Ability of endophytic fungi associated with *Withania somnifera* L. to control Fusarium Crown and Root Rot and to promote growth in tomato

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Abstract

Fusarium crown and root rot (FCRR), caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), is a soilborne tomato disease of increased importance worldwide. In this study, *Withania somnifera* was used as a potential source of biological control and growth-promoting agents. Seven fungal isolates naturally associated with *W. somnifera* were able to colonize tomato seedlings. They were applied as conidial suspensions or a cell-free culture filtrate. All isolates enhanced treated tomato growth parameters by 21.5–90.3% over FORL-free control and by 27.6–93.5% over pathogen-inoculated control. All tested isolates significantly decreased by 28.5–86.4% disease severity over FORL-inoculated control. The highest disease suppression, by 86.4–92.8% over control and by 81.3–88.8% over hymexazol-treated control, was achieved by the I6 isolate. FORL radial growth was suppressed by 58.5–82.3% versus control when dual cultured with tested isolates and by 61.8–83.2% using their cell-free culture filtrates. The most active agent was identified as *Fusarium* sp. I6 (MG835371), which displayed chitinolytic, proteolytic, and amylase activities. This has been the first report on the potential use of fungi naturally associated with *W. somnifera* for FCRR suppression and for tomato growth promotion. Further investigations are required in regard to mechanisms of action involved in disease suppression and plant growth promotion.

Keywords Antifungal activity · Associated fungi · *Fusarium oxysporum* f. sp. *radicis-lycopersici* · *W. somnifera* · Tomato growth

Introduction

Fusarium Crown and Root Rot (FCRR), caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), is a severe fungal disease of tomato (*Solanum lycopersicum* L.) [1, 2]. In Tunisia, it occurs both in open-field and greenhouse cropping systems where losses have been reported to reach 90% [3].

Disease management is complicated by the soilborne behavior of the causal agent, the possible aerial dissemination of its conidia developing on crown cankers [4] and the lack of

resistance [5]. Thus, FCRR is mainly managed based on cultural practices and chemical control [6]. However, research on more environmental friendly control methods, such as biological control, is needed [7].

The plant-associated microbiome plays an essential role in stabilizing the ecosystem and in promoting host growth [8]. Endophytic fungi, in particular, are able to colonize the internal plant tissues without inducing any pathogenic effects [9] and establish a mutualistic and symbiotic relationship with their hosts [10]. Furthermore, they are known as potent sources of secondary metabolites, which are bioactive in plant growth promotion and its protection against various biotic and abiotic stresses [11, 12].

In previous studies, bacterial endophytes belonging mainly to *Bacillus*, *Pseudomonas*, and *Achromobacter* genera have been explored as potent antagonists for FCRR biocontrol [13, 14]. However, few studies have been focused on the use of endophytic fungi for the management of this disease. For instance, the endophytic *Fusarium solani* was able to efficiently suppress the FCRR disease by inducing tomato resistance through ethylene and jasmonic acid signaling pathways [15],

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whereas *Fusarium equiseti* GF191 actively synthesized antifungal compounds [16].

Endophytic fungi are also involved in the promotion of plant growth [17]. This effect may be directly achieved through the release of bioactive compounds, such as phytohormones, through phosphorus and nitrogen mobilization [18], or indirectly by releasing enzymes able to degrade the pathogen cell wall [19]. For instance, *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 have promoted plant growth through the production of gibberellins [20], whereas the dark septate endophytic fungus has facilitated micronutrients (Fe, Mn, Zn) and macronutrients (N, K, P) uptake.

Recent studies have demonstrated that *Withania somnifera*, commonly known as Ashwagandha or Indian ginseng, harbors high biodiversity of endophytes able to produce a variety of bioactive compounds of medical and agriculture interests [21, 22]. For example, ethyl acetate, hexane, and methanol extracts of *Chaetomium globosum* EF18 have suppressed *Sclerotinia sclerotiorum* [22], whereas *Corynespora cassicola*, *F. solani*, and *Penicillium chrysogenum* have displayed antibacterial activity against various human pathogenic bacteria [22].

In addition, the endophytic fungus *Fusarium semitectum* has been able to produce silver nanoparticles (AgNps) with antimicrobial activity [23].

The current study aims to isolate *W. somnifera*-associated fungal endophytes and to evaluate their ability to suppress FCRR severity, to promote tomato growth, and to inhibit FORL in vitro growth.

Materials and methods

Pathogen inoculum production

The FORL isolate used in this study was provided by the Laboratory of Phytopathology of the Regional Research Center on Horticulture and Organic Agriculture at Chott-Mariem, Sousse, Tunisia. It was originally recovered from wilted tomato plants showing severe vascular brown discoloration associated with crown and root rots.

FORL was cultured on potato dextrose agar (PDA) medium supplemented with streptomycin sulfate (300 mg/L) and incubated at 25 °C for 5 days before use.

Inoculum production was initiated by suspending a mycelial plug (5 mm in diameter), cut from 5-day-old cultures in a potato dextrose broth (PDB) medium. After an incubation period of 5–7 days, under continuous shaking at 150 rpm, the suspension was filtered through sterile Whatman no. 1 and the conidial concentration was adjusted to 10^7 conidia/mL using a hemocytometer [24].

Seedling growth conditions

Tomato cv. Rio Grande seeds were soaked for 5 min in 70% (v/v) ethanol, for 20 min in 0.9% (v/v) sodium hypochlorite (NaOCl), and washed thrice with sterile distilled water (SDW) [25]. Afterward, the seeds were planted in alveolus trays (7 × 7 cm) containing sterilized peat TM (Floragard VertriebsGmbH für gartenbau, Oldenburg, Germany). Seedlings were grown until the two-true-leaf stage, under controlled conditions (24–26 °C, 12-h photoperiod, and 70% relative humidity), for about 28 days, and watered when needed.

W. somnifera plant sampling and fungal isolation

Healthy *W. somnifera* plants were collected from the Tunisian coastal area near littoral, Chott-Mariem (latitude 35° 56' 20.451" N, longitude E10° 33' 32.028") in November 2013. Leaves, stems, fruits, and flowers were washed under running tap water to remove any adhering dust and soil particles.

The samples were surface-sterilized according to Cao et al. [26] protocol. They were first soaked for 30 s in 70% (v/v) ethanol, for 2–3 min in 0.5% (v/v) sodium hypochlorite, for 2 min in a 70% (v/v) ethanol, and finally rinsed 2–3 times in SDW. Sterility checks were performed for each sample to ensure the efficiency of the disinfecting process. For these tests, 0.1 mL from the final rinsing water was spread on PDA plates. Cultures were incubated for 6 days and checked daily for any eventual growth of fungal colonies [27].

The disinfected segments were blotted dry on sterilized filter papers and sectioned using a sterile razor blade. Ten pieces were plated out in each plate containing a PDA medium and three plates were used per sample. Cultures were incubated at 25 °C and surveyed daily for any emerging fungal growth. Individually, growing fungal colonies were selected and subcultured onto new PDA plates at 25 °C. The collected isolates were purified using the single-spore isolation technique and stored at 4 °C or in 20% glycerol (v/v) at –20 °C.

Pure colonies were morphologically characterized for taxonomic purposes. Fungal isolates were divided into morphotypes, and one representative isolate from each morphotype was screened for endophytic colonization ability.

Preparation of conidial suspensions and cell-free culture filtrates of endophytic fungi

Conidia were harvested from 7-day-old cultures and suspended in 100 mL of a PDB medium. Liquid cultures were incubated at 25 °C for 12 days under continuous shaking at 150 rpm. They were filtered through Whatman no. 1 filter paper and the concentration of the conidial suspension was adjusted to 10^6 conidia/mL [28].

Fungal cultures, previously grown for 15 days at 28 °C in a PDB medium, were filtered through Whatman no. 1. filter

paper and centrifuged thrice at 10,000 rpm for 10 min. The obtained cell-free culture supernatants were subjected to microfiltration through millipore membrane filters (0.22 µm pore size) before use [29].

Assessment of endophytic colonization ability

For each individual fungal isolate, five tomato cv. Rio Grande seedlings (at the two-true leaf stage) were dipped for 30 min into 25 mL of the fungal conidial suspension [34] and controls were dipped in equal volume of SDW. Tomato seedlings were transplanted in individual pots (12.5 × 14.5 cm) containing commercialized peat. They were maintained for 60 days at 20–25 °C, 70–85% relative humidity, and for a 12-h photoperiod.

To ensure their ability to colonize tomato tissues, tested fungal isolates were recovered from tomato roots, crowns, and stems following the methodology described by Hallmann et al. [31]. Colonies exhibiting similar morphological traits as the wild-type were selected and considered as endophytes.

The colonization frequency (F) was calculated according to the Kumareson and Suryanarayanan [32] formula as follows:

$$FC (\text{in}\%) = \frac{\text{number of segments colonized by the tested fungus}}{\text{total number of segments plated}} \times 100.$$

Arcsine transformation was applied to the percentage data before performing statistical analysis.

Assessment of FCRR suppression ability

Conidial suspensions and cell-free culture filtrates of seven fungal isolates were tested for their ability to suppress the FCRR disease on tomato cv. Rio Grande under greenhouse conditions.

Tomato seedlings (at the two-true leaf stage) were transplanted into pots (12.5 × 14.5 cm) filled with commercialized peat. They were individually watered with 20 mL of a conidial suspension (10^6 conidia/mL), cell-free supernatant or SDW (control). For fungicide-treated control (FC), seedlings were drenched each with 20 mL of 30% hymexazol (active ingredient of the fungicide Tachigazole). Pathogen inoculation was carried out 7 days post-treatment as a substrate drench using 20 mL of FORL conidial suspension (10^7 conidia/mL) [16]. Uninoculated control seedlings were watered with 20 mL of SDW.

Seedlings were grown in a greenhouse at 20–25 °C, with 70–85% relative humidity and a 12-h photoperiod. Five replicates of one seedling each were used for each individual treatment. The whole experiment was repeated twice.

Disease severity, root length, shoot height, roots and shoot fresh weights, and FORL re-isolation frequency (percentage

of pathogen isolation from roots, collars, and stems on PDA medium) were assessed at 60 days post-inoculation with FORL.

FCRR severity was measured based on the extent, in centimeters (cm), of the brown vascular discoloration (collar area), and on the intensity of above and below ground symptoms according to a 0–3 scale, where 0 = no symptoms and 3 = dead seedlings [33].

The frequency of FORL re-isolation was calculated using the following formula:

$$IF (\%) = f / F \times 100$$

where f = number of fragments showing pathogen growing colonies and F = total number of fragments plated on a PDA medium.

Assessment of growth-promoting ability

Conidial suspensions or cell-free culture filtrates from tested fungal isolates were tested in vivo for their ability to enhance tomato growth.

Treatments were applied by dipping roots of a set of five tomato cv. Rio Grande seedlings (at two-true leaf stage) for 30 min into 20 mL of fungal conidial suspensions (10^6 conidia/mL) and another group into 20 mL cell-free filtrates [30]. Control plants were similarly challenged using SDW. Afterward, seedlings were transplanted into individual pots (12.5 × 14.5 cm) containing commercial peat and grown under greenhouse conditions as described above. They were regularly watered with tap water to avoid water stress. All treatments were replicated five times and the whole experiment was repeated twice. The root length, shoot height, and fresh weight of roots and shoots were assessed at 60-day post-treatment.

Assessment of in vitro antagonistic activity

The endophytic isolates were tested for their antagonistic activity against FORL using the dual culture technique. Two agar plugs (6 mm in diameter), one colonized by FORL (removed from a 5-day-old culture at 25 °C), and a second by the endophytic fungus (removed from a 7-day-old culture at 25 °C) were placed 2 cm apart on a PDA medium supplemented with streptomycin sulfate (300 mg/L) [34]. Control plates were inoculated with FORL plugs only. Three replications were used for each individual treatment. The whole experiment was repeated twice. Plates were incubated at 25 °C for 5–6 days. The mean diameter (cm) of the FORL colony was measured when pathogen reached the center of the control plates. The growth inhibition of FORL was calculated according to the following formula [35].

$$\text{Growth inhibition } (\%) = [(DC - DT) / DC] \times 100$$

where DC = mean FORL colony diameter in control plates; DT = mean FORL colony diameter in treated plates (dual culture).

Assessment of the *in vitro* antifungal activity of cell-free culture filtrates

Fungal isolates were grown on a PDB medium and incubated under continuous shaking at 150 rpm at 25 °C for 30 days [36].

A 2-mL sample of each liquid culture was centrifuged thrice at 10,000 rpm for 10 min. Collected cell-free supernatants were filtered through a syringe filter (0.22- μ m pore size). A PDB filtrate was considered as the control treatment. Filtrates were injected at the concentration of 10% (v/v) to Petri plates containing a molten PDA medium amended with streptomycin sulfate (300 mg/mL) (*w/v*). After medium solidification, three agar plugs (6 mm in diameter) containing FORL mycelia were placed equidistantly in each Petri plate. Three replicate plates were used for each individual treatment and the whole trial was repeated twice. Cultures were maintained at 25 °C for 5 days. FORL growth inhibition was calculated as described above.

Identification of the most active fungal isolates

The genomic DNA extraction of selected fungal isolates was performed using the DNA Mini Kit (Analytik Jena, Biometra, Germany) according to the manufacturer instructions. The ITS region was amplified by polymerase chain reaction (PCR) using two universal fungal primers: ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) [37]. The PCR reaction was performed in a total reaction volume of 25 μ l containing 5 μ l of buffer (5 $\times$), 2.5 μ l of dNTP (2 mM), 1.5 μ l of MgCl₂ (25 mM), 0.25 μ l Taq polymerase (5 U/ μ l), 2.5 μ l of each primer (6 μ M), 5.75 μ l of ultra-pure water, and 5 μ l of genomic DNA templates (10 ng).

The amplification program, performed in an Opticon II (Bio-Rad) thermal cycle, included an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min. Amplification ended in a final extension step of 7 min at 72 °C. The obtained PCR products were visualized through agarose gel electrophoresis (1%) stained with ethidium bromide, under UV light. Gene sequencing was carried out in a private laboratory (Biotools, Tunisia). ITS sequences were analyzed using Basic Local Alignment Search Tool (BLAST) through GenBank (<http://www.blast.ncbi.nlm.nih.gov/>).

Screening of enzymatic activity of selected endophytic isolates

The most effective fungi in suppressing FCRR disease and in promoting tomato growth were screened for their ability to produce the extracellular enzymes described below. All assays were carried out in triplicates.

Amylase activity

Amylase activity was tested by growing fungal isolates on a glucose yeast extract peptone agar medium amended with 0.2 g starch. After incubation at 25 °C for 4 days, the plates were flooded with 1% iodine in 2% potassium iodide and the formation of white zones around fungal colonies, induced by the digestion of starch added to the medium, indicated a positive reaction [38].

Lipolytic activity

For lipase activity, fungal isolates were grown on a peptone agar medium amended with sterilized tween 20 diluted at 1% (v/v). Plates were incubated at 25 °C for 3–7 days. The presence of a visible precipitate around the colony, due to the formation of calcium salts of the lauric acid released by the enzyme, indicated positive lipase activity [39].

Proteolytic activity

For protease activity, fungal agar plugs (3 mm in diameter) cut from 10-day-old cultures were spot inoculated on casein starch agar with 1% skimmed milk and incubated at 25 °C for 96 h. After incubation, the formation of clear halos around fungal colonies indicated positive proteolytic activity [40].

Chitinolytic activity

Chitinase activity was tested by inoculating fungal plugs on a chitin-based medium. Cultures were maintained at 25 $\pm$ 2 °C for 10 days. Isolates displaying chitinolytic activity were able to grow on the chitin medium [41].

Statistical analysis

Data were subjected to one-way analysis of variance using the Statistical Package for the Social Sciences (SPSS) software for Windows version 20.0. Each experiment was repeated twice. Data were analyzed according to a completely randomized design. The means were separated using LSD or Duncan multiple range tests (at *p* < 0.05).

Results

Diversity of endophytic fungi recovered from *W. somnifera*

The surface sterilization protocol was found to be effective as no growing colonies of epiphytic fungi were observed on the plates inoculated with the final rinsing water after 10 days of incubation at 25 °C on a PDA medium. Moreover, isolations from *W. somnifera* leaves, stems, flowers, and fruits recovered a total of 37 fungal isolates after 15 days of incubation at 25 °C. There was a difference in the isolation frequency of isolates depending on the plant parts used. In fact, 12 fungal isolates (32.43% of the total collected) were recovered from leaves as compared to 10 (27.03%), 9 (24.32%), and 6 (16.2%) isolated from fruits, stems, and flowers, respectively (Table 1).

Based on morphological traits, the isolates were affiliated to five genera, namely *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus*, and *Trichoderma*. Most of the fungal isolates recovered from *W. somnifera* belonged to the genera *Fusarium* (32.4%) and *Aspergillus* (21.6%) genera. The isolation frequencies of *Trichoderma*, *Penicillium*, and *Alternaria* were 18.9, 16.2, and 10.8%, respectively (Table 1).

Based on their morphological characteristics, the 37 isolates recovered from *W. somnifera* were grouped into seven distinct morphotypes. One representative isolate from each morphotype was used for the screening of endophytic colonization ability.

Endophytic ability of collected fungal isolates

The results indicated that all tomato seedlings inoculated with the seven tested isolates remained healthy until 60 days post-treatment. Thus, they were considered non-pathogenic and selected for further screenings.

Tomato colonization frequency, at 60 days post-treatment, varied significantly (at $p < 0.05$) depending on the treatment. The parameter ranged between 33.3 and 76.6%

for roots, between 50 and 83.3% for crowns, and between 60 and 96.6% for stems. The highest colonization frequency was on tomato plants inoculated with the I6 isolate, which was recovered at 96.6, 83.3, and 76.6% from roots, crowns, and stems, respectively. The lowest colonization frequencies (33.3–60%) were recorded for the I3 and I5 isolates (Table 2).

The seven isolates inoculated in tomato seedlings were successfully re-isolated onto the PDA medium and exhibited similar traits like the wild-types. Thus, they were considered as endophytes and selected for further screening of their ability to suppress the FCRR disease and to promote tomato growth.

Effects of selected endophytic isolates on disease severity

Disease-suppressive effects of conidia-based preparations

FCRR severity, at 60 days post-inoculation with FORL, varied significantly (at $p < 0.05$) depending on fungal treatment. Data given in Table 3 showed that all tested isolates decreased the leaf and root damage index by 28.5–92.8% relative to pathogen-inoculated and untreated control (IC). The treatment with an I6 conidia-based preparation was the most effective in suppressing disease severity by 92.8% over inoculated control (IC) and by 88.8% over FORL-inoculated and hymexazol-treated control (FC). The lowest disease-suppressive effect (28.5%) was noted on plants treated with I3 and I5 conidial suspensions.

All tested treatments significantly (at $p < 0.05$) lowered the extent of the vascular brown discoloration (from collar) by 9.7 to 86.4% over IC. Treatment with the I6 conidia-based preparation exhibited the highest ability to decrease this parameter by 86.4% over control. The lowest disease suppression effect (9.7%) was expressed by I5 conidial suspensions (Table 3).

The FORL re-isolation frequency on the PDA medium from roots, crowns, and stems varied upon tested treatment. The FORL re-isolation frequency was lowered by 33.3–46.6,

Table 1 Diversity of endophytic fungi recovered from *Withania somnifera* on PDA medium and their relative isolation frequency

Identification	Leaf		Stem		Flower		Fruit		N total (%)	F total (%)
	N	F (%)	N	F (%)	N	F (%)	N	F (%)		
<i>Fusarium</i>	5	13.51	3	8.11	1	2.70	3	8.11	12	32.4
<i>Alternaria</i>	2	5.41	1	2.70	1	2.70	0	0	4	10.8
<i>Penicillium</i>	2	5.41	1	2.70	1	2.70	2	5.41	6	16.2
<i>Aspergillus</i>	2	5.40	2	5.40	1	2.70	3	8.11	8	21.6
<i>Trichoderma</i>	1	2.70	2	5.41	2	5.40	2	5.41	7	18.9
N total	12	–	9	–	6	–	10	–	37	–
F total	–	32.43	–	24.32	–	16.2	–	27.03	–	100

N, number of isolates; F, isolation frequency (%)

Table 2 Re-isolation frequency (%) of endophytic fungal isolates from tomato cv. Rio Grande roots, crowns, and stems noted 60 days post-inoculation

Isolate	Genera	Roots	Crowns	Stems
NC	—	—	—	—
I1	<i>Alternaria</i>	63.33 b	73.33 b	83.33 b
I2	<i>Aspergillus</i>	63.33 b	73.33 b	83.33 b
I3	<i>Fusarium</i>	33.33 d	50.00 c	60.00 c
I4	<i>Aspergillus</i>	63.33 b	73.33 b	83.33 b
I5	<i>Penicillium</i>	33.33 d	50.00 c	60.00 c
I6	<i>Fusarium</i>	76.66 a	83.33 a	96.67 a
I7	<i>Trichoderma</i>	46.67 c	66.67 b	83.33 b

NC, untreated control; no isolation; I1, isolate from flowers; I2, I6, I4, isolates from leaves; I5, isolates from stems; and I3, I7, isolates from fruits. Values followed by the same letter are not significantly different according to the Duncan multiple range test (at $p < 0.05$)

40–60, and 44.8–96.5% for tomato roots, crowns, and stems, respectively, as compared to control (96.6–100%) (Table 3).

Disease-suppressive effects of cell-free culture filtrates

FCRR severity, at 60 days post-inoculation with FORL, varied significantly (at $p < 0.05$) depending on tested fungal treatment. The leaf and root damage index decreased by 35.7–92.8% versus control following treatment with the different cell-free filtrates. The highest FCRR-suppressive potential was expressed by the cell-free filtrate of the I6 isolate where disease severity was 92.8% lower than control. This treatment suppressed disease more efficient than hymexazol (88.8%) used as chemical control treatment (Table 3). Interestingly, I1, I4, and I7 cell-free filtrates suppressed disease severity by 71.4% compared to control (IC) and by 55.5% compared to hymexazol (FC).

Data presented in Table 3 revealed that the extent of the vascular brown discoloration was significantly (at $p < 0.05$) lowered by 24.2–86.4% compared to FORL-inoculated and untreated control (IC). The most efficient treatment was the I6 cell-free culture filtrate where the disease was suppressed by 86.4% relative to control. Also, treatment with I1, I4, and I7 cell-free filtrates led to a significant decrease in disease severity by 46.6–50.4% over control (IC) and by 27.6–32.8%, over hymexazol (FC).

The pathogen re-isolation frequency performed on the PDA medium for all tomato plants varied upon tested cell-free filtrates. In fact, the recorded decrease in this parameter following treatment ranged between 30–60, 43.3–70, and 44.8–96.5% for roots, crown, and stems, respectively, as compared to 96.6–100% for isolations made from control plants (Table 3).

Effects of selected endophytic isolates on growth of FORL-inoculated tomato plants

Growth-promoting effects of conidia-based preparations

Growth parameters of tomato plants (root length, plant height, root, and aerial part fresh weights), at 60 days post-inoculation, varied significantly (at $p < 0.05$) depending on the tested treatment. As illustrated in Table 4, all tested conidial suspensions enhanced root length by 44.4–80.1% over IC where the I6 treatment exhibited the highest growth-promoting effect (80.1%). Interestingly, all tested treatment enhanced this parameter by 11.8–39.5% over hymexazol.

As for the root fresh weight, data provided in Table 4 revealed a significant enhancement in this parameter following treatment where the highest increment (80.5% over control) was noted on plants treated with I6 conidial suspensions. It should be highlighted that all tested treatment improved this parameter by 8.5–47.5% relative to hymexazol. Importantly, the root fresh weight of tomato plants already inoculated with FORL and treated with I1, I2, I4, I6, and I7 conidial suspensions was 1.1–35.9% higher than that of pathogen-free control (NC).

The shoot height was enhanced following treatment by 33.1–86.5% versus control where the highest increment was induced by I6 conidial suspensions. Moreover, all tested conidial suspensions significantly improved this parameter by 10.2–54.4% over hymexazol-based treatment (FC). Furthermore, plants inoculated with FORL and treated with I1, I2, I4, I6, and I7 conidial suspensions were 9.7–29.7% taller than the NC.

Based on the shoot fresh weight, all tested biological treatment improved this parameter 36.1–85.8% over control with the I6 conidial suspension being the most efficient one. It should be also highlighted that all tested conidial suspensions enhanced the shoot fresh weight by 2.9–40.4% over hymexazol (Table 4).

Growth-promoting effects of cell-free culture filtrates

All measured growth parameters, at 60-day post-inoculation with FORL, varied significantly depending on tested treatment. As shown in Table 4, all tested cell-free culture filtrates improved the root length of FORL-inoculated and treated tomato seedlings by 45.4–90.9% over control (IC) with the I6 filtrate expressing the highest growth-promoting effect (90.9%). All tested treatment, except the I5 filtrate, improved the root extent by 7–26.1% over hymexazol (FC).

All tested cell-free culture filtrates promoted the root fresh weight over control. The recorded increments ranged between 27.6 and 87.6%, and the highest one was obtained on tomato plants treated with the I6 filtrate

Table 3 Effects of endophytic fungi recovered from *Withania somnifera* and their cell-free culture filtrates on *Fusarium* crown and root rot severity and pathogen re-isolation frequency, as compared to controls, noted 60 days post-inoculation

Leaf and root damage index (0–3)		Vascular browning extent (cm)	FORL isolation frequency (%)		
			Roots	Crowns	Stems
Conidia-based preparations					
NC	—	—	—	—	—
IC	2.8 ± 0.14 a	2.06 ± 0.09 a	10 ± 0.25 a	10 ± 0 a	9.66 ± 0.25 a
FC	1.8 ± 0.14 b (35.7)	1.50 ± 0.04 c (27.1)	7.66 ± 0.25 b (23.3)	5.0 ± 0 c (50)	4.66 ± 0.25 b (51.7)
I1	1.4 ± 0.21 c (50)	1.44 ± 0.05 c (30)	5.66 ± 0.14 d (43.3)	4.0 ± 0.3 d (60)	2.66 ± 0.14 c (72.4)
I2	1.4 ± 0.21 c (50)	1.50 ± 0.03 c (27.1)	5.66 ± 0.14 d (43.3)	4.66 ± 0.25 c (53.3)	2.66 ± 0.14 c (72.4)
I3	2.0 ± 0.17 b (28.5)	1.56 ± 0.05 c (24.2)	5.66 ± 0.14 d (43.3)	4.66 ± 0.25 c (53.3)	2.66 ± 0.14 c (72.4)
I4	0.8 ± 0.14 d (71.4)	1.04 ± 0.02 d (49.5)	5.33 ± 0.25 d (46.6)	4.0 ± 0 d (60)	2.66 ± 0.14 c (72.4)
I5	2.0 ± 0.17 b (28.5)	1.86 ± 0.12 b (9.7)	6.66 ± 0.25 c (33.3)	6.0 ± 0 b (40)	5.33 ± 0 b (44.8)
I6	0.2 ± 0.14 e (92.8)	0.28 ± 0.06 e (86.4)	5.33 ± 0.14 d (46.6)	4.0 ± 0 d (60)	0.33 ± 0.25 d (96.5)
I7	0.8 ± 0.14 d (71.4)	1.02 ± 0.01 d (50.4)	5.33 ± 0.25 d (46.6)	4.0 ± 0 d (60)	2.66 ± 0.25 c (72.4)
Cell-free culture filtrate					
NC	—	—	—	—	—
IC	2.8 ± 0.14 a	2.06 ± 0.09 a	10 ± 0 a	10 ± 0 a	9.66 ± 0.25 a
FC	1.8 ± 0.14 b (35.7)	1.52 ± 0.02 b (26.2)	7.66 ± 0.25 b (23.3)	5 ± 0 c (50)	5.33 ± 0.25 b (44.8)
I1	0.8 ± 0.14 c (71.4)	1.1 ± 0.03 c (46.6)	5.0 ± 0 e (50)	4.66 ± 0.25 cd (53.3)	3.66 ± 0.14 c (62)
I2	1.8 ± 0.14 b (35.7)	1.56 ± 0.05 b (24.2)	6.0 ± 0 d (40)	4.66 ± 0.25 cd (53.3)	3.66 ± 0.14 c (62)
I3	1.8 ± 0.21 b (35.7)	1.56 ± 0.02 b (24.2)	6.0 ± 0 d (40)	4.66 ± 0.25 cd (53.3)	3.66 ± 0.14 c (62)
I4	0.8 ± 0.21 c (71.4)	1.04 ± 0.05 c (49.5)	5.0 ± 0 e (50)	4.0 ± 0 d (60)	2.66 ± 0.14 c (72.4)
I5	1.8 ± 0.14 b (35.7)	1.52 ± 0.02 b (26.2)	7.0 ± 0 c (30)	5.66 ± 0.25 b (43.3)	5.33 ± 0 b (44.8)
I6	0.2 ± 0.14 c (92.8)	0.28 ± 0.01 d (86.4)	4.0 ± 0 f (60)	3.0 ± 0 e (70)	0.33 ± 0.25 d (96.5)
I7	0.8 ± 0.21 c (71.4)	1.02 ± 0.06 c (50.4)	5.0 ± 0 e (50)	4.0 ± 0 d (60)	2.66 ± 0.25 c (72.4)

NC, uninoculated and untreated. No disease symptoms observed on pathogen-free and untreated tomato plants; IC, inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) and untreated; FC, inoculated with FORL and treated with hymexazol-based fungicide; I1, isolate from flowers; I2, I6, I4, isolates from leaves; I5, isolates from stems; and I3, I7, isolates from fruits

FORL isolation was performed on PDA medium and the frequency was noted after 5 days of incubation at 25 °C. For each column, values followed by the same letter are not significantly different according to a Duncan multiple range test at $p < 0.05$

Values in parentheses indicate the percentage (in %) of decrease on disease severity parameters as compared to FORL-inoculated and untreated control (IC)

(Table 4). All tested filtrates, except that of the I5 isolate, increased the root fresh weight by 20.7–48.7% over hymexazol-treated control (FC), while the I6 and I7 filtrates promoted this parameter by 16.8–28.4% over NC.

All tested filtrates induced a decrease by 36.8–93.5% in the height of tomato shoots, as compared to the IC with the I6 filtrate being the most effective one, leading to a 20.4% increase in this parameter over NC. Moreover, all tested filtrates, except that of I5, enhanced this growth parameter by 9.3–30.9% over hymexazol control (FC) (Table 4).

The shoot fresh weight was also improved following treatment with the cell-free filtrates of tested isolates where the highest improvement (90.2%) was induced by the I6 isolate filtrate. More interestingly, all tested filtrates, except those from the I90 and I93 isolates, promoted this parameter in treated tomato plants by 8.5–46.5% over hymexazol (Table 4).

Effects of selected endophytic isolates on growth of pathogen-free tomato plants

Tested fungal isolates did not induce any phytotoxic symptoms when inoculated to tomato plants which remained healthy till 60 days post-inoculation. As they were found to be non-pathogenic, their conidial suspensions and their cell-free culture filtrates were further screened for their ability to promote growth on pathogen-free tomato plants.

All growth parameters (root length, root fresh weight, shoot height, and shoot fresh weight), noted 60-day post-treatment, varied significantly (at $p < 0.05$) depending on tested treatment.

Growth-promoting effects of conidia-based preparations

All tested treatment significantly improved the root length, the root fresh weight, the shoot height, and the shoot weight by

Table 4 Effects of conidia-based preparations and cell-free culture filtrates from endophytic fungi obtained from *Withania somnifera* on tomato cv. Rio Grande growth parameters recorded 60 days post-inoculation with *Fusarium oxysporum* f. sp. *radicis-lycopersici* as compared to controls

	Roots length (cm)	Roots fresh weight (g)	Shoots height (cm)	Shoots fresh weight (g)
Conidia-based preparations				
NC	30.5 ± 0.20 c	17.8 ± 0.28 c	37.1 ± 0.23 d	44.2 ± 0.43 d
IC	21.7 ± 0.45 e	13.4 ± 0.21 e	25.8 ± 0.14 g	31 ± 0.18 g
FC	28.0 ± 0.31 d (29.1)	16.4 ± 0.21 d (22.3)	31.1 ± 0.27 f (20.7)	41 ± 0.36 f (32.2)
I1	35.8 ± 0.27 b (65.2)	22.2 ± 0.14 b (65.6)	40.7 ± 0.38 c (57.7)	46.8 ± 0.14 c (50.9)
I2	35.8 ± 0.42 b (65.1)	22.2 ± 0.14 b (65.6)	41.16 ± 0.22 c (59.5)	47.2 ± 0.43 c (52.2)
I3	31.4 ± 0.24 c (45)	18 ± 0.17 c (34.3)	34.36 ± 0.28 e (33.1)	42.2 ± 0.43 ef (36.1)
I4	35.7 ± 0.13 b (64.7)	22 ± 0.17 b (64.1)	44 ± 0.29 b (70.5)	52 ± 0.36 b (67.7)
I5	31.3 ± 0.40 c (44.4)	17.8 ± 0.28 c (32.8)	34.42 ± 0.22 e (33.4)	42.8 ± 0.29 e (38)
I6	39.1 ± 0.29 a (80.1)	24.2 ± 0.28 a (80.5)	48.14 ± 0.13 a (86.5)	57.6 ± 0.39 a (85.8)
I7	36.2 ± 0.14 b (66.8)	21.8 ± 0.28 b (62.6)	44.22 ± 0.36 b (71.3)	51.2 ± 0.47 b (65.1)
Cell-free culture filtrates				
NC	36.5 ± 0.26 b	19 ± 0.14 c	41.7 ± 0.13 c	46.0 ± 0.43 d
IC	20.3 ± 0.20 g	13 ± 0.21 f	26.0 ± 0.14 e	30.8 ± 0.18 g
FC	30.7 ± 0.20 e (51.3)	16.4 ± 0.21 d (26.1)	38.4 ± 0.15 d (47.8)	40 ± 0.36 f (29.8)
I1	33.6 ± 0.30 d (65.1)	19.8 ± 0.17 c (52.3)	42.0 ± 0.18 c (61.6)	50.8 ± 0.14 c (64.9)
I2	33.2 ± 0.16 d (63.8)	20.2 ± 0.21 c (55.3)	42.32 ± 0.16 c (62.7)	52.0 ± 0.43 c (68.8)
I3	33.1 ± 0.23 d (63.1)	20.0 ± 0.14 c (53.8)	42.42 ± 0.03 c (63.1)	50.2 ± 0.43 c (62.9)
I4	34.6 ± 0.18 c (70.5)	20.0 ± 0.21 b (53.8)	44.66 ± 0.05 c (71.7)	54.0 ± 0.36 b (75.3)
I5	29.5 ± 0.43 f (45.4)	16.6 ± 0.14 e (27.6)	35.58 ± 0.05 d (36.8)	43.4 ± 0.29 e (40.9)
I6	38.8 ± 0.28 a (90.9)	24.4 ± 0.21 a (87.8)	50.32 ± 0.07 a (93.5)	58.6 ± 0.39 a (90.2)
I7	34.6 ± 0.43 c (70.3)	22.2 ± 0.14 b (70.7)	44.8 ± 0.16 b (72.3)	54.0 ± 0.47 b (75.3)

NC, uninoculated and untreated; IC, inoculated with *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) and untreated; FC, inoculated with FORL and treated with hymexazol-based fungicide; I1, isolate from flowers; I2, I6, I4, isolates from leaves; I5, isolates from stems; and I3, I7, isolates from fruits

For each column, values followed by the same letter are not significantly different according to a Duncan multiple range test at $p < 0.05$

Values in parentheses indicate the percentage (in %) of increment on tomato growth parameters as compared to the inoculated with FORL and untreated control (IC)

32.3–81.8, 25.5–84.3, 31–87.6, and 32–81%, respectively, over control (NC). Treatment with the I6 conidial suspension exhibited the highest growth-promoting potential by increasing these parameters by 81.8, 84.3, 87.6, and 81%, respectively (Table 5).

Growth-promoting effects of cell-free culture filtrates

The root length, the root fresh weight, the shoot height, and the shoot weight were significantly increased by 21.5–90.3, 25.6–87.1, 30.4–88.5, and 28.9–83.1%, respectively, over NC. Plants treated with the I6 cell-free filtrate exhibited the highest growth-promoting potential where these parameters were improved by 90.3, 87.1, 88.5, and 83.1%, respectively, over control.

Comparative antagonistic activity of selected endophytic isolates against FORL

The FORL colony diameter, noted after 5 days of incubation at 25 °C, varied significantly (at $p < 0.05$) depending on the tested biological treatment as compared to control (IC).

Pathogen growth was inhibited by 58.1–82.3% relative to control depending on tested treatment where the I6 isolate displayed the highest antagonistic activity (82.3%) (Fig. 1a). Interestingly, I1, I4, and I7 isolates suppressed FORL mycelial growth by 66.6–67.3% over control (Table 6).

Antifungal activity of cell-free culture filtrates of selected endophytic isolates toward FORL

FORL mycelial growth inhibition induced by the tested cell-free filtrates ranged between 61.8 and 83.2% (Table 6) with the highest antifungal potential expressed by the I6 cell-free filtrate. Interestingly, I1, I2, I4, and I7 filtrates also inhibited pathogen radial growth by 73.5% over control (Fig. 1b).

Screening of enzymatic activity of selected endophytic isolates

All isolates were found able to produce the chitinase enzyme while only I6 and I7 isolates had proteolytic activity. Among

Table 5 Comparative plant growth-promoting ability of conidia-based preparations and cell-free culture filtrates of endophytic fungal isolates recovered from *Withania somnifera* recorded on tomato cv. Rio Grande plants 60 days post-treatment

	Roots length (cm)	Roots fresh weight (g)	Shoots height (cm)	Shoots fresh weight (g)
Conidia-based preparations				
NC	13.9 ± 0.15 e	6.4 ± 0.21 d	17.4 ± 0.18 e	15.0 ± 0.32 e
I1	21.3 ± 0.15 c (52.3)	9.6 ± 0.21 b (50)	27.2 ± 0.12 c (56.1)	22.8 ± 0.17 c (52)
I2	21.4 ± 0.21 c (53.2)	10.0 ± 0.17 b (56.2)	26.8 ± 0.36 c (53.7)	22.6 ± 0.21 c (50.6)
I3	21.3 ± 0.18 c (52.8)	9.8 ± 0.17 b (53.1)	22.8 ± 0.34 d (31)	19.8 ± 0.21 d (32)
I4	22.5 ± 0.30 bc (61.6)	10.6 ± 0.21 ab (65.6)	27.2 ± 0.35 c (55.7)	24.6 ± 0.32 b (64)
I5	18.4 ± 0.27 d (32.3)	8.0 ± 0.14 c (25.5)	23.7 ± 0.34 d (35.8)	20.0 ± 0.17 d (33.3)
I6	25.3 ± 0.19 a (81.8)	11.8 ± 0.14 a (84.3)	32.7 ± 0.21 a (87.6)	27.2 ± 0.17 a (81.3)
I7	23.1 ± 0.17 b (65.7)	10.7 ± 0.21 b (67.9)	30.0 ± 0.26 b (72.1)	25.0 ± 0.21 b (66.6)
Cell-free culture filtrates				
NC	14.1 ± 0.28 e	7.8 ± 0.28 d	17.6 ± 0.28 e	16.6 ± 0.43 d
I1	24.3 ± 0.35 b (72.2)	12.8 ± 0.39 b (64.1)	30.4 ± 0.35 b (72.9)	26.6 ± 0.18 b (60.2)
I2	20.8 ± 0.27 c (47.4)	11.0 ± 0.28 c (41)	28.1 ± 0.27 c (59.4)	22.8 ± 0.11 c (37.3)
I3	20.9 ± 0.17 c (48.3)	11.4 ± 0.11 c (46.1)	23.0 ± 0.17 d (30.4)	23.0 ± 0.14 c (38.5)
I4	24.1 ± 0.14 b (71.2)	12.8 ± 0.21 b (64.1)	28.0 ± 0.14 c (58.8)	26.8 ± 0.17 b (61.4)
I5	17.1 ± 0.11 d (21.5)	9.8 ± 0.17 d (25.6)	23.1 ± 0.11 d (31.5)	21.4 ± 0.28 c (28.9)
I6	26.8 ± 0.21 a (90.3)	14.6 ± 0.14 a (87.1)	33.2 ± 0.10 a (88.5)	30.4 ± 0.14 a (83.1)
I7	24.1 ± 0.12 b (70.9)	13.0 ± 0.21 b (66.6)	30.5 ± 0.28 b (73.2)	28.0 ± 0.21 b (68.6)

NC, uninoculated and untreated; I1, isolate from flowers; I2, I6, I4, isolates from leaves; I5, isolates from stems; and I3, I7, isolates from fruits

For each column, values followed by the same letter are not significantly different according to a Duncan multiple range test at $p < 0.05$

Values in parentheses indicate the percentage (in %) of increment on tomato growth parameters as compared to the uninoculated and untreated control (NC)

the tested isolates, I1, I2, and I5 were lipase-producing isolates. Except for I2 and I5, the remaining isolates were amylase positive (Table 7).

Characterization of most active endophytic isolate

Isolate I6 was selected based on its ability to suppress pathogen in vitro growth and disease severity and its interesting growth-promoting potential on infected or disease-free plants. Its morphological identification was based on various macroscopic and microscopic features. Macroscopically, colonies of I6, with cottony aerial mycelium, showed a rapid growth (about 6 mm/day) on the PDA medium. Colonies are initially white becoming pink after 2 days of incubation. The plate reverse color is also pink. As for its micro-morphological traits, hyphae are septate and hyaline. Micro- and macroconidia are oval and septate. The size of macro- and microconidia was of about $13.2\text{--}36.3 \times 3\text{--}4.2$ and $4\text{--}11 \times 2\text{--}3.2$ μm , respectively (Fig. 2).

The BLAST analysis of the sequenced rDNA gene homology and the phylogenetic analysis based on the neighbor-joining (NJ) method with 1000 bootstrap sampling revealed that isolate I6 belonged to genus *Fusarium* with 100% of similarity with *Fusarium* sp. (MG835371) (Table 8).

Discussion

In modern agriculture, endophytic fungi have received considerable attention as they are considered as promising organisms due to their ability to produce novel and natural bioactive compounds [12]. In fact, previous investigations have reported that endophytic fungi could enhance the growth of various crops by solubilizing phosphate synthesizing phytohormones and controlling several phytopathogens [30, 42].

W. somnifera was qualified as being the most valued species regarding its greatest richness and diversity of beneficial endophytic fungi [20, 21]. In a previous work, we demonstrated the ability of aqueous and organic extracts from this species to suppress FORL growth. However, this study was more focused on the valorization of fungal agents naturally associated with *W. somnifera* as biocontrol and growth-promoting agents.

A total of 37 fungal isolates were recovered from *W. somnifera* leaves, stems, flowers, and fruits. The frequency of isolation seemed to be organ-specific where the highest richness in fungal species (32.4%) was noted in leaves. These results were in agreement with other findings indicating that the distribution of endophytic fungi was higher in leaves than in the other organs [43, 44]. Possible reasons for these differences could be attributed to the wide surface of leaves

Fig. 1 Inhibition of *Fusarium oxysporum* f. sp. *radicis-lycopersici* mycelial growth when dual cultured with *Fusarium* sp. (I6) recovered from *Withania somnifera* (a) or grown on PDA amended with 1 mL of I6 cell-free culture filtrates (b) noted after 5 days of incubation at 25 °C

which would facilitate the penetration of fungi and also because leaves were more rich in cellulose [45]. In contrast, Khan et al. [46] found that *W. somnifera* stems harbored the highest frequency of endophytic fungi since they were persistent as compared to the other organs.

Based on their morphological traits, fungal isolates recovered from *W. somnifera* belonged to five genera with *Fusarium* and *Aspergillus* being the most commonly recovered ones. Similarly, Qadri et al. [47] found that *Fusarium* species were the dominant taxa obtained from *W. somnifera*. *Fusarium* species have been also known by their capacity to endophytically colonize wild [48] and cultivated plants such as tomato [15].

Table 6 Comparative antifungal activity of endophytic fungal isolates recovered from *Withania somnifera* and their cell-free culture filtrates toward *Fusarium oxysporum* f. sp. *radicis-lycopersici* noted after 5 days of incubation at 25 °C compared to control

Fungal treatments	FORL colony diameter (cm)	
	Dual culture	Cell-free culture filtrates
IC	4.9 ± 0.01 a	5.2 ± 0.01 a
I1	1.6 ± 0.05 c (67.3)	1.4 ± 0.01 c (73.5)
I2	1.9 ± 0.03 b (59.5)	1.4 ± 0.03 c (73.5)
I3	2.0 ± 0.03 b (58.5)	2.0 ± 0.03 b (61.8)
I4	1.6 ± 0.02 c (67)	1.4 ± 0.02 c (73.5)
I5	2.5 ± 0.01 b (58.1)	1.9 ± 0.01 b (62.7)
I6	0.8 ± 0.02 d (82.3)	0.8 ± 0.02 d (83.2)
I7	1.6 ± 0.03 c (66.6)	1.4 ± 0.03 c (73.5)

IC, untreated control; I1, isolate from flowers; I2, I6, I4, isolates from leaves; I5, isolates from stems; and I3, I7, isolates from fruits

FORL isolation was performed on PDA medium and the frequency was noted after 5 days of incubation at 25 °C. For each column, values followed by the same letter are not significantly different according to Duncan multiple range test at $p < 0.05$

Values in parentheses indicate the percentage (in %) of the mycelial growth inhibition of *Fusarium oxysporum* f. sp. *radicis-lycopersici* as compared to the untreated control

In the present study, conidia-based preparations or cell-free culture filtrates of seven fungal isolates recovered from *W. somnifera* were shown effective in suppressing the FCRR disease. Isolate I6 was selected as it exhibited not only the highest disease-suppressive potential but also the most growth-promoting effect on tomato plants inoculated or not with FORL. This fungal isolate was identified based on rDNA sequencing as *Fusarium* sp. (I6). In fact, endophytic fungi have been investigated as potential sources of biologically active compounds useful for agricultural applications [21, 30]. Endophytic *F. solani* and *F. equiseti*, recovered from healthy tomato root tissues, were shown able to colonize root tissues of cultivated tomato seedlings and to protect them from FORL infections [15, 16]. Interestingly, endophytic

Table 7 Enzymatic activity displayed by the endophytic fungi recovered from *Withania somnifera*

Isolate	Amylase	Lipase	Protease	Chitinase
I1	+	+	–	+
I2	–	+	–	+
I3	+	–	–	+
I4	+	–	–	+
I5	–	+	–	+
I6	+	–	+	+
I7	+	–	+	+

+, presence of enzymatic activity; –, absence of enzymatic activity

Fig. 2 Macroscopic and microscopic features of the most bioactive endophytic isolate (I6) recovered from *Withania somnifera* and grown on PDA medium for 7 days at 25 °C

Fusarium species have been investigated as potential producers of antimicrobial secondary metabolites. For instance, Ratnaweera et al. [49] proved that *Fusarium* sp., naturally associated with *Opuntia dillenii*, was able to produce an antimicrobial metabolite named equisetin. *F. solani*, recovered from the roots of *Coscinium fenestratum*, was capable of producing an antimicrobial substance named berberine [50]. Sanguinarine is an antimicrobial benzyloquinoline alkaloid which was released by *F. proliferatum*, an endophyte inhabiting *Macleaya cordata* leaves [51].

In the current study, the ability of conidia-based preparations and cell-free culture filtrates from the tested endophytic fungi to promote tomato growth was clearly demonstrated on pathogen-free and pathogen-inoculated plants with I6 (*Fusarium* sp.) being the most active agent. Fungal endophytes may promote the growth of their host either directly by the synthesis of growth regulators phytohormones [52], siderophores, phosphate solubilization, nitrogen fixation, or indirectly through the suppression of pathogenic agents [53]. For example, Khan et al. [52] demonstrated that *F. tricinctum*, recovered from *Solanum nigrum* leaves, was a great producer of the well-characterized plant growth regulator indole-3-acetic acid. Plant growth promotion conferred by *Fusarium* sp. colonization has been also previously reported in Saldajeno and Hyakumachi [54] study. Yong et al. [55] demonstrated that endophytic *Fusarium* spp. E4 and E5 could induce diterpene and triterpene production in *Euphorbia pekinensis* leading to improved plant growth and an increase in its terpenoid contents. *F. equiseti* was also shown to be able to colonize various hosts such as *Cucumis sativus* [56] and *Pisum sativum* L. roots [57]. The present study has been, to the best of our knowledge, the first report demonstrating the possible exploration and valorization of some *W. somnifera*-

associated fungi, like *Fusarium* sp. (I6), as growth-promoting agents or “biofertilizers.”

Screened using the dual culture method, conidial suspensions of the tested fungal isolates displayed significant antifungal activity against FORL where the most active agent was isolate I6 identified as *Fusarium* sp.. This endophytic fungus was proved to be able to produce extracellular cell wall-degrading enzymes such as protease, chitinase, and amylase, which could explain the antifungal activity registered toward FORL growth. In this regard, previous findings showed the ability of endophytic *Fusarium* species such as *F. oxysporum* (Ci16), *F. solani* (Ci24), *F. chlamydosporum*, and *Fusarium* sp. to synthesize extracellular hydrolases including chitinases, cellulases, β -1,3-glucanases, proteases, or lipases, which were involved in the biocontrol mechanism against phytopathogens [58]. Such hydrolases could lyse fungal cells and consequently suppress their growth.

Our results revealed that extracellular metabolites present in the cell-free culture filtrate of endophytic fungi, applied at 10% (v/v), were also found effective in suppressing FORL in vitro growth, with the filtrates of *Fusarium* sp. (I6), showing the highest decrease (by 83.2%) in pathogen mycelial growth. Previous reports demonstrated that *Fusarium* were widely reported as producers of various antifungal metabolites [59, 60]. For instance, the endophytic *Fusarium* sp. isolated from the *Taxus baccata* bark was able to produce an antifungal metabolite identified as hydrocarbon which strongly inhibited *F. oxysporum*, *Aspergillus niger*, and *Rhizopus stolonifera* [61]. Additionally, two polyhydroxysterols were produced by *F. solani*, an endophytic fungus from *Chloranthus multistachys* which significantly reduced the mycelial growth of *Penicillium digitatum*, *Alternaria brassicae*, and *Botrytis cinerea* [62].

Table 8 Identification of the most bioactive endophytic isolate (I6) by 16S rDNA sequencing gene

Isolate	Accession number	Most related species	Sequence homology (%)
I6	MG835371	Uncultured <i>Fusarium</i> sp. C1206	100

I6, fungal isolate recovered from surface-sterilized *Withania somnifera* leaves

Conclusion

Considering their diverse distribution and association, fungal endophytes might benefit their plant hosts through the synthesis of natural bioactive secondary metabolites which could serve as great promise in sustainable agriculture. To the best of our knowledge, the present paper is a first report which highlights the exploration of the wild *Solanaceae* *W. somnifera* as a promising source of isolation of endophytic fungal agents with antifungal potential against FORL. In this study, we have clearly demonstrated that there are diverse endophytic fungi harboring the different *W. somnifera* tissues. Conidial suspensions and cell-free culture sequencing data have been the most effective treatments in suppressing FCRR and in promoting tomato growth. Our study suggests that *W. somnifera* constitutes an interesting source of isolation of potent endophytic fungal isolates with FCRR suppression and biofertilizing abilities. Further studies are required to characterize and identify the bioactive compounds involved in biocontrol effect and growth promotion.

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Compliance with ethical standards

All the experiments undertaken in this study comply with the current law of the country where they were performed.

Conflict of interest The authors declare that they have no conflicts of interest.

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