philosophiae-doctoris* after 15 days.

4. Discussion

In this study, three different species of the *Nigrospora* genus, i.e., *N. gorlenkoana*, *N. osmanthi*, and *N. philsophiae-doctoris*, were detected on olive trees in Croatia. These species were the causal agents of leaf spots on olive trees in Istria and in Kvarner Gulf (Croatia).

Nigrospora species are cosmopolitan, filamentous, dematiaceous taxa distributed on various hosts, including crops with economic importance [15,16]. These species were isolated from different hosts around the world, such as *Cirsium setosum*, *Nelumbo* sp., *Oryza sativa*, *Vitis vinifera*, etc. [26]. They are known as plant pathogens, endophytes, and saprobes [35,36]. *Nigrospora* species known as plant pathogens cause many diseases, but

the most common disease is leaf spot. A list of reported *Nigrospora* pathogens, diseases, and distributions is represented in Table 6. *Nigrospora* sp. are also known as contaminants on farm-stored maize [37] and stored wheat [38] and are the causative agent of the post-harvest rot of ginger rhizomes [39] and post-harvest black rot of kiwifruit [40,41]. There are records of *N. oryzae* infecting the roots of 21 plant species [42] and *N. sphaerica* isolated from diseased grapevines [43], but there are no data on the pathogenicity of this fungus on the mentioned plant species.

Based on GenBank® data, *N. philosophiae-doctoris* was first isolated from the plant species *Disporum sessile* (Thunb.) D.Don ex Schult. & Schult.f., but there are no reports of this fungus as a plant pathogen. *N. philosophiae-doctoris* clustered in a well-supported clade closely related to *N. sacchari-officinarum* and *N. gorlenkoana*. *N. philosophiae-doctoris* produces smaller conidiogenous cells, when compared to those in *N. sacchari-officinarum* and *N. gorlenkoana*, and smaller conidia than those of *N. sacchari-officinarum* [28]. The conidial sizes frequently overlap among morphologically similar, but phylogenetically distinct, species of *Nigrospora*, and identification based on molecular and phylogenetic data for this fungal species is crucial [15]. In this research for molecular identification, consensus sequences were made of ITS1, ITS5, and ITS4 sequence data for the ITS gene region, Btub2Fd, and Btub4Rd sequence data for the TUB gene region and EF-728F and EF-986R sequence data for the EF1α gene region. In our research, *Nigrospora* species were distinguished based on morphological and molecular data, four phylogenetic trees were made, and they support three separate species.

Sporulation in fungi usually occurs when the growth rate is reduced and is hampered under conditions that favor rapid mycelial growth [44]. Many techniques are used for inducing the sporulation of fungi, such as slide culture [45], low-nutrient media [46], sporulation on host tissue, etc. In our survey, several techniques were performed for inducing the sporulation of the JA20 NP isolate (Table 5) in order to carry out morphological research. Banana peel culture is used for inducing the sporulation of *Nigrospora sphaerica* [21] but was not effective for the *Nigrospora osmanthi* JA20 NP isolate. Interestingly, this isolate sporulates only on PDA + banana medium. Spore dispersal in *Nigrospora* is aided by the wind, rain splashes, and insect vectors [47], so it can be easily spread around the orchard and create defoliation and economical losses. In some cases, an irregular string of a mucilaginous substance (small hyaline drop) was found to be attached to the spore [48]. It has been hypothesized that this substance facilitates adherence to the host substrate or to a vector as a successful spore-dispersal mechanism [26]. The moth *Sitotroga cerealella* transports the spores of *Nigrospora oryzae*, which adhere to its body [49]. Alfaro [50] has described how the non-gravid females of the mite *Pediculopsis graminum* transport the conidia of *Nigrospora oryzae* in their abdominal sacks. Webster [48] states that the spores can be transmitted while adhered to the body of the mite. A study of airborne fungal spores carried out at nine locations in Nigeria showed that the numbers of *Nigrospora* spores significantly correlate with the relative humidity, light intensity, and temperature [51]. Research conducted on bananas has shown that with an increase in the temperature, the rotting of bananas caused by *Nigrospora* species speeds up, with maximum infection recorded at 30 °C [52]. In our survey, out of the four temperatures at which all three isolates were kept on PDA (represented in Table 5), the fastest growth was recorded at 28 °C. Therefore, this temperature was chosen as the incubation temperature for morphological analysis of the isolates.

Regarding protection measures, for *N. oryzae*, Azoxystrobin (EC50 = 0.0001 mg/L) had the most significant fungal-controlling effect, followed by Prochloraz (copper salt), 15% Difenoconazole + 15% Propiconazole, Difenoconazole, Pyraclostrobin, and Myclobutanil [53]. Lignans, isopicropodophyllone, and dehydropodophyllotoxin, isolated from the leaves of *Podophyllum hexandrum* (Royle) T. S. Ying of Pakistani origin showed strong antifungal activity against *N. oryzae* [54]. *Bacillus thuringiensis* var. *israelensis* has shown inhibitory effects against *Nigrospora* sp. [55]. The mushroom species *Coprinellus disseminates* (isolate 12b), *Marasmiellus palmivorus* (isolate 42b), *Trametes maxima* (isolate 56e), and *Lenti-*

nus sajor-caju (isolate 60a) have potential antagonistic effects on *Nigrospora* species via the production of secondary metabolites and mycoparasitic interactions [56]. Unfortunately, there are no data about protection measures for these three species identified in this study.

Some *Nigrospora* species can act as an antagonist against fungal and bacterial plant pathogens [57,58]. Phomalactone from *Nigrospora sphaerica* exhibits a broad spectrum of antimicrobial activity against human and phytopathogenic bacteria and fungi [59], such as *Phytophthora infestans* (Mont.) de Bary, which causes tomato late blight [60]. An ethyl acetate extract of *Nigrospora sphaerica* affects the cell wall in growing methicillin-resistant *Staphylococcus aureus* Rosenbach and *Klebsiella pneumonia* (Schroeter 1886) Trevisan [61].

Table 6. A list of reported *Nigrospora* pathogens, diseases, and distributions.

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
Nigrospora aurantiaca	Chinese chestnut	*Castanea mollissima* Blume	Leaf spot	China	[62]
	Pandan rampeh	*Pandanus amaryllifolius* Roxb.	Leaf spot	Malaysia	[63]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
	Tobacco	*Nicotiana tabacum* L.	Leaf spot	China	[64]
Nigrospora brasiliensis	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown leaf spot	Brazil	[31]
Nigrospora camelliae-sinensis	Black tea	*Camellia sinensis* L.	Leaf blight	China	[65]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora chiensis	Tea-oil plant	*Camellia oleifera* C. Abel	Leaf blight	China	[66]
Nigrospora falsivesicularis	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora guiliensis	Chinese corktree	*Phellodendron chinense* C. K. Schneid.	Leaf spot	China	[67]
Nigrospora hainanensis	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown spot	Brazil	[68]
	Pink wood sorrel	*Oxalis corymbosa* DC.	Leaf spot	China	[69]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora lacticolonia	Date palm	*Phoenix dactylifera* L.	Leaf spot	Oman	[70]
	Dragon fruit	*Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose	Reddish-brown spot	Malaysia	[71]
	Great Bougainvillea	*Bougainvillea spectabilis* Raeusch. Willd.	Leaf spot	China	[72]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora oryzae	Aloe-vera	*Aloe vera* var. *chinensis* (Haw.) A.Berger	Leaf spot	China	[73]
			Leaf spot	Pakistan	[74]
			Leaf spot	Bangladesh	[75]
	Asiatic dayflower	*Commelina communis* L.	Leaf spot	China	[76]
	Bayberry	*Morella rubra* Lour.	Twig blight	China	[53]
	Blueberry	*Vaccinium corymbosum* L.	Leaf spot	China	[77]
	Chinese photinia	*Photinia serratifolia* (Desf.) Kalkman (syn. *Photinia serrulata* Lindl.)	Leaf spot	China	[78]
	Cotton	*Gossypium hirsutum* L.	Leaf spot	China	[79]

Table 6. *Cont.*

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
	Cotton-rose	*Hibiscus mutabilis* Mill.	Black leaf spot	China	[80]
	Crepe-ginger	*Hellenia speciosa* (J.Koenig) Govaerts (syn. *Costus speciosus* (J.Koenig) Sm.)	Leaf spot	China	[36]
	Dendrobium (Shi Hu)	*Dendrobium candidum* Wall. ex Lindl.	Leaf spot	China	[81]
	Dove tree	*Davidia involucrata* Baill.	Leaf blight	China	[82]
	Dryland winter wheat	*Triticum* L.	Crown and rot root	Azerbaijan	[83]
	Giant red	*Arundo donax* L.	Foliar and cane rot	Europe	[84]
	Ginger	*Zingiber officinale* Roscoe	Leaf spot	China	[85]
	Indian lotus	*Nelumbo nucifera* Gaertn.	Leaf spot	China	[86]
	Indian mustard	*Brassica juncea* (L.) Czern.	Stem blight	India	[87]
	Kentucky bluegrass	*Poa pratensis* L.	Leaf spot	Ontario	[88]
	Kidney bean	*Phaseolus vulgaris* L.	Leaf spot	China	[89]
	Kiwifruit	*Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson	Brown/black spot	China	[90]
	Million bells	*Calibrachoa* hybrid cultivar	Leaf spot	Argentina	[91]
	Pearl millet	*Cenchrus americanus* (L.) Morrone (syn. *Pennisetum americanum* (L.) Leeke)	Leaf spot	Iran	[92]
	Peppermint	*Mentha spicata* L.	Brown leaf spot	Iran	[93]
	Poplar	*Populus alba* L. × *P. berolinensis* Dipp. (hybrid poplar)	Leaf blight	China	[94]
	Rice	*Oryza sativa* L.	Sheaths and grains of sheath rot	Bangladesh	[95]
	Tobacco	*Nicotiana tabacum* L.	Leaf spot	China	[96]
	Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Leaf spot	China	[97]
	Wheat	*Triticum aestivum* Vill.	Dark brown to black lesions	Kazahstan	[98]
			Crown and root rot	Kazahstan	[99]
	Wild rice	*Oryza rufipogon* Griff.	Leaf spot	China	[100]
	Zebra leaf aloe	*Aloe zebrina* Baker	Flower malformation	Namibia	[101]
Nigrospora osmanthi	Fiddle-leaf fig	*Ficus pandurata* Hance	Leaf blight	China	[102]
	Java tea	*Orthosiphon stamineus* Benth.	Leaf blight	Malaysia	[103]
	St. Augustine grass	*Stenotaphrum secundatum* (Walter) Kuntze	Leaf blight	China	[104]
	Tartary buckwheat	*Fagopyrum tataricum* (L.) Gaertn.	Leaf spot	China	[105]
Nigrospora panici	Big marigold	*Tagetes erecta* L.	Leaf blight	Bangladesh	[106]
	French marigold	*Tagetes patula* L.	Leaf blight	Bangladesh	[106]
Nigrospora pyriformis	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
	White goosefoot	*Chenopodium album* L.	Leaf spot	China	[107]

Table 6. *Cont.*

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
Nigrospora sacchari-officinarum	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora saccharicola	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora singularis	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora sp.	Black tea	*Camellia sinensis* L.	Blister blight lesions	India	[108]
	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown spot	Brazil	[68]
	Maize	*Zea mays* L.	Weight/discoloration/necrosis of grains	Brazil	[109]
Nigrospora sphaerica	Balloon flower	*Platycodon grandiflorus* (Jacq.) A. DC.	Necrosis	China	[28]
	Black tea	*Camellia sinensis* L.	Leaf blight	India	[110]
				China	[111]
	Blueberry	*Vaccinium corymbosum* L.	Leaf spot, twig and shoot blight	Argentina	[112]
	Calabash	*Lagenaria siceraria* (Molina) Standl.	Leaf spot	Georgia	[113]
	China fir	*Cunninghamia lanceolata* (Lamb.) Hook.	Leaf blight	China	[114]
	Chinese Wisteria	*Wisteria sinensis* (Sims) DC., 1825	Leaf spot	Turkey	[115]
	Cochineal cactus	*Nopalea cochenillifera* (L.) Salm-Dyck	Brown spot	Brazil	[68]
	Corn mint	*Mentha canadensis* L.	Leaf blight	China	[116]
	Cowpea	*Vigna unguiculata* (L.) Walp.	Leaf spot	India	[117]
	Curcuma	*Curcuma wenyujin* Y.H.Chen & C.Ling	Leaf blight	China	[118]
	Date palm	*Phoenix dactylifera* L.	Not applicable	Iraq	[119]
			Root disease	Oman	[120]
			Leaf spot	Pakistan	[121]
	Devilpepper	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Leaf spot and antracnose	Bangladesh	[122]
	Dragon fruit	*Selenicereus monacanthus* (hort. ex Lem.) D.R.Hunt (syn. *Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose)	Reddish-brown spot	Malaysia	[71]
	Dragon fruit (pitaya)	*Selenicereus undatus* (Haw.) D.R.Hunt (syn. *Hylocereus undatus* (Haw.) Britton & Rose)	Reddish-brown spot	Philippines	[123]
			Reddish-brown spot	China	[124]
	Elephant grass	*Cenchrus purpureus* (Schumach.) Morrone	Leaf blight	China	[125]
	European nettle tree	*Celtis australis* L., 1753	Leaf spot	India	[126]
	False Daisy	*Eclipta prostrata* (L.) L.	Leaf spot	China	[127]
	Kinnow mandarin	hybrid: *Citrus nobilis* Lour. × *Citrus deliciosa* Ten.	Leaf spot	Pakistan	[128]
	Kiwifruit	*Actinidia deliciosa* (A. Chev.) C.F.Liang & A.R.Ferguson	Leaf spot	China	[129]
	Liqourice	*Glycyrrhiza glabra* L.	Leaf spot	India	[130]

Table 6. *Cont.*

SPECIES	HOST (Common Name)	TAXONOMY ID	DISEASE SYMPTOMS	DISTRIBUTION	REFERENCES
	Mango	*Mangifera indica* L.	Leaf spot	India	[131]
			Twig dieback and leaf spot	Egypt	[132]
	Moonlight cactus	*Selenicereus monacanthus* (hort. ex Lem.) D.R.Hunt (syn. *Hylocereus monacanthus* (hort. ex Lem.) Britton & Rose)	Reddish-brown spot	Philippines	[123]
	Mulberry	*Morus alba* Hort. ex Loudon L	Shot hole	China	[133]
				India	[134]
	Passion fruit	*Passiflora edulis* Sims	Leaf blight	China	[135]
	Peanut	*Arachis hypogaea* L.	Leaf blight	China	[136]
	Pitaya	*Selenicereus megalanthus* (K.Schum. ex Vaupel) Moran (syn. *Hylocereus megalanthus* (K.Schum. ex Vaupel) Ralf Bauer)	Reddish-brown spot	Philippines	[123]
	Purging nut	*Jatropha curcas* L.	Necrosis, chlorosis	India	[137]
	Qing qian liu	*Cyclocarya paliurus* (Batalin) Iljinsk.	Leaf blight	China	[138]
	Sesame	*Sesamum indicum* L.	Leaf blight	China	[139]
			Leaf blight	Pakistan	[140]
	Sugarcane	*Saccharum* spp.	Leaf blight	China	[141]
	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
	Three-leaf Akebia	*Akebia trifoliata* (Thunb.) Koidz.	Dried-shrink fruit	China	[142]
	Tea-oil plant	*Camellia oleifera* C. Abel	Leaf blight	China	[143]
	Watermelon (wild melon)	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Leaf spot	Malaysia	[144]
	White moho	*Heliocarpus americanus* L.	Leaf spot	Brazil	[145]
Nigrospora vesicularifera	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]
Nigrospora zimmermani	Sugarcane	*Saccharum officinarum* L.	Leaf spot	China	[30]

5. Conclusions

This study identified, described and characterized three fungal species that caused leaf spot symptoms on olive trees in Croatia. *Nigrospora* species can be economically significant as plant pathogens, causing crop loses in agriculture. Early detection can help prevent the spreading, so it is important to identify and manage leaf spot promptly to prevent it from causing damage to olive trees. Over time, repeated infections can weaken the olive tree. Weakened trees are more susceptible to other diseases and environmental stressors, which can lead to a decline in the tree's overall health and longevity. Also, fungi can spread to other parts of the tree and neighboring trees, which can lead to more widespread infections and increased management challenges for growers. Additionally, the cost of managing and treating leaf spot diseases can add to production expenses. In conclusion, leaf spot diseases on olive trees are important because they can negatively affect the tree's health, fruit quality, and overall productivity. Olive growers need to monitor for leaf spot diseases and implement effective management strategies to minimize their impact and ensure a healthy and productive orchard. It is also necessary to conduct further research

that will include monitoring these fungal diseases and studying the effectiveness of various substances or treatments in inhibiting the growth and reproduction of these fungi.

To our knowledge, this paper is the first report of *Nigrospora* species causing diseases on olives and the first report of *Nigrospora philosophiae-doctoris* causing plant disease.

Author Contributions: Conceptualization, E.P. and S.G.; methodology, E.P. and S.G.; investigation, E.P. and S.G., writing—original draft preparation, E.P.; writing—review and editing, S.G., K.V., J.Ć. and E.Đ. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Croatian Science Foundation Installation Research Project "Natural bioactive compounds as a source of potential antimicrobial agents in the control of bacterial and other fungal pathogens of olives", Anti-Mikrobi-OL (AMO), UIP-2020-02-7413, and "Young Researchers' Career Development Project" DOK-2021-02-2882.

Data Availability Statement: All sequence data are available in NCBI GenBank in accordance with the accession numbers in the manuscript.

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

References

1. FAO. Food and Agriculture Organization of the United Nations. Crop and Livestock Products. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 19 January 2023).
2. FAO. Food and Agriculture Organization of the United Nations. Land & Water. Available online: https://www.fao.org/land-water/databases-and-software/crop-information/olive/en/ (accessed on 19 January 2023).
3. Varanda, C.M.R.; Materatski, P.; Landum, M.; Campos, M.D.; Rosário Félix, M.D. Fungal communities associated with peacock and cercospora leaf spots in olive. *Plants* **2019**, *8*, 169. [CrossRef] [PubMed]
4. Figueres, G. *Repilos del Olivo: Ataque en Fruto*; Phytoma España: Valencia, Spain, 1991; pp. 31–36.
5. Civantes, M. *Olive Pest and Disease Management*; IOCC: Towson, MD, USA, 1999.
6. Benitez, Y.; Botella, M.A.; Trapero, A.; Alsalimiya, M.; Caballero, J.L.; Dorado, G.; Muñoz-Blanco, J. Molecular analysis of the interaction between *Olea europaea* and the biographic fungus *Spilocaea oleagina*. *Mol. Plant Pathol.* **2005**, *6*, 425–438. [CrossRef] [PubMed]
7. Obanor, F.; Jaspers, M.; Jones, E.E.; Walter, M. Greenhouse and field evaluation of fungicides for control of olive leaf spot in New Zealand. *Crop Prot.* **2008**, *27*, 1335–1342. [CrossRef]
8. Sanei, S.; Razav, S. Survey of *Spilocaea oleagina*, causal agent of olive leaf spot, in North of Iran. *J. Yeast Fungal Res.* **2011**, *2*, 33–38. [CrossRef]
9. Basim, E.; Basim, H.; Abdulai, M.; Baki, D.; Ozturk, N. Identification and characterization of *Alternaria alternata* causing leaf spot of olive tree (*Olea europaea*) in Turkey. *Crop Prot.* **2017**, *92*, 79–88. [CrossRef]
10. Sergeeva, V.; Spooner-Hart, R.; Nair, N.G. First report of *Colletotrichum acutatum* and *C. gloeosporioides* causing leaf spots of olives (*Olea europaea*) in Australia. *Australas. Plant Dis. Notes* **2008**, *3*, 143–144.
11. Trouillas, F.P.; Nouri, M.T.; Lawrence, D.P.; Moral, J.; Travadon, R.; Aegerter, B.J.; Lightle, D. Identification and Characterization of *Neofabraea kienholzii* and *Phlyctema vagabunda* Causing Leaf and Shoot Lesions of Olive in California. *Plant Dis.* **2019**, *103*, 3018–3030. [CrossRef]
12. Zimmerman, A. Ueber einige an tropischen Kulturpfianzen beobachtete Pilze III. *Zentralblatt Bakteriol. Parasitenkd.* **1992**, *8*, 216–221.
13. Barnett, H.L.; Hunter, B.B. *Illustrated Genera of Imperfect Fungi*, 4th ed.; APS Press: St. Paul, MN, USA, 1998.
14. Kirk, P.M.; Cannon, P.F.; Minter, D.W.; Stalpers, J.A. Dictionary of the Fungi. *Mycol. Res.* **2008**, *113*, 908–910.
15. Wang, M.; Liu, F.; Crous, P.W.; Cai, L. Phylogenetic reassessment of *Nigrospora*: Ubiquitous endophytes, plant and human pathogens. *Persoonia* **2017**, *39*, 118–142. [CrossRef]
16. Hyde, K.D.; Norphanphoun, C.; Maharachchikumbura, S.S.N.; Bhat, D.J.; Jones, E.B.G.; Bundhun, D.; Chen, Y.J.; Bao, D.F.; Boonmee, S.; Calabon, M.S.; et al. Refiend families of *Sordariomycetes*. *Mycosphere* **2020**, *11*, 305–1059. [CrossRef]
17. MycoBank Database. Available online: https://www.mycobank.org/Basic%20names%20search (accessed on 18 September 2023).
18. Fisher, P.J.; Petrini, O.; Petrini, L.E.; Descals, E. A preliminary study of fungi inhabiting xylem and whole stems of Olea europaea. *Sydowia* **1992**, *44*, 117–121.
19. Landum, M.C.; Félix, M.d.R.; Alho, J.; Garcia, R.; Cabrita, M.J.; Rei, F.; Varanda, C.M. Antagonistic activity of fungi of *Olea europaea* L. against *Colletotrichum acutatum*. *Microbiol. Res.* **2016**, *183*, 100–108. [CrossRef] [PubMed]
20. Su, Y.-Y.; Qi, Y.-L.; Cai, L. Induction of sporulation in plant pathogenic fungi. *Mycology* **2012**, *3*, 195–200.
21. Kindo, A.J.; Tupaki-Sreepurna, A.; Yuvaraj, M. Banana peel culture as an indigenous medium for easy identification of late-sporulation human fungal pathogens. *Indian J. Med. Microbiol.* **2016**, *34*, 457–461. [CrossRef] [PubMed]

22. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. 38—Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In *PCR—Protocols and Applications—A Laboratory Manual*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press, Inc.: Cambridge, MA, USA, 1990; pp. 315–322.
23. EPPO. European and Mediterranean Plant Protection Organization. PM 7/129 (1) DNA barcoding as an identification tool for a number of regulated pests. *Bull. OEPP* **2016**, *46*, 501–537. [CrossRef]
24. Woudenberg, J.H.C.; Aveskamp, M.M.; de Grutyer, J.; Spiers, A.G.; Crous, P.W. Multiple *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. *Persoonia* **2009**, *22*, 56–62. [CrossRef]
25. Carbone, I.; Kohn, L.M. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* **1995**, *91*, 553–556. [CrossRef]
26. Hao, Y.; Aluthmuhandiram, J.V.S.; Chethana, K.W.T.; Manawasinghe, I.S.; Li, X.; Liu, M.; Hyde, D.K.; Phillips, A.J.L.; Zhang, W. *Nigrospora* Species Associated with Various Hosts from Shandong Peninsula, China. *Mycobiology* **2020**, *48*, 169–183. [CrossRef]
27. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425.
28. Chen, Q.; Bakhshi, M.; Balci, Y.; Broders, K.D.; Cheewangkoon, R.; Chen, S.F.; Fan, X.L.; Gramaje, D.; Halleen, F.; Jung, M.H.; et al. Genera of phytopathogenic fungi: GOPHY 4. *Stud. Mycol.* **2022**, *101*, 417–564. [CrossRef] [PubMed]
29. Slippers, B.; Crous, P.W.; Denman, S.; Coutinho, T.A.; Wingfield, B.D.; Wingfield, M. Combined multiple gene genealogies and phenotypic characters differentiate several species previously identified as *Botryosphaeria dothidea*. *Mycologia* **2004**, *96*, 83–101. [CrossRef] [PubMed]
30. Raza, M.; Zhang, Z.-F.; Hyde, K.D.; Diao, Y.-Z.; Cai, L. Culturable plant pathogenic fungi associated with sugarcane in southern China. *Fungal Diver* **2019**, *99*, 1–104. [CrossRef]
31. Crous, P.W.; Carnegie, A.J.; Wingfield, M.J.; Sharma, R.; Mughini, G.; Noordeloos, M.E.; Santini, A.; Shouche, Y.S.; Bezerra, J.D.P.; Dima, B.; et al. Fungal Planet description sheets: 868–950. *Persoonia* **2019**, *42*, 291–473. [CrossRef] [PubMed]
32. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [CrossRef]
33. Tamura, K.; Nei, M.; Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11030–11035. [CrossRef] [PubMed]
34. Tamura, K.; Stecher, G.; Kumar, S. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Mol. Biol. Evol.* **2021**, *38*, 3022–3027. [CrossRef]
35. Rashmi, M.; Kushveerm, J.; Sarma, V. A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere* **2019**, *10*, 798–1079. [CrossRef]
36. Sun, X.D.; Cai, X.L.; Pang, Q.Q.; Zhou, M.; Zhang, W.; Chen, Y.S.; Bian, Q. First record of leaf spot disease on *Costus speciosus* caused by *Nigrospora oryzae* in Hainan, China. *Plant Dis.* **2021**, *105*, 506. [CrossRef]
37. Ngoko, Z.; Marasas, W.F.O.; Rheeder, J.P.; Shephard, G.S.; Wingfield, M.J.; Cardwell, K.F. Fungal infection and mycotoxin contamination of maize in the humid forest and the western highlands of Cameroon. *Phytoparasitica* **2001**, *29*, 352–360. [CrossRef]
38. Barkat, E.H.; Hardy, G.E.S.J.; Ren, Y.; Calver, M.; Bayliss, K.L. Fungal contaminants of stored wheat vary between Australian states. Australas. *Plant Pathol.* **2016**, *45*, 621–628.
39. Moreira, S.I.; Dutra, D.; Rodrigues, A.; de Oliveira, J.; Dhingra, O.; Pereira, O. Fungi and bacteria associated with post-harvest rot of ginger rhizomes in Espírito Santo, Brazil. *Trop. Plant Pathol.* **2013**, *38*, 218–226.
40. Kwon, Y.; Kim, M.; Kwack, Y.-B.; Kwak, Y.-S. First report of *Nigrospora* sp. causing kiwifruit postharvest black rot. *N. Z. J. Crop Hortic. Sci.* **2016**, *45*, 75–79. [CrossRef]
41. Li, L.; Pan, H.; Chen, M.Y.; Zhang, S.J.; Zhong, C.H. First report of *Nigrospora oryzae* causing brown/black spot disease of kiwifruit in China. *Plant Dis.* **2018**, *102*, 243. [CrossRef]
42. Shahzad, S.; Ghaffar, A. New records of soilborne root infecting fungi in Pakistan. *Pak. J. Bot.* **1995**, *27*, 209–216.
43. Lucca, A.J.D.; Mich, M.; Boue, S.; Cleveland, T.E.; Sien, T.; Walsh, T.J. Fungicidal activity of plant saponin CAY-i for fungi isolated from diseased vitis fruit and stems. *Am. J. Enol. Vitic.* **2008**, *59*, 67–72. [CrossRef]
44. Dahlberg, K.R.; Etten, J. Physiology and biochemistry of fungal sporulation. *Annu. Rev. Phytopathol.* **1982**, *20*, 281–301. [CrossRef]
45. Riddell, R.W. Permanent stained mycological preparations obtained by slide culture. *Mycologia* **1950**, *42*, 265–270. [CrossRef]
46. Masangkay, R.F.; Paulitz, T.C.; Hallett, S.G.; Watson, A.K. Characterization of sporulation of *Alternaria alternata* f. sp. *sphenocleae*. *Biocontrol Sci. Technol.* **2000**, *10*, 385–397. [CrossRef]
47. Wu, P.-C.; Tsai, J.-C.; Li, F.-C.; Lung, S.-C.; Su, H.-J. Increased levels of ambient fungal spore in Taiwan are associated with dust events from China. *Atmos. Environ.* **2004**, *38*, 4879–4886. [CrossRef]
48. Webster, J. Spore projection in hyphomycete *Nigrospora sphaerica*. *New Phytol.* **1952**, *51*, 229–235. [CrossRef]
49. Savulescu, T.; Rayss, T. Contribution a la connaissance de la hiologie de *Nigrospora oryzae* (B. et Br.) Petch Parasite du mats. *Rec. Trav. Cryptogam. dédiés à Louis Mangin. Muséum Nat. d'Hist. Nat. Paris* **1931**, 233–240.
50. Alfaro, A. El Ácara *Pediculopsis graminum* Reut. y el hongo *Nigrospora oryzae* (Berk, et Br.). Petch en asociación parasitaria sobre Tigos arogeneses. *Bol. Pathol. Veg. Entomol. Agric.* **1946**, *16*, 321.
51. Njokuocha, R.C.; Agwu, C.O.C.; Okezie, C.E.A. Effects of weather conditions on selected airborne fungal spores in the southern part of the state of Enugu, Nigeria. *Grana* **2017**, *56*, 263–272. [CrossRef]
52. Sarkar, S.; Girisham, S.; Reddym, S.M. Influence of temperature and relative humidity on the development of post-harvest rot of banana. *Proc. Natl. Acad. Sci. India Sect.* **2011**, *81*, 285–287.

53. Li, W.J.; Hu, M.; Xue, Y.; Li, Z.; Zhang, Y.; Zheng, D.; Lu, G.; Wang, J.; Zhou, J. Five fungal pathogens are responsible for bayberry twig blight and fungicides were screened for disease control. *Microorganisms* **2020**, *8*, 689. [CrossRef] [PubMed]
54. Rahman, A.U.; Ashraf, M.; Choudary, M.I.; Rehman, H.U.; Kazmi, M.H. Antifungal aryltetralin lignans from leaves of *Podophyllum-hexandrum*. *Phytochemistry* **1995**, *40*, 427–431. [CrossRef]
55. Reyes-Ramirez, A.; Escudero-Abarca, B.I.; Aguilar-Uscanga, G.; Hayward-Jones, P.M.; Barboza-Corona, J.E. Antifungal activity of *Bacillus thuringiensis* chitinase and its potential for the biocontrol of phytopathogenic fungi in soybean seeds. *J. Food Sci.* **2020**, *69*, M131–M134. [CrossRef]
56. Mohd-Baseri, N.; Anuar, M.S.K.; Shamsuhazli, N.A.S.; Zulkifli, M.A.F.; Wasoh, H.; Yusof, M.T. Antagonistic activity of wild growing mushrooms against various fungal rice pathogen. *Int. Microbiol.* **2023**, *26*, 91–98. [CrossRef]
57. Sornakili, A.; Thankappan, S.; Sridharan, A.P.; Nithya, P.; Uthandi, S. Antagonistic fungal endophytes and their metabolite-mediated interactions against phytopathogens in rice. *Physiol. Mol. Plant Pathol.* **2020**, *112*, 101525. [CrossRef]
58. Lu, L.; Karunarathna, S.C.; Hyde, K.D.; Suwannarach, N.; Elgorban, A.M.; Stephenson, S.L.; Al-Rejaie, S.; Jayawardena, R.S.; Tibpromma, S. Endophytic fungi associated with coffee leaves in China exhibited in vitro antagonism against fungal and bacterial pathogens. *J. Fungi* **2022**, *8*, 698. [CrossRef]
59. Ramesha, K.P.; Mohana, N.C.; Nuthan, B.R.; Rakshith, D.; Satish, S. Antimicrobial metabolite profiling of *Nigrospora sphaerica* from *Adiantum philippense* L. *J. Genet. Eng. Biotechnol.* **2020**, *18*, 66. [CrossRef] [PubMed]
60. Kim, J.-C.; Choi, G.J.; Park, J.-H.; Kim, H.T.; Cho, K.Y. Activity against plant pathogenic fungi of phomalactone isolated from *Nigrospora sphaerica*. *Pest Manag. Sci.* **2001**, *57*, 554–559. [CrossRef] [PubMed]
61. Ibrahim, D.; Lee, C.C.; Yenn, T.W.; Zakaria, L.; Sheh-Hong, L. Effect of the extract of endophytic fungus, *Nigrospora sphaerica* CL-OP 30, against the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumonia* cells. *Trop. J. Pharm. Res.* **2015**, *14*, 2091–2097. [CrossRef]
62. Luo, F.; Li, W.; Zhu, T.; Han, S.; Qiao, T.; Li, S. First report of *Nigrospora aurantiaca* causing leaf spot disease of *Castanea mollissima* in China. *Plant Dis.* **2020**, *104*, 2730. [CrossRef]
63. Khoo, Y.W.; Tan, H.T.; Khaw, Y.S.; Li, S.-F.; Chong, K.P. First report of *Nigrospora aurantiaca* causing leaf spot on *Pandanus amaryllifolius* in Malaysia. *J. Plant Pathol.* **2022**, *104*, 1205–1206. [CrossRef]
64. Huang, Y.; Li, Z.; Wang, H.-C.; Chen, Q.; Li, W.H. First report of leaf spot caused by *Nigrospora aurantiaca* in tobacco in China. *Plant Dis.* **2021**, *105*, 1569. [CrossRef]
65. Manawasinghe, I.S.; Jayawardena, R.S.; Li, H.L.; Zhou, Y.Y.; Zhang, W.; Phillips, A.J.L.; Wanasinghe, D.N.; Dissanayake, A.J.; Li, X.H.; Li, Y.H.; et al. Microfungi associated with *Camellia sinensis*: A case study of leaf and shoot necrosis on Tea in Fuijan, China. *Mycosphere* **2021**, *12*, 430–518. [CrossRef]
66. Qin, S.; Chen, X.; Zhou, X.; Zhao, J.; Baccelli, I.; Cernava, T. First report of *Camellia oleifera* leaf blight caused by *Nigrospora chiensis*. *J. Plant Pathol.* **2021**, *103*, 711–712. [CrossRef]
67. Zeng, Y.; Li, L.; Zhu, T.; Han, S.; Li, S. First report of brown leaf spot disease caused by *Nigrospora guiliensis* on *Phellodendron chinense* in China. *Plant Disease* **2020**, *104*, 2518. [CrossRef]
68. Conforto, C.; Lima, N.B.; Silva, F.J.A.; Câmara, M.P.S.; Macharachchikumbura, S.; Michereeff, S.J. Characterization of fungal species associated with cladode brown spot on *Nopalea cochenillifera* in Brazil. *Eur. J. Plant Pathol.* **2019**, *155*, 1179–1194. [CrossRef]
69. Zheng, T.; Zhao, L.; Huang, M.-G.; Deng, J.-X.; Wang, Y.-H. First report of leaf spot caused by *Nigrospora hainanensis* on *Oxalis corymbose*—China. *Plant Dis.* **2022**, *106*, 1986. [CrossRef]
70. Al-Nadabi, H.; Maharachchikumbura, S.S.N.; Al-Gahaffi, Z.S.; Al-Hasani, A.S.; Velazhahan, R.; Al-Sadi, A.M. Molecular identification of fungal pathogens associated with leaf spot disease of date palms (*Phoenix dactylifera*). *All Life* **2020**, *13*, 587–597. [CrossRef]
71. Kee, Y.J.; Hafifi, A.B.M.; Huda-Shakirah, A.R.; Wong, K.L.; Jin, X.L.; Nordahliawate, M.S.S.; Zakaria, L.; Mohd, M.H. First report of reddish brown spot disease of red-fleshed dragon fruit (*Hypocereus polyrhizus*) caused by *Nigrospora lacticolonia* and *Nigrospora sphaerica* in Malaysia. *Crop Prot.* **2019**, *122*, 165–170. [CrossRef]
72. Li, M.; Gao, Z.; Wang, Y.; Zhang, W.; Yang, J.; Gong, D.; Ma, Y.; Li, Y.; Hu, M. First report of *Nigrospora lacticolonia* causing leaf spot of *Bougainvillea spectabilis* in China. *Can. J. Plant Pathol.* **2022**, *44*, 695–701. [CrossRef]
73. Zhai, L.F.; Liu, J.; Zhang, M.X.; Hong, N.; Wang, G.P.; Wang, L.P. The first report of leaf spot in *Aloe vera* caused by *Nigrospora oryzae* in China. *Plant Dis.* **2013**, *97*, 1256. [CrossRef]
74. Alam, M.W.; Rehman, A.; Saira, M.; Khan, N.A.; Aslam, S.; Fiaz, M.; Muhammad, S. First report of leaf spots in *Aloe vera* caused by *Nigrospora oryzae* in Pakistan. *Plant Dis.* **2017**, *101*, 841. [CrossRef]
75. Begum, M.; Hamza, A.; Tanny, T.; Das, K.C.; Mahmud, M.T.; Salimullah, M.; Alam, I. First report of leaf spot disease in *Aloe vera* caused by *Nigrospora oryzae* in Bangladesh. *Plant Dis.* **2018**, *102*, 1461. [CrossRef]
76. Qiu, C.; Zhu, W.; Niu, T.; Liu, Z. *Nigrospora oryzae* causing leaf spot on asiatic dayflower on Chongqing, China. *Plant Dis.* **2022**, *106*, 763. [CrossRef]
77. Zhang, L.Q.; Jiang, S.; Meng, J.J.; An, H.S.; Zhang, X.Y. First report of leaf spot caused by *Nigrospora oryzae* on blueberry in Shangai, China. *Plant Dis.* **2019**, *103*, 2473. [CrossRef]
78. He, B.Y.; Cernava, T.; He, H.D.; Li, H.X.; Chen, X.Y.L.; Yang, H. First report of leaf spon on *Photinia serrulata* caused by *Nigrospora oryzae* in China. *Plant Dis.* **2019**, *103*, 2480. [CrossRef]

79. Zhang, L.X.; Li, S.S.; Tan, G.J.; Shen, J.T.; He, T. First report of *Nigrospora oryzae* causing leaf spot on cotton in China. *Plant Dis.* **2012**, *96*, 1379. [CrossRef] [PubMed]
80. Han, S.; Yu, S.T.; Zhu, T.; Li, S.; Qiao, T.; Liu, Y.; Lin, T.; Yang, C. *Nigrospora oryzae* causing black leaf spot disease of *Hibiscus mutabilis* in China. *Plant Dis.* **2021**, *105*, 2255. [CrossRef]
81. Wu, J.B.; Zhang, C.L.; Mao, P.P.; Qian, Y.S.; Wang, H.Z. First report of leaf spot caused by *Nigrospora oryzae* on *Dendrobium candidum* in China. *Plant Dis.* **2014**, *98*, 996. [CrossRef] [PubMed]
82. Yang, H.L.; Du, C.M.; Liang, L.Y.; Qin, Q.Y.; Wang, C.; Cui, D.X.; Wu, Q.G.; Zou, L. First report of *Davida involucrata* leaf blight caused by *Nigrospora oryzae* in Sichuan, China. *Plant Dis.* **2022**, *106*, 2520. [CrossRef] [PubMed]
83. Özer, G.; Paulitz, T.C.; Imren, M.; Alkan, M.; Muminjanov, H.; Dababat, A.A. Identity and pathogenicity of fungi associated with crown and root rot of dryland winter wheat in Azerbaijan. *Plant Dis.* **2020**, *104*, 2149–2157. [CrossRef]
84. Widmer, T.; Kirk, A.; Kirk, G.; Guermache, F. Foliar and cane rot of *Arundo donax* caused by *Nigrospora oryzae* in Europe. *Plant Dis.* **2006**, *90*, 1107. [CrossRef]
85. Liu, Z.; Zhou, S.; Qi, L.; Wang, X.; Song, J.; Li, D.; Chen, T.; Wang, Q. First report of *Nigrospora oryzae* causing leaf spot on ginger in China. *Plant Dis.* **2022**, *106*, 316. [CrossRef]
86. Zhang, Q.H.; Huang, L.L.; Liu, Y.J.; Ai, Y.; Pend, D.H. First report of leaf spot of lotus (*Nelumbo nucifera*) caused by *Nigrospora oryzae* in China. *Plant Dis.* **2018**, *102*, 1038. [CrossRef]
87. Sharma, P.; Meena, P.D.; Chauhan, J.S. First report of *Nigrospora oryzae* (Berk. & Broome) petch causing stem blight on *Brassica juncea* in India. *J. Plant Pathol.* **2013**, *161*, 439–441.
88. Zheng, L.; Shi, F.; Kelly, D.; Hsiang, T. First report of leaf spot of Kentucky bluegrass (*Poa pratensis*) caused by *Nigrospora oryzae* in Ontario. *Plant Dis.* **2012**, *96*, 909. [CrossRef] [PubMed]
89. Luo, M.-Y.; Jiang, Y.-L. First report of leaf spot on kidney bean caused by *Nigrospora oryzae* in China. *Plant Dis.* **2022**, *106*, 1064. [CrossRef] [PubMed]
90. Li, L.; Pan, H.; Liu, Y.F.; Li, D.W.; Zhang, Q.; Deng, L.; Chen, M.Y.; Zhong, C.H. First report of *Nigrospora sphaerica* causing kiwifruit postharvest rot disease in China. *Plant Dis.* **2018**, *102*, 1666. [CrossRef]
91. Borrelli, N.P.; Stancanelli, S.; Papone, M.L.; Moreno, M.V.; Stenglein, S.; Wright, E.R.; Hagiwara, J.C.; Rivera, M.C. Leaf spots on calibrachoa caused by *Nigrospora oryzae*. *Ornam. Hortic.* **2020**, *26*, 591–597. [CrossRef]
92. Kalati, T.H.; Jahani, M.; Zare, R.; Mirzaee, M. First report of Nigrospora leaf spot on *Pennisetum americanum* in Iran. *J. Plant Pathol.* **2014**, *96*, 606.
93. Farid, K.; Zafari, D.; Soleimani, M.J.; Bagherabadi, S. First report of *Nigrospora oryzae* causing brown leaf spot on *Mentha spicata*. *J. Plant Pathol.* **2020**, *102*, 1281. [CrossRef]
94. Zhang, H.; Kong, N.; Ji, S.; Liu, B.; Tian, Z.; Qi, J.; Liu, Z. First report of leaf blight caused by *Nigrospora oryzae* on Poplar in China. *Plant Dis.* **2022**, *106*, 1063. [CrossRef]
95. Shamsi, S.; Khan, A.; Shahjahan, A.K.M.; Miah, S.A. Fungal species associated with sheaths and grains of sheath rot affected rice varietes from Bangladesh. *Bangladesh J. Bot.* **2003**, *32*, 17–22.
96. Wang, D.; Li, Y.; Sun, G.; Ai, Y.F.; Wang, F.; Wang, X. First report of leaf spot disease caused by *Nigrospora oryzae* on *Nicotiana tabacum* in China. *Plant Dis.* **2022**, *106*, 2526. [CrossRef]
97. Chen, X.; Wang, N.; Yang, M.F.; Li, H.-X. First report of *Nigrospora* leaf spot caused by *Nigrospora oryzae* on watermelon in China. *Plant Dis.* **2019**, *103*, 1019. [CrossRef]
98. Eken, C.; Spanbayev, A.; Tulegenova, Z.; Yechshzhanov, T. First report of *Nigrospora oryzae* on wheat iz Kazahstan. *Plant Dis.* **2016**, *100*, 861. [CrossRef]
99. Bozoğlu, T.; Dervis, S.; Imren, M.; Amer, M.; Özdemir, F.; Paulitz, T.C.; Morgounov, A.; Dababat, A.A.; Özer, G. Fungal pathogens associated with crown and root rot of wheat in Central, Eastern, and Southeastern Kazahstan. *J. Fungi* **2022**, *8*, 417. [CrossRef] [PubMed]
100. Liu, Y.L.; Tang, J.R.; Li, Y.; Zhou, H.K. First report of leaf spot caused by *Nigrospora oryzae* in wild rice in China. *Plant Dis.* **2021**, *105*, 3293. [CrossRef] [PubMed]
101. Kido, L.R.; Uzabakirihno, J.D.; Chimwamourombe, P.M. Isolation and identification of pathogenic fungi associated with *Aloe zebrina* flower malformation—First report. *J. Pure Appl. Microbiol.* **2012**, *6*, 125–129.
102. Liu, J.; Yang, L.; Miao, P.; Wu, D.; Cai, G.; Li, X.; Lu, J. First report of leaf blight on *Ficus pinduara* caused by *Nigrospora osmanthi* in China. *Plant Dis.* **2019**, *103*, 2685. [CrossRef]
103. Ismail, S.I.; Norzaki, N.A.M.; Ya'acob, M.E.; Jamian, S. First report of *Nigrospora osmanthi* causing leaf blight on *Orthosiphon stamineus* in Malaysia. *Plant Dis.* **2022**, *106*, 770. [CrossRef] [PubMed]
104. Mei, S.S.; Wang, Z.Y.; Zhang, J.; Rong, W. First report of leaf blight on *Stenotaphrum secundatum* caused by *Nigrospora osmanthi* in China. *Plant Dis.* **2019**, *103*, 1783. [CrossRef]
105. Shen, Q.; Peng, X.; He, F.; Li, S.; Xiao, Z.; Wang, H.; Tang, X.; Zhou, M. First report of *Nigrospora osmanthi* causing leaf spot on *Tartary* buckwheat in China. *Plant Dis.* **2021**, *105*, 1227. [CrossRef]
106. Aktar, M.; Shamsi, S. Mycloflora associated with infected plant parts of *Tagetes erecta* L. and *Tagetes patula* L. *Bangladesh J. Bot.* **2021**, *50*, 131–140. [CrossRef]
107. Chen, X.-Y.-L.; Zhang, C.; Yang, H.; Yang, M.-F.; Cernava, T. First report of leaf spot on *Chenopodium album* caused by *Nigrospora pyriformis* in China. *Plant Dis.* **2020**, *104*, 1872. [CrossRef]

108. Barman, A.; Nath, A.; Thakur, D. Identification and characterization of fungi associated with blister blight lesion of tea (*Camellia sinensis* L. Kuntze) isolated from Meghalaya, India. *Microbiol. Res.* **2020**, *240*, 126561. [CrossRef] [PubMed]
109. Szilagyi-Zecchin, V.J.; Adamoski, D.; Rodrigues Gomes, R.; Hungria, M.; Ikeda, A.C.; Kava-Cordeiro, V.; Glienke, C.; Galli-Terasawa, L.V. Composition of endophytics fungal community associated with leaves of maize cultivated in south Brazilian field. *Acta Microbiol. Immunol. Hung.* **2016**, *63*, 449–466. [CrossRef] [PubMed]
110. Dutta, J.; Gupta, S.; Thakur, D.; Handique, P.J. First report of *Nigrospora* leaf blight on tea caused by *Nigrospora sphaerica* in India. *Plant Dis.* **2015**, *99*, 417. [CrossRef]
111. Liu, Y.J.; Tang, Q.; Fang, L. First report of *Nigrospora sphaerica* causing leaf blight on *Camellia sinensis* in China. *Plant Dis.* **2016**, *100*, 221. [CrossRef]
112. Wright, E.R.; Folgado, M.; Rivera, M.C.; Crelier, A.; Vasquez, P.; Lopez, S.E. *Nigrospora sphaerica* causing leaf spot and twig and shoot blight on blueberry: A new host of the pathogen. *Plant Dis.* **2008**, *92*, 171. [CrossRef]
113. Li, Y.G.; Huang, M.H.; Sun, L.P.; Ji, P. Occurence of leaf spot on calabash caused by *Nigrospora sphaerica* in Georgia. *Plant Dis.* **2016**, *100*, 1506.
114. Xu, Y.M.; Liu, Y.J. First report of *Nigrospora sphaerica* causing leaf bligh on *Cunninghamia lanceolata*—China. *Plant Dis.* **2017**, *101*, 389. [CrossRef]
115. Soylu, S.; Dervis, S.; Soylu, E.M. First report of *Nigrospora sphaerica* causing leaf spots on Chinese Wisteria: A new host of the pathogen. *Plant Dis.* **2011**, *95*, 219. [CrossRef]
116. Sun, X.D.; Cai, X.L.; Pang, Q.Q.; Zhou, M.; Zhang, W.; Chen, Y.S.; Bian, Q. First report of leaf blight on *Mentha canadensis* caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2020**, *104*, 3059. [CrossRef]
117. Deepika, Y.S.; Mahadevakumar, S.; Amruthesh, K.N.; Lakshmidevi, N. First report of *Nigrospora sphaerica* associated with leaf spot disease of Cowpea (*Vigna unguiculata*) from India. *Plant Dis.* **2020**, *105*, 506. [CrossRef]
118. Zhang, L.X.; Song, J.H.; Tan, G.J.; Li, S.S. First report of leaf blight caused by *Nigrospora sphaerica* on Curcuma in China. *Plant Dis.* **2011**, *95*, 1190. [CrossRef]
119. Abass, M.H.; Hameed, M.A.; Ahmed, A.N. First report of *Nigrospora sphaerica* (Sacc.) Mason as potential pathogen on date palm (*Phoenix dactylifera* L.). *Can. J. Plant Pathol.* **2012**, *35*, 75–80. [CrossRef]
120. Al-Sadi, A.M.; Al-Jabri, A.H.; Al-Mazroui, S.S.; Al-Mahmooli, I.H. Characterization and pathogenicity of fungi and oomycetes associated with root disease of date palm in Oman. *Crop Prot.* **2012**, *37*, 1–6. [CrossRef]
121. Alam, M.W.; Rehman, A.; Ahmad, S.; Sarwar, M.; Nawaz, A.; Khan, S.M.; Ali, S.; Aslam, S.; Mannan, A. First report of *Nigrospora sphaerica* causing leaf spot of date palm in Pakistan. *J. Plant Pathol.* **2020**, *102*, 223. [CrossRef]
122. Yasmin, Z.; Shamsi, S. Mycoflora associated with symptomatic leaves of *Rauvolfia serpentina* (L.) Benth. Ex. Kurz. in Bangladesh. *Bangladesh J. Plant Taxon.* **2020**, *27*, 129–136. [CrossRef]
123. Taguiam, J.D.; Evallo, E.; Bengoa, J.; Maghirang, R.; Balendres, M.A. Detection of *Nigrospora sphaerica* in the Philippines and the suscpetibility of three *Hylocereus* species to reddish-brown spot disease. *J. Prof. Assoc. Cactus Dev.* **2020**, *22*, 49–61. [CrossRef]
124. Liu, F.; Wu, J.B.; Zhan, R.L.; Ou, X.C. First report of reddish brown spot disease on pitaya caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2016**, *100*, 1792. [CrossRef]
125. Han, Y.Z.; Fan, Z.W.; Wu, C.F.; Li, M.Y.; Zhou, D.D. First report of *Nigrospora* leaf blight on elephant grass caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2019**, *103*, 2681. [CrossRef]
126. Gautam, A.K. First report of *Nigrospora sphaerica* causing leaf spot on *Celtis australis* from Himachal Pradesh, India. *Int. Lett. Nat. Sci.* **2015**, *40*, 16–18. [CrossRef]
127. Qiu, C.; Liu, C.; Niu, T.; Zhu, W.; Liu, Z. *Nigrospora sphaerica* causing leaf spot in a new host, *Eclipta prostrata* (False Daisy), in China. *J. Phytopathol.* **2022**, *170*, 242–246. [CrossRef]
128. Alam, M.W.; Rehman, A.; Gleason, M.L.; Riaz, K.; Saira, M.; Aslam, S.; Rosli, H.; Muhammad, S. First report of *Nigrospora sphaerica* causing leaf spot on Kinnow mandarin in Pakistan. *J. Plant Pathol.* **2017**, *99*, 295.
129. Chen, Y.; Yang, X.; Zhang, A.F.; Zhang, H.Y.; Gu, C.Y.; Hameed, U.; Qi, Y.J.; Xu, Y.L. First report of leaf spot caused by *Nigrospora sphaerica* on kiwifruit in China. *Plant Dis.* **2016**, *100*, 2326. [CrossRef]
130. Verma, O.P.; Gupta, R.B.L. A new host for *Nigrospora sphaerica* causing leaf spots on *Glycyrrhiza glabra*. *Plant Pathol.* **2008**, *57*, 782. [CrossRef]
131. Pandey, A.; Pandey, S.; Awasthi, A.K. A new host record of *Nigrospora sphaerica* on *Mangifera indica* from Jabalpur, India. *J. Mycol. Plant Pathol.* **2013**, *43*, 255–256.
132. Youssef, K.; Mosa, M.A.; Kamhawy, M.A. First report of *Nigrospora sphaerica* causing twig dieback and leaf spot of mango in Egypt. *J. Plant Pathol.* **2022**, *104*, 1571. [CrossRef]
133. Chen, J.; Xiang, T.T.; Liu, X.Y.; Wang, W.H.; Zhang, B.L.; Liu, J.; Zhou, W.; Wan, Y.J.; Chen, G.; Zhu, H.S. First report of *Nigrospora sphaerica* causing shot hole disease on mulberry in China. *Plant Dis.* **2018**, *102*, 245. [CrossRef]
134. Arunakumar, G.S.; Gnanesh, B.N.; Supriya, M.; Sivaprasad, V. First report of *Nigrospora sphaerica* causing shot hole disease on mulberry in India. *Plant Dis.* **2019**, *103*, 1783. [CrossRef]
135. Wang, Y.; Cernava, T.; Zhou, X.; Yang, L.; Baccelli, I.; Wang, J.; Gou, Y.; Sang, W.; Chen, X. First report of passion fruit leaf blight caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2022**, *106*, 323. [CrossRef]
136. Liu, X.; Yu, F.; Fu, D.; Yang, W. First report of leaf blight on peanut caused by *Nigrospora sphaerica* in China. *J. Plant Pathol.* **2020**, *102*, 1269. [CrossRef]

137. Hernández-Cubero, L.C.; Ampofo, P.; Montes, J.M.; Voegele, R.T. Identification of pathogenic fungi and preliminary screening for resistance in *Jatropha curcas* L. germplasm. *Eur. J. Plant Pathol.* **2017**, *149*, 325–336. [CrossRef]
138. Zheng, X.; Liu, C.; Zhang, M.; Shang, X.; Fang, S.; Chen, F. First report of leaf blight of *Cyclocarya paliurus* caused by *Nigrospora sphaerica* in China. *Crop Prot.* **2021**, *140*, 105453. [CrossRef]
139. Zhao, H.; Liu, H.Y.; Yang, X.S.; Liu, Y.X.; Ni, Y.X.; Wang, F.; Tang, L. First report of *Nigrospora* leaf blight on sesame caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2014**, *98*, 842. [CrossRef]
140. Rehman, A.; Alam, M.W.; Saira, M.; Naz, S.; Mushtaq, R.; Chohan, T.A.; Din, S.U.; Noureen, A.; Gilani, K.; Hussain, D. *Nigrospora sphaerica* causing leaf blight disease on sesame in Palkistan. *Plant Dis.* **2022**, *106*, 317. [CrossRef]
141. Cui, Y.P.; Wu, B.; Peng, A.T.; Li, Z.L.; Lin, J.F.; Song, X.B. First report of *Nigrospora* leaf blight on sugarcane caused by *Nigrospora sphaerica* in China. *Plant Dis.* **2018**, *102*, 824. [CrossRef]
142. Hong, X.; Chen, S.; Wang, L.; Liu, B.; Yang, Y.; Tang, X.; Liu, Y.S.; Huang, S. First report of *Nigrospora sphaerica* causing fruit dried-shrink disease in *Akebia trifolitata* from China. *Plant Dis.* **2021**, *105*, 2244. [CrossRef]
143. Liu, Y.J.; Hu, F.; Chen, L.S.; Xu, S.W. First report of *Nigrospora sphaerica* causing leaf blight on oil tea (*Camellia oleifera*) in China. *Plant Dis.* **2020**, *104*, 3252. [CrossRef]
144. Ismail, S.I.; Razak, N.F.A. First report of *Nigrospora sphaerica* causing leaf spot on watermelon (*Citrullus lanatus* L.) in Malaysia. *Plant Dis.* **2020**, *105*, 488. [CrossRef]
145. Bernardi, C.; Busso, C.; Borin, R.C.; Mazaro, S.M.; Saburo, R.S.S. The first report of *Nigrospora sphaerica* associated with *Helicarpus americanus* seeds in Brazil. *Floresta Ambiente* **2021**, *28*, e20190103. [CrossRef]