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Fungal endophytes from wild *Solanum torvum* seeds: diversity and antagonism against plant pathogens

Tiziana Irdani[1] · Ilaria Cutino[1] · Agostino Strangi[1] · Renato Torre[2]

Abstract

This study investigates the endophytic microbial communities from the seeds of two *Solanum* species, selected for their contrasting resistance to the plant pathogen *Meloidogyne incognita* (Kofold & White) Chitwood: *Solanum torvum* Sw., which is highly resistant, and *Solanum melongena* L., which is highly susceptible. Aseptic extractions from individual seeds, followed by molecular analysis, revealed a variety of culturable microbial strains. Our results focused on the differential culturable microorganisms associated with two genotypes. Notably, significant differences were observed in the fungal communities, with the identification of the fungus *Lecanicillium aphanocladii* and the yeast *Papiliotrema flavescens* (formerly *Cryptococcus flavescens*) exclusively in *S. torvum*. This represents the first report of these fungal species associated with *Solanum* seeds. We characterized the micro-morphology, growth patterns, and molecular phylogenetic relationships of *L. aphanocladii*, based on two independent genetic loci. In vitro bioassays demonstrated the biocontrol potential of *P. flavescens* which inhibited *Botrytis cinerea* mycelial growth up to 60% and reduced the viability of *M. incognita* juveniles by 35%. Similarly, *L. aphanocladii* exhibited antagonistic activity against *B. cinerea* and was capable of capturing and digesting the plant-parasitic nematode *M. incognita* and the plant-associated *Bursaphelenchus eremus* Ruhm. These findings suggest that the unique endophytic microorganisms isolated from *S. torvum* may play a role in its natural resistance and have potential as biological control agents, supporting their use in integrated pest and disease management strategies.

Keywords *Lecanicillium aphanocladii* · *Papiliotrema flavescens* · Plant-nematode resistance · *Botrytis cinerea* · *Meloidogyne incognita* · *Bursaphelenchus eremus*

Introduction

Various microorganisms, e.g., bacteria, yeasts, and fungi, have been identified as specific endophytes associated with different crop ecosystems. Among these diverse groups, endophytic fungi are noteworthy for their biological role in association with their host plants. Generally, these fungi contribute to plant development by producing plant growth hormones (e.g., IAA) and lytic enzymes, enhancing the bidirectional nutrient transfer, and promoting plant health. Additionally, endophytic fungal associations protect against adverse environmental conditions such as heavy metal tolerance, e.g., Cu, Pb, and Zn, increased resistance to drought, and protection against pathogens and parasites (Pratap et al. 2024). Endophytic fungi are not simply considered saprophytes since they are associated with living tissues, thus contributing to the fitness of the plant. It is conceivable that the seed microbiota shaped by plant genotype, environmental conditions, and agricultural management (Berg and Raaijmakers 2018) serves as a reservoir of beneficial microbes that enhance offspring fitness. The microbial community may contribute to seed preparation, germination environment, seedling development, and overall plant growth (Abdelfattah et al. 2023; Afridi et al. 2023). Although the seeds host a wide range of microbial communities, their inhabitants remain less studied that microbes associated with other parts of the plant. The seed endophyte diversity associated with *Solanum torvum* has not been analyzed yet. *S. torvum* Sw. is a wild robust member of the Solanaceae family that exhibits remarkable resilience

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to climatic adversities and common pests affecting the domesticated eggplant (Gousset et al. 2005; Rotino et al. 1997). Its resistance encompasses a broad spectrum of soil-borne pathogens, including *Verticillium dahlia*, *Ralstonia solanacearum*, *Fusarium oxysporum*, and root-knot nematode *Meloidogyne incognita* (Bagnaresi et al. 2013; Bletsos et al. 2003; Gisbert et al. 2011; Gousset et al. 2005; Singh and Gopalakrishnan 1997). *S. torvum* is also recognized for its health benefits (Arthan et al. 2002; Lu et al. 2011; Yousaf et al. 2013), including antioxidant and antimicrobial properties, derived from various plant parts like roots, leaves, and fruits. Various studies have explored this species to discover useful compounds from different parts of the plant (Barbosa et al. 2012; Muthezhilan 2012; Lakshmi et al. 2013; Yousaf et al. 2013; Senizza et al. 2021). In a recent study, Irdani et al. 2023 (Irdani et al. 2023) demonstrated that *S. torvum* has developed intricate defense mechanisms against the root-knot nematodes. The root exudates of plants significantly shape the microbial populations in the rhizosphere, affecting the proliferation of soil-borne diseases and pests (Afridi et al. 2023; Zhou et al. 2011). Notably, the root exudates of resistant species like *S. torvum* exhibit variations in the types and amounts of amino acids, amines, or phenols compared to those of *S. melongena*, thus contributing to these observed effects (Afridi et al. 2023; Zhou et al. 2011). Consequently, these alterations influence the growth, richness, and diversity of microorganisms in the rhizosphere, thereby impacting the colonization of plant roots (Gómez-Vidal et al. 2006; Lopez-Llorca et al. 2007). An earlier study on the endophytic microbiota of *S. torvum* has revealed the presence of *Phoma medicaginis*, *Colletotricum spp.* (Coelomycetes) on the petiole, and the presence of *Cladosporium cladosporioides*, *Curvularia tuberculate*, *Aspergillus* spp. (Ascomycetes) on the stem (Kannan and Muthumary 2012). However, no other study has explored the microbes of *S. torvum* seeds yet.

This is the first study to report on the presence of endophytic microbes in *S. torvum* seeds. We documented several isolates obtained from the seeds of two *Solanum* species: the resistant *S. torvum,* collected from two different locations, and the susceptible *S. melongena*. The objectives of the current research were (i) to explore the endophytes of *S. torvum* seeds and compare them among different Solanaceae species, (ii) to characterize the fungal strains found in *S. torvum*, (iii) to investigate in vitro the potential antagonism with a plant–pathogen such as *Botrytis cinerea*, the plant-parasitic nematode *M. incognita*, and the plant-associated *Bursaphelenchus eremus*. The results revealed previously unknown endophytic fungi inhabiting *S. torvum* seeds and help to understand their interactions with significant plant pathogens.

Materials and methods

Seed sampling, sterilization, and endophytes isolation

Twenty-five seeds per genotype of *Solanum torvum* (TG1, from CREA-GB Lombardy 2008; CREA-DC Tuscany 2020) and *S. melongena* (1F5-9, from CREA-GB Lombardy 2008) were investigated. Seeds were washed twice in distilled sterile water, pulling up and down several times with a Gilson pipette. Subsequently, they were immersed in 5% sodium hypochlorite for 5 min, twice, and rinsed three times in distilled sterile water. After sterilization, an aliquot of rinsed water was controlled for sterility on agar plates overnight at 28 °C. Each seed was then cultured singly for 48 h on MS agar plates at 25 °C. The isolates grown around seeds were considered epiphytic. Only those seeds without any epiphytic growth were considered sterile and individually homogenized in 1.5-ml Eppendorf tube containing 50 µl of yeast peptone dextrose broth (YPD, Sigma), or Luria Broth bacterial medium (LB, Sigma). The resulting suspension was then spread on TSA agar (Difco) and incubated until colonies were formed. Each microorganism isolated, presumed endophyte was subsequently cultured on new plates of Malt-agar (MAD, Difco; MAA, Acumedia) or potato dextrose agar (PDA, Difco) for fungi and LB agar for bacteria. The incubation temperatures were maintained at 28 °C for yeasts/fungi or 36 °C for bacteria. Each isolate was then subjected to molecular identification and morphological analysis.

Nematode populations

The nematode populations used in this study are stored in CREA-DC Collection (Italy). Root-knot nematode *M. incognita* IT30 (Irdani et al 2011) was maintained on tomato (cv. Roma) in a greenhouse. *B. eremus* IT17 (Carletti et al. 2008) was maintained on *B. cinerea* Malt agar plates, at 25 °C.

Amplification, sequencing, and molecular identification

DNA extraction from bacterial isolates was performed with QIAmp DNA Mini Kit (QIAGEN). The amplification of 16S rDNA locus was obtained using primer pairs P0/P6 according to protocol by Chiellini (Chiellini et al. 2014). DNA isolation from fungal isolates was performed using DNeasy Plant Mini Kit (QIAGEN) following the standard protocol suggested by the manufacturer. For molecular identification of fungi (dos Reis et al. 2022), the ITS locus between 3'end of 18S rDNA and 5'end of 28S rDNA genes was amplified using primers

ITS5 and ITS4 according to White (White et al. 1990). All the resulting amplicons were sequenced. Bacterial and fungal isolates were identified via homology search in the NCBI database using the current version of BLAST online tool. For analysis of the Italian (IT) isolate of *Lecanicillium aphanocladii* IT45 two additional loci, the 5' regions of 18S rDNA and 28S rDNA were amplified according to White et al., (1990) and Vilgalyslab (https://sites.duke.edu/vilgalyslab/files/2017/08/rDNA-primers-for-fungi.pdf), and the resulting amplicons were sequenced. Orthologous sequences for both rDNA loci belonging to phylogenetically close species were obtained from GenBank. Sequences were concatenated to construct a total alignment 1669 position long with 61 polymorphic positions which 21 were parsimony informative sites with at least two variants. *Aphanocladium album* strain CBS 401.70 was included in the alignment as outgroup. The most suitable substitution matrix was evaluated using JmodelTest2 v. 2.1.10 (Darriba et al. 2012). The phylogenetic tree was computed using MEGA X (Tamura et al. 2021) with maximum likelihood approach, assuming Kimura-2 parameters as substitution matrix and the resulting reconstruction was tested with 1000 pseudo-replicates.

Each culture is stored as glycerol stocks at -80 °C at the CREA-DC (Firenze-Italy). The fungal isolates IT45 and IT53 were deposited in the CBS culture collection (Netherlands) with the accession number CBS 151552 and CBS 18623, respectively.

Microscopical and morphometrical analysis of strains

The identification of endophytic fungus IT45 was conducted following standard methods based on macroscopic and micro-morphological features (Gams and Zare 2003; Zare and Gams 2001). A Zeiss Axiolab microscope, equipped with a VisiCam TC10 tablet, was utilized for capturing microscopic images. Photographic pictures were realized with a Samsung camera. All isolated yeast and fungi were cultured on PDA agar plates (Difco) at 28 °C for a period ranging from 3 to 7 days. Successively, these strains were further cultivated on various media like MAA/MAV (MA-Acumedia; MA-VWR) and MAD (MA-Difco), with or without agar, to promote their different growth forms. Additionally, NGM plates (Stiernagle 2006) were employed for the production of blastospores/chlamydospores. Yeast cultures were grown in liquid PD broth (Sigma) and maintained in a shaker at 28 °C.

Pathogenicity assay: endophyte interaction with *B. cinerea*

The fungal *L. aphanocladii* IT45 and the yeast *P. flavescens* IT53 were tested against the pathogen gray mold *B. cinerea*

IT48. A week before trials, each strain was refreshed on media with agar at 25 °C.

In the *L. aphanocladii test,* the antagonistic potential of fungal was evaluated through dual culture technique. Plates were symmetrically inoculated with a 0.5-cm^2 plugs of sporulating mycelium of both fungi, on top of MAD plates. Control plates inoculated with each strain alone. Plates kept two weeks at 25 °C and ca. 60% HR in the dark. The experiment was performed three times. Observations were made at 7 and 10 day post-inoculation (dpi) to assess the growth of mycelia. Antagonistic activity was evaluated based on visual inspection of the inhibition zone between the colonies and overgrowth of the endophyte onto the pathogen.

The *P. flavescens test* followed a lawn-plate method as by Fernandez-San Millan et al. (2021). The yeast was cultured in PD broth up to 10^8 cell/ml, plated on MAD for 24 h at 28 °C in the dark. The yeast plates were centrally inoculated with *B. cinerea*, in direct contact with the yeast-lawn below. Mycelial growth of *B. cinerea* was monitored at 1, 3, and 18 dpi. Control plates containing each strain alone were included. All plates were incubated for two weeks at 25 °C and approximately 60% relative humidity in the dark. The experiment was conducted three times. Reported data represent the means of the radial growth (in cm) of *B. cinerea* mycelium, in the presence and absence of the endophytic yeast.

Pathogenicity assay: endophyte interaction with plant-parasitic nematodes

To perform the test of *P. flavescens* with the root-knot nematode *M. incognita*, several egg masses of *M. incognita* IT30 monoxenic culture were collected from tomato plants (cv. Roma), washed with tap water, immersed three times with 0.5% sodium hypo-chloride, and rinsed several times with sterile water.

Approximately 200 s-stage juveniles (J2) were transferred into individual wells of 12-well culture plates (Corning-Costar, 2.5 cm diameter), each containing 1 mL of sterile tap water. Experimental wells received a suspension of *P. flavescens* at 10^7 cells/mL, while control wells contained only sterile water. Nematode viability was assessed after 48 h at room temperature (≈ 20 °C), based on mobility and evidence of entrapment documented through microscopic imaging. Observations began soon after the inoculations and then daily for a week. For each well, the percentage of motile (viable) juveniles was calculated and compared to control values. The experiment was conducted in triplicate, and results are expressed as mean $\pm$ standard deviation.

For test with *L. aphanocladii* and *M. incognita*, isolate IT45 was subcultured on MAD and incubated at 25 °C in the dark one month prior to the trials. The test was conducted at room temperature (approximately 20 °C) using

Table 1 Culturable endophytic yeast and fungal strains isolated from seeds of resistant and susceptible *Solanum* species. The data include both previously reported endophytes and novel isolates identified in this study

Solanum spp.		Yeast	Fungi	Role
Susceptible	*S. melongena* 1F5(9) Lombardy-Italy [**this study**]		*Aspergillus niger* (1)	Saprophyte/Pathogen
			Aspergillus niger (1), *Cladosporium sphaerospermum* (1)	Saprophyte/Pathogen
	S. *melongena* [a]		*Alternaria alternata* (2)	Saprophyte/Pathogen
			Arthrinium sp. (4)	Mycoparasite
			Aspergillus paradoxus (30)	Mycoparasite
			Cladosporium cladosporoides (1) *Cladosporium oxysporum* (3) *Cladosporium sphaerospermum* (1) *Hormonema* sp. (4) *Penicillum solitum* (1) *Penicillum waksmanni* (1) *Penicillum* sp. (1) *Rhizopus stolonifer* (1) *Trichoderma harzianum* (1)	
	S. *lycopersicum* [b]		*Alternaria alternata* (12)	Mycoparasite
			Aspergillus spp. (1)	Mycoparasite
			Cladorrhinum spp. (8)	Pathogen
			Cladosporium cladosporoides (11), *Fusarium verticillioides* (2), *Penicillum* sp. (2), *Rhizopus stolonifer* (17), *Tritirachium* sp. (1)	
Resistant	*S. torvum* TG1 [**this study**] Lombardy-Italy		*Lecanicillium aphanocladii* IT45 (1)	Mycoparasite & Nematoparasite [**this study**] Entomofagous [c]
			Aspergillus niger (1)	Saprophyte/Pathogen
			Aspergillus niger (1)	Saprophyte/Pathogen
	S. torvum TG1 [**this study**] Tuscany-Italy	*Papiliotrema flavescens* IT53 (1)		Mycoparassite & Nematoparasite [**this study**]
			Penicillium olsonii IT55 (1)	Plant growth promoting, Inducing resistance [d]
			Penicillium sp. IT54 (1)	
			Penicillium sp. IT56 (1)	
			Aspergillus sp. IT51 (1)	Saprophyte/Pathogen

In brackets, the number of seeds where the species were found

[a](Nishikawa et al. 2006)

[b](Abdel-Azeem et al. 2021)

[c](Nedveckytė et al. 2021)

[d](Adeleke and Babalola 2021; Thambugala et al. 2020)

MAD plates colonized by one-month-old fungal mycelium. This period enables fully colonization of the agar medium, ensuring abundant conidial production, before introducing the nematodes. Approximately 200 freshly hatched second-stage juveniles (J2) of *M. incognita* were inoculated onto each plate. Observations were initiated immediately after inoculation and continued daily for one week to monitor nematode behavior and potential fungal interaction.

For the test of *L. aphanocladii* and *B. eremus,* a week before trials the fungus *L. aphanocladii* IT45 was refreshed on MAD at 25 °C, in the dark. The test was carried out at 25 °C on MAD agar inoculated with pool (≈500 animals) of *B. eremus* IT17. The plates were kept at 25 °C for 4 weeks. Observations began daily and then at weekly intervals over approximately 1 month. The experiment was performed three times.

Statistical analysis

All the bioassay data were analyzed by independent samples and compared per pair based on the Student's t-test. The significant differences ($p < 0.01$ or $p < 0.05$) were marked (*) (**) in the figures, as reported in the captions.

Fig. 1 Morphological features of *Lecanicillium aphanocladii* (**A**). Micro-morphologies of fungus grown on MAD. (**B**, **C**) Thin aerial mycelium with aphano-phialides with microconidia and verticillate phialides, (**D**) crystals developed on MAD, (**E**) pseudo-hyphae, (**F**) macroconidia, (**G**) a cluster of blastospores and chlamydospores on NGM

Fig. 2 Phylogenetic tree inferred with nuclear 18S rDNA small subunit (SSU) and 28S rDNA large subunit (LSU) fragments. In red, the *Lecanicillium* strain isolated in this work. *Aphanocladium album* strain was included in the alignment as outgroup

Table 2 Micromorphological comparisons among *Lecanicillium* species. The *L. aphanocladii* isolated from *Solanum torvum* in this study and from earlier studies

Strain/Isolate	Host	Aphaphialides		Phialides		Microconidia		Macroconidia		Chlamydospores
		Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	
Lecanicillium aphanocladii IT45 (=*Aphanocladium arearum*) (Italy) [**This study**]	*Solanum torvum*	5.1 ± 1.0	0.8 ± 0.6	25 ± 11	1.9 ± 0.5	3.0 ± 0.8	1.5 ± 0.1	6.0 ± 0.6	2.9 ± 0.5	Yes
Lecanicillium aphanocladii[a]	Fungi; Insects; Plants	4.5–11	0.9–1.8	n. r	n. r	2.7–4.0	1.4–2.2	n. r	n. r	Unknown or absent
Lecanicillium aphanocladii[b] UMC11913 (Egypt)	Air contaminant	3.6–11.5	1.5–2.6	n. r	n. r	n. r	n. r	n. r	n. r	n. r
Lecanicillium lecanii[c] (=*Verticillium lecanii*)	Insects	n. r	n. r	11–30	1.3–2.0	2.5–3.5	1.0–1.5	6–9	1.5–2.5	Unknown or absent
Lecanicillium psalliotae[d] (=*Lecanicillium saksenae*)	Fungi; Arthropoda Nematoda; Plants	n. r	n. r	25–35	1.0–1.4	2.7–3.7	1.0–1.5	5–10 (curved usually pointed ends)	1.2–1.7	Unknown or absent

n. r., not reported; data ± standard deviation

[a](Gams and Zare 2003; Zare and Gams 2001)

[b](El-Kader El-Debaiky 2017)

[c](Diaz et al. 2009; Zare and Gams 2001, 2008)

[d](Senthil Kumar et al. 2015; Zare and Gams 2001)

Fig. 3 *Lecanicillium aphanocladii* growth on solid and liquid media: (**A**) White cream mycelium on solid and (**C**) in liquid MAA media. (**B**) Reddish mycelium on solid and (**D**) in liquid MAD media

Data sequence

All sequences were deposited in GenBank under the accession number indicated in Supplementary Table 2. ITS sequences were deposited under the accession number OR131317, OR131318, OR131325, OR131326, and OR131318.

Results

Isolation and characterization of endophytes from *Solanum* seeds

This study provides microbiological and molecular analysis of single seeds of two different *Solanum* species, *S. torvum* (wild and resistant to soil pathogens) and *S. melongena* (domesticated and susceptible to soil pathogens). Overall, 23 endo- and 6 epiphytic culturable strains were isolated, identified by PCR molecular analysis and sequence of the internal transcribed spacer 1–5,8S-internal transcribed spacer 2 of ribosomal DNA (Supplementary Table 1). Each single seed contained a single culturable fungal/yeast along with various bacterial strains.

Overall, the isolates included both bacterial and fungal/yeast strains, spanning seven genera of endophytes and four genera of epiphytes (Supplementary Table 1). Among the endophytic bacteria, *Bacillus* and *Staphylococcus* species were identified (Supplementary Table 1), while the endophytic fungi and yeasts comprised *Aspergillus*, *Cladosporium*, *Papiliotrema*, *Lecanicillium*, and *Penicillium* species are shown in Table 1. The epiphytic microbiota included bacterial genera such as *Methylorubrum* and *Bacillus*, along with fungal genera including *Cladosporium* and *Alternaria* (Supplementary Table 1). The seed-associated microbiota showed variability among the *Solanum* genotypes analyzed. *S. torvum* shared several microbial genera with other *Solanum* species such as *Aspergillus* spp., *Penicillium* spp., *Bacillus* sp., and *Staphylococcus* spp. yet also revealed distinct endophytic profiles. Notably, *L. aphanocladii* and *P.*
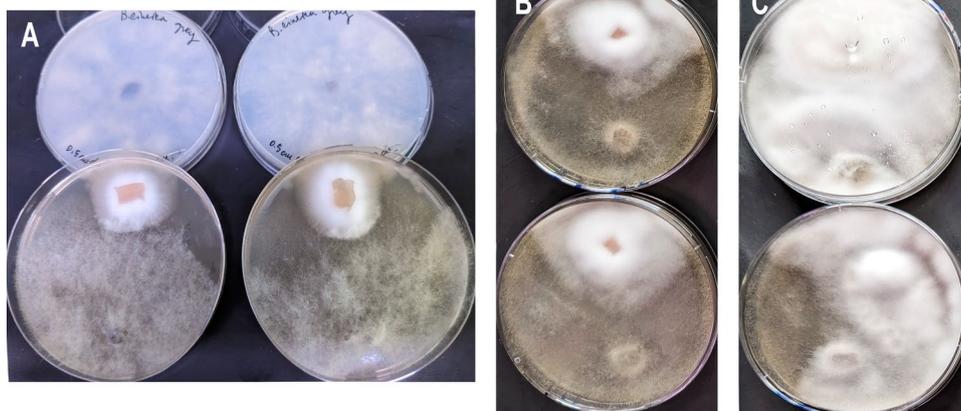

Fig. 4 In vitro competition between *Lecanicillium aphanocladii* and *Botrytis cinerea*. (**A**, top) Two plates of the pathogen *B. cinerea* as control. (**A**, bottom) Two dual culture plates with *L. aphanocladii* (up) and *B. cinerea* (below), after a week. (**B**) Two dual culture plates, the *L. aphanocladii* (up) starts to surmount the dark pathogenic mycelium of *B. cinerea* (below), after 10 days. (**C**) Two dual culture plates, the *L. aphanocladii* (up) replaces completely the dark pathogenic mycelium of *B. cinerea* (below), in two weeks
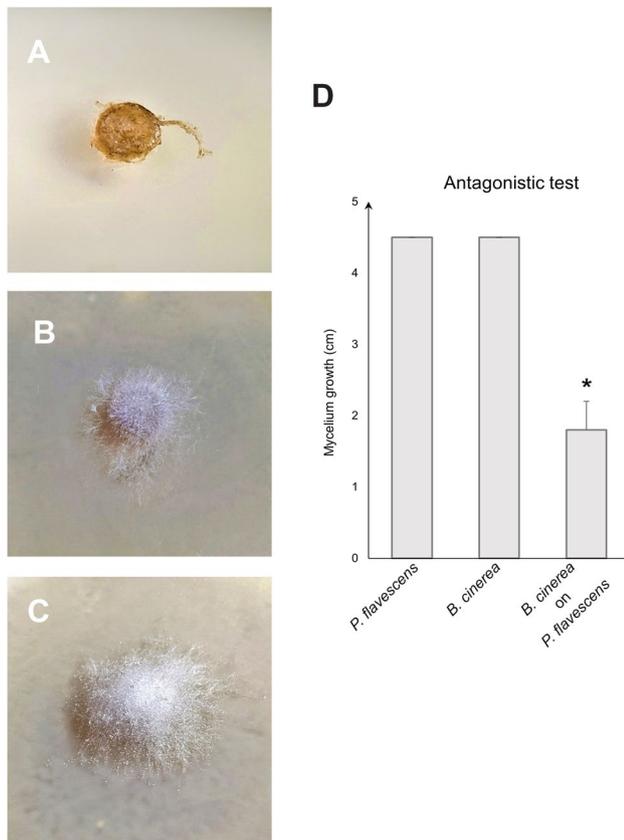
Fig. 5 Development of *Botrytis cinerea* on a *Papiliotrema flavescens* lawn. (**A**) *B. cinerea* mycelium on day 1, (**B**) after 3 days, and (**C**) after 18 days at 28 °C. (**D**) Quantitative analysis of mycelial growth reduction after 18 days in the presence of *P. flavescens*. Data represent the average of three independent replicates. Error bars indicate standard deviation (SD). No visible bars are shown for the control due to minimal variability among replicates. An asterisk (*) indicates a statistically significant difference (Student's t-test, $P < 0.01$) in co-culture conditions

flavescens were found exclusively among the endophytes. Among the endophytic fungi isolated from *S. torvum* seeds, *L. aphanocladii* IT45 and *P. flavescens* IT53 emerged as uncommon residents when compared to seed microbiota from another Solanaceae, including *S. melongena*. Table 1 presents the complete list of fungal and yeast isolates, including comparisons with other Solanaceae species.

Morphological and molecular characterization of *L. aphanocladii*

For the first time, a strain of *L. aphanocladii* was isolated from a sterile seed of Solanaceae. The isolate was observed to colonize seeds as unicellular developing either puffy cloud in liquid or a fungal mycelium on solid agar, as illustrated in Fig. 1A. Rapid fungal growth was noted, with color changes on the reverse side of culture plate, to reddish- or

light-cream color dependently by the media used, as shown in Fig. 2. The anamorph state of fungus exhibited various micromorphologies, such as thin aerial hyphae and aphanophialides, each bearing single microconidia (Fig. 1B, C). The fungus also developed conidiophores producing clusters of dense microconidia (Fig. 1B, C). Successively, several octahedral crystals were produced on MAD (Fig. 1D; pseudo-hyphae Fig. 1E), and oval/ellipsoidal shaped macroconidia could also be observed (Fig. 1F). The production of chlamydospores on NGM plates and cluster of blastospores/yeast-like cells are shown in Fig. 1G.

Table 2 shows the micrometric characters of IT45 strain to outline the differences with the other strains/species described (Diaz et al. 2009; El-Kader El-Debaiky 2017; Gams and Zare 2003; Senthil Kumar et al. 2015; Zare and Gams 2001, 2008). *L. aphanocladii* IT45 produces two types of conidia: micro- and macroconidia. Microconidia were globose and measured ($n = 10$) on average of 3 ± 0.8 in length and 1.5 ± 0.1 μm in width. The macroconidia were oval/ellipsoidal and measured ($n = 10$) on average of 6 ± 0.6 in length and 2.9 ± 0.5 μm in width. The phialides measured ($n = 10$) on average of 25 ± 11 in length and 1.9 ± 0.5 μm in width. The aphano-phialides measured ($n = 10$) on average of 5.1 ± 1 in length and 0.8 ± 0.6 μm in width. Multi-locus analysis was performed using concatenated SSU and LSU gene fragments to confirm the morphological identification as *L. aphanocladii* (Fig. 2).

L. aphanocladii pigment production

The *L. aphanocladii* IT45 isolate exhibited a red–purple pigmentation on the reverse side of mycelium when grown on MAD, as depicted in Fig. 3B, D. This pigmentation did not occur when the fungus was grown on MAA, despite similar compositions (Fig. 3A, C). Similarly to other *L. aphanocladii* isolates, the IT45 showed a reddish pigmentation after a few weeks on MAD (Fig. 3B). A light-red color developed when the fungus was grown in liquid MAD (Fig. 3D).

Pathogenicity assay: endophyte interactions with *B. cinerea*

Experiments have been carried out to reveal the mode of interaction between the endophyte *L. aphanocladii* and the pathogenic fungus *B. cinerea*. Dual culture assays were conducted on MAD inoculated with same plugs of both fungi. The mycelia growth checked daily, for two weeks. After one week, the mycelia of both species approached each other but maintained a distinct separation, forming a clear inhibition zone or halo at the interface, as depicted in Fig. 4 (A, bottom). In contrast, control plates with single-species cultures showed uninhibited radial growth (Fig. 4 (A, top). By the

Fig. 6 In vitro parasitism of *Meloidogyne incognita* by *Papiliotrema flavescens* (**A**) Interaction between *P. flavescens* and *M. incognita* second-stage juveniles (J2). (**B**) Percentage of free versus yeast-parasitized nematodes 48 h post-inoculation. Bars represent standard deviation (SD). Asterisks indicate statistically significant differences: P < 0.05 (*) and P < 0.01 (**), respectively
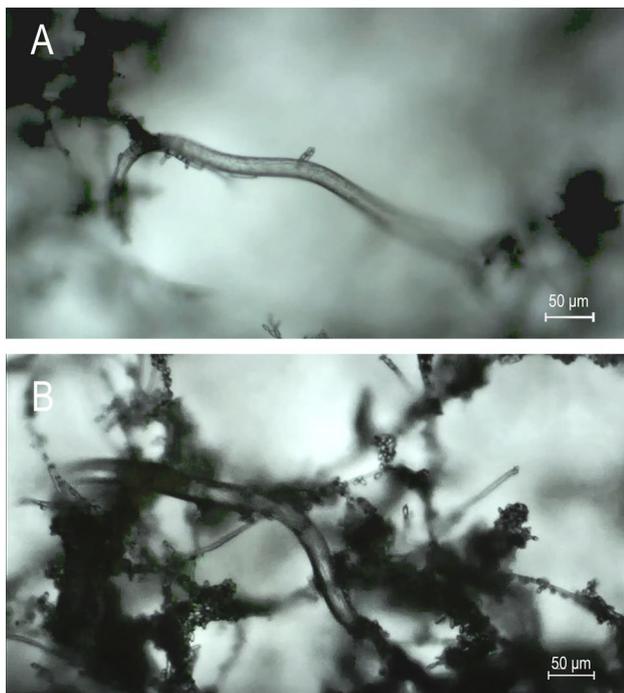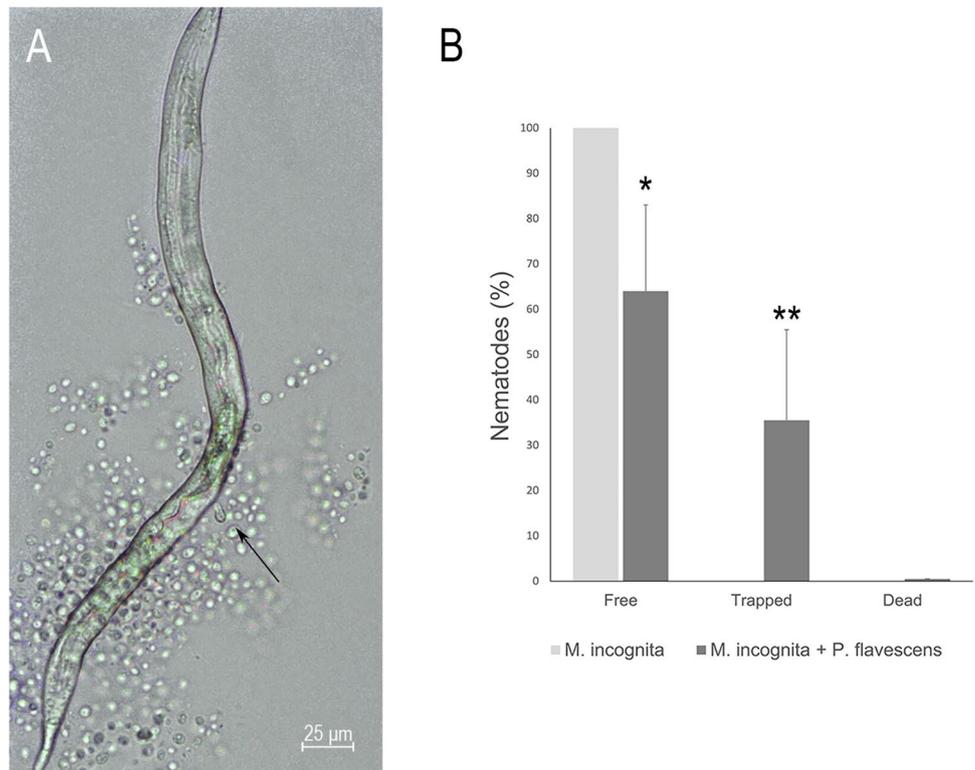

Fig. 7 Interaction between *Lecanicillium aphanocladii* and *Meloidogyne incognita* second-stage juveniles (J2). (**A**) Juveniles captured at their extremities by adhesive conidia from a well-developed aerial mycelium; additional conidia are seen adhering to the central body cuticle. (**B**) A juvenile fully entrapped within a dense fungal mycelium

tenth day, the mycelia established contact (Fig. 4B), and over the subsequent week, *L. aphanocladii* IT45 progressively overgrew and replaced *B. cinerea* IT48, culminating in the complete colonization of the plate surface (Fig. 4C). The experiments were repeated three times to validate data. However, further analysis is needed to interpret the nature of this interaction as mycoparasitism.

In a second test, the interaction between the yeast *P. flavescens* IT53 and *B. cinerea* was evaluated as shown in Fig. 5. Yeast plates were inoculated with *B. cinerea*, and growth was monitored at specific intervals of 1, 3, 18 dpi (Fig. 5A, B, C). Data of growth (cm) of the pathogenic mycelium when grown alone (control) and when placed above the yeast lawn were reported, as shown in Fig. 5D. A significant reduction in pathogenic fungal growth, up to 60% reduction (P < 0.01) compared to control, was observed in the presence of isolate IT53 (Fig. 5D).

Pathogenicity assay: endophyte interaction with plant parasitic nematodes

Several dual tests were performed separately with *P. flavescens* IT53 and *L. aphanocladii* IT45 against the juveniles of *M. incognita* IT30. Aliquots of fresh juveniles' larvae of *M. incognita* were incubated with *P. flavescens* IT53 and kept at 20 °C. Quickly, yeast cells adhere to the nematode cuticles, and larvae appear surrounded and immobilized by yeast cells, as shown in Fig. 6A. Percentages of J2, free
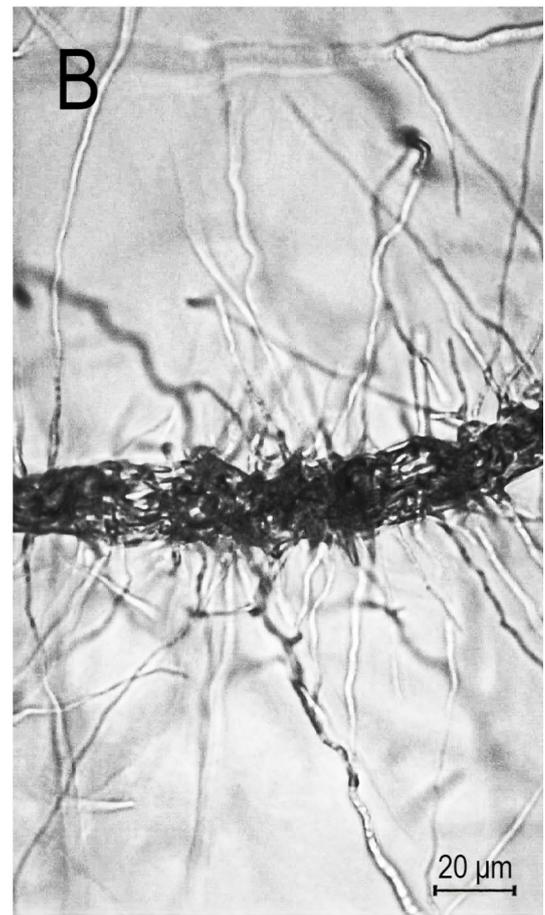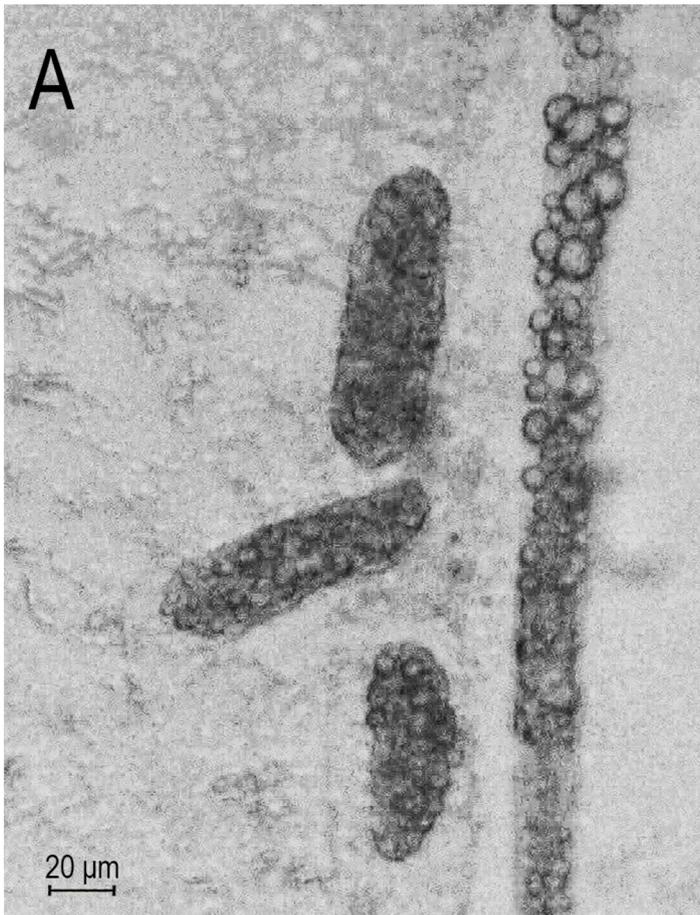
◄**Fig. 8** Interaction between *Lecanicillium aphanocladii* and *Bursaphelenchus eremus*. (**A**) Eggs and adult nematodes are digested by fungal activity. (**B**) Close-up of a nematode body colonized by *L. aphanocladii* hyphae. (**C**, center) *B. eremus* adult infected at the genital opening; (left) hypha emerging from the nematode body. Further visual details of *B. eremus* enwrapped by *L. aphanocladii* are available in Supplementary Fig. 1

or trapped by yeast, are reported in Fig. 6B after 48 h at room temperature. The results show that yeast significantly ($P < 0.01$) reduces the juveniles' movement by 35% compared to controls, trapping them in tangles of larvae. *P. flavescens* IT53 show an antagonistic effect against the plant-parasitic nematode.

In our study, we assessed the antagonistic potential of *L. aphanocladii* IT45 against the root-knot nematode *M. incognita*. This nematode is an obligatory plant pest unable to survive without a host plant. A well-developed, one-month-old mycelium of *L. aphanocladii* IT45 was observed to form a physical barrier that can potentially prevent the movement of second-stage juveniles (J2) toward the roots. In vitro, the assays demonstrated that the microconidia of *L. aphanocladii* adhered to the cuticle of *M. incognita* juveniles, particularly at the extremities, leading to J2 immobilization (Fig. 7 (A, B). Microscopic observations revealed that the conidia not only adhered to but also penetrated the nematode cuticle as shown in Fig. 7A and in the animation (Supplementary Video 1) https://doi.org/10.6084/m9.figshare.27878553.v1.

The time-lapse video shows the interaction between the endophyte *L. aphanocladii* IT45 and juveniles of the *M. incognita*.

In this study, we assessed the interaction between the endophytic fungus *L. aphanocladii* IT45 and the fungivores *B. eremus* IT17. Although *B. eremus* does not exhibit the same level of pathogenicity as *B. xylophilus*, previous studies have reported its association with wilting and declining *Quercus* species (Carletti et al. 2007; Čermák et al. 2013). Moreover, *L. aphanocladii* IT45 culture plate was capable to sustain the growth of *B. eremus*, albeit less effective than *B. cinerea* (data not shown). Notably, nematode reproduction was particularly affected when populations were maintained on red–purple MAD, over successive generations. In adult nematodes, conidia penetration was frequently facilitated through natural openings, particularly the genital pore. This mode of entry was evidenced by localized swelling and hyphal emergence, as depicted in Fig. 8(C). Following penetration, the fungus exhibited internal colonization, leading to the digestion of nematode tissues (Fig. 8A). Hyphal growth was observed emerging from the nematode body, indicating successful internal proliferation of the fungus (Fig. 8B, C).

Discussion and conclusions

This study explored the microbial culturable endophytes extracted from single seeds of two *Solanum* species: *S. torvum* and *S. melongena*. Our findings demonstrate that they are colonized by a few bacterial strains. Seeds were shown to be colonized by a few bacterial strains, especially *Staphylococcus* spp. or *Bacillus* spp., and by a fungal strain. This may suggest that a selective mechanism might be exerted by roots for fungal colonization. The fungal endophytic strains in common to *S. melongena* and *S. torvum* seed biomes were *Aspergillus niger*, *Cladosporium sphaerospermum*, and *Penicillium* spp. (Nishikawa et al. 2006), the same found by other authors in *S. lycopersicum* seeds (Abdel-Azeem et al. 2021). Some distinctive variations were observed in the *S. torvum* seed biome, especially among saprophytic, plant-growth promoting, and perhaps mycoparasites fungi. Interestingly, two rarely encountered species, *L. aphanocladii* and *P. flavescens*, appeared among the culturable endophytic community of *S. torvum*, highlighting their unique association compared to other Solanaceae species, such as *S. melongena*. These fungi appear to be specific to *S. torvum* seeds and show limited distribution in the broader environment. Only once *L. aphanocladii* was annotated on germinated maize kernels (Rijavec et al. 2007). Thus, *L. aphanocladii* strain IT45 (CBS 1515152) was microscopically and phylogenetically investigated in detail. The genus *Lecanicillium* including species like *L. lecanii*, is renowned for its role in biological controlling pests such as aphids, whiteflies, and thrips (Goettel et al. 2005, 2008). Its presence is relatively rare and predominantly isolated from environmental samples of soil/air (El-Kader El-Debaiky 2017; Pozdnyakova et al. 2019; Rijavec et al. 2007), or associated with spiders and insects (Gharsallah et al. 2020; Manfrino et al. 2017; Pečiulytė D, Kačergius 2012). Therefore, the isolation of *L. aphanocladii* from a Solanaceae species marks a novel discovery, particularly from a species resistant to soil pathogens such as *S. torvum*.

This study demonstrates the antagonistic behavior of *L. aphanocladii* IT45 against the pathogen *B. cinerea* IT48 and two important plant-parasitic and plant-associated nematodes, *M. incognita* and *B. eremus*. In dual culture assays, IT45 initially inhibited the radial growth of *B. cinerea* through the production of a red–purple pigment, presumably oosporein. Oosporein, a benzoquinone derivative (2,5-dihydroxybenzoquinone), is a known secondary metabolite with antifungal activity. Its presence suggests a biochemical mechanism contributing to the suppression of *B. cinerea* by IT45 (Cardoso et al. 2019; Ramesha et al.

2015; Taniguchi et al. 1984). However, following prolonged incubation *L. aphanocladii* IT45 progressively overgrew and ultimately replaced the pathogen's mycelium, indicating a possible antagonistic interaction. These findings support the potential use of *L. aphanocladii* IT45 as a biocontrol agent against *B. cinerea*. However, further investigations in greenhouse or field conditions are required to confirm its efficacy and stability.

Furthermore, our results demonstrate that conidia of *L. aphanocladii* IT45 exhibit antagonistic activity against the plant-parasitic nematode *M. incognita* through two complementary mechanisms. First, the conidia adhere to the nematode cuticle and likely damage it via enzymatic action (Li et al. 2010; Yang et al. 2005), as suggested by the known production of cuticle-degrading proteases in *Lecanicillium* species, similar to other nematophagous fungi (Li et al. 2010; Yang et al. 2005). Additionally, species of this genus have been reported to express enzymes involved in the degradation of xenobiotics and other complex molecules (Pozdnyakova et al. 2019), which may contribute to their nematoxic potential. Second, conidia were observed to physically trap juveniles, impairing their motility thus, a crucial factor for successful root infection. This dual mode of action suggests that IT45 may reduce nematode infectivity both by direct parasitism and mechanical immobilization.

In addition, *L. aphanocladii* IT45 exhibits similar antagonistic strategies against the fungivores *B. eremus*. Although *B. eremus* was able to grow on the fungal mycelium to some extent, its reproduction might also be limited by the activity of fungal conidia. These conidia were observed to penetrate through the genital openings of adult nematodes or to infiltrate the eggshells, ultimately leading to their complete digestion. Moreover, the conidia readily adhered to the nematode cuticle and effectively trapped individuals at their extremities, thereby immobilizing larvae within the mycelial network. Collectively, these results highlight the capacity of *L. aphanocladii* IT45 to interfere with nematode motility and reproduction, supporting its potential as a biocontrol agent against both plant-parasitic and fungivores nematodes.

P. flavescens IT53, identified in this study, has already been recognized for its antagonistic activity against a range of plant pathogens. Its efficacy in controlling *Fusarium* head blight (FHB) in wheat has been previously demonstrated, with reductions in disease severity observed under field conditions (Khan et al. 2004; Shude et al. 2022). Phylogenetic analyses have recently confirmed a close relationship between *P. flavescens* and *P. terrestris* (Palmieri et al. 2021). Furthermore, *P. terrestris* and *P. laurentii* have frequently been reported as effective biocontrol agents against several post-harvest and field pathogens (Lima et al. 1998). These strains have been shown to inhibit economically significant fungi such as *Penicillium expansum*, *B. cinerea*, *Rhizopus stolonifer*, *Aspergillus niger*, and *Monilia fructigena* on various fruits (Palmieri et al. 2021).

In the present work, *P. flavescens* IT53 effectively limited the development of *B. cinerea* in vitro. When the pathogenic fungus IT48 was grown on a lawn of *P. flavescens*, a significant reduction of approximately 60% in mycelial growth was observed. Moreover, IT53 also demonstrated notable antagonism against the root-knot nematode *Meloidogyne incognita*. In vitro*,* assays revealed that juveniles were rapidly immobilized, with conidia attached to their cuticle within 48 h. Overall, these results suggest that *P. flavescens* IT53 is a promising candidate for biological control applications, with dual activity against both fungal and nematode plant pathogens. Its potential utility in sustainable agriculture is further supported by its likely low environmental impact and minimal toxicological risk.

In this study, we investigated the culturable endophytic microbiota associated with seeds of *S. torvum* and *S. melongena*, revealing a diverse assemblage of yeast and fungal strains. Notably, certain isolates from *S. torvum* seeds exhibited unique antagonistic properties, underscoring the potential ecological and biotechnological relevance of seed-associated endophytes in Solanaceae. Among these, *L. aphanocladii* IT45 and *P. flavescens* IT53 demonstrated significant biocontrol potential, showing inhibitory effects against both plant-pathogenic fungi and parasitic nematodes in vitro. These findings highlight two key outcomes: (i) the identification of endophytic strains with dual antagonistic activity and (ii) the discovery of novel microbe-plant associations that may contribute to plant resilience. Such insights not only expand our understanding of the ecological roles of seed endophytes but also support their future application in sustainable agriculture as environmentally friendly biocontrol agents.

OR131325/OR131326 and OR131318, respectively. In GenBank also, the sequences of all the other isolates are deposited (accession number as reported in Supplementary Table 2). The time-lapse video is shown in Supplementary Video 1 https://doi.org/10.6084/m9.figshare.27878553.v1. All data and materials generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or no-financial interests to disclose.

References

Abdel-Azeem MA, El-Maradny YA, Othman AM, Abdel-Azeem AM (2021) Endophytic fungi as a source of new pharmaceutical biomolecules. In: Abdel-Azeem AN, Yadav NY, Sharma M (eds) Industrially important fungi for sustainable development bioprospecting for biomolecules, vol 2. Springer International Publishing, Cham, pp 115–151. https://doi.org/10.1007/978-3-030-85603-8_3

Abdelfattah A, Tack AJM, Lobato C, Wassermann B, Berg G (2023) From seed to seed: the role of microbial inheritance in the assembly of the plant microbiome. Trends Microbiol 31:346–355. https://doi.org/10.1016/j.tim.2022.10.009

Adeleke BS, Babalola OO (2021) The endosphere microbial communities, a great promise in agriculture. Int Microbiol 24:1–17. https://doi.org/10.1007/s10123-020-00140-2

Afridi MS, Kumar A, Javed MA, Dubey A, de Medeiros FHV, Santoyo G (2023) Harnessing root exudates for plant microbiome engineering and stress resistance in plants. Microbiol Res. https://doi.org/10.1016/j.micres.2023.127564

Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y (2002) Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochemistry 59:459–463. https://doi.org/10.1016/S0031-9422(01)00417-4

Bagnaresi P, Sala T, Irdani T, Scotto C, Lamontanara A, Beretta M, Rotino GL, Sestili S, Cattivelli L, Sabatini E (2013) *Solanum torvum* responses to the root-knot nematode *Meloidogyne incognita*. BMC Genomics. https://doi.org/10.1186/1471-2164-14-540

Barbosa QPS, Da Camara CAG, Silva TMS, Ramos CS (2012) Chemical constituents of essential oils from *solanum torvum* leaves, stems, fruits, and roots. Chem Nat Compd 48:698–699. https://doi.org/10.1007/s10600-012-0355-5

Berg G, Raaijmakers JM (2018) Saving seed microbiomes. ISME J 12:1167–1170. https://doi.org/10.1038/s41396-017-0028-2

Bletsos F, Thanassoulopoulos C, Roupakias D (2003) Effect of grafting on growth, yield, and verticillium wilt of eggplant. HortScience 38(2):183–186. https://doi.org/10.21273/hortsci.38.2.183

Cardoso MGB, Trento MVC, Reis CH, Marcussi S, Cardoso PG (2019) *Lecanicillium aphanocladii* : snake venom phospholipases A2 and proteases as tools to prospect enzymatic inhibitors. Lett Appl Microbiol 69:88–95. https://doi.org/10.1111/lam.13171

Carletti B, Ambrogioni L, Irdani T, Al E (2007) Morphometrics and molecular identification of some italian populations of Bursaphelenchus eremus Ruhm (Goodey) associated with quercus spp. Redia 90:3–21

Carletti B, Irdani T, Ambrogioni LA, Pennacchio FA, Francardi VA (2008) New occurrences of Bursaphelenchus species (nematoda parasitaphelenchidae) phoretic associate of bark beetles in Italy. Redia 91:67–71

Čermák V, Vieira P, Gaar V, Čudejková M, Foit J, Zouhar M, Douda O, Mota M (2013) On the genus *Bursaphelenchus* Fuchs, 1937 (Nematoda: Parasitaphelenchinae) associated with wood and insects from declining forest trees in the Czech Republic. For Pathol 43:306–316. https://doi.org/10.1111/efp.12031

Chiellini C, Maida I, Emiliani G, Mengoni A, Mocali S, Fabiani A, Biffi S, Maggini V, Gori L, Vannacci A, Gallo E, Firenzuoli F, Fani R (2014) Endophytic and rhizospheric bacterial communities isolated from the medicinal plants *Echinacea purpurea* and *Echinacea angustifolia*. Int Microbiol 17(3):165–174

Darriba D, Taboada GL, Doallo R, Posada D (2012) Jmodeltest 2: more models, new heuristics and parallel computing. Nat Methods 9:772. https://doi.org/10.1038/nmeth.2109

Diaz BM, Oggerin M, Lastra CCL, Rubio V, Fereres A (2009) Characterization and virulence of *Lecanicillium lecanii* against different aphid species. Biocontrol 54:825–835. https://doi.org/10.1007/s10526-009-9218-9

El-Kader El-Debaiky SA (2017) New record of *Lecanicillium aphanocladii* family: Cordycipitaceae from Egypt. J Bacteriol Mycol Open Access 5:430–432. https://doi.org/10.15406/jbmoa.2017.05.00161

Fernandez-San Millan A, Larraya L, Farran I, Ancin M, Veramendi J (2021) Successful biocontrol of major postharvest and soil-borne plant pathogenic fungi by antagonistic yeasts. Biol Control 160:104683. https://doi.org/10.1016/j.biocontrol.2021.104683

Gams W, Zare R (2003) A taxonomic review of the clavicipitaceous anamorphs parasitizing nematodes and other microinvertebrates. Clavicipitalean Fungi 18:26–81. https://doi.org/10.1201/9780203912706.pt1

Gharsallah H, Ksentini I, Naayma S, Hadj Taieb K, Abdelhedi N, Schuster C, Triki MA, Ksantini M, Leclerque A (2020) Identification of fungi in Tunisian olive orchards: Characterization and biological control potential. BMC Microbiol 20:1–13. https://doi.org/10.1186/s12866-020-01997-z

Gisbert, C., Prohens, J., and Nuez, F. 2011. Performance of eggplant grafted onto cultivated, wild, and hybrid materials of eggplant and tomato

Goettel MS, Koike M, Kim JJ, Aiuchi D, Shinya R, Brodeur J (2008) Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. J Invertebr Pathol 98:256–261. https://doi.org/10.1016/j.jip.2008.01.009

Goettel MS, Eilenberg J, Glare T (2005) 6.11 - Entomopathogenic Fungi and their Role in Regulation of Insect Populations. In: Science CMI (ed) Lawrence I Gilbert. Elsevier, Amsterdam, pp 361–405. https://doi.org/10.1016/B0-44-451924-6/00088-0

Gómez-Vidal S, Lopez-Llorca LV, Jansson H-B, Salinas J (2006) Endophytic colonization of date palm (*Phoenix dactylifera* L.) leaves by entomopathogenic fungi. Micron 37:624–632. https://doi.org/10.1016/j.micron.2006.02.003

Gousset C, Collonnier C, Mulya K, Mariska I, Rotino GL, Besse P, Servaes A, Sihachakr D (2005) *Solanum torvum*, as a useful source of resistance against bacterial and fungal diseases for improvement of eggplant (*S. melongena* L.). Plant Sci 168:319–327. https://doi.org/10.1016/j.plantsci.2004.07.034

Irdani T et al (2011) Low cryoprotectant concentrations and fast cooling for nematode cryostorage. Cryobiology 63:12–16. https://doi.org/10.1016/j.cryobiol.2011.04.003

Irdani T, Tarchi F, Cutino I, Sala T (2023) *Solanum torvum* mediates protection against the nematode *Meloidogyne incognita* in neighboring plants. J Plant Dis Prot. https://doi.org/10.1007/s41348-023-00778-w

Kannan K, Muthumary J (2012) Comparative analysis of endophytic mycobiota in different tissues of medicinal plants. Afr J Microbiol Res 6:4219–4225. https://doi.org/10.5897/AJMR11.530

Khan NI, Schisler DA, Boehm MJ, Lipps PE, Slininger PJ (2004) Field testing of antagonists of Fusarium head blight incited by *Gibberella zeae*. Biol Control 29:245–255

Lakshmi D, Prasanna N, Ponmurugan N (2013) In vitro interactions between *Solanum torvum* extracts and microbes. Acad J Plant Sci 6:47–51. https://doi.org/10.5829/idosi.ajps.2013.6.1.325

Li J, Yu L, Yang J, Dong L, Tian B, Yu Z, Liang L, Zhang Y, Wang X, Zhang K (2010) New insights into the evolution of subtilisin-like serine protease genes in Pezizomycotina. BMC Evol Biol 10:68. https://doi.org/10.1186/1471-2148-10-68

Lima G, Curtis FDE, Castoria R, Cicco VDE (1998) Activity of the yeasts *Cryptococcus laurentii* and *Rhodotorula glutinis* against post-harvest rots on different fruits. Biocontrol Sci Tech 8:257–267. https://doi.org/10.1080/09583159830324

Lopez-Llorca LV, Jansson HB, Vicente JG, Salinas J (2007) Nematophagous Fungi as Root Endophytes. Microbial Root Endophytes. https://doi.org/10.1007/3-540-33526-9_11

Lu Y-Y, Luo J-G, Kong L-Y (2011) Chemical constituents from *Solanum torvum*. Chin J Nat Med 9:30–32. https://doi.org/10.1016/S1875-5364(11)60015-0

Manfrino RG, González A, Barneche J, Tornesello Galván J, Hywell-Jones N, López Lastra CC (2017) Contribution to the knowledge of pathogenic fungi of spiders in Argentina. Southernmost record in the world. Rev Argent Microbiol 49:197–200. https://doi.org/10.1016/j.ram.2016.10.007

Muthezhilan R (2012) Fatty acid composition and antimicrobial activity of solanum torvum SW. J Modern Biotechnol 1:75–78

Nedveckytė I, Pečiulytė D, Būda V (2021) Fungi associated with horse-chestnut leaf miner moth *Cameraria ohridella* mortality. Forests 12:58. https://doi.org/10.3390/f12010058

Nishikawa J, Kobayashi T, Shirata K, Chibana T, Natsuaki KT (2006) Seedborne fungi detected on stored solanaceous berry seeds and their biological activities. J Gen Plant Pathol 72:305–313. https://doi.org/10.1007/s10327-006-0289-5

Palmieri D, Barone G, Cigliano RA, de Curtis F, Lima G, Castoria R, Ianiri G (2021) Complete genome sequence of the biocontrol yeast *Papiliotrema terrestris* strain LS28. G3 Genes Genomes Genet 11:0–4. https://doi.org/10.1093/g3journal/jkab332

Pečiulytė D, Kačergius A (2012) *Lecanicillium aphanocladii*—a new species to the mycoflora of lithu- ania and a new pathogen of tree leaves mining insects. Bot Lith 18:133–146

Pozdnyakova NN, Varese GC, Prigione V, Dubrovskaya EV, Balandina SA, Turkovskaya OV (2019) Degradative properties of two newly isolated strains of the ascomycetes *Fusarium oxysporum* and *Lecanicillium aphanocladii*. Int Microbiol 22:103–110. https://doi.org/10.1007/s10123-018-0032-z

Ramesha A, Venkataramana M, Nirmaladevi D, Gupta VK, Chandranayaka S, Srinivas C (2015) Cytotoxic effects of oosporein isolated from endophytic fungus Cochliobolus kusanoi. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00870

dos Reis JBA, Lorenzi AS, do Vale HMM (2022) Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips. Arch Microbiol 204:1–30. https://doi.org/10.1007/s00203-022-03283-0

Rijavec T, Lapanje A, Dermastia M, Rupnik M (2007) Isolation of bacterial endophytes from germinated maize kernels. Can J Microbiol 53(6):802–808. https://doi.org/10.1139/W07-048

Rotino GL, Perri E, Acciarri N, Sunseri F, Arpaia S (1997) Development of eggplant varietal resistance to insects and diseases via plant breeding. Adv Hortic Sci 11:193–201

Senizza B, Rocchetti G, Sinan KI, Zengin G, Mahomoodally MF, Glamocilja J, Sokovic M, Lobine D, Etienne OK, Lucini L (2021) The phenolic and alkaloid profiles of *Solanum erianthum* and *Solanum torvum* modulated their biological properties. Food Biosci. https://doi.org/10.1016/j.fbio.2021.100974

Senthil Kumar CM, Jacob TK, Devasahayam S, D'Silva S, Kumar NKK (2015) Isolation and characterization of a *Lecanicillium psalliotae* isolate infecting cardamom thrips (*Sciothrips cardamomi*) in India. Biocontrol 60:363–373. https://doi.org/10.1007/s10526-015-9649-4

Shude SPN, Mbili NC, Yobo KS (2022) Epiphytic yeasts as potential antagonists against Fusarium head blight of wheat (*Triticum aestivum* L.) caused by *Fusarium graminearum* sensu stricto. J Saudi Soc Agric Sci 21:404–411. https://doi.org/10.1016/j.jssas.2021.11.001

Singh PK, Gopalakrishnan TR (1997) Grafting for wilt resistance and productivity in brinjal (*Solanum melongena* L.). Hortic J 10:57–64

Singh BP, Abdel-Azeem AM, Gautam V, Singh G, Singh SK (2024). In: Singh BP, Abdel-Azeem AM, Gautam V, Singh G, Singh SK (eds) Endophytic Fungi. Springer, Cham. https://doi.org/10.1007/978-3-031-49112-2

Stiernagle T (2006) Maintenance of *C. elegans*. WormBook, ed. The C. elegans Research Community, 1–11. https://doi.org/10.1895/wormbook.1.101.1

Tamura K, Stecher G, Kumar S (2021) MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120

Taniguchi M, Kawaguchi T, Tanaka T, Oi S (1984) Antimicrobial and respiration-inhibitory activities of oosporein. Agric Biol Chem 48:1065–1067. https://doi.org/10.1080/00021369.1984.10866268

Thambugala KM, Daranagama DA, Phillips AJL, Kannangara SD, Promputtha I (2020) Fungi vs. fungi in biocontrol: an overview of fungal antagonists applied against fungal plant pathogens. Front Cell Infect Microbiol. https://doi.org/10.3389/fcimb.2020.604923

White TJ, Bruns T, Lee SJ, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. PCR Protoc Guide Methods Appl 18(1):315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Yang J, Huang X, Tian B, Wang M, Niu Q, Zhang K (2005) Isolation and characterization of a serine protease from the nematophagous fungus, *Lecanicillium psalliotae*, displaying nematicidal activity. Biotechnol Lett 27:1123–1128. https://doi.org/10.1007/s10529-005-8461-0

Yousaf Z, Wang Y, Baydoun E (2013) Phytochemistry and pharmacological studies on *Solanum torvum* Swartz. J Appl Pharm Sci 3:152–160. https://doi.org/10.7324/JAPS.2013.3428

Zare R, Gams W (2001) A revision of *Verticillium* section *Prostrata*. IV. The genera *Lecanicillium* and *Simplicillium* gen. nov.°). Nova Hedwigia 73:1–50. https://doi.org/10.1127/nova.hedwigia/73/2001/1

Zare R, Gams W (2008) A revision of the *Verticillium fungicola* species complex and its affinity with the genus *Lecanicillium*. Mycol Res 112:811–824. https://doi.org/10.1016/j.mycres.2008.01.019

Zhou BL, Chen ZX, Du L, Xie Y, Zhang Q, Ye X (2011) Allelopathy of root exudates from different resistant eggplants to Verticillium dahliae and the identification of allelochemicals. Afr J Biotech 10:8284–8290. https://doi.org/10.5897/AJB10.2300