Shahid Bahonar University of Kerman

Journal of Advances in Plant Protection

JAPP 2023

Association of Iranian Plant Protection Societies

Evaluation of endophytic fungi as biological control agents of broomrape (*Phelipanche aegyptiaca* Pers.) in tomato (*Solanum lycopersicum* L.) cv. Karoon

Elahe Mohammadi[1], Samad Jamali[1]✉, Bhagirath S. Chauhan[2], Rouhallah Sharifi[1]

1 Department of Plant Protection, College of Agriculture, Razi University, Kermanshah, Iran.

2 Queensland Alliance for Agriculture and Food Innovation (QAAFI) and School of Agriculture and Food Sciences (SAFS), The University of Queensland, Gatton, Queensland 4343, Australia.

✉ Corresponding authors: s.jamali@razi.ac.i

Article Info.

Article type:
Original article

Article history:
Received 09 Jun. 2026
Received in revised form 21 Jun. 2026
Accepted 26 Jun. 2026
Available Online 27 Jun. 2026

Keywords:
Biological control, Broomrape management, Kermanshah, Mycoherbicide, Phelipanche.

Abstract

Egyptian broomrape (*Phelipanche aegyptiaca* Pers.) is a devastating root-parasitic weed that poses a severe threat to global tomato production, particularly within the arid and semi-arid agroecosystems of Iran. Due to the parasite's subterranean life cycle, conventional chemical and mechanical control strategies are limited in efficacy. This study evaluated the biocontrol potential and plant-growth-promoting efficacy of eighteen indigenous endophytic fungal isolates on tomato (*Solanum lycopersicum* L.) cv. 'Karoon' is infested with *P. aegyptiaca* under controlled greenhouse conditions. Fungal inoculation was performed utilizing colonized sterile wheat seed matrices arranged in a completely randomized design. Comprehensive morphological and developmental traits encompassing root/shoot fresh and dry weights, and root length were systematically analyzed. The quantitative data revealed that select endophytic mutualists significantly mitigated parasitic stress and augmented host tolerance. Specifically, the endophytic fungus *Epicoccum nigrum* significantly increased root fresh weight compared to the infested control. *Trichoderma viride* exhibited the highest shoot fresh weight, while *Microdochium bolleyi* enhanced shoot dry weight, both reaching levels comparable to the uninfested control. *Ascotricha funiculosa* exhibited the highest root dry weight, while *Fusarium tricinctum* and *Ascotricha chartarum* produced the greatest root length, representing up to 57% increase compared to the infested control. These findings underscore that targeted endophytic fungi can serve as pivotal components in integrated pest management frameworks aimed at suppressing broomrape infestations.

Cite this article: Mohammadi, E., Jamali, S., Chauhan, B. S., & Sharifi, R. (2026). Evaluation of endophytic fungi as biological control agents of broomrape (*Phelipanche aegyptiaca* Pers.) in tomato (*Solanum lycopersicum* L.) cv. Karoon. *Journal of Advances in Plant Protection*, *3*(1), 41–52.

Publisher: Shahid Bahonar University of Kerman

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically vital vegetable crops globally. According to recent FAO statistics, annual global tomato production exceeds 188 million tons (FAOSTAT, 2024). In Iran, tomato cultivation occupies approximately 68,000 hectares, yielding 2.3 million tons; however, both cultivated area and total yield have declined significantly over the past decade from peak levels of 158,000 ha (FAOSTAT, 2024). Iran ranks seventh in average tomato production (3.4 million tons) after China (40 million tons), India, the United States, Turkey, Egypt, and Italy (FAOSTAT, 2024).

A primary driver of this agricultural constriction is the pervasive infestation of root-parasitic weeds belonging to the Orobanchaceae family, specifically broomrapes (*Orobanche* and *Phelipanche* species) (Nosratti et al., 2020). Within these regions, Egyptian broomrape (*Phelipanche aegyptiaca* Pers.) induces severe phytotoxic constraints, aggressively penetrating host roots *via* specialized evolutionary structures known as

haustoria to draw photoassimilates, water, and essential minerals (Mamnooi, 2017). This parasitic interaction triggers profound metabolic imbalances, culminating in systemic host degradation and crop yield liquidations ranging from 30% to total crop failure in highly susceptible cultivars.

Traditional mitigation methodologies encompass mechanical extraction, crop rotations, chemical soil fumigation, and synthetic herbicides. For instance, split post-emergence applications of sulfosulfuron (75% WG) at 30 g/ha have been utilized to suppress parasite biomass (Modares Najafabadi & Minbashi Moeini, 2017). Similarly, integrated chemical programs combining nitrogen fertilizers like ammonium sulfate with low-dose glyphosate applications have been deployed to minimize broomrape density (Bazobandi et al., 2015). Cultural practices, such as pot intercropping with leguminous species (*Phaseolus vulgaris*), have also demonstrated potential in reducing broomrape branching (Baneshi et al., 2016). However, these strategies frequently fall short due to the microscopic proportions, high persistence, and prolonged dormancy of the subterranean seed bank, alongside risks of host phytotoxicity (Nosratti et al., 2020).

Consequently, research attention has shifted toward identifying sustainable biological alternatives (Habimana et al., 2013). Microorganisms, particularly root-associated endophytic fungi which colonize internal plant tissues without instigating pathology, offer a dual advantage. These microbes mitigate parasitic stress by synthesizing herbicidal phytotoxins, degrading host-derived germination stimulants (e.g., strigolactones) and reinforcing host defensive mechanisms through systemic induced resistance (SIR) (Chen et al., 2018).

Among endophytic microorganisms, dark septate endophytes (DSE) represent a highly important group that form distinct dark-pigmented and septate hyphal structures within plant roots and play multiple beneficial roles (Shadmani et al., 2021 a, b). These fungi, mainly belonging to the phylum Ascomycota, not only suppress plant pathogens and promote growth but also play an essential role in enhancing plant tolerance to abiotic stresses such as heavy metals (Barkhordari et al., 2024). By colonizing roots, DSE fungi help the host plant cope with stress through mechanisms such as increasing chlorophyll content, photosynthetic rate, production of plant hormones, and inducing defense mechanisms (Barkhordari et al., 2024). However, despite the high potential of this group of fungi in the biological control of pathogens and abiotic stresses, few studies have addressed their direct role in suppressing root parasitic weeds such as broomrape. Therefore, in this study, using various endophytic fungal isolates (including DSE and non-DSE strains), we evaluated their ability to reduce damage caused by Egyptian broomrape and to improve the growth of tomato cv. Karoon.

Understanding how these fungal mutualists interact with specific commercial cultivars, such as Karoon, is essential to optimizing targeted biocontrol applications. This investigation determines the effects of eighteen distinct endophytic fungal isolates on the phenotypic development and root architectural resilience of tomato cultivar 'Karoon' subjected to *P. aegyptiaca* parasitism.

Material and Methods

Endophytic fungi

A total of 18 fungal isolates, including both dark septate endophytes (DSE) and non-DSE, were obtained from various sources (the in-house Razi University fungal culture collection and previous published studies) and used in this study. The complete list of isolates, their species identification, accession numbers, and DSE/Non-DSE status is provided in Table 1.

Parasite identification and inoculum preparation

The parasitic agent was harvested from infested tomato fields in Sarab Niloofar (47°01′58.75″ E; 34°22′04.91″ N) and Mahi Dasht (46°49′48.06″ E; 34°16′35.16″ N) in Kermanshah Province, Iran. Pure *P. aegyptiaca* seeds were separated using a 200-mesh sieve. The internal transcribed spacer (ITS) region of the ribosomal DNA was amplified using ITS1 (5′-TCCTCCGCTTATTGATATGC-3′) (and ITS7A (5′-GGAAGGAGAAGTCGTAACAAGG-3′) primers (White et al., 1990; Blattner, 1990) and sequenced for genetic validation (GenBank accession numbers: ON738595 and ON738596). Fungal endophyte inocula were produced by incubating mycelial plugs on sterile autoclaved wheat grains at 25 °C for 14 days to achieve dense colonization.

Greenhouse bioassays

The trial was conducted under controlled greenhouse conditions organized in a completely randomized design (CRD) with three replications. Potting soil was sterilized at 121 °C (15 psi) for 45 minutes. Each pot was filled with 2.5 kg of sterilized soil (Texture: 21.8% sand, 38.2% clay, 40% silt; pH: 7.94; EC: 2.5 dS/m; Field Capacity: 38.4%). Pots were infested with a standardized concentration of 0.05 g of *P. aegyptiaca* seeds alongside 10–15 g of the respective colonized

wheat matrix (containing the endophyte). Seedlings of *S. lycopersicum* cv. 'Karoon' were transplanted into the pots (thinned to one uniform plant per pot). To maintain uniform carrier conditions across all treatments, 10–15 g of sterile (non-colonized) wheat matrix was also added to all control pots. Accordingly, the negative control (healthy plants without parasite or endophyte) received only the sterile wheat matrix, whereas the positive control (plants challenged with *P. aegyptiaca* alone) received both 0.05 g of *P. aegyptiaca* seeds and the sterile wheat matrix (without the endophyte). Irrigation was maintained at field capacity, and fertilization with micronutrients was applied every three days.

Table 1. Endophytic fungi utilized for the suppression of broomrape (*Phelipanche aegyptiaca*) in tomato (*Solanum lycopersicum* L.) cv. Karoon

Species	Isolate	DSE/Non-DSE	Accession numbers	References
Ascotricha chartarum	RU-AsCh	Non-DSE	MW916314	Jamali (2021a)
Microdochium bolleyi	RU-MiBo	DSE	KX343031	Shadmani et al., (2021a)
Rhodotorula toruloides	RU-RoTo	Non-DSE	KP324973	Razi University fungal culture collection
Alternaria sp.	TC-26-6	DSE	KX061184	Shadmani et al., (2021b)
Fusarium tricinctum	RU-FuTr	Non-DSE	KX343029	Shadmani et al., (2021b)
Alternaria sp.	TBL-33	DSE	KX061186	Shadmani et al., (2021b)
Chaetomium elatum	RU-ChEl	DSE	MK459302	Mehrmoradi et al., (2020)
Beauveria bassiana	RU-BeBa	Non-DSE	MK651117	Mehrmoradi et al., (2020)
Alternaria sp.	TW-24-2	DSE	KX061191	Shadmani et al., (2021)
Trichothecium roseum	RU-TrRo	Non-DSE	-*	Razi University fungal culture collection
Trichoderma koningii	RU-TrKo	Non-DSE	-	Razi University fungal culture collection
Trichoderma koningiopsis	RU-TrKon	Non-DSE		Razi University fungal culture collection
Trichoderma atroviride	RU-TrAt	Non-DSE	-	Razi University fungal culture collection
Clonostachys rosea	RU-ClRo	Non-DSE	-	Shadmani et al., (2021)
Epicoccum nigrum	RU-EpNi	Non-DSE	-	Razi University fungal culture collection
Trichoderma viride	RU-TrVi	Non-DSE	-	Razi University fungal culture collection
Trichoderma aeruginosum	RU-TrAe	Non-DSE	-	Razi University fungal culture collection
Ascotricha funiculosa	RU-AsFu	Non-DSE	OK324153	Jamali (2021b)

DSE: Dark septate endophytes; Non-DSE: Non-dark septate endophytes.*Isolates obtained from the in-house Razi University fungal culture collection have not been deposited in NCBI GenBank; therefore, accession numbers are indicated by dashes (—).

Growth and morphological parameters

Plants were harvested 70 days post-transplantation. Shoot fresh weight (SFW) and root fresh weight (RFW) were recorded immediately. Shoot dry weight (SDW) and root dry weight (RDW) were determined after oven-drying at 70 °C for 48 hours until a constant mass was achieved. Root length (RL) was quantified using a digital caliper.

Statistical analysis

Data normality was verified *via* the Shapiro–Wilk test. A one-way analysis of variance (ANOVA) was executed using JMP statistical software. Mean comparisons were performed using Duncan's Multiple Range Test at a significance level of alpha = 0.05.

Results

Soil physicochemical properties

The detailed physicochemical properties of the experimental soil substrate are presented in Table 2. The soil texture was classified as clay loam (21.8% sand, 38.2% clay, 40% silt) with a saturated paste pH of 7.95

and electrical conductivity of 2.5 dS/m. Field capacity was 38.4%, and soil organic carbon content was 0.99%.

Table 2. Soil physicochemical properties

Soil Property	Unit	Value
Sand	%	21.8
Clay	%	38.2
Silt	%	40
Electrical conductivity	ds/m	2.5
Calcium carbonate	%	25
Sodium (Na)	mg/kg	0.66
Saturated paste pH	_	7.95
Soil organic carbon	%	0.99
Soluble calcium	mg/kg	15
Magnesium	mg/kg	3.5
Phosphorus	mg/kg	25
Ca/Mg	mg/kg	5
Bulk density	g/cm^3	1.27
Saturation moisture	%	52
Field capacity	%	38.4
Permanent wilting point	%	23.3

Effect of endophytic fungi on shoot fresh weight

Shoot fresh weight (SFW) varied significantly among treatments ($P < 0.05$, Fig. 1). The uninfested control (healthy plants) showed an SFW of 37.66 ± 2.81 g. Infestation with *P. aegyptiaca* drastically reduced SFW to 3.83 ± 0.99 g. Among fungal treatments, *Trichoderma viride* produced the highest SFW (43.43 ± 7.78 g), which was statistically similar to the uninfested control. *Microdochium bolleyi* also restored SFW to 37.66 ± 11.92 g, while *Fusarium tricinctum* reached 32.43 ± 5.42 g. In contrast, *Rhodotorula toruloides* (9.16 ± 3.19 g) and *Alternaria* sp. (TC-26-6) (12.6 ± 3.09 g) performed poorly, not differing significantly from the infested control (Table 3).

Effect of endophytic fungi on shoot dry weight

Shoot dry weight (SDW) followed a similar pattern (Fig. 2). The uninfested control had an SDW of 11.6 ± 0.87 g, while the infested control dropped to 4.76 ± 1.58 g (59.0% reduction). *Microdochium bolleyi* exhibited the highest SDW (12.66 ± 1.48 g), surpassing even the uninfested control. *Trichoderma viride* (10.36 ± 0.89 g) and *Fusarium tricinctum* (8.33 ± 1.19 g) also performed well. Several isolates, including *Rhodotorula toruloides* (4.4 ± 1.0 g) and *Alternaria* sp. (TC-26-6) (4.4 ± 0.1 g), were statistically indistinguishable from the infested control (Table 3).

Effect of endophytic fungi on root fresh weight

Root fresh weight (RFW) was severely suppressed by *P. aegyptiaca* (Fig. 3). The infested control yielded only 1.76 ± 1.12 g, compared to 11.73 ± 0.81 g in the uninfested control. *Epicoccum nigrum* produced the highest RFW (22.3 ± 7.8 g). *Microdochium bolleyi* (16.96 ± 5.7 g) and *Trichoderma viride* (9.36 ± 1.12 g) also significantly exceeded the uninfested control. In contrast, *Rhodotorula toruloides* (2.2 ± 0.7 g) and *Alternaria* sp. (TC-26-6) (2.83 ± 0.36 g) showed no improvement over the infested control (Table 3).

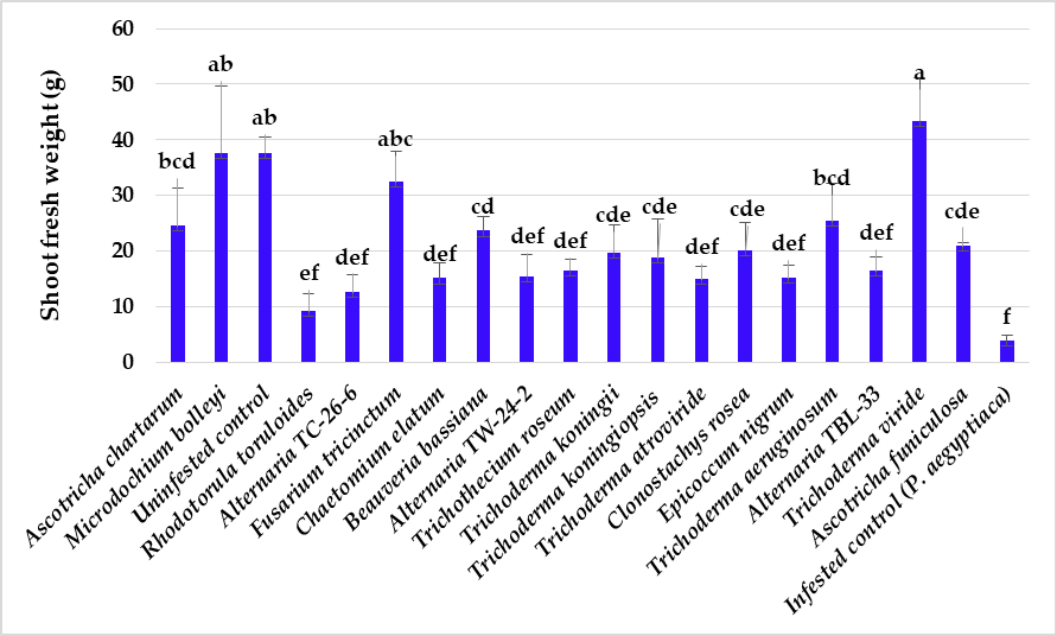

Fig. 1. Effect of endophytic fungal isolates on shoot fresh weight (g) of tomato (*Solanum lycopersicum* L.) cv. 'Karoon' under *Phelipanche aegyptiaca* infestation. Values represent means ± standard error. Different letters indicate significant differences according to Duncan's Multiple Range Test ($P < 0.05$). *Trichoderma viride* and *Microdochium bolleyi* produced the highest shoot fresh weights (43.43 g and 37.66 g, respectively), statistically similar to the uninfested control (37.66 g).

Table 3. Growth parameters of tomato (*Solanum lycopersicum* L.) cv. 'Karoon' inoculated with endophytic fungi under *Phelipanche aegyptiaca* infestation (values are mean ± SD).

Species	**Shoot fresh weight** (g)	**Root length** (cm)	**Root dry weight** (g)	**Shoot dry weight** (g)	**Root fresh weight** (g)
Ascotricha chartarum	24.56 ± 6.78	30.00 ± 6.8	4.03 ± 0.81	7.50 ± 1.48	7.76 ± 2.32
Microdochium bolleyi	37.66 ± 11.92	24.66 ± 4.84	4.16 ± 1.65	12.66 ± 1.48	16.96 ± 5.7
Uninfested control	37.66 ± 2.81	31.00 ± 1.2	4.40 ± 0.47	11.60 ± 0.87	11.73 ± 0.81
Rhodotorula toruloides	9.16 ± 3.19	22.33 ± 1.45	0.73 ± 0.24	4.40 ± 1.0	2.20 ± 0.7
Alternaria TC-26-6	12.60 ± 3.09	17.33 ± 2.66	0.80 ± 0.11	4.40 ± 0.1	2.83 ± 0.36
Fusarium tricinctum	32.43 ± 5.42	30.33 ± 2.18	2.70 ± 0.17	8.33 ± 1.19	9.13 ± 1.09
Chaetomium elatum	15.1 ± 2.86	28.33 ± 3.28	2.23 ± 0.73	6.70 ± 2.55	6.56 ± 2.2
Beauveria bassiana	23.66 ± 2.56	18.66 ± 1.85	1.80 ± 0.25	6.43 ± 0.5	5.7 ± 0.78
Alternaria TW-24-2	15.43 ± 4.03	20.66 ± 0.66	1.53 ± 0.29	5.23 ± 1.28	4.26 ± 0.82
Trichothecium roseum	16.53 ± 1.87	24.00 ± 2.3	0.96 ± 0.12	2.53 ± 1.07	4.46 ± 0.67
Trichoderma koningii	19.73 ± 5.03	19.00 ± 2.51	1.40 ± 0.2	4.96 ± 1.06	4.63 ± 0.63
Trichoderma koningiopsis	18.8 ± 6.86	26.00 ± 3.46	1.00 ± 0.35	6.76 ± 0.92	5.50 ± 2.0
Trichoderma atroviride	14.96 ± 2.31	26.00 ± 3.51	2.03±1.18	7.43 ± 2.61	3.63 ± 0.61
Clonostachys rosea	20.2 ± 4.85	23.66 ± 2.9	1.00 ± 0.35	5.63 ± 0.6	6.10 ± 2.18
Epicoccum nigrum	15.13 ± 2.41	21.66 ± 1.2	4.16 ± 1.71	7.93 ± 1.71	22.3 ± 7.8
Trichoderma aeruginosum	25.4 ± 6.83	28.66 ± 2.18	1.90 ± 0.4	6.36 ± 0.92	7.7 ± 1.05
Alternaria TBL-33	16.43 ± 2.49	23.33 ± 1.76	1.30 ± 0.1	4.76 ± 0.29	5.50 ± 0.4
Trichoderma viride	43.43 ± 7.78	28.66 ± 2.33	3.56 ± 0.66	10.36 ± 0.89	9.36 ± 1.12
Ascotricha funiculosa	20.93 ± 0.47	21.00 ± 2.51	5.46 ± 0.32	1.73 ± 0.17	7.16 ± 1.07
Infested control (*P. aegyptiaca*)	3.83 ± 0.99	19.33 ± 1.76	1.00 ± 0.5	4.76 ± 1.58	1.76 ± 1.12

All values are presented as mean ± standard deviation (SD).

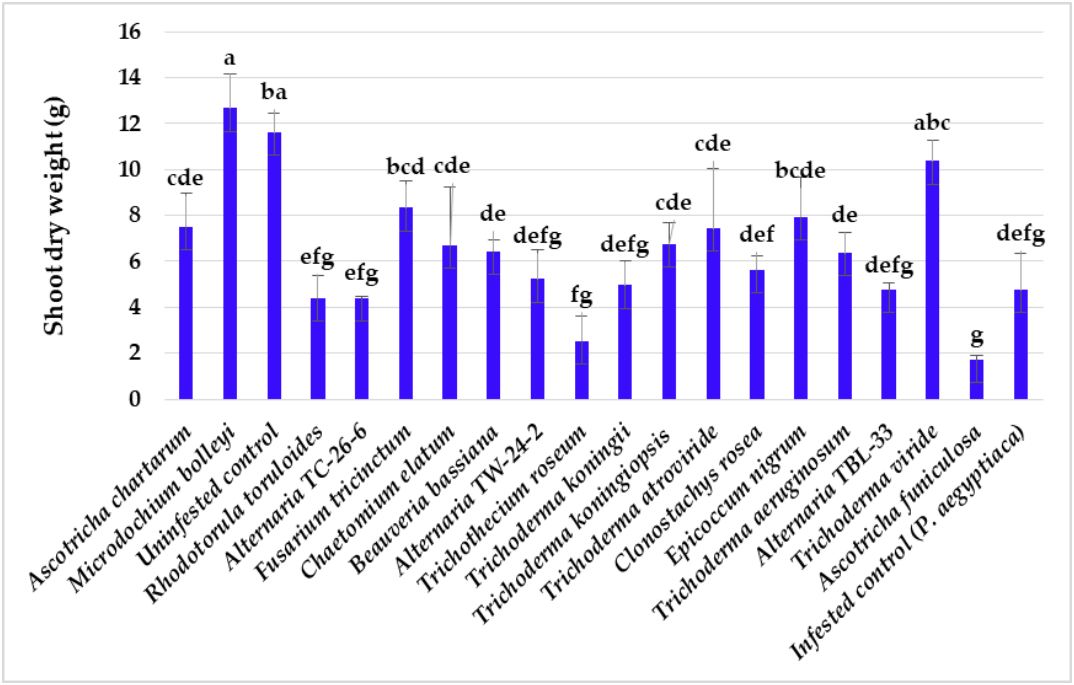

Fig. 2. Effect of endophytic fungal isolates on shoot dry weight (g) of tomato (*Solanum lycopersicum* L.) cv. 'Karoon' under *Phelipanche aegyptiaca* infestation. Values represent means ± standard error. Different letters indicate significant differences ($P < 0.05$). *Microdochium bolleyi* exhibited the highest shoot dry weight (12.66 g), exceeding the uninfested control (11.6 g).

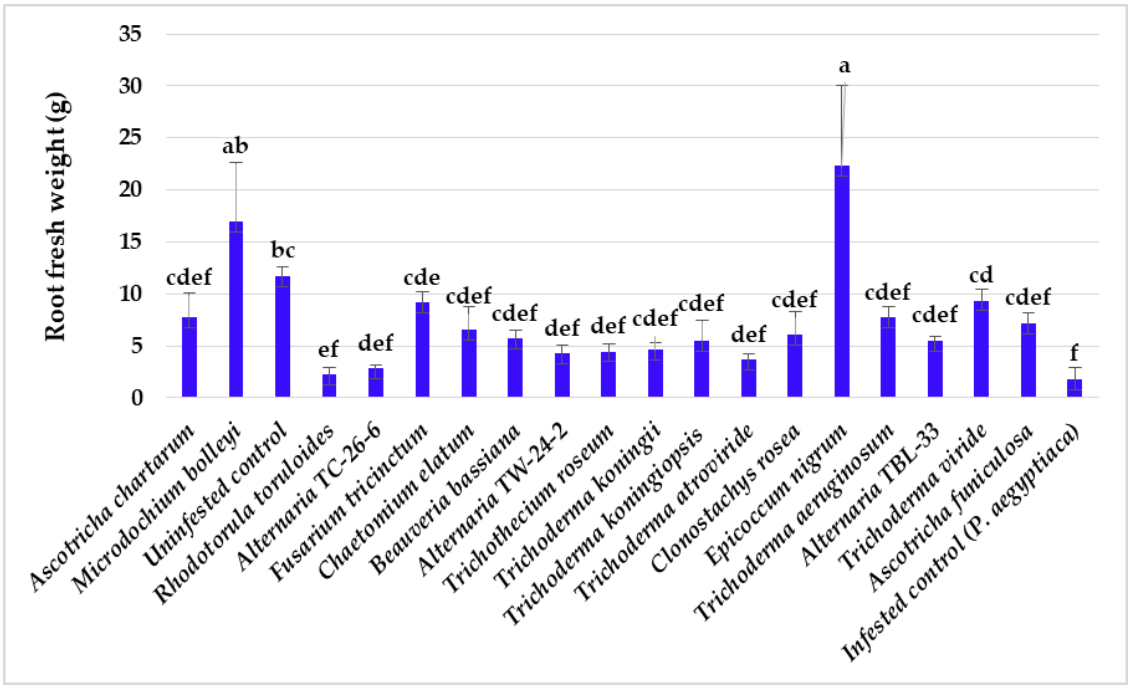

Fig. 3. Effect of endophytic fungal isolates on root fresh weight (g) of tomato (*Solanum lycopersicum* L.) cv. 'Karoon' under *Phelipanche aegyptiaca* infestation. Values represent means ± standard error. Different letters indicate significant differences ($P < 0.05$). *Epicoccum nigrum* produced the highest root fresh weight (22.3 g), followed by *Microdochium bolleyi* (16.96 g), both significantly exceeding the uninfested control (11.73 g). The infested control had the lowest root fresh weight (1.76 g).

Effect of endophytic fungi on root dry weight

Root dry weight (RDW) followed a similar trend (Fig. 4). The infested control had an RDW of 1.0 ± 0.5 g, while the uninfested control reached 4.4 ± 0.47 g. *Ascotricha funiculosa* exhibited the highest RDW (5.46 ± 0.32 g), statistically similar to the uninfested control. *Microdochium bolleyi* (4.16 ± 1.65 g) and *Epicoccum nigrum* (4.16 ± 1.71 g) also performed well. Notably, *Clonostachys rosea* (1.0 ± 0.35 g) and *Trichothecium roseum* (0.96 ± 0.12 g) showed no significant difference from the infested control (Table 3).

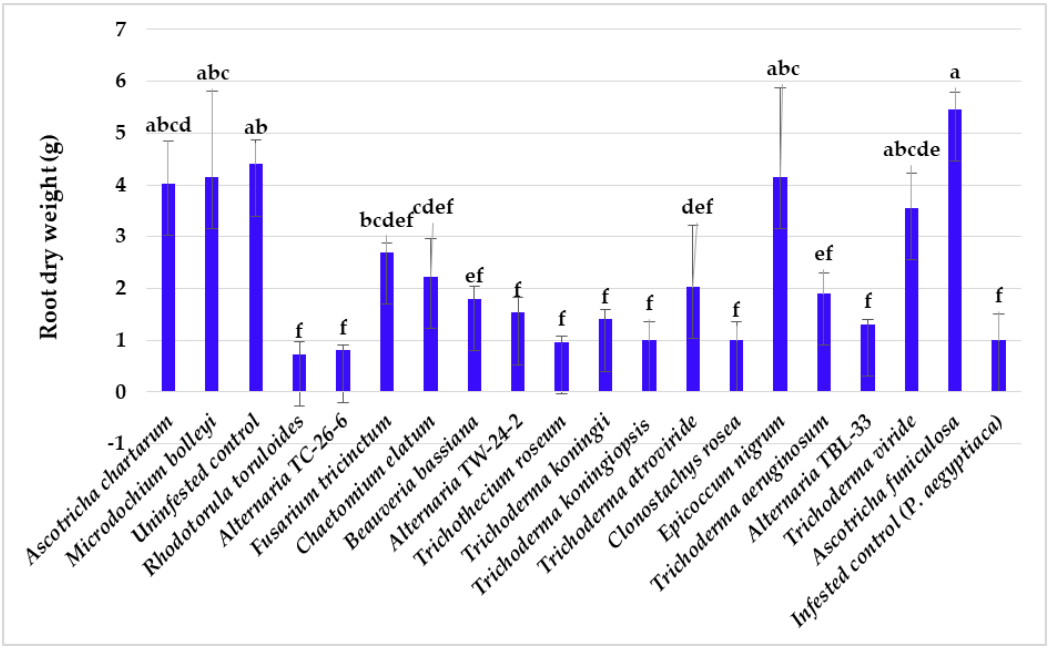

Fig. 4. Effect of endophytic fungal isolates on root dry weight (g) of tomato (*Solanum lycopersicum* L.) cv. 'Karoon' under *Phelipanche aegyptiaca* infestation. Values represent means ± standard error. Different letters indicate significant differences ($P < 0.05$). *Ascotricha funiculosa* showed the highest root dry weight (5.46 g), statistically similar to the uninfested control (4.4 g) and significantly higher than the infested control (1.0 g).

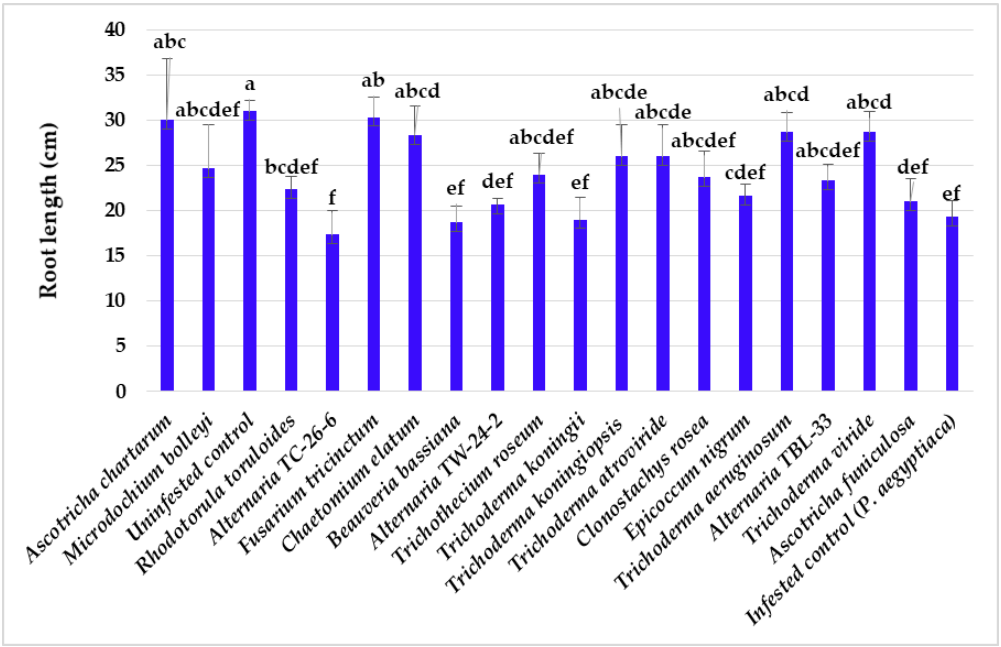

Fig. 5. Effect of endophytic fungal isolates on root length (cm) of tomato (*Solanum lycopersicum* L.) cv. 'Karoon' under *Phelipanche aegyptiaca* infestation. Values represent means ± standard error. Different letters indicate significant differences ($P < 0.05$). *Fusarium tricinctum* (30.33 cm) and *Ascotricha chartarum* (30.0 cm) produced root lengths statistically indistinguishable from the uninfested control (31.0 cm), representing a ~57% increase over the infested control (19.33 cm).

Comparative summary of DSE versus non-DSE performance

Dark septate endophytes (DSE), including *Microdochium bolleyi* and *Alternaria* spp. (TC-26-6, TBL-33, TW-24-2), showed variable performance. *Microdochium bolleyi* consistently ranked among the top performers for shoot fresh weight, shoot dry weight, and root fresh weight. However, non-DSE isolates such as *Epicoccum nigrum* (root fresh weight), *Trichoderma viride* (shoot fresh weight), *Ascotricha funiculosa* (root dry weight), and *Fusarium tricinctum* (root length) outperformed DSE isolates in specific parameters (Table 3).

Discussion

The quantitative data obtained from this investigation provide compelling evidence that specific root-associated fungal endophytes can significantly improve the phenotypic performance of *Solanum lycopersicum* cv. 'Karoon' under severe stress from the parasitic weed *Phelipanche aegyptiaca* in Iran. To our knowledge, this is among the first studies to demonstrate the potential of endophytic fungi, particularly isolates from the genera *Epicoccum*, *Microdochium*, *Fusarium* and *Ascotricha*, in mitigating broomrape infestation in tomato (Cignitas et al., 2024). This finding extends the known biocontrol potential of these microorganisms from traditional phytopathogens and abiotic stressors- to the challenging context of root-parasitic plants.

The substantial increase in root fresh weight (RFW) mediated by *Epicoccum nigrum* indicates that this endophyte acts as an effective biostimulant, enhancing nutrient uptake and photosynthetic allocation towards the roots, thereby countering the carbon drain imposed by *P. aegyptiaca*. This aligns with previous reports showing that *E. nigrum* possesses multiple plant-beneficial traits. Beyond our previous findings on its role in suppressing wheat bacterial leaf streak (Barkhordari et al., 2024), other studies have shown *E. nigrum* can produce antifungal compounds, induce root growth, and exhibit various extracellular enzyme activities (Li et al., 2023). Recent work has also demonstrated its ability to disrupt pathogen membranes and induce oxidative stress *via* secondary metabolites (Fu et al., 2025). The convergent results suggest *E. nigrum* possesses broad-spectrum stress mitigation capabilities, likely mediated by secondary metabolites and growth regulators that enhance host tolerance across diverse challenges.

The restoration of shoot biomass by *Microdochium bolleyi* (SFW: 37.66 g vs. 3.83 g in infested control) aligns with previous reports showing that this fungus can improve plant growth and act as a biocontrol agent (Shadmani et al., 2018, 2021a, b). Notably, *M. bolleyi* is a well-studied dark septate endophyte (DSE) in cereal roots and has been proposed as an ideal model organism for studying plant-endophyte interactions. This functional conservation across different stress regimes from heavy metal toxicity and fungal pathogens to parasitic weeds, strongly suggests *M. bolleyi* possesses a robust and versatile suite of plant-beneficial traits. These likely include the production of siderophores, phytohormones, and volatile organic compounds (VOCs), as well as the induction of systemic resistance. The consistent high performance of *M. bolleyi* across multiple growth parameters in our study reinforces its potential as a key component of microbial consortia for integrated stress management.

Our findings on the efficacy of endophytic fungi are consistent with a growing body of literature on their use in sustainable agriculture. Endophytic fungi can promote plant growth through increased micronutrient acquisition, root development, and production of VOCs (Beniwal et al., 2026). They induce plant immune responses and enhance tolerance to various abiotic stresses (Beniwal et al., 2026). A recent systematic review confirms that endophytes play a crucial role in enhancing plant resistance against pathogens and insect pests while simultaneously promoting growth through induced systemic resistance (ISR), entomopathogenicity, and phytohormone production (Hendra et al., 2026). Specifically, in the context of parasitic weed control, soil microorganisms can suppress *Phelipanche* spp. through multiple mechanisms: direct antagonism of the parasite, enhancement of the host's defense responses, and interference with chemical signaling between host and parasite, such as strigolactone degradation (Pongpamorn et al., 2026).

A notable observation from our study is the differential performance between DSE and non-DSE isolates. While *Microdochium bolleyi* (a DSE) consistently ranked among the top performers across multiple shoot and root parameters, specific non-DSE isolates such as *Trichoderma viride* (SFW), *Ascotricha funiculosa* (RDW), and *Fusarium tricinctum* (root length) outperformed DSE isolates in certain traits. For instance, *Trichoderma* species are renowned for their biocontrol capabilities, which include suppressing a wide range of phytopathogens through mycoparasitism, antibiosis, and the induction of systemic resistance (Guzmán-Guzmán et al., 2025). They also promote plant growth by producing phytohormones such as IAA, gibberellins, and cytokinins (Guzmán-Guzmán et al.,

2025). This suggests that, in the context of biotic stress from a parasitic weed, non-DSE isolates offer comparable or even superior growth-promoting benefits, underscoring that the functional advantages of endophytic fungi are context-dependent, varying with the nature of the stressor and the host-endophyte compatibility.

Regarding host-endophyte compatibility, we acknowledge that direct evidence such as microscopy, TEM, or serological assays was not obtained in this study; instead, compatibility was assessed indirectly through significant growth promotion and the consistent absence of disease symptoms, which are widely accepted as strong circumstantial indicators of successful endophytic establishment (Card et al., 2016; Hardoim et al., 2015). We explicitly recognize this as a limitation. It is noteworthy that *Ascotricha chartarum* has recently been reported as an endophytic fungus from healthy tissues of damask rose (*Rosa damascena*) in Iran for the first time (Atghia et al., 2025). This finding supports the endophytic nature of this species, at least in the context of Rosaceous hosts. However, the specific mechanisms by which *A. chartarum* and *A. funiculosa* exert their growth-promoting effects in tomato under *Phelipanche* stress remain to be elucidated. These may include rhizosphere competition, production of volatile organic compounds, or other beneficial plant-microbe interactions that do not necessarily require extensive intracellular colonization. The context-dependent nature of fungal lifestyles where a single species can exhibit endophytic, saprobic, or even pathogenic behaviors depending on the host and environmental conditions should also be considered.

We have added direct colonization assays (e.g., qPCR, FISH, or confocal microscopy) to our future research priorities to unequivocally confirm the presence of these isolates inside tomato root tissues. Furthermore, we agree that competitive interactions between the parasite and biocontrol agents in the rhizosphere could have contributed to the observed effects, and we cannot entirely rule out this possibility. However, the differential performance among isolates with some consistently enhancing biomass while others specifically stimulated root elongation suggests that the benefits are not solely due to uniform soil-level competition, as such competition would likely produce more homogeneous responses. The well-documented endophytic lifestyle of key genera such as *M. bolleyi* and *E. nigrum* further supports our interpretation that endophytic behaviors are at least partially involved. Finally, we fully concur that final yield (fruit/tuber weight) would have provided the most robust criterion for ranking overall efficacy, but our greenhouse study focused on vegetative parameters under controlled conditions. We have now acknowledged this gap and emphasized that future field trials must include yield data to validate the practical superiority of the most promising isolates.

Nevertheless, the beneficial effect of *F. tricinctum* calls for caution. This species is recognized as a weak pathogen with a broad host range and is known to produce mycotoxins, including enniatins, moniliformin, and T-2 toxin, which pose risks to crop safety (Wang et al., 2022). However, pathogenicity and mycotoxin production are highly strain-dependent, and several endophytic strains have demonstrated strong biocontrol activity without causing disease symptoms (Niu et al., 2026). Our isolate, obtained from healthy wheat, did not induce any disease symptoms in tomato under the tested conditions. Nonetheless, comprehensive safety assessments including host pathogenicity tests, mycotoxin profiling, and evaluation of non-target effects remain essential prior to any field application.

The observed variations among endophyte genera where specific strains maximize biomass accumulation while others stimulate structural elongation highlight the complexity of these symbiotic relationships. These findings suggest that a multi-species consortia approach could provide comprehensive defense by simultaneously enhancing root biomass and linear root development, laying the groundwork for a more robust integrated pest management (IPM) framework for Iranian tomato growers. However, future research should focus on: (i) elucidating the specific molecular mechanisms employed by the most effective isolates, such as their VOC profiles, phytohormone biosynthesis, and ability to degrade strigolactones or directly inhibit haustorium development; (ii) evaluating the compatibility and synergistic effects of these strains in defined consortia under field conditions; and (iii) conducting comprehensive biosafety and ecotoxicological assessments, including strain-level pathogenicity tests, mycotoxin profiling, and evaluation of non-target effects, to ensure the safe deployment of these promising biocontrol agents, particularly for *Fusarium* isolates.

Acknowledgments

The authors express their gratitude to the Razi University of Kermanshah, Iran.

Ethical Approval and Consent

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT author statement

E. Mohammadi: Provided laboratory support, Analysed the sequence data. **S. Jamali:** Designed the project, Provided laboratory support, Analysed the sequence data and drafted the manuscript, Writing – review, **B.S. Chauhan:** Designed the project. **R. Sharifi:** Provided laboratory support.

Funding

The author(s) received no financial support for the research.

Data Availability

All datasets generated during this study are available from the corresponding author upon request.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Atghia, O., Fotouhifar, K. B., Farzaneh, M., & Javan Nikkhah, M. (2025). Evaluation of total phenolic and flavonoid content in some endophytic fungi of Damask Rose (*Rosa damascena* Mill.) in Iran. *Iranian Journal of Plant Protection Science*, 55(2), 295–321.

Baneshi, H., Mohammadi, S., & Basirnia, T. (2016). Effect of pot intercropping of bean types and tomato on Egyptian broomrape control and growth indices improvement of tomato. *Journal of Agroecology*, 12(3), 63–69.

Barkhordari, K., Sharifi, R., Jamali, S., Moarrefzadeh, N., & Hosseini, S. (2024). Biological control of Xanthomonas translucens, the causal agent of bacterial leaf streak of wheat, by some endophytic fungi. *Plant Protection (Scientific Journal of Agriculture)*, 47(2), 35–53. Doi: 10.22055/ppr.2024.46960.1747

Bazobandi, M., Hadizadeh, M. H., & Sadraei Far, A. (2015). Integrated management of Egyptian broomrape (*Orobanche aegyptiaca*) in tomato (*Solanum lycopersicum*) with ammonium sulfate fertilizer and glyphosate herbicide. *Journal of Plant Protection*, *29*(2), 265–272.

Beniwal, R., Ror, P., & Ramakrishna, W. (2026). Endophytic fungi as partners in a holistic approach to enhance plant growth and stress tolerance for sustainable agriculture. *Fungal Biology Reviews*, 56, 100483. DOI: 10.1016/j.fbr.2026.100483.

Blattner, F. R. (1999). Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. *BioTechniques*, *27*(6), 1180–1186.

Card, S., Johnson, L., Teasdale, S., & Caradus, J. (2016). Deciphering endophyte behaviour: the link between endophyte biology and efficacious biological control agents. *FEMS Microbiology Ecology*, *92*(8), fiw114. DOI: 10.1093/femsec/fiw114.

Chen, J., Ma, Y. Q., & Xue, Q. H. (2018). Use of microorganisms in controlling parasitic root weed *Orobanche* spp. *Chinese Journal of Eco-Agriculture*, 26(1), 49–56.

Cignitas, E., Basbagci, G., Sulu, G., & Kitis, Y. E. (2024). *Fusarium fujikuroi* as a potential biocontrol agent of the parasitic weed *Phelipanche aegyptiaca* in tomato. *Journal of Phytopathology*, 172(3), e13344. DOI: 10.1111/jph.13344.

FAOSTAT. (2024). Statistical Database of the Food and Agriculture Organization of the United Nations.

Fu, S. Q., Cao, F., Pang, S., Qi, X. Q., Xu, Z. H., Zhang, Y. H., Liu, Z. C., Luo D. Q., & Liu, Y. F. (2025). The biocontrol effect and mechanism of the marine-derived fungus *Epicoccum nigrum* against *Alternaria alternata*, the causal agent of tomato black spot disease. *Pesticide Biochemistry and Physiology*, *213*, 106552. DOI: 10.1016/j.pestbp.2025.106552.

Guzmán-Guzmán, P., Etesami, H., & Santoyo, G. (2025). *Trichoderma*: a multifunctional agent in plant health and microbiome interactions. *BMC Microbiology*, *25*(1), 434. DOI: 10.1186/s12866-025-04158-2.

Habimana, S., Murthy, K. N. K., & Hatti, V. (2013). Management of orobanche in field crops - a review. *Scientific Journal of Crop Science*, *2*(11), 144–158. Doi: 10.14196/sjcs.v2i11.1024.

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., & Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews*, *79*(3), 293–320. DOI: 10.1128/MMBR.00050-14.

Hendra, Y., Ulpah, S., Trizelia, T., Syahrawati, M., & Saragih, M. (2026). Triple functional roles of endophytic fungi in pest management: a systematic review. *Egyptian Journal of Biological Pest Control*, *36*(1), 1. https://doi.org/10.1186/s41938-025-00874-z.

Jamali, S. (2021a). *Ascotricha funiculosa* a new species for the funga of Iran. *Mycologia Iranica*, *8*(1), 81–85.

Jamali, S. (2021b). First report of *Ascotricha chartarum* for funga of Iran. *Rostaniha*, *22*(1), 147–149.

Li, Q., Wang, S., Chen, S., Shang, Y., Zhou, F., Yu, J., Ding, Z., & Ye, X. (2023). Identification of volatile components and growth promoting effects of endophytes of *Tinospora capillipes* Gagnep. *Journal of Plant Pathology*, *105*(1), 57–68. DOI: 10.1007/s42161-022-01282-0.

Mamnooi, E. (2017). *Broomrape Management*. Extension Publication, Agricultural Research, Education and Extension Organization (AREEO), South Kerman Agricultural and Natural Resources Research Center, Iran.

Mehrmoradi, H., Jamali, S., & Pourian, H. R. (2020). Isolation and identification of entomopathogenic fungi from cultivated and natural soils in Kermanshah Province (West of Iran). *Rostaniha*, *21*(1), 49–64

Modares Najafabadi, S. S., & Minbashi Moeini, M. (2017). Chemical control of Egyptian broomrape (*Phelipanche aegyptiaca*) with sulfosulfuron (75% WG) in tomato fields of Hormozgan Province. *Pesticides in Plant Protection Sciences*, *6*(2), 140–148.

Mohammadi, E., (2025). Evaluation of endophytic fungi as biological control agents of broomrape in tomato and rapeseed plants. M.Sc. Thesis, Faculty of Agricultural Sciences and Engineering, Razi University, Kermanshah, Iran.

Nosratti, I., Mobli, A., Mohammadi, G. R., Yousefi, A. R., Sabeti, P., & Chauhan, B. S. (2020). The

Problem of *Orobanche* and *Phelipanche* spp. and Their Management in Iran. *Weed Science*, *68*(6), 555–564. DOI: 10.1017/wsc.2020.61.

Niu, Y. J., Liu, L. B., Yang, Z. D., Xu, C. J., Zhao, T. K., Chen, L. L., Wang, Q. Q., Sun, B., & Kim, S. (2026). The antimicrobial arsenal of endophytes in *Lilium pumilum*: active components of *Fusarium tricinctum* and antifungal mechanisms. *Archives of Microbiology*, *208*(7), 363. DOI: 10.1007/s00203-026-04913-7.

Pongpamorn, P., Zwart, M., & Bouwmeester, H. J. (2026). The role of soil microbiota in the control of parasitic weeds. *Plant and Cell Physiology*, *67*(4), 403-413. DOI: 10.1093/pcp/pcaf125.

Shadmani, L., Jamali, S., & Fatemi, A. (2018). Biocontrol activity of endophytic fungus of barley, *Microdochium bolleyi*, against *Gaeumannomyces graminis* var. *tritici*. *Mycologia Iranica 5*(1), 7 – 14.

Shadmani, L., Jamali, S., & Fatemi, A. (2021a). Effects of root endophytic fungus, *Microdochium bolleyi* on cadmium uptake, translocation and tolerance by *Hordeum vulgare* L. *Biologia*, *76*(2), 711–719. DOI: 10.2478/s11756-020-00598-5.

Shadmani, L., Jamali, S., & Fatemi, A. (2021b). Isolation, identification, and characterization of cadmium-tolerant endophytic fungi isolated from barley (*Hordeum vulgare* L.) roots and their role in enhancing phytoremediation. *Brazilian Journal of Microbiology*, *52*(3), 1097–1106. DOI: 10.1007/s42770-021-00493-4.

Wang, Y., Wang, R., & Sha, Y. (2022). Distribution, pathogenicity and disease control of *Fusarium tricinctum*. *Frontiers in Microbiology*, *13*, 939927. Doi: 10.3389/fmicb.2022.939927.

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. L. (1990). Amplified and direct sequencing of fungal ribosomal RNA genes for phylogenies. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). San Diego: Academic.