Biologia (2023) 78:361–371
https://doi.org/10.1007/s11756-022-01218-0

ORIGINAL ARTICLE

Endophytic fungi from the common walnut and their in vitro antagonistic activity against *Ophiognomonia leptostyla*

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Received: 6 April 2022 / Accepted: 13 September 2022 / Published online: 28 October 2022
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Abstract

The common (or English) walnut (*Juglans regia* L.) is an economically important hardwood tree species cultivated worldwide for its edible nuts and high-quality wood. However, walnut trees are attacked by many pathogens causing economic damage. Among these pathogens, the most important fungal disease agent of walnut is *Ophiognomonia leptostyla* (Fr.) Sogonov (formerly *Gnomonia leptostyla* (Fr.) Ces. et de Not), which causes walnut anthracnose. In this study, endophytic fungi from various walnuts tissues (leaves, roots, twigs, shoots, fruits, and petioles) were isolated and molecularly identified by ITS gene sequencing. Additionally, the isolated fungi were tested for their in vitro antagonistic potential against *O. leptostyla*. A total of 35 endophytic fungi were isolated and species richness of endophytic fungi in roots was found to be higher than in aboveground organs. The most frequently isolated species was *Fusarium* sp. Seven isolates (*Alternaria* sp. CC-3, *A. chlamydosporigena* CC-8 and CC-9, *Fusarium acuminatum* CC-11, unidentified CC-16, *Neonectria* sp. CC-22 and unidentified CC-44) showed the antagonistic effects on *O. leptostyla* at different rates and the highest inhibition rate was obtained from *Alternaria* sp. CC-3 with 52.5%. Also, the presence of polyketide synthase I-II and non-ribosomal peptide synthase genes (possible antifungal-related genes) was investigated by PCR. From seven isolates showing antagonistic activity, PKS-I gene was detected in 3 isolates (*Alternaria* sp. CC-3, *A. chlamydosporigena* CC-8 and CC-9), PKS-II in one isolate (*F. acuminatum* CC-11), NRPS in two isolates (unidentified CC-16 and *Neonectria* sp. CC-22). None of three genes was detected in one isolate (unidentified CC-44). This is the first study to determine the antagonistic activity of the endophytic fungi isolated from walnuts against *O. leptostyla*. It is thought that the results will be beneficial in the future biological control programs for walnut anthracnose disease.

Keywords *Alternaria chlamydosporigena* · Anthracnose · Antifungal genes · Biological control · Endophyte · *Juglans regia*

Abbreviations

ANOVA	Analysis of Variance
BLAST	The Basic Local Alignment Search Tool
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphates
GPS	Global Positioning System
ITS	Internal transcribed spacer
LSD	Fisher's least significant difference
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	The National Center for Biotechnology Information
NJ	Neighbor joining
NRPS	Non-ribosomal peptide synthase
PCR	Polymerase chain reaction
PDAY	Potato dextrose agar + 1% yeast extract
PKS	Polyketide synthase
PKS-I	Polyketide synthase I
PKS-II	Polyketide synthase II
rRNA	Ribosomal ribonucleic acid
USA	The United States of America

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Introduction

According to taxonomic studies, the genus *Juglans* of family the *Juglandaceae* has a total of around 20 recognized species. The members of this genus are mostly distributed in the temperate and subtropical regions of the Northern Hemisphere (Gray 2013; Wani et al. 2016). Among the walnut trees, the common walnut (*Juglans regia* L.), also called the English or the Persian walnut, is widely cultivated and one of the most important horticultural crops grown worldwide (Akça et al. 2015). The common walnut is a type of tree providing multifaceted benefits to human being with mainly its high-quality timber and edible nuts (McGranahan and Leslie 1991; Pollegioni et al. 2017). Also, walnuts are a rich source of tocopherol, potassium, and polyunsaturated fatty acids. This rich nutrient profile is important for human nutrition and contributes to the many health benefits (Segelke et al. 2020). It is thought that the common walnut first originated in ancient Persia and was later brought to Greece and from here it was spread throughout the Roman Empire (Gray 2013). Walnut is an important agricultural product for Turkey and Turkey is in the fourth place in the world walnut production after China, Iran, and USA (Anwar et al. 2020).

One of the most important negative factors affecting walnut production in the world is various diseases causing damage to walnut trees and fruits. Among walnut diseases, walnut anthracnose, also called leaf blotch, is considered the most important fungal foliar disease of walnuts globally. The disease attacks mainly the black walnut (*J. nigra*), the common walnut (*J. regia*) and other species of the *Juglans* genus (Mudasir and Khurshid 2017). It is caused by the ascomycetous fungus *Ophiognomonia leptostyla* (formerly *Gnomonia leptostyla* and anamorph *Marssonina juglandis* (Lib.) Magn.) and it was first reported in Europe in 1815 (Woeste and Beineke 2001; Belisario 2002; Walker et al. 2012). The disease can be seen more severely in wet and rainy weather, and usually causes damage to the leaves, twigs, fruits, and rarely shoots (Medic et al. 2021). Walnut production was reported to decreased up to 50% due to this disease (Yang et al. 2021). Although cultural control methods are generally recommended in the control of walnut anthracnose, chemical control is required in places where the disease is severe every year (T.C. Ministry of Agriculture and Forestry, 2017). However, due to the negative effects of chemicals used in agriculture and forestry on human and environmental health, the research of novel environmental-friendly methods and biological control agents has become a desirable topic.

Endophytes are widely found in plants and form an important group of plant symbiosis. It has been shown by many studies that fungal endophytes can form a beneficial relationship with plants and have a wide variety of functions in plants (Aamir et al. 2020). In addition, endophytic fungi protect plants against especially pathogenic microorganisms through various mechanisms such as competition, antibiosis, mycoparasitism and induced resistance. This knowledge reveals the potential for these microorganisms to be used as biological control agents against plant pathogens (Latz et al. 2018). Although endophytic fungi are usually found in aboveground plant organs (leaves, stems, bark, petioles, and reproductive organs) and tissues, some endophytes can also be found in root tissues and the number of their hosts is quite large. Especially, woody plants are known to contain many endophytes (Faeth and Fagan 2002; Ivanova et al. 2017; Nishad et al. 2021; Zhou et al. 2021). By now, fungal endophytes belonging to many plants have been isolated and characterized, and most of these plants are agriculturally important plant species (Gimenez et al. 2007; Vega et al. 2018; Shadmani et al. 2021). In the literature, there are also some studies on the isolation and characterization of endophytic fungi from walnuts. For example, Xiaoyue et al. (2020) studied on the isolation of endophytic fungi from different tissues of walnuts, and they determined that the most frequently isolated species was *Alternaria* sp. Also, Pardatscher and Schweigkofler (2009) isolated and identified many fungal endophytes from walnuts and found a high species diversity. In addition, Rang et al. (2019) studied endophytic fungi on Yili wild walnuts and determined the promising antibacterial and antioxidant activity from some fungi. However, to our knowledge, there is no study showing antagonistic activity of fungal endophytes from walnuts against plant pathogenic fungi (especially *O. leptostyla*).

The species diversity and richness of fungal endophytes might differ according to the host plant and even in the same plant species due to ecological and environmental differences. Therefore, in this study, we aimed to isolate and characterize endophytic fungal species from different tissues of the common walnut in Turkey. The isolated fungi were identified by ITS gene sequencing and their in vitro antagonistic activities against *O. leptostyla* were determined. The antagonistic activity of fungal endophytes was also correlated by the presence of possible antifungal genes. The obtained results should be beneficial for future biological control programs of walnut anthracnose.

Materials and methods

Collection of samples

Different organs (leaves, roots, twigs, shoots, fruits, and petioles) of the common walnut trees were collected from the vicinity of Kırşehir, Turkey between May and July in 2020.

Table 1 PCR conditions and primers used in this study and their references

Primers	Sequence (5'◊3')	Trial	PCR conditions	Reference
ITS5 ITS4	5'- GGAAGTAAAAGTCGTAACAAGG-3' 5'-TCCCGCTTATTGATATCG- 3'	Fungal identification	94 °C for 5 min; 95 °C for 1 min, 55 °C for 45 s, 72 °C for 1 min for 35 cycles and 72 °C for 10 min for 1 cycle	White et al. (1990)
K1F M6R	5'-TSAAGTCSAACATCGGBCA-3' 5'-CGCAGGTTSCSGTACCAGTA-3'	PKS-I gene	94 °C for 5 min; 95 °C for 1 min, 57 °C for 45 s, 72 °C for 1 min for 35 cycles and 72 °C for 10 min for 1 cycle	Ayuso-Sacido and Genilloud (2005)
KSα KSβ	5'-TSGCSTGCTTGGAYGCSATC-3' 5'-TGGAANCCG CCGAABCCTCT-3'	PKS-II gene	94 °C for 5 min; 95 °C for 1 min, 57 °C for 45 s, 72 °C for 1 min for 35 cycles and 72 °C for 10 min for 1 cycle	Metsa-Ketela et al. (1999)
A3F A7R	5'-GCSTACSYSATSTACACSTCSGG-3' 5'-SASGTCVCCSGTSCGGTAS-3'	NRPS gene	94 °C for 5 min; 95 °C for 1 min, 58 °C for 45 s, 72 °C for 1 min for 35 cycles and 72 °C for 10 min for 1 cycle	Ayuso-Sacido and Genilloud (2005)

In total, 10 mono-cultured walnut plantations and 2 trees for each were randomly selected and sampled. GPS coordinates of the sampling points are 39°27'47.6"N 33°45'45.6"E (Darıözü), 39°24'22.9" N 33°39'52.3"E (Ömerkahya), 39°19'36.7"N 33°43'20.5"E (Savcılı), 39°02'50.7"N 34°26'50.9"E (Kurugöl), 39°26'11.0"N 34°06'47.1"E (Tatarilyasyayla), 39°30'59.6"N 34°10'12.5"E (Dulkadirli), 39°20'13.5"N 33°43'15.5"E (Kaman), 39°22'37.2"N 33°42'49.9"E (Kırşehir, city center), 39°23'17.1"N 33°41'59.1"E (Kırşehir, city center), 39°05'58.9"N 34°12'43.5"E (Kırşehir, city center). In selection of trees, completely healthy trees without any disease symptom were selected. All sampled trees were 15–25 years old mature trees. During the collection of leaves, twig, fruit and petiole, samples were taken from the lower crown of trees. Root samples adjacent to the rootstock were collected using pruning shears and a chisel. All materials were cleaned with 50% bleach between uses to prevent possible contamination. Collected samples were put into a plastic bag and brought to the laboratory for endophytic fungal isolation.

Endophytic fungal isolation

The samples were first surface sterilized in tap water, followed by sterile deionized water, 2% sodium hypochlorite for 3 min and 70% ethanol for 5 min. Finally, they were washed with sterile deionized water and left to dry in laminar cabinet (Arnold et al. 2001). After removing dead tissues, the healthy tissues were cut with a sterile surgical knife with a size of 1 cm^2 and they were placed on PDAY (potato dextrose agar + 1% yeast extract) (Merck, Darmstadt, Germany) containing 50 µg/mL tetracycline and 75 µg/mL ampicillin to prevent bacterial growth. All petri dishes were incubated at 25–28 °C in the dark for 20 days (Allegrucci et al. 2018). A different PDAY was used for each sample. Petri dishes were monitored daily, and growing fungal colonies were transferred to another antibiotic-free PDAY. To prove the accuracy of the surface sterilization, 100 µL from the last water sample used in washing was plated on PDAY and incubated in the dark at 25–28 °C for 20 days. Non-growing specimens were considered successful (Gurulingappa et al. 2010). Purified fungi were stocked in 15% glycerol for use in subsequent studies.

Molecular identification

Fungal isolates were molecularly identified by ITS gene sequencing. Genomic DNA extraction was performed with the E.Z.N.A. Soil DNA kit (OMEGA-BIO-TEK) according to the manufacturer's recommendations. Isolated DNAs were preserved at – 20 °C until use.

PCR conditions and primers for amplifying ITS gene region are given in Table 1. 5 µL from each PCR product was electrophoresed for 45 min at 90 V on 1% agarose gel containing 0.5 µg/mL ethidium bromide. The remaining PCR products were sent to MACROGEN (The Netherlands) for sequencing. The resulting DNA sequences were compared with the most related fungal species or isolates at NCBI GenBank to perform species identification (Altschul et al. 1990; Benson et al. 2012).

Antagonistic activity test

O. leptostyla was isolated from symptomatic walnut leaves according to the study of Jamshidi et al. (2012) and identified based on ITS sequence as described in the previous section. The symptomatic leaves were collected from Kırşehir (39°10'49.2"N 34°09'08.9"E) in the summer of 2020. The antagonistic activities were determined according to the direct opposition method described by Dennis and Webster (1971). 5 mm diameter mycelial disc of *O. leptostyla* actively growing on PDA + 7 gr/L oatmeal was cut and placed 1 cm from the edge of the fresh PDA + 7 gr/L oatmeal plate (9 cm). Likewise, the same diameter mycelial discs of the endophytic fungi were cut and placed 1 cm from the opposite edge of the plate. Different PDA + 7 gr/L oatmeal

was used for each fungal endophyte. All petri dishes were incubated at 25 °C for 20 days in the dark. The control group contained only *O. leptostyla*. To calculate the percentage of inhibition, the radial growth of fungi in the control group and the inhibition tests were measured by a caliper at the 20th day of incubation. The inhibition percentages were corrected using the following formula indicated in the studies of Royse and Ries (1977) and Landum et al. (2016). Antagonistic activity tests were repeated three times.

$$I\,(\text{Inhibitionpercentage}) = \left(\frac{\text{R1}\,(\text{colonyradiusincontrol}) - \text{R2}\,(\text{colonyradiusintest})}{\text{R1}}\right) \times 100$$

The inhibition rate was assessed using a scale from 1 to 4, in which 1 = 0–24% (low inhibition), 2 = 25–49% (middle-low inhibition), 3 = 50–74% (medium inhibition), 4 = 75–100% (high inhibition).

Determination of antifungal-activity-related genes

The presence of PKS (polyketide synthase I and II) and NRPS (nonribosomal peptide synthase) genes in the isolates showing antagonistic activity was investigated to indicate the relationship of isolates with antifungal activity (Kampapongsa and Kaewkla 2016; Zhao et al. 2022). The PCR conditions, primers and their references used in the study are given in Table 1. PCR products were analyzed as described above.

Data analysis

All DNA sequences were edited with BioEdit 7.09 software, and they were blasted at NCBI GenBank to determine their similarities with the most related fungal species or isolates (Altschul et al. 1990; Hall 1999; Benson et al. 2012). The percentage data from the antagonistic tests was analyzed using SPSS 16.0 statistical software. The difference among the fungal isolates with respect to percentage inhibition was determined by One-way Analysis of Variance (ANOVA) followed by LSD multiple comparison test. All data was tested using Levene statistics with respect to variance homogeneity.

Results

In total, 35 endophytic fungi were isolated from various tissues of the common walnut. Of these, 26 were isolated from the root, 5 from twigs, 2 from leaves, 1 from the petiole and 1 from the fruit. Based on ITS gene sequencing, 16 species belonging to 11 genera were identified. Nine isolates (CC-6, CC-10, CC-13, CC-16, CC-19, CC-27, CC-38, CC-39 and, CC-44) couldn't be identified. The most frequently isolated species was *Fusarium* sp. (9). The details about the isolated fungi were given in Table 2.

Among the endophytic fungi, seven isolates (CC-3, CC-8, CC-9, CC-11, CC-16, CC-22, and CC-44) from three genera (*Alternaria*, *Fusarium* and *Neonectria*) and two unidentified genera showed the in vitro antagonistic activity against *O. leptostyla*. There was a significant difference amongst isolates with respect to percent inhibition against *O. leptostyla* ($df = 6, 14$, $F = 128.95$, $p < 0.001$). The highest inhibition rate was obtained from *Alternaria* sp. CC-3 with 52.5% inhibition rate ($df = 6, 14$, $F = 128.95$, $p < 0.001$). The inhibition rates for the other isolates were ranged from 8.96 to 36.33% (Fig. 1).

Within in the isolates showing the antagonistic activity, PKS-I gene was detected in CC-3, CC-8, and CC-9, PKS-II in CC-11 and NRPS gene in CC-16 and CC-22 (Fig. 1).

Discussion

We isolated and identified 35 fungal endophytes from different tissues of the common walnut and determined their in vitro antagonistic activities against *O. leptostyla* which is the most important fungal pathogen of walnut. Species diversity was relatively high and some of fungal isolates examined had some degree of the antagonistic activity for *O. leptostyla* with considerable variability. Three antibiosis related genes (PKS-I, II and NRPS) were detected in some of antagonistically active isolates.

In this study, the overall endophytic species diversity in different tissues of walnut was relatively high and 16 species belonging to 11 genera were identified. The most frequently isolated genus was *Fusarium* with 9 isolates. Xiaoyue et al. (2020) isolated a total of 64 endophytic fungal isolates from different organs and tissues of walnuts such as roots, leaves, fruits, and shoots in China and observed that *Alternaria* sp. was the most frequent species. In a study conducted in Italy, Pardatscher and Schweigkofler (2009) isolated endophytic fungi from different tissues of walnuts and showed that the most common genera were *Alternaria, Botryosphaeria, Cladosporium, Epicoccum, Fusarium, Penicillium, Phoma* and *Phyllosticta*. Rang et al. (2019) also isolated a total of 49 endophytic fungal isolates from Yili wild walnuts and found that *F. tricinctum* YHT-4 showed strong antibacterial and antioxidant activity. It is seen that the species diversity obtained from our study differs moderately from these studies, which are also different among themselves. This might be because some endophytes are specific to the host, and some even colonize only in certain plant tissues (Boyle et al. 2001; Zhou and Hyde 2001). In addition, it is known that geographical and environmental factors have an impact on endophyte communities and fungal endophytes isolated

Table 2 Percentage similarities of the endophytic fungi with their the most closely related fungal species based on the Blast search in NCBI GenBank (Altschul et al. 1990; Benson et al. 2012) using ITS gene sequences with their GenBank accession numbers and the isolation source

Isolate	The most related species	Percent identity (%)	Query coverage (%)	GenBank accession number	GenBank accession number for ITS	Source	Suggested identification
CC-1	*Ulocladium* sp. MAB-2010a	99.82	100	HQ829119	OM903048	Twig	*Alternaria* sp.
	Alternaria sp. CMED5rs1aP4	99.82	99	MT444989			
	Alternaria multiformis GBC-Fungus	99.82	99	MN077466			
	Alternaria sp. D21	99.82	99	MH029120			
CC-2	*Aspergillus flavus* IFM 42,127	99.83	100	LC602023	OM903049	Twig	*Aspergillus flavus*
	Aspergillus flavus Af-1	99.66	100	MH127459			
	Aspergillus flavus IFM 42,150	99.66	100	LC602026			
	Aspergillus flavus IFM 42,130	99.66	100	LC602025			
CC-3	*Embellisia astragali* WH2-*1*	99.49	100	KX213847	OM903050	Root	*Alternaria sp.*
	Alternaria sp. C6_169-E9_612	99.49	99	MW729200			
	Alternaria chlamydosporigena 17MQ-2-6	99.32	100	MH384943			
	Alternaria chlamydosporigena CBS 125,833	98.99	99	MH863800			
CC-4	*Fusarium oxysporum* JJF2	99.81	100	MN626452	OM903051	Root	*Fusarium oxysporum*
	Fusarium oxysporum JJF1	99.81	100	MN626451			
	Fusarium oxysporum KEMS_4a	99.81	100	MK922065			
	Fusarium oxysporum LD200518	99.81	100	MW073409			
CC-5	*Fusarium equiseti* YT2	99.26	100	KX576658	OM903052	Root	*Fusarium equiseti*
	Fusarium equiseti UgF11	99.81	98	MW486520			
	Fusarium equiseti UgC09	99.81	98	MW486514			
	Fusarium equiseti CC1-3	99.81	99	MT428184			
CC-6	*Fungal sp.* NLEndoHerit 017_2008N7-06-3 J	99.47	100	JX978246	OM903053	Leaf	Unidentified
	Uncultured fungus clone 4_52 18 S	99.81	95	KC884299			
	Fungal sp. 44	98.91	97	MN534799			
	Tricharina sp. SAA16	100	88	MF398839			
CC-8	*Alternaria chlamydosporigena* 17MQ-2-6	99.49	100	MH384943	OM903054	Root	*Alternaria chlamydosporigena*
	Alternaria chlamydosporigena CBS 125,833	99.49	99	MH863800			
	Alternaria chlamydosporigena CBS 125,829	99.49	99	MH863797			
	Alternaria chlamydosporigena CK1261+	99.49	99	MH473921			
CC-9	*Alternaria chlamydosporigena* 17MQ-2-6	99.32	100	MH384943	OM903055	Root	*Alternaria chlamydosporigena*
	Alternaria chlamydosporigena CBS 125,833	99.49	99	MH863800			
	Alternaria chlamydosporigena CBS 125,829	99.49	99	MH863797			
	Alternaria chlamydosporigena CK1261+	99.49	99	MH473921			
CC-10	*Fusarium acuminatum* GC-1	99.62	100	MK583543	OM903056	Root	Unidentified
	Dactylonectria torresensis CUZF132Trs	99.81	99	MN294554			
	Hypocreales F249 JA-2017	100	99	LT821507			
	Dactylonectria novozelandica 418	99.81	99	MN817697			
CC-11	*Fusarium* sp. Y2	99.64	100	MH383177	OM903057	Root	*Fusarium acuminatum*
	Fusarium acuminatum N-51-1	99.64	99	MT566456			
	Fusarium acuminatum N-43-1	99.64	99	MT560377			
	Fusarium acuminatum KRA_6	99.64	99	MT514382			
CC-12	*Paraphoma chrysanthemicola* 8924	99.80	100	MK647980	OM903058	Root	*Paraphoma chrysanthemicola*
	Uncultured fungus clone 4248_210	99.80	100	MT236451			
	Paraphoma chrysanthemicola IHBF 2210	99.80	100	MF326621			
	Paraphoma sp. P1878	99.80	100	KT269147			
CC-13	*Diaporthe columnaris*	99.29	100	MN540315	OM903059	Root	Unidentified
	Fungal sp. MG206Sc2R1x	99.12	100	KF752695			
	Phomopsis columnaris PA544RZ	99.11	99	KM519653			
	Phomopsis sp. Phom1	99.28	98	MN450640			
CC-14	Fungal sp. NLEndoHerit_022_2008N2-33-3G	99.81	100	JX978251	OM903060	Twig	*Microsphaeropsis olivacea*
	Microsphaeropsis olivacea D4/2c	99.62	100	MG020349			
	Microsphaeropsis olivacea D4/2b	99.62	100	MG020348			
	Microsphaeropsis olivacea D4/3b	99.62	100	MG020342			
CC-15	*Fusarium* sp. NRS-9	99.82	100	MW067648	OM903061	Root	*Fusarium* sp.
	Uncultured Fusarium clone D1579ITS	99.82	100	MK407351			
	Uncultured Fusarium clone D1578ITS	99.82	100	MK407350			
	Uncultured Fusarium clone D1576ITS	99.82	100	MK407348			

Table 2 (continued)

Isolate	The most related species	Percent identity (%)	Query coverage (%)	GenBank accession number	GenBank accession number for ITS	Source	Suggested identification
CC-16	*Fusarium acuminatum* GC-1	99.44	100	MK583543	OM903062	Root	Unidentified
	Ilyonectria sp. C9. endophyte	99.44	99	MK990631			
	Ascomycota sp. X47	99.62	98	FJ999637			
	Dactylonectria torresensis CUZF132Trs	99.44	99	MN294554			
CC-17	*Fusarium solani* CBS 140,079	99.82	100	NR_163531	OM903063	Root	*Fusarium* sp.
	Fusarium sp. FSSC_5bb GJS 09-1470	99.82	100	KT313637			
	Fusarium sp. FSSC_5q GJS 09-1468	99.82	100	KT313635			
	Fusarium sp. FSSC_5pp GJS 09-1466	99.82	100	KT313633			
CC-19	*Diaporthe columnaris*	99.46	100	MN540315	OM903064	Root	Unidentified
	Fungal sp. MG206Sc2R1x	99.29	100	KF752695			
	Phomopsis columnaris PA544RZ	99.11	100	KM519653			
	Phomopsis sp. Phom1	99.4	98	MN450640			
CC-21	*Fusarium oxysporum* JJF2	99.63	100	MN626452	OM903065	Root	*Fusarium oxysporum*
	Fusarium oxysporum JJF1	99.63	100	MN626451			
	Fusarium oxysporum KEMS_4a	99.63	100	MK922065			
	Fusarium oxysporum LD200518	99.63	100	MW073409			
CC-22	*Neonectria* sp. JZB3210004	99.44	100	MN988722	OM903066	Root	*Neonectria* sp.
	Neonectria sp. BV-2682	99.44	100	MK602792			
	Nectriaceae sp. B55	99.44	100	MF615035			
	Uncultured *Neonectria* clone D2170ITS	99.44	100	MK407939			
CC-23	*Penicillium philippinense* CBS 623.72	98.79	100	MH860600	OM903067	Root	*Penicillium* sp.
	Penicillium chalabudae CBS 219.66	98.79	100	NR_144845			
	Penicillium sp. M13003	98.79	100	KU365879			
	Penicillium chalabudae CBS 219.66	98.79	100	KP016811			
CC-24	*Fusarium oxysporum* JJF2	99.81	100	MN626452	OM903068	Root	*Fusarium oxysporum*
	Fusarium oxysporum JJF1	99.81	100	MN626451			
	Fusarium oxysporum KEMS_4a	99.81	100	MK922065			
	Fusarium oxysporum LD200518	99.81	100	MW073409			
CC-25	Uncultured fungus clone RFLP25	96.92	100	FJ528699	OM903069	Leaf	*Myriodontium keratinophilum*
	Myriodontium keratinophilum CBS 256.81	96.89	99	MH861337			
	Myriodontium keratinophilum CBS 947.73	99.43	90	NR157454			
	Myriodontium keratinophilum S4-P-2-4	96.45	96	KP216891			
CC-26	*Tritirachium* sp. IAM 14,522	100	100	AB109761	OM903070	Fruit	*Tritirachium* sp.
	[*Tritirachium*] sp. (in: Ascomycota) MEFC052	100	99	MK732104			
	[*Tritirachium*] sp. (in: Ascomycota) MEFC055	99.83	100	MK732106			
	Engyodontium sp. FP-027-B9	99.83	100	MH102090			
CC-27	*Fusarium acuminatum* GC-1	99.63	100	MK583543	OM903071	Root	Unidentified
	Ilyonectria sp. C9. endophyte	99.44	99	MK990631			
	Neonectria radicicola Cyl17	99.62	99	CQ131875			
	Ascomycota sp. X47	99.81	98	FJ999637			
CC-28	*Fusarium oxysporum* f. sp. *dianthi* 10-ITS4-H06.ab1	99.63	99	MW800331	OM903072	Twig	*Fusarium oxysporum*
	Fusarium oxysporum JJF2	99.26	100	MN626452			
	Fusarium oxysporum JJF1	99.26	100	MN626451			
	Fusarium oxysporum KEMS_4a	99.26	100	MK922065			
CC-30	Fungal sp. NLEndoHerit_022_2008N2-33-3G	99.25	100	JX978251	OM903073	Twig	*Microsphaeropsis olivacea*
	Microsphaeropsis olivacea D4/2c	99.06	100	MG020349			
	Microsphaeropsis olivacea D4/2b	99.06	100	MG020348			
	Microsphaeropsis olivacea D4/3b	99.06	100	MG020342			
CC-31	*Dactylonectria novozelandica* 4181	100	100	MN817697	OM903074	Root	*Dactylonectria* sp.
	Dactylonectria macrodidyma GFR05	100	100	MT447510			
	Dactylonectria torresensis JZB33100012	100	100	MN988721			
	Dactylonectria torresensis JZB33100011	100	100	MN988720			
CC-35	*Paraphoma radicina* 16EDSHB2	99.82	100	KY810511	OM903075	Root	*Paraphoma radicina*
	Pleosporales sp.18EDS-1-4	99.82	99	MK564739			
	Paraphoma radicina 16ALSHB1	99.64	100	KY810506			
	Phoma radicina VB1-2	99.46	100	MK764998			

Table 2 (continued)

Isolate	The most related species	Percent identity (%)	Query coverage (%)	GenBank accession number	GenBank accession number for ITS	Source	Suggested identification
CC-38	*Thielaviopsis basicola* SE112RZ 18 S	99.44	100	KM519645	OM903076	Root	Unidentified
	Setophoma sp. DS782	99.44	99	MK808904			
	Uncultured Ascomycota voucher CIAT544	99.44	99	KP012903			
	Uncultured Ascomycota clone 308	99.44	99	HM162069			
CC-39	Uncultured Pleosporales clone 8WF2cg1	99.45	100	GU910826	OM903077	Petiole	Unidentified
	Uncultured Pleosporales clone 8WF0cc06	99.45	100	GU910617			
	Uncultured Pleosporales clone 8WF2cc07	99.26	100	GU910783			
	Uncultured Pleosporales clone 8WF3ce01	99.62	97	GU910879			
CC-41	*Periconia macrospinosa* ZMXR37	99.63	100	MT446142	OM903078	Root	*Periconia macrospinosa*
	Periconia macrospinosa ZMXR16	99.63	100	MT446121			
	Periconia macrospinosa ZMQR17	99.63	100	MT446098			
	Periconia sp. DS963	99.63	100	MK809044			
CC-42	*Alternaria chlamydosporigena* 17MQ-2-6	99.16	100	MH384943	OM903079	Root	*Alternaria chlamydosporigena*
	Alternaria chlamydosporigena MQ-ZMC-1	99.66	98	KY420915			
	Alternaria chlamydosporigena CBS 125,833	99.49	99	MH863800			
	Alternaria chlamydosporigena CBS 125,829	99.49	99	MH863797			
CC-43	*Fusarium oxysporum* JJF2	99.44	100	MN626452	OM903080	Root	*Fusarium oxysporum*
	Fusarium oxysporum JJF1	99.44	100	MN626451			
	Fusarium oxysporum KEMS_4a	99.44	100	MK922065			
	Fusarium oxysporum LD200518	99.44	100	MW073409			
CC-44	*Dactylonectria torresensis* CUZF132Trs	100	100	MN294554	OM903081	Root	Unidentified
	Ilyonectria macrodidyma MBAi42CL	100	100	KF460429			
	Neonectria radicicola Cyl19	100	100	GQ131874			
	Dactylonectria torresensis CBS 129,086	100	99	MH865183			
CC-45	*Paraphoma radicina* 16EDSHB2	99.46	100	KY810511	OM903082	Root	*Paraphoma radicina*
	Paraphoma radicina 16ALSHB1	99.82	98	KY810506			
	Leptosphaeria sclerotioides VB1-1, VB1-2, VB1-2	99.64	98	MK764998			
	Leptosphaeria sp. P1004	99.64	98	KT268323			

from different plants and geographical regions are expected to differ in terms of species diversity and richness (Jia et al. 2016; Huang 2020). In this sense, it is possible to say that endophytic fungi might adapt to various environmental factors and the selection of endophytic fungi to be used in biological control (or other purposes such as plant growth promoting) from indigenous isolates might increase the chances of success.

In this study, 26 fungal endophytes were obtained only from the walnut roots along with high species diversity. In general, systemic, and comprehensive colonization of fungal endophytes are known to form mostly in the roots rather than above-ground organs because the roots are an interface between plants and microorganisms living in the soil (Xia et al. 2019; Alam et al. 2021). For example, Doolotkeldieva and Bobusheva (2014) investigated the presence of fungal endophytes in 255 wild medicinal plants and showed that the fungal endophytes were the most frequent in the roots. Jin et al. (2013) isolated and identified endophytic fungi from *Stellera chamaejasme* L. (toxic weed) and found that the frequency and the diversity of endophytic fungi was greater in the roots rather than in leaves and stems. However, contrary to these studies, Xiaoyune et al. (2020) showed that endophytic fungal diversity was higher in branch tissues of walnut, followed by leaf, fruit and root tissues. In another study related to walnut, Rang et al. (2019) studied the isolation of endophytic fungi from Yili wild walnut and determined that the most isolates came from the stem and the roots, respectively. Based on these studies, it is possible to say that there is no general rule showing endophytic fungi are more abundant only in certain plant tissues (especially in roots).

Seven isolates (three of them are in the genus of *Alternaria*) showed the in vitro antagonistic activity against *O. leptostyla* and *Alternaria* sp. CC-3 caused the highest activity with 52.5% inhibition rate. The other two isolates (*A. chlamydosporigena* CC-8 and CC-9) caused the moderate activity with 34.76 and 36.33%, respectively. *Alternaria* genus (or alternarioid hyphomycetes) constitutes biologically a rich group of fungi, and the members of this genus are in a wide range of ecological classes such as saprophytic, endophytic, and pathogenic (Lawrence et al. 2016). In addition, some *Alternaria* species were isolated from asymptomatic plant tissues (tomatoes, wheat, maple, etc.) and can live endophytically with these plants (Larran et al. 2001, 2007; Qi et al. 2009; Lawrence et al. 2016). Likewise,

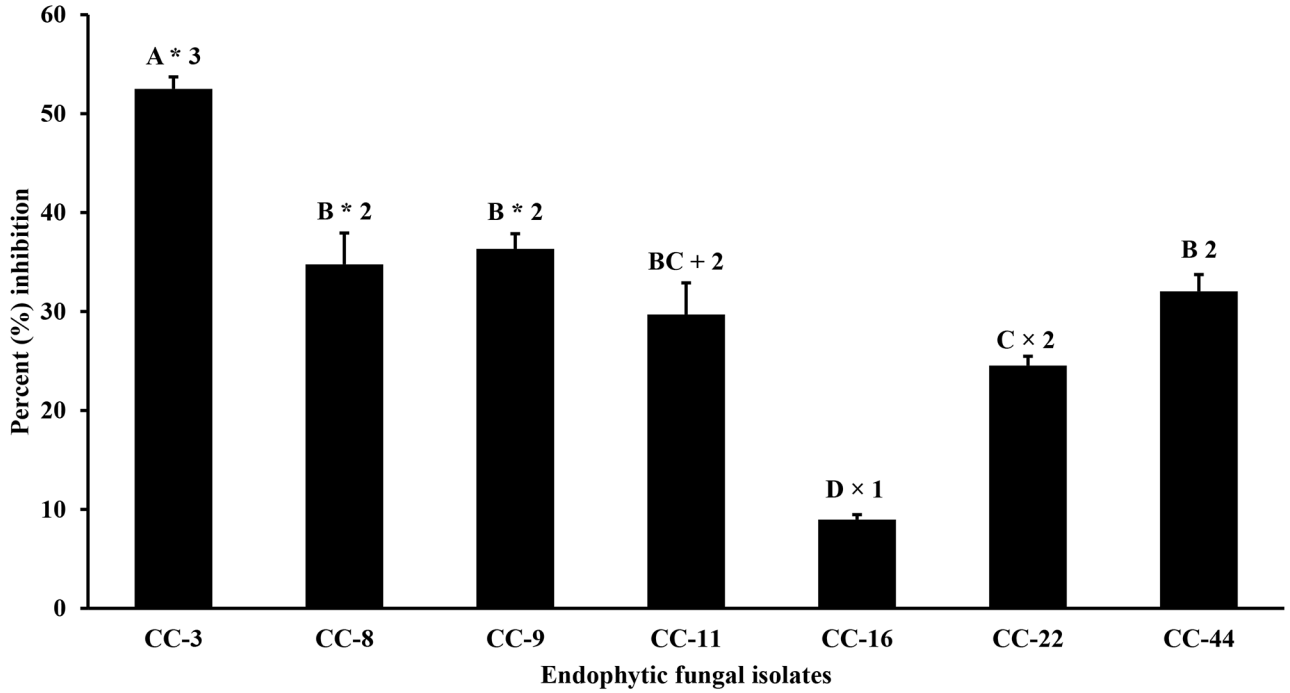

Fig. 1 Percent (%) inhibition of the fungal endophytes against *Ophiognomonia leptostyla* according to the method of Dennis and Webster (1971). Inhibition values were calculated using the formula described by Royse and Ries (1977). The different uppercase letters indicated on the columns show the statistical difference in terms of percent inhibition amongst isolates. Comparisons amongst the isolates were performed with ANOVA analysis followed by LSD multiple comparison test ($p < 0.001$). Bars show standard deviation. CC-3, *Alternaria* sp.; CC-8 and CC-9, *Alternaria chlamydosporigena*; CC-11, *Fusarium acuminatum*; CC-16, unidentified; CC-22, *Neonectria* sp.; CC-44, unidentified. * indicates the presence of PKS-I gene, + indicates the presence of PKS-II gene and × indicates the presence of NRPS gene. The numbers upon the columns show the inhibition rate based on the scale

it was also shown that some *Alternaria* species can live endophytically with walnuts (Pardatscher and Schweigkofler 2009; Xiaoyue et al. 2020). Since all these studies involve only isolation experiments, more experimental and the detailed studies are needed to understand type of the relationship between these *Alternaria* species and the host plant (especially walnut). With this study, the antagonistic effects of endophytic fungi isolated from walnuts against *O. leptostyla* were investigated for the first time and *Alternaria* sp. CC-3 demonstrated promising results.

According to the literature, the other fungal species determined in this study (such as *F. acuminatum* CC-11, unidentified CC-16, *Neonectria* sp. CC-22 and unidentified CC-44) which had the antagonistic activity against *O. leptostyla* were shown that they can live endophytically within various plant species such as *Geum macrophyllum* Willd, *Meconopsis grandis* Prain and some conifer trees (Clark et al. 2018; Rigerte et al. 2019; Lin et al. 2020). They also might have a potential to be used against walnut anthracnose, but fields studies are needed to prove this.

We also investigated the presence of PKS I-II and NRPS genes in the isolates showing the antagonistic activity to indicate possible association with their antibiosis activity against *O. leptostyla*. Large numbers of biologically active molecules (or secondary metabolites) are synthesized in metabolic pathways involving polyketide synthases or owing to reactions catalyzed by non-ribosomal peptide synthases (Wawrik et al. 2005; Le Govic et al. 2019). Products synthesized via these enzymes may have a wide range of biological functions such as antimicrobial, antagonism, antiviral, antifungal, phytotoxic, insecticidal, and antibiotic (Wawrik et al. 2005; Süssmuth et al. 2011; Fatema et al. 2018; Le Govic et al. 2019). For example, Fatema et al. (2018) showed that the PKS genes in *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W.Gams were associated with a degree of antagonism against *Botrytis cinerea* Pers. (1974) and *F. graminearum* (Schwabe). Although PKS and NRPS genes were detected in some species of *Alternaria*, *Fusarium*, *Neonectria* and unidentified genera (Hansen et al. 2015; Gramaje et al. 2020; Lin et al. 2020; Creamer et al. 2021), the biological activities of these genes in these species were not yet studied. In this study, PKS I-II and NRPS genes were determined in some of the isolates showing the antagonistic activity against *O. leptostyla* and this might be evaluated the data confirming the antagonistic activity even if there was no direct correlation. However,

functional genomics experiments such as gene expression and gene knockout are needed to fully prove the relationship of these genes to antagonism.

In conclusion, we isolated and molecularly identified fungal endophytes from various tissues of the common walnut. Also, the isolated fungal endophytes were investigated in terms of the antagonistic activity against *O. leptostyla*. Some isolates showed the inhibition (especially *Alternaria* sp. CC-3) at good level. It is thought that the results can be useful in biological control of walnut anthracnose. However, further studies are needed to prove the field efficacy of the isolate CC-3 against *O. leptostyla*. In addition, further experimental studies are needed to prove the endophytic properties of these fungi.

Acknowledgements Not applicable.

Authors' contributions Ali Sevim performed the study conception and design. Cafer Yabaneri performed biological material collection, fungal isolation, gene sequencing and antagonistic activity tests. Ali Sevim and Cafer Yabaneri performed PCR and data analysis. Ali Sevim wrote the manuscript. All authors read and approved the final version manuscript.

Funding This study was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) under the project application number of 1919B012000116.

Data Availability Data and materials are available upon request.

Declarations

Conflicts of interest/Competing interests The authors have no conflicts of interest to declare.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

References

Aamir M, Rai KK, Zehra A, Kumar S, Yadav M, Shukla V, Upadhyay RS (2020) Fungal endophytes: Classification, diversity, ecological role, and their relevance in sustainable agriculture. In: Kumar A, Singh VK (eds) Microbial Endophytes. Woodhead Publishing, Cambridge, pp 291–323

Akça Y, Bilgen Y, Ercisli S (2015) Selection of superior persian walnut (*Juglans regia* L.) from seedling origin in Turkey. Acta Sci Pol Hortorum Cultus 14:103–114

Alam B, Lǐ J, Gě Q, Khan MA, Gōng J, Mehmood S, Yuán Y, Gǒng W (2021) Endophytic fungi: From symbiosis to secondary metabolite communications or vice versa? Front Plant Sci 12:791033. https://doi.org/10.3389/fpls.2021.791033

Allegrucci N, Velázquez MS, Russo ML, Perez EP, Scorsetti AC (2018) Endophytic colonisation of tomato by the entomopathogenic fungus *Beauveria bassiana*: The use of different inoculation techniques and their effects on the tomato leafminer *Tuta absoluta* (Lepidoptera: Gelechiidae). J Plant Prot Res 57(4):205–211. https://doi.org/10.1515/jppr-2017-0045

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Anwar F, Qadir R, Abbas A (2020) Cold pressed walnut (*Juglans regia* L.) oil. In: Ramadan MF (ed) Cold pressed oils. Academic Press, Amsterdam, pp 491–495

Arnold AE, Maynard Z, Gilbert GS (2001) Fungal endophytes in dicotyledonous neotropical trees: patterns of abundance and diversity. Mycol Res 105(12):1502–1507. https://doi.org/10.1017/S0953756201004956

Ayuso-Sacido A, Genilloud O (2005) New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: Detection and distribution of these biosynthetic gene sequences in major taxonomic groups. Microb Ecol 49:10–24. https://doi.org/10.1007/s00248-004-0249-6

Belisario A (2002) Anthracnose In: Teviotdale BL, Michailides TJ, Pscheidt JW (eds) Compendium of nut crop diseases in temperate zones, The American Phytopathological Society, Minnesota, pp 77–78

Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW (2012) GenBank. Nuc Acids Res 40 (Database issue): D48-D53. https://doi.org/10.1093/nar/gkr1202

Boyle C, Dammann-Tugend M, Schulz B (2001) Endophyte-host interactions III. Local vs. systemic colonization. Symbiosis 31(4):259–281

Clark TN, Carroll M, Ellsworth K, Guerrette R, Robichaud GA, Johnson JA, Gray CA (2018) Antibiotic mycotoxins from an endophytic *Fusarium acuminatum* isolated from the medicinal plant *Geum macrophyllum*. Nat Prod Commun 13(10):1301–1304. https://doi.org/10.1177/1934578X1801301017

Creamer R, Hille DB, Neyaz M, Nusayr T, Schardl CL, Cook D (2021) Genetic relationships in the toxin-producing fungal endophyte, *Alternaria oxytropis* using polyketide synthase and non-ribosomal peptide synthase genes. J Fungi (Basel) 7(7):538. https://doi.org/10.3390/jof7070538

Dennis C, Webster J (1971) Antagonistic properties of species groups of *Trichoderma* III. hyphal interaction. Trans Br Mycol Soc 57:363–369. https://doi.org/10.1016/S0007-1536(71)80050-5

Doolotkeldieva T, Bobusheva S (2014) Endophytic fungi diversity of wild terrestrial plants in Kyrgyzstan. Glo Adv Res J Microbiol 3(9):163–176

Faeth SH, Fagan WF (2002) Fungal endophytes: Common host plant symbionts but uncommon mutualists. Integr Comp Biol 42(2):360–368. https://doi.org/10.1093/icb/42.2.360

Fatema U, Broberg A, Jensen DF et al (2018) Functional analysis of polyketide synthase genes in the biocontrol fungus *Clonostachys rosea*. Sci Rep 8:15009. https://doi.org/10.1038/s41598-018-33391-1

Gimenez C, Cabrera R, Reina M, Gozales-Coloma A (2007) Fungal endophytes and their role in plant protection. Curr Org Chem 11:707–720. https://doi.org/10.2174/138527207780598765

Gramaje D, Berlanas C, Martínez-Diz MDP, Diaz-Losada E, Antonielli L, Beier S, Gorfer M, Schmoll M, Compant S (2020) Comparative genomic analysis of *Dactylonectria torresensis* strains from grapevine, soil and weed highlights potential mechanisms in pathogenicity and endophytic lifestyle. J Fungi (Basel) 29;6(4): 255. https://doi.org/10.3390/jof6040255

Gray J (2013) Nuts and seeds. In: Caballero B, Allen L, Prentice A (eds) Encyclopedia of human nutrition. Academic Press, Amsterdam, pp 329–335

Gurulingappa P, Sword GA, Murdoch G, Mc Gee P (2010) Colonization of crop plants by fungal entomopathogens and their effects

on two insect pests when in plant. Biol Cont 55(1):34–41. https://doi.org/10.1016/j.biocontrol.2010.06.011
Hansen FT, Gardiner DM, Lysøe E, Fuertes PR, Tudzynski B, Wiemann P, Sondergaard TE, Giese H, Brodersen DE, Sørensen JL (2015) An update to polyketide synthase and non-ribosomal synthetase genes and nomenclature in *Fusarium*. Fungal Genet Biol 75:20–29. https://doi.org/10.1016/j.fgb.2014.12.004
Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nuc Acids Symp 41:95–98
Huang YL (2020) Effect of host, environment, and fungal growth on fungal leaf endophyte communities in Taiwan. J Fungi 6(4):244. https://doi.org/10.3390/jof6040244
Ivanova H, Hamarova L, Pristas P (2017) *Clonostachys rosea* associated with ponderosa and Coulter pine needles in Slovakia. Biologia 72(11):1258–1263. https://doi.org/10.1515/biolog-2017-0145
Jamshidi S, Salahi S, Jamshidi S (2012) Genetic diversity of Iranian *Ophiognomonia leptostyla* (Fr.) populations using RAPD and ISSR markers. Ann Biol Res 3(2):890–898
Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP (2016) A friendly relationship between endophytic fungi and medicinal plants: A systematic review. Front Microbiol 7:906. https://doi.org/10.3389/fmicb.2016.00906
Jin H, Yan Z, Liu Q, Yang X, Chen J, Qin B (2013) Diversity and dynamics of fungal endophytes in leaves, stems and roots of *Stellera chamaejasme* L. in northwestern China. Antonie Van Leeuwenhoek 104(6):949–963. https://doi.org/10.1007/s10482-013-0014-2
Kampapongsa D, Kaewkla O (2016) Biodiversity of endophytic actinobacteria from jasmine rice (*Oryza sativa* L. KDML 105) grown in Roi-Et Province, Thailand and their antimicrobial activity against rice pathogens. Ann Microbiol 66:587–595. https://doi.org/10.1007/s13213-015-1140-z
Landum MC, Felix MR, Alho J, Garcia R, Cabrita MJ, Rei F, Varanda CMR (2016) Antagonistic activity of fungi of *Olea europaea* L. against *Colletotrichum acutatum*. Microbiol Res 183:100–108. https://doi.org/10.1016/j.micres.2015.12.001
Larran S, Monaco C, Alippi H (2001) Endophytic fungi in leaves of *Lycopersicon esculentum* Mill. World J Microbiol Biotechnol 17:181–184. https://doi.org/10.1023/A:1016670000288
Larran S, Perelló A, Simón MR, Moreno V (2007) The endophytic fungi from wheat (Triticum aestivum L.). World J Microbiol Biotechnol 23:565–572. https://doi.org/10.1007/s11274-006-9266-6
Latz MAC, Jensen B, Collinge DB, Jørgensen HJL (2018) Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. Plant Ecol Diver 11(5–6):555–567. https://doi.org/10.1080/17550874.2018.1534146
Lawrence DP, Rotondo F, Gannibal PB (2016) Biodiversity and taxonomy of the pleomorphic genus *Alternaria*. Mycol Prog 15:3. https://doi.org/10.1007/s11557-015-1144-x
Le Govic Y, Papon N, Le Gal S, Bouchara JP, Vandeputte P (2019) Non-ribosomal Peptide Synthetase Gene Clusters in the Human Pathogenic Fungus *Scedosporium apiospermum*. Front Microbiol 10: 2062. https://doi.org/10.3389/fmicb.2019.02062
Lin X, Xu H, Liu L, Li H, Gao Z (2020) Draft genome sequence of *Neonectria* sp. DH2 isolated from *Meconopsis grandis* Prain in Tibet. 10(8): 346. https://doi.org/10.1007/s13205-020-02345-8
McGranahan G, Leslie CA (1991) Walnuts (*Juglans*). In: Moore JN, Ballington JR Jr (eds) Genetic resources of temperate fruit and nut crops. International Society for Horticultural Science, Wageningen, pp 907–951
Medic A, Solar A, Hudina M, Veberic R (2021) Phenolic response to walnut anthracnose (*Ophiognomonia leptostyla*) infection in different parts of *Juglans regia* husks, using HPLC-MS/MS. Agric 11:659. https://doi.org/10.3390/agriculture11070659
Metsa-Ketela M, Salo V, Halo L, Hautala A, Hakala J, Mantsala P, Ylihonko K (1999) An efficient approach for screening minimal PKS genes from *Streptomyces*. Fems Microbiol Lett 180:1–6. https://doi.org/10.1111/j.1574-6968.1999.tb08770.x
Mudasir H, Khurshid A (2017) Anthracnose disease of walnut-A review. Int J Environ Agric Biotechnol 2(5):2319–2327. https://doi.org/10.22161/ijeab/2.5.6
Nishad JH, Singh A, Gautam VS, Kumari P, Kumar J, Yadav M, Kharwar RN (2021) Bioactive potential evaluation and purification of compounds from an endophytic fungus *Diaporthe longicolla*, a resident of *Saraca asoca* (Roxb.) Willd. Arch Microbiol 203:4179–4188. https://doi.org/10.1007/s00203-021-02390-8
Pardatscher R, Schweigkofler W (2009) Microbial biodiversity associated with the walnut *Juglans regia* L. in South Tyrol (Italy). Mitt Klosterneuburg 59:24–30
Pollegioni P, Woeste K, Chiocchini F, Del Lungo S, Ciolfi M, Olimpieri I et al (2017) Rethinking the history of common walnut (*Juglans regia* L.) in Europe: Its origins and human interactions. PLoS ONE 12(3):e0172541. https://doi.org/10.1371/journal.pone.0172541
Qi FH, Jing TZ, Wang ZX, Zhan YG (2009) Fungal endophytes from *Acer ginnala* Maxim: Isolation, identification, and their yield of gallic acid. Lett Appl Microbiol 49:9. https://doi.org/10.1111/j.1472-765X.2009.02626.x
Rang FJ, Ren YL, Zhang W, Ouyang Y (2019) Isolation, screening, and identification of active endophytic fungi from Yili wild walnut. Biotechnol Bull 35(9):218–223. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2019-0443
Republic of Turkey Ministry of Agriculture and Forestry (2017) Ceviz entegre mücadele teknik talimatı. General Directorate of Agricultural Research and Policies. https://www.tarimorman.gov.tr/TAGEM/Belgeler/Entegre/ceviz%20entegre.pdf. Accessed 04 March 2020
Rigerte L, Blumenstein K, Terhonen E (2019) New R-Based methodology to optimize the identification of root endophytes against *Heterobasidion parviporum*. Microorganisms 7(4):102. https://doi.org/10.3390/microorganisms7040102
Royse D, Ries S (1977) The influence of fungi isolated from peach twigs on the pathogenicity of *Cytospora cincta*. Pyhtopathol 68:603–607. https://doi.org/10.1094/Phyto-68-603
Segelke T, von Wuthenau K, Kuschnereit A, Müller MS, Fischer M (2020) Origin determination of walnuts (*Juglans regia* L.) on a worldwide and regional level by inductively coupled plasma mass spectrometry and chemometrics. Foods 9(11):1708. https://doi.org/10.3390/foods9111708
Shadmani L, Jamali S, Fatemi A (2021) Effects of root endophytic fungus, *Microdochium bolleyi* on cadmium uptake, translocation and tolerance by *Hordeum vulgare* L. Biologia 76:711–719. https://doi.org/10.2478/s11756-020-00598-5
Süssmuth R, Müller J, von Döhren H, Molnár I (2011) Fungal cyclooligomer depsipeptides: From classical biochemistry to combinatorial biosynthesis. Nat Prod Rep 28:99–124. https://doi.org/10.1039/c001463j
Vega FE (2018) The use of fungal entomopathogens as endophytes in biological control: A review. Mycologia 110(1):4–30. https://doi.org/10.1080/00275514.2017.1418578
Walker DM, Castlebury LA, Rossman AY, Mejia LC, White JF (2012) Phylogeny and taxonomy of *Ophiognomonia* (Gnomoniaceae, Diaporthales), including twenty-five new species in this highly diverse genus. Fungal Divers 57:85–147. https://doi.org/10.1007/s13225-012-0200-y
Wani MS, Hussain A, Ganie SA, Munshi AH, Lal EP, Gupta RC (2016) *Juglans regia* in a review. Int J Latest Res Sci Technol 5(1):90–97
Wawrik B, Kerkhof L, Zylstra GJ, Kukor JJ (2005) Identification of unique type II polyketide synthase genes in soil. Appl

Environ Microbiol 71(5):22322238. https://doi.org/10.1128/AEM.71.5.2232-2238.2005

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a Guide to Methods and Applications. Academic Press, New York, pp 315–322

Woeste KE, Beineke WF (2001) An efficient method for evaluating black walnut for resistance to walnut anthracnose in filed plots and the identification of resistant genotypes. Plant Breed 120:454–456. https://doi.org/10.1046/j.1439-0523.2001.00632.x

Xia Y, Sahib MR, Amna A, Opiyo SO, Zhao Z, Gao YG (2019) Culturable endophytic fungal communities associated with plants in organic and conventional farming systems and their effects on plant growth. Sci Rep 9:1669. https://doi.org/10.1038/s41598-018-38230-x

Xiaoyue W, Kehang L, Mengmeng H, Wenyu Z, Xiaoyan L, Dongshuo M, Fang W, Meixia P, Jinghua Q (2020) Isolation and identification of endophytic fungi in walnut. IOP Conf Series: Earth Environ Sci 508:012138. https://doi.org/10.1088/1755-1315/508/1/012138

Yang H, Cao G, Jiang S, Han S, Yang C, Wan X, Zhang F, Chen L, Xiao J, Zhu P et al (2021) Identification of the anthracnose fungus of walnut (*Juglans* spp.) and resistance evaluation through physiological responses of resistant vs. susceptible hosts. Plant Pathol 70:1219–1229. https://doi.org/10.1111/ppa.13354

Zhao X, Hou D, Xu J, Wang K, Hu Z (2022) Antagonistic activity of fungal strains against *Fusarium* crown rot. Plants 11:255. https://doi.org/10.3390/plants11030255

Zhou D, Hyde KD (2001) Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. Mycol Res 105(12):1449–1457. https://doi.org/10.1017/S0953756201004713

Zhou W, Wei Q, Feng R, Liu Y, Liang H, Li J, Yan K (2021) Diversity and spatial distribution of endophytic fungi in *Cinnamomum longepaniculatum* of Yibin, China. Arch Microbiol 203:3361–3372. https://doi.org/10.1007/s00203-021-02325-3

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