Harnessing the biocontrol and phytostimulatory potential of endophytic fungi against damping -off disease in okra

Pragati Gautam
CSK Himachal Pradesh Krishi Vishvavidalaya Palampur
Joginder Pal
jpal15889@gmail.com
CSK Himachal Pradesh Krishi Vishvavidalaya Palampur
Twinkle Gujjar
CSK Himachal Pradesh Krishi Vishvavidalaya Palampur
Shiv Shanker Pandey
CSIR-Institute of Himalayan Bioresource Technology (IHBT)
Surinder Paul
ICAR-Indian Grassland and Fodder Research Institute, Centre for Indian Himalayan Grasslands Palampur
Nikhil Rawat
CSIR-Institute of Himalayan Bioresource Technology (IHBT)
Abhishek Walia
CSK Himachal Pradesh Krishi Vishvavidalaya Palampur
Rishi Mahajan
CSK Himachal Pradesh Krishi Vishvavidalaya Palampur
Shalini Chandel
Sri Sai University
Kavita Kushwaha
Rani Lakshmi Bai Central Agricultural University
Rishita Kapoor
CSK Himachal Pradesh Krishi Vishvavidalaya Palampur
Tanish Dhiman
CSK Himachal Pradesh Krishi Vishvavidalaya Palampur

Research Article

Keywords: Okra, damping-off, endophytic fungi, Rhizoctonia solani, root colonization, biocontrol

Posted Date: May 21st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9166059/v1

Additional Declarations: No competing interests reported.

Harnessing the biocontrol and phytostimulatory potential of endophytic fungi against damping -off disease in okra

Pragati Gautam[a], Joginder Pal[a*], Twinkle Gujjar[a], Shiv Shanker Pandey[b], Surinder Paul[c], Nikhil Rawat[b] Abhishek Walia[d], Rishi Mahajan[d], Shalini Chandel[e], Kavita Kushwaha[f], Rishita Kapoor[g] and Tanish Dhiman[a]

[a]Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidalaya Palampur-176062, Himachal Pradesh, India

[b]Fermentation and Phytofarming Technology Division, CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur-176061, Himachal Pradesh, India

[c]Department of Biotechnology, ICAR-Indian Grassland and Fodder Research Institute, Centre for Indian Himalayan Grasslands Palampur-176061, Himachal Pradesh, India

[d]Department of Microbiology, College of Basic Sciences, CSK Himachal Pradesh Krishi Vishvavidalaya Palampur-176062, Himachal Pradesh, India

[e]Department of Botany, Sri Sai University, Baijnath Road, Palampur, Distt. Kangra-176081, Himachal Pradesh, India

[f]Department of Plant Pathology, Rani Lakshmi Bai Central Agricultural University, Jhansi-284003, Uttar Pradesh, India

[g] Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishvavidalaya Palampur-176062, Himachal Pradesh, India

[*]**Correspondence**: jpal15889@gmail.com

ORCIDs: Joginder Pal: orcid.org/0000-0001-6318-1556

Abstract

Okra (*Abelmoschus esculentus* L. Moench) is an economically important vegetable crop, with summer cultivation critical for early market supply. Seedling damping-off, primarily caused by *Rhizoctonia solani*, poses a major constraint, often leading to severe yield losses. This study evaluated the potential of fungal endophytes for managing damping-off under summer cultivation in Himachal Pradesh, India. Field surveys (2023–2024) across Kangra, Mandi, and Hamirpur districts recorded disease incidence of 31.7–84.3%. Among 44 isolates recovered, *R. solani* JPO1 was identified as the predominant pathogen via morpho-molecular characterization. Forty-two fungal endophytes were screened for antagonism and plant growth-promoting (PGP) traits; eight isolates with strong activity were further characterized: *Coniothyrium* sp. E1P, *Xylaria juruensis* E4P, *Aspergillus terreus* E10P, *Epicoccum* sp. E13P, *Aspergillus welwitschiae* E16P, *Fusarium proliferatum* E25P, *F. solani* E26P, and *Epicoccum* sp. JPE2. Glasshouse and field evaluations identified *F. proliferatum* E25P as the most effective isolate, significantly enhancing plant growth and suppressing damping-off. Root colonization, confirmed by plate counts and confocal laser scanning microscopy, demonstrated robust endophytic establishment in treated plants. These

results highlight the potential of fungal endophytes as sustainable, eco-friendly alternatives to chemical fungicides for effective damping-off management in okra.

Keywords: Okra; damping-off; endophytic fungi; *Rhizoctonia solani*; root colonization; biocontrol

Statements and Declarations

Availability of data and material: All sequences generated in this study are available in GenBank with accession numbers cited in Table 1. The remaining datasets not included here will be made available from the corresponding author on reasonable request

Competing interests: The authors declare that they have no competing interests

Funding: Not applicable

Acknowledgements: The first author gratefully acknowledges the help granted by Directorate of Research, Professor & Head, Dept. of Plant Pathology CSK Himachal Pradesh Krishi Vishvavidalaya Palampur-176061, (H.P.) India for permitting the field and laboratory facilities and other assistance. Authors also gratefully acknowledge the CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur, Himachal Pradesh (India) for providing Confocal Microscopy facility to carry out the root colonization studies

Introduction

Okra (*Abelmoschus esculentus* L.) is an important vegetable crop cultivated widely in tropical and subtropical regions, particularly across the Indian subcontinent, including Himachal Pradesh (Gaur et al. 2025; Kumar et al. 2025). The crop is known by several local names such as “lady’s finger” in England, “gumbo” in the United States, “bhindi” in India, and “bamia” in Ethiopia (Benchasri 2012; Gemede et al. 2015). Okra pods are valued for their nutritional richness, containing carbohydrates, proteins, dietary fiber, vitamins, and essential minerals. They also possess bioactive compounds associated with antioxidant and other health-promoting properties (Mishra et al. 2017; Wu et al. 2022; Gaur et al. 2025). Owing to its nutritional value and low caloric content, okra contributes significantly to dietary health and food security (Obomeghei et al. 2022). India is the largest producer of okra in the world, contributing more than 72% of global production, with an annual output of about 6.35 million tonnes cultivated over 0.52 million hectares (FAOSTAT 2020; Karmakar et al. 2025). The crop can be grown during both summer and rainy seasons, although summer cultivation is often preferred because early harvests generally fetch better market prices (Rubatzky and Yamaguchi 1997).

Okra cultivation is affected by several diseases incited by fungi, bacteria, and viruses, including root rot, wilt, leaf spot, anthracnose, powdery mildew, and yellow vein mosaic virus (Patel et al. 2019). Among these, damping-off is particularly damaging during the early stages of crop establishment. The disease affects seeds and young seedlings and is caused by a complex of soil-borne pathogens, including *Pythium* spp., *Phytophthora nicotianae*, *Rhizoctonia solani*, and *Macrophomina phaseolina* (Diwakar et al. 1986; Jiskani et al. 2007; Matny 2013; Jukte et al. 2016). Infection at this stage often results in seed rot, poor germination, and collapse of young seedlings, leading to significant crop loss in the field.

Among the pathogens associated with damping-off, *Rhizoctonia solani* Kühn is considered one of the most aggressive, particularly under warm conditions typical of summer cultivation. The pathogen causes seed rot as well as pre- and post-emergence damping-off, resulting in rapid seedling collapse and reduced plant stand (Abbas et al. 2017; Muimba-Kankolongo 2018; Patel et al. 2019; Nasimi et al. 2024). In later stages of growth, *R. solani* may also cause root rot, further affecting crop productivity (Mimma et al. 2023). Its persistence in soil is aided by the formation of sclerotia, which enable the pathogen to survive in soil and crop residues for extended periods, making disease management difficult (Akber and Fang 2024).

Damping-off in okra is commonly managed through fungicidal seed treatments or soil applications. Although these treatments can suppress the disease, their frequent use has raised concerns about environmental contamination, development of resistant pathogen populations, adverse effects on beneficial soil microorganisms, and possible chemical residues in edible produce (Pandey et al. 2018; Bhattarai et al. 2022). These limitations have encouraged the search for safer and more sustainable approaches to disease management.

Biological control using beneficial microorganisms has gained increasing attention as an alternative to chemical control. Among these, fungal endophytes have attracted particular interest. Endophytic fungi colonize internal plant

tissues without causing visible harm and often form beneficial associations with their host plants (Murthy et al. 2014; Alam et al. 2021; Attia et al. 2022a, b; Abd Alhakim et al. 2022). By occupying internal plant niches, these microorganisms can limit the establishment of invading pathogens through competition for space and nutrients (de Lamo et al. 2018).

Endophytic fungi may also contribute to plant protection by enhancing plant defense responses. They can stimulate defense-related enzymes, promote the accumulation of phenolic compounds, strengthen plant cell walls, and improve the plant's ability to cope with oxidative stress (Llorens et al. 2017; Constantin et al. 2019; Daroodi et al. 2021; El-Sharkawy et al. 2022). In addition, they may inhibit pathogens through mechanisms such as nutrient competition, production of antimicrobial metabolites, induction of systemic resistance, and mycoparasitic interactions involving hyphal coiling, penetration, and secretion of cell wall–degrading enzymes (Waghunde et al. 2017; Sharaf et al. 2022). Many endophytic fungi also produce diverse secondary metabolites that contribute to disease suppression and plant growth promotion (El Enshasy et al. 2019). Their potential for managing plant diseases has been reported in several crop systems (Rashad et al. 2020; Pal et al. 2020; Campos and Jacob 2021; Kushwaha et al. 2024).

Despite these promising attributes, the role of endophytic fungi in managing damping-off in okra has received limited attention, particularly under the agro-climatic conditions of the Himalayan region. Considering the economic importance of okra and the destructive nature of damping-off pathogens such as *R. solani*, identifying effective endophytic fungi that can suppress disease while promoting plant growth is important for sustainable crop production. The present study therefore aimed to isolate and characterize fungal endophytes associated with okra and to evaluate their biocontrol and phytostimulatory potential against the damping-off pathogen of summer-grown okra. The findings are expected to contribute to the development of environmentally sound strategies for managing this disease.

Materials and methods

Sampling, isolation and purification of the microbial culture (s)

Extensive surveys were conducted in three districts of Himachal Pradesh (Kangra, Mandi, and Hamirpur) during the summer season (April–June 2023) to determine the prevalence of damping-off in okra. Surveyed locations ranged in altitude from 350 to 2251 m.a.s.l. Healthy and symptomatic okra roots exhibiting typical damping-off symptoms (water-soaked lesions or brown discoloration at the collar region) were carefully uprooted, placed in sterilized polythene bags, and transported to the laboratory for processing within 24–48 hours. These samples were used to isolate both the causal pathogen(s) and fungal endophytes.

Isolation and purification of causative pathogen(s)

Infected root segments were washed under running water, dried on Whatman No.1 filter paper, and dissected into 0.5–1 cm pieces from the junction of healthy and diseased tissue. Tissue fragments were surface-sterilized with 70% ethanol for 1 min, followed by 1% NaOCl for 1 min, and rinsed four times with sterile distilled water. Excess

moisture was removed on sterilized filter paper, and fragments were placed (four per plate) onto Potato Dextrose Agar (PDA) supplemented with antibiotics to suppress bacterial growth. Plates were incubated at 25 ± 2°C in a BOD incubator for 4 days. Emerging mycelia were purified using the hyphal tip method, and axenic cultures were maintained on PDA slants at 4°C (Ghaffar et al. 2018).

Colonization rate (CR) of pathogens was calculated as per Madhi et al. (2020):

$$\text{Colonization Rate (CR)} = \frac{\text{Total number of segments yielding fungus}}{\text{Total number of segments plated}} \times 100$$

Morpho-cultural identification was performed using a compound microscope (Olympus BX50) and reference descriptions (Waterhouse 1967; Parmeter 1970; Booth 1971; Ho 1981; Nelson et al. 1983; Boerema et al. 2004; Samson et al. 2011).

Pathogenicity bioassay

To confirm pathogenicity, inoculum of *R. solani* was prepared on sand–wheat meal (8:2) medium in 500 ml Erlenmeyer flasks (Sharma 2011). Ten mycelial discs (5 mm) from 3-day-old cultures were inoculated into sterilized medium and incubated at 25 ± 2°C for 12 days, with shaking every 2–3 days. The colonized medium was air-dried and stored at 4°C for subsequent use.

Glasshouse bioassays were conducted using the susceptible okra cultivar 'Palam Komal'. Sterilized pots (20 cm diameter) were filled with clay:sand:FYM (2:1:1) mixture and inoculated with *R. solani* inoculum (100 g/kg). Surface-sterilized seeds were sown, and disease development was monitored. Pathogen re-isolation from diseased seedlings fulfilled Koch's postulates. The most aggressive isolate from Palampur was selected for further experiments.

Isolation and quantification of fungal endophytes

Fungal endophytes were isolated from asymptomatic roots following Sharma and Roy (2015). Roots were washed, cut into 0.5 cm fragments, and surface-sterilized sequentially with 70% ethanol (1 min), 4% NaOCl (5 min), 70% ethanol (30 s), and rinsed five times with sterile distilled water. Fragments were macerated in 10 ml sterile water and serially diluted to 10^{-5}. Aliquots (100 µl) were plated on PDA (10^{-1} to 10^{-5}), in triplicate, and incubated at 25 ± 2°C for 2 weeks. Outgrowing hyphal tips were purified, maintained on PDA slants at 4°C, and sub-cultured every 9–10 weeks. Fungal populations were quantified using the plate count technique (Agarwal and Hasija 1986):

$$\text{Cfu/g} = \frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Weight of root sample taken (g)}}$$

Morpho-molecular characterization

Morpho-cultural identification

Pure cultures of pathogens and selected endophytes were assessed on PDA after 7 days for colony color, hyphal septation, spore morphology, pigmentation, and sclerotia formation. Microscopic observations were performed using

slide culture stained with lactophenol cotton blue at 40× magnification (Olympus CX51) and compared with standard keys (Christensen and Raber 1978; Parmeter 1970; Booth 1971; Nelson et al. 1983; Samson et al. 2011; Mathur and Olga 2003; Hussain et al. 2014; Song et al. 2016; Ying et al. 2019; Shehabeldine et al. 2024).

Molecular identification

Pure cultures of *R. solani* and seven efficient endophytes (excluding JPE2) were sequenced for 18S rRNA (Eurofins Genomics, India). Sequences were analyzed using BLAST (NCBI), and Maximum Likelihood phylogeny was constructed in MEGA v.11 (Tamura et al. 2021). Consensus sequences were submitted to GenBank.

***In vitro* confrontation assay of fungal endophytes**

Dual culture assays were performed to assess antagonism of 42 fungal endophytes against *R. solani* (Coskuntuna and Ozer 2008). Agar plugs (5 mm) of both pathogen and endophyte were placed diametrically opposite on PDA plates (6 cm apart, 1 cm from edge) and incubated at 25 ± 2°C for 7 days. Pathogen radial growth was measured, and inhibition (%) was calculated (Gaigole 2011):

$$\text{Inhibition (\%)} = \frac{\text{R1} - \text{R2}}{\text{R1}} X\ 100$$

Where;

R1 = Radial growth of pathogen in control and R2 = Radial growth of pathogen in the presence of endophyte

Microscopic observation of pathogen-fungal endophyte interactions

Fungal endophytes exhibiting significant mycelial inhibition of *Rhizoctonia solani* under *in vitro* were examined under compound microscopy to evaluate their interaction with the pathogen (Yurnaliza et al. 2014; Kushwaha et al. 2024). Mycelium from the interaction zone was collected using a sterilized needle and placed onto a clean microscope slide with a drop of distilled water. A coverslip was gently applied, and alterations in hyphal growth at the interaction zone were observed at 40× magnification, comparing treated and control samples.

Characterization of endophytic fungi for plant growth promotion, stress tolerance, and phytochemical profiling

Endophytic fungi exhibiting ≥40% *in vitro* antifungal activity were selected for further characterization.

In Vitro Plant Growth-Promoting Profiling

Siderophore Production: Isolates were precultured on PDA for 7–10 days at 25 ± 2°C. Agar plugs (3 mm) were placed on Chrome Azurol S (CAS) agar and incubated at 25 ± 2°C for 5–6 days. Color changes from blue to yellow, purple, red, or orange indicated positive siderophore production. Zone diameters were measured by subtracting colony diameter from total halo zone (Schwyn and Neilands 1987).

Phosphate Solubilization: Fungal stubs were inoculated onto Pikovskaya's agar (Himedia, India) containing $Ca_3(PO_4)_2$ and incubated for 5–6 days at 25 ± 2°C. Formation of a clear halo indicated phosphate solubilization; zone diameter calculated as total zone minus colony diameter (Potshangbam et al. 2017).

Cellulase Activity: Cultures were inoculated on YEPD medium with 0.5% Na-carboxymethyl cellulose (CMC), incubated at 25 ± 2°C for 5–6 days, stained with 0.2% Congo red, and destained with 1 M NaCl for 15 min. Clear zones indicated cellulase production (Toghueo et al. 2017).

Amylase Activity: Fungi were grown on Glucose YEPD agar amended with 0.2 g starch, incubated at 25 ± 2°C, and flooded with 1% iodine solution. Clear zones indicated starch hydrolysis (Hankin and Anagnostakis1975).

Protease Activity: 3 mm agar plugs from 10-day-old cultures were inoculated onto Casein Starch Agar with 1% skimmed milk, incubated at 25 ± 2°C for 5–6 days. Clear halos indicated proteolytic activity (Hankin and Anagnostakis 1975).

HCN Production: Isolates were inoculated on PDA slants supplemented with 4.4 g/L glycine. Whatman No.1 filter paper saturated with 2% sodium carbonate in 0.5% picric acid was placed in tubes, sealed, and incubated at 28°C for 7 days. Yellow discoloration indicated HCN production (Lorck 1948).

Abiotic Stress Tolerance

Salt Stress: Isolates were spot-inoculated on PDA amended with 1–5% NaCl and incubated at 25 ± 2°C for 2 weeks; growth was recorded.

pH Tolerance: Potato Dextrose Broth adjusted to pH 2, 5, and 8 using glacial acetic acid; cultures incubated for 2 weeks; growth monitored via optical density.

Temperature Stress: Isolates incubated at 4°C, 10°C, and 40°C on PDA; growth observed after 2 weeks.

Phytochemical profiling

Crude fungal extracts were screened for alkaloids, phenols, tannins, triterpenoids, saponins, and flavonoids following standard protocols (Bhardwaj et al. 2015; Thorati and Mishra 2017; Jagadevi Shivaputrappa 2018). Reactions were visualized by colorimetric changes as described in the original protocols.

***In vivo* Biocontrol Efficacy**

Eight fungal endophytes with highest *in vitro* antagonistic activity were selected for *in vivo* testing.

Preparation of liquid spore suspensions and seed treatment with endophytic fungi

Spore Suspension and Seed Treatment: One-week-old cultures were transferred aseptically to PDB (300 ml in 500 ml flasks) and incubated on a rotary shaker at 180 rpm, 25 ± 2°C for 7 days. Spore suspensions were filtered, quantified using a Neubauer's haemocytometer, and standardized to 1×10^6 spores/ml. Surface-sterilized okra seeds (*Palam Komal*) were soaked overnight and air-dried for 3 h. Soil in pots was inoculated with *R. solani* mass culture at 100 g/kg.

Glasshouse Evaluation

Treated seeds were sown in 20 cm diameter pots containing sterilized clay:sand:FYM (2:1:1). Soil sterilization involved 5% formaldehyde treatment under polythene cover for 10–15 days, followed by aeration for 1 week. Six seeds were sown per pot; experiment arranged in CRD with five replicates. Glasshouse conditions: 25°C, 14/10 h light/dark, 85% RH. Regular irrigation was provided. Data recorded at 10 days' post-inoculation The following parameters were recorded:

Disease Incidence

$$Disease\ incidence\ (\%) = \frac{Number\ of\ diseased\ plants}{Total\ number\ of\ plants\ observed} \times 100$$

Disease Control

$$Disease\ control\ (\%) = \frac{[Incidence\ in\ control - Incidence\ in\ treatment]}{Incidence\ in\ control} \times 100$$

Plant growth parameters

Germination (%): Germination was recorded 10 days after sowing and per cent was estimated as the number of seeds sown and the number of seeds germinated expressed in percentage.

Vigour index (cm): Vigour index (%) = (Mean root length + Mean shoot length) x Germination (%)

Shoot length (cm): Shoot length was recorded in centimeters from the ground level to the apical bud stem.

Root length (cm): The length of tap root was recorded in centimeters using measuring scale by placing it horizontally on the ground.

Field Evaluation

Field trials were conducted at CSKHPKV Research Farm (2024) using RBD with three replications, plot size 2.25 m^2 (4 rows × 20 plants). Seeds were soaked in endophytic spore suspensions (1×10^6 spores/ml). Natural epiphytotic conditions were utilized; PDB-treated seeds served as control. Disease and growth parameters were recorded as described above.

Root Colonization Assay

Colonization of roots by representative endophytes was assessed through re-isolation at 7 and 14 days after inoculation (DAI). The fungal population was enumerated at 10^{-2} dilution (cfu/g) on PDA following the methods of Agarwal and Hasija (1986), Barretti et al. (2008), and Miles et al. (2012). Further, for confirmation of endophytic colonization using laser scanning confocal microscopy (LSCM), the fungal treated roots were rinsed 3–4 times with autoclaved distilled water and then treated with 10% KOH solution overnight at 4°C, followed by washing with 1× PBS (2–3 times), the roots were then kept at RT for 10 minutes, followed by vacuum infiltration for 30 minutes after being incubated in 1mL of staining solution containing aniline blue, alexafluor 488-WGA, propidium iodide &

Tween 20 in PBS buffer and images were captured using STELLARIS 5, Leica Microsystems, Germany and represented following the protocol of Bhardwaj et al. (2025).

Statistical Analysis

Laboratory and field data were analyzed using ANOVA (OPSTAT software; Gomez and Gomez, 1984). CRD and RBD designs with a minimum of three replicates were applied. Treatment means were compared using LSD at 5% significance level ($p < 0.05$).

Results

Isolation and Identification of Pathogens Associated with Damping-Off of Okra

Surveys conducted during April–June 2023 across Kangra, Mandi, and Hamirpur districts of Himachal Pradesh revealed widespread damping-off in all okra-growing localities, predominantly at post-emergence stages. The highest disease incidence was observed in Tal, Hamirpur (84.33%), followed by Bhota, Hamirpur (81.67%) and Kanaid, Mandi (81.00%), whereas the lowest incidence was recorded in Naun, Mandi (31.67%). District-wise, Hamirpur showed the highest mean incidence (65.46%), followed by Mandi (59.04%), with Kangra having the lowest (53.33%). Overall, the incidence ranged from 31.67% to 84.33%, and no surveyed fields were disease-free, indicating a critical constraint on okra cultivation in Himachal Pradesh.

A total of 44 pathogenic isolates were recovered. *Rhizoctonia solani* exhibited the highest colonization frequency (16.67%), followed by *Fusarium solani* (9.37%). In total, eight fungal genera were associated with okra damping-off (Fig. 1), including *R. solani* (16 isolates), *F. solani* (8), *Pythium* spp. (6), *F. oxysporum* (4), *Phytophthora* sp. (4), *Aspergillus* sp. (3), *Didymella* sp. (2), and *Sclerotium rolfsii* (1). These data indicate *R. solani* as the predominant causal agent of damping-off in the region.

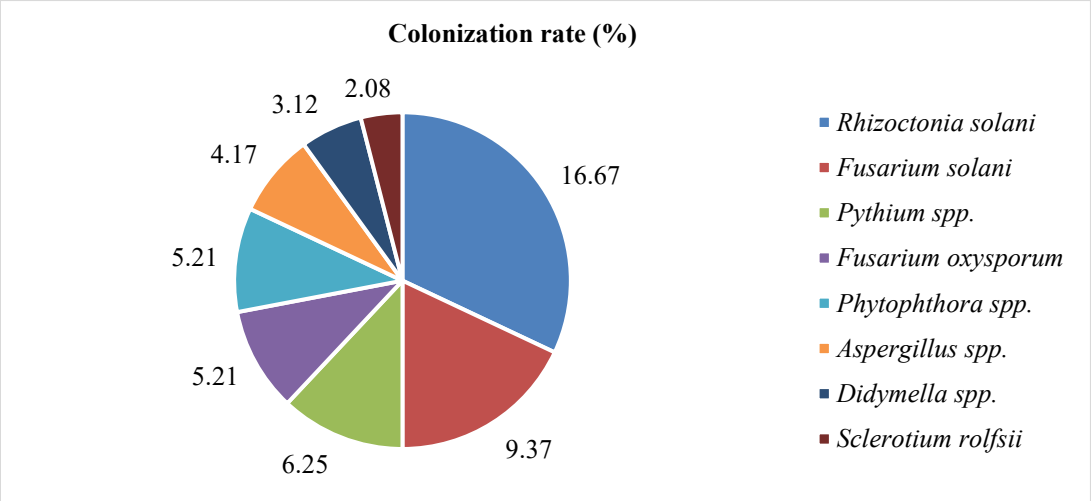


Fig. 1 Frequency percentage of pathogenic fungal isolates associated with okra damping-off disease in different districts of Himachal Pradesh

289 **Pathogenicity Bioassay**

290 Among the 44 isolates, *R. solani* was selected for further experiments. Under controlled pot conditions using the
291 susceptible cultivar '*Palam Komal*', soil inoculated with *R. solani* produced typical damping-off symptoms within
292 seven days' post-inoculation, primarily at the 2–3 leaf stage. Infected seedlings showed irregular, water-soaked,
293 brown lesions at the basal stem, leading to wilting, leaf drooping, and seedling collapse. Koch's postulates were
294 fulfilled through re-isolation, and morphological comparison confirmed the pathogenicity of *R. solani*.

295 **Isolation and Quantification of Root Endophytic Fungi**

296 Healthy okra roots harbored diverse indigenous fungal populations. Maximum endophyte densities were recorded in
297 Palampur and Sunehar (Kangra) and Naun (Mandi) at 4.33×10^2 cfu/g root, followed by Tikkar, Mandi (4×10^2
298 cfu/g root). The lowest populations were recorded in Tal, Nalti, and Bhota (Hamirpur; 1×10^2 cfu/g root). Overall,
299 Kangra contributed the highest number of isolates (18), followed by Mandi (13) and Hamirpur (10), yielding 41
300 endophytic isolates. These findings suggest that host genotype and geographical location influence root-associated
301 microbial communities.

302 **Identification of Predominant Pathogen and Selected Fungal Endophytes**

303 **Morpho-Molecular Identification of *Rhizoctonia solani***

304 The predominant isolate of *R. solani* formed cream to light brown colonies with floccose aerial hyphae on PDA after
305 5–6 days at 25 ± 1°C. Within 20 days, dark brown sclerotia (0.2–1.0 mm) developed. Microscopically, hyphae
306 exhibited right-angled branching with constriction and septation (width 6.7–7.9 µm; mean 7.3 µm). ITS sequence
307 analysis confirmed 99.36% identity with *R. solani* (isolate JPO1; GenBank PQ605661). Phylogenetic analysis
308 (Maximum Likelihood, MEGA 11) revealed close relation to *R. solani* isolate GPB (MK621284.1) (Fig. 2).

309 **Morpho-Cultural and Molecular Identification of Selected Endophytes**

310 Eight endophytic fungi exhibiting ≥40% pathogen inhibition were characterized. Colony morphologies ranged from
311 creamish white to dark brown or dull green, with septate hyphae and varying sporulation. Growth rates ranged from
312 6 to 24 days, fastest in E16P (6 days), slowest in E4P (24 days). The isolates E10P and E16P were marked as highly
313 sporulating, E13P, E25P, E26P and JPE2, were recorded as less sporulating. However, E1P and E4P were
314 categorized in to very less sporulating group of endophytic fungi. The sequence data from these fungal isolates were
315 deposited in GenBank (the accession numbers are provided in Table 1). Phylogenetic analysis confirmed
316 evolutionary relationships with top BLAST hits (Fig. 2).

317 **Table 1** Identification of endophytic fungi recovered from different districts of Himachal Pradesh
318

Isolate	Identified fungi	Closest Neighbour	GenBank accession	Match identity (%)
E1P	*Coniothyrium* sp. isolate E1P	*Coniothyrium* sp. isolate k31 ON643525.1	PQ634823	98.28

E4P	*Xylaria juruensis* isolate E4P	*Xylaria juruensis* isolate DQS67F MZ735435.1	PQ634822	99.12
E10P	*Aspergillus terreus* isolate E10P	*Aspergillus terreus* isolate E3 ON532722.1	PQ634826	98.03
E13P	*Epicoccum* sp. isolate E13P	*Epicoccum* sp. strain AM581 OP113922.1	PQ634828	98.31
E16P	*Aspergillus welwitschiae* isolate E16P	*Aspergillus welwitschiae* strain RCBBR_AEAMCB3 MN603499.1	PQ634825	99.42
E25P	*Fusarium proliferatum* isolate E25P	*Fusarium proliferatum* strain GMBCC1191 PQ573371.1	PQ634824	92.13
E26P	*Fusarium solani* isolate E26P	*Fusarium solani* isolate F5 OR123272.1	PQ634827	98.60
JPE2	*Epicoccum* sp. isolate JPE2	*Epicoccum sorghinum* OR397965.1	OR632996	100

319

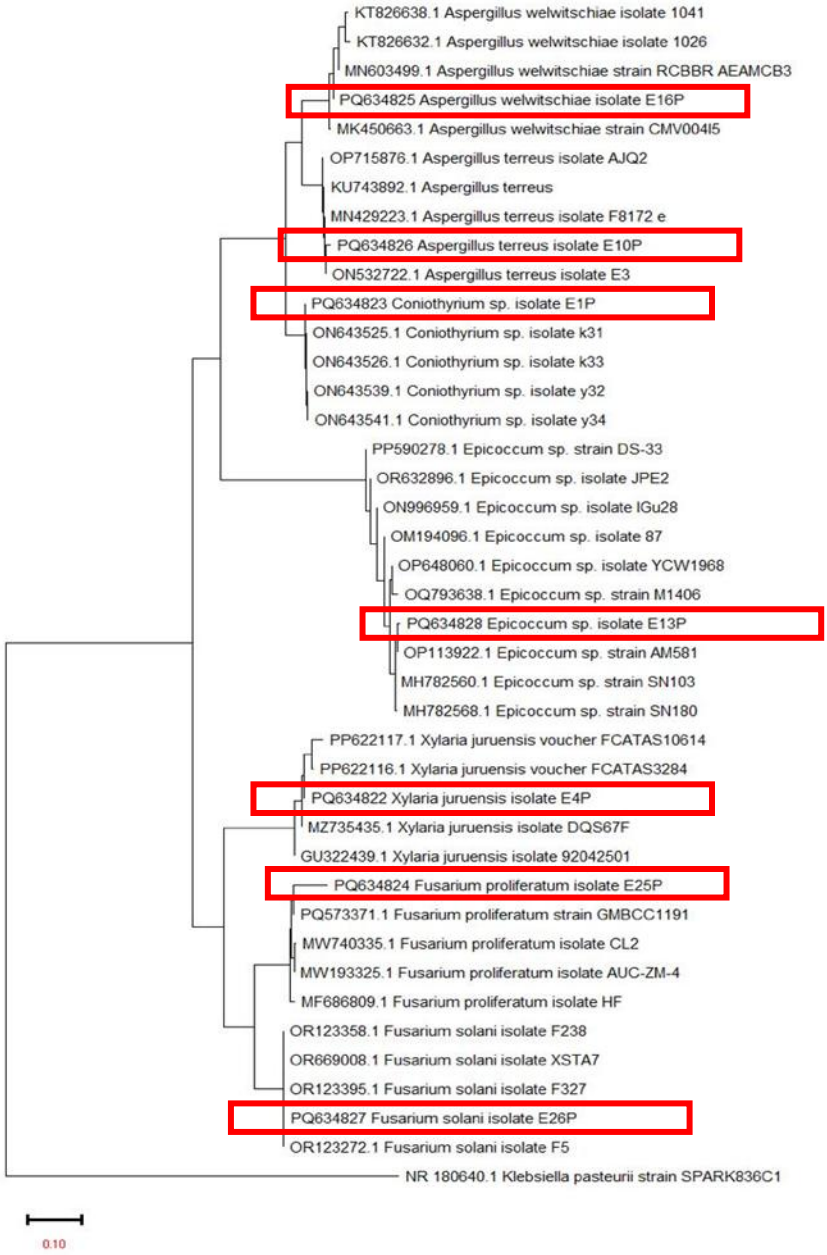


Fig. 2 Phylogenetic tree drawn using the Maximum Likelihood method showing relationship between selected endophytic fungi and similar other sequences deposited in the NCBI Genbank. (Evolutionary analysis were conducted in MEGA vs. 11)

In Vitro Antagonism of Fungal Endophytes

Dual culture assays of 42 endophytes against *R. solani* showed varying degrees of inhibition (Fig. 3, Table 2). Maximum inhibition was observed with E16P (79.63%), followed by E25P (63.70%). The lowest inhibition was E36P (15.93%). Furthermore, the representative fungal antagonists (showing ≥40% inhibition) were subjected to microscopic examination of the interaction zone formed during the *in vitro* confrontation assay. Various

morphological abnormalities and alterations were observed in the mycelium of test pathogen due to its interaction with the fungal endophytes (Supplementary file 1).

Table 2 *In vitro* antagonism of efficient fungal root endophytes against *Rhizoctonia solani,* causing damping-off of okra

Isolate	**Mycelial growth (mm)**	**Mycelial growth inhibition (%) ***	**Changes in mycelial growth at interaction zone**
Coniothyrium sp. isolate E1P	44.00^{d}	51.11^{de} (45.62)	Swelling and deformation of the pathogen mycelium
Xylaria juruensis isolate E4P	47.33^{e}	47.41^{e} (43.50)	Abnormalities and penetration in the pathogen hyphae
Aspergillus terreus isolate E10P	40.33^{c}	55.19^{cd} (47.96)	Penetration of the endophytic conidia inside the pathogen hyphae
Epicoccum sp. isolate E13P	42.67^{cd}	52.59^{d} (46.47)	Swelling and deformity of the pathogen mycelium
Aspergillus welwitschiae isolate E16P	18.33^{a}	79.63^{a} (63.18)	Breakage and encircling over the pathogen hyphae
Fusarium proliferatum isolate E25P	32.67^{b}	63.70^{b} (52.94)	Distortion and penetration in the pathogen mycelium
Fusarium solani isolate E26P	51.33^{f}	42.96^{f} (40.93)	Penetration in the pathogen mycelium
Epicoccum sp. isolate JPE2	37.67^{bc}	58.15^{c} (49.68)	Bending and twisting of the pathogen mycelium
Control	90.00^{g}	0.00^{g}	
CD (p=0.05)	4.54	3.00	
SE(m)	0.913	1.688	
SE(d)	1.291	2.387	
C.V.	3.740	5.837	

**Figures in parentheses are arc sine transformed value*

Figures denoted by same letter do not differ significantly

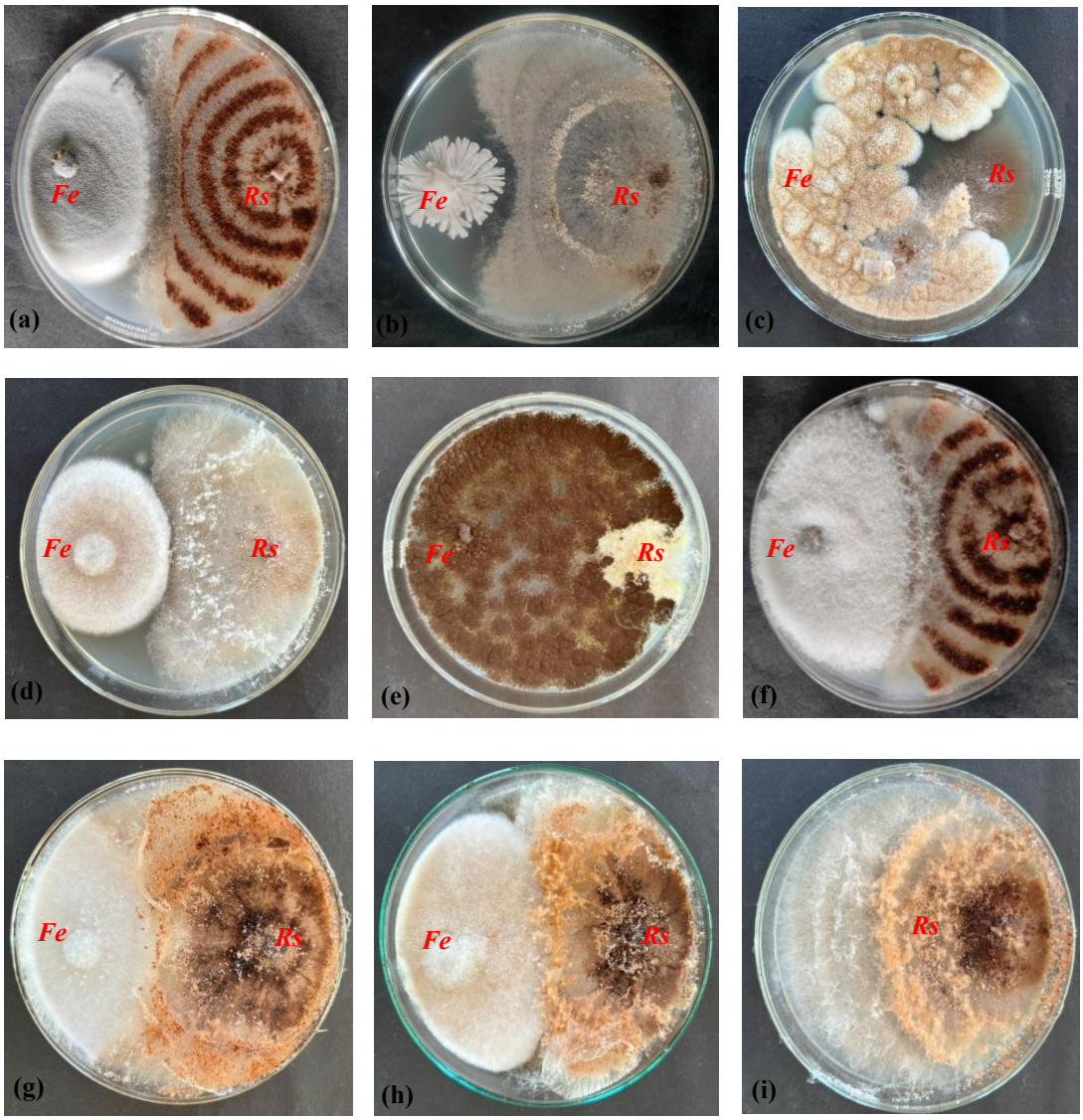


Fig. 3 *In vitro* confrontation activity of endophytic fungi [a. *Coniothyrium* sp. isolate E1P, b. *Xylaria juruensis* isolate E4P, c. *Aspergillus terreus* isolate E10P, d. *Epicoccum* sp. isolate E13P, e. *Aspergillus welwitschiae* isolate E16P, f. *Fusarium proliferatum* isolate E25P, g. *Fusarium solani* isolate E26P, h. *Epicoccum* sp. isolate JPE2, i. Control] against *Rhizoctonia solani* isolate JPO1, inciting okra damping off (Left side-Fungal endophyte (*Fe*); Right side-*Rhizoctonia solani (Rs*)

Plant Growth-Promoting Traits, Stress Tolerance, and Phytochemical Profiling

Selected endophytes exhibited variable PGP traits: all isolates solubilized phosphate, with *F. solani* E26P showing maximum activity (29.67 mm). Siderophore production ranged 7.67–18.33 mm; HCN production was observed in 62.5% of isolates. Lytic enzyme activities among various isolates varied: cellulase (87.5%), protease (62.5%), amylase (50%). Notably, 50% of the isolates exhibited both PGP traits and lytic enzyme activities (Figs. 3–4). Stress tolerance assays indicated growth at pH 2–8, NaCl 1–5% (maximum at 1%), and temperature up to 40°C for most

isolates. *E. juruensis* E4P and *F. solani* E26P showed reduced tolerance at extreme pH/salt. From the results obtained on stress tolerance assay (Table 3), it is inferred that all representative fungal antagonists were able to withstand temperature and salinity stress which could enhance the plant growth under drastic climatic conditions. Phytochemical screening of crude extracts revealed alkaloids, phenols, tannins, triterpenoids, saponins, and flavonoids. *A. terreus* E10P and *A. welwitschiae* E16P contained all tested metabolites; other isolates varied in compound profiles (Fig. 5).

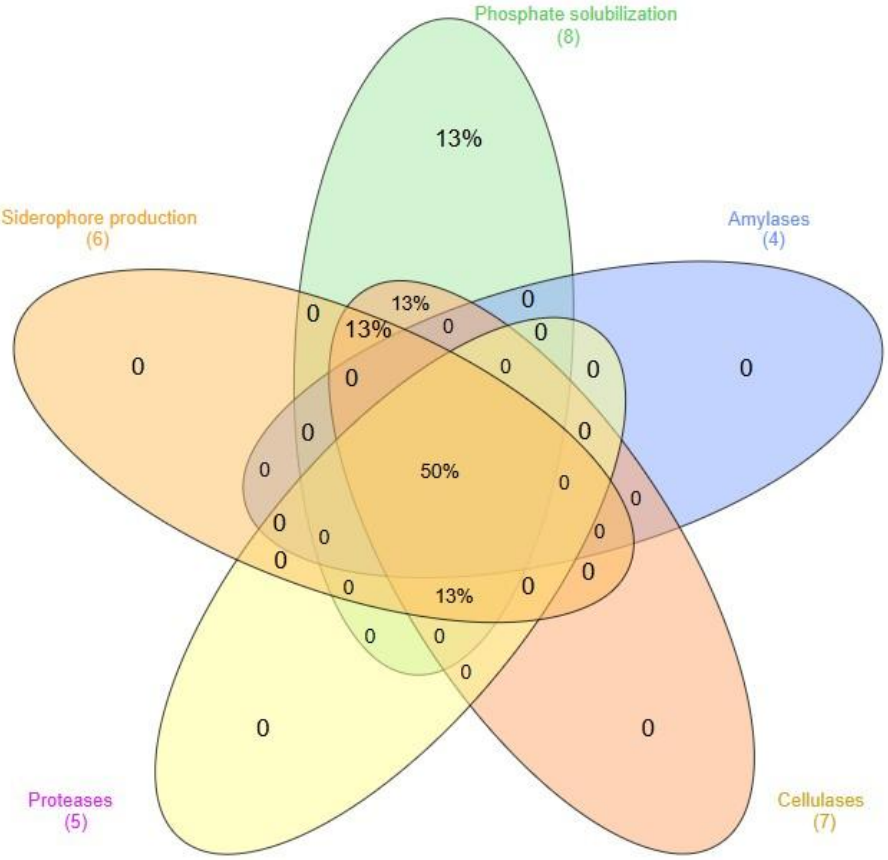


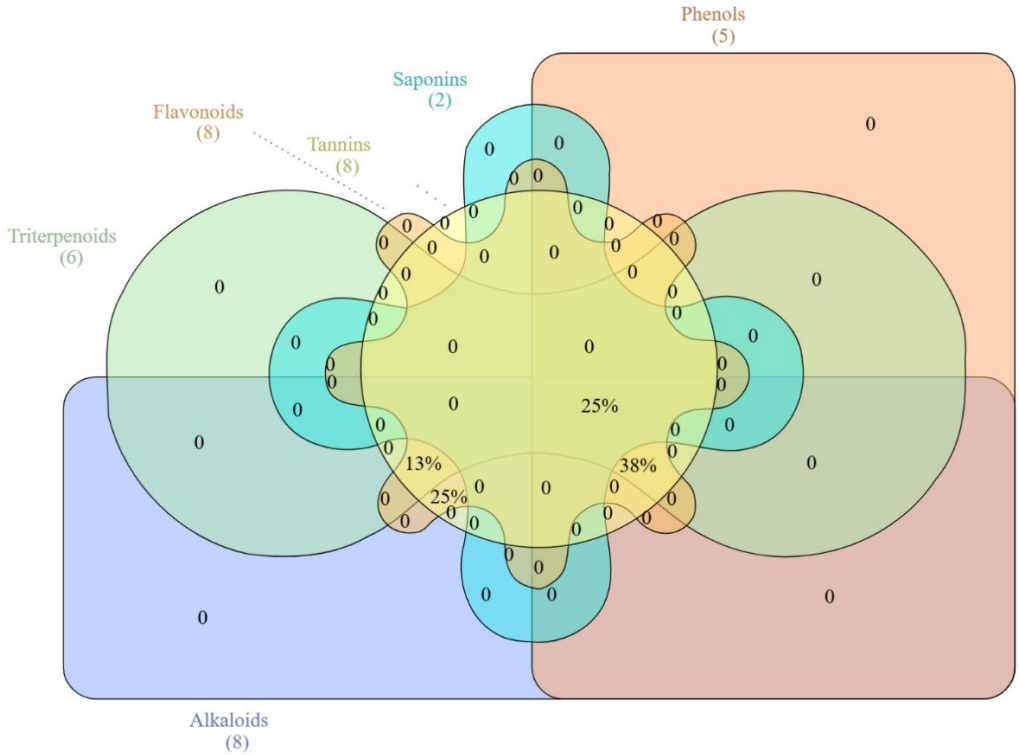
Fig. 4 Venn diagram representing various plant growth promoting (PGP) traits and lytic enzymes activity of selected endophytic fungi of okra

Table 3 *In vitro* screening of selected fungal root endophytes for stress tolerance traits

Isolate	HCN production	Stress Tolerances								
		pH			NaCl (%)			Temperature (°C)		
		2	5	8	1	3	5	4	10	40
Coniothyrium sp. isolate E1P	-	+	+	+	++	+	+	+	+	-
Xylaria juruensis isolate E4P	-	-	+	-	+	+	-	+	+	-

Aspergillus terreus isolate E10P	+		++	+++	++	+++	++	+	+	+	+
Epicoccum sp. isolate E13P	+		+	++	+	++	+	+	-	+	+
Aspergillus welwitschiae isolate E16P	+		++	+++	++	+++	++	+	+	++	+
Fusarium proliferatum isolate E25P	+		+	++	++	++	+	+	+	+	+
Fusarium solani isolate E26P	-		+	++	++	++	+	-	-	+	-
Epicoccum sp. isolate JPE2	+		+	++	+	++	+	+	-	+	+
CD (p=0.05)											
SE(m)											
SE(d)											
C.V.											

393 *-=No activity; +=Low activity; ++=Moderate activity; +++=High activity



394

395 **Fig. 5** Venn diagram illustration of distribution of various phytochemicals/secondary metabolite present in crude
396 extracts of selected endophytic fungi of okra

397 **Biocontrol Potential of Fungal Endophytes Against Damping-Off Under Glasshouse Conditions**

The eight most promising fungal endophytes identified *in vitro* were evaluated for their effectiveness against *Rhizoctonia solani* under glasshouse conditions (Supplementary file 2). All isolates significantly reduced disease incidence compared to the untreated control.

Pre- and post-emergence damping-off: *Fusarium proliferatum* E25P exhibited the lowest disease incidence (16.66% and 11.66%, respectively) at 10 days' post-inoculation. For pre-emergence damping-off, E25P was statistically similar to *Coniothyrium* sp. E1P (20.00%) and *Epicoccum* sp. E13P (21.66%). For post-emergence damping-off, E13P (16.66%) was comparable with E1P (18.33%).

Disease control: Maximum disease suppression was observed with *Fusarium proliferatum* E25P (76.2% pre-emergence, 82.06% post-emergence), followed by *Coniothyrium* sp. E1P (71.43% pre-emergence) and *Epicoccum* sp. E13P (74.37% post-emergence). *Fusarium solani* E26P was the least effective (35.72% and 41.03%, respectively) (Table 4).

Table 4 Efficacy of fungal endophytic formulations against okra damping-off caused by *Rhizoctonia solani* under glass house conditions

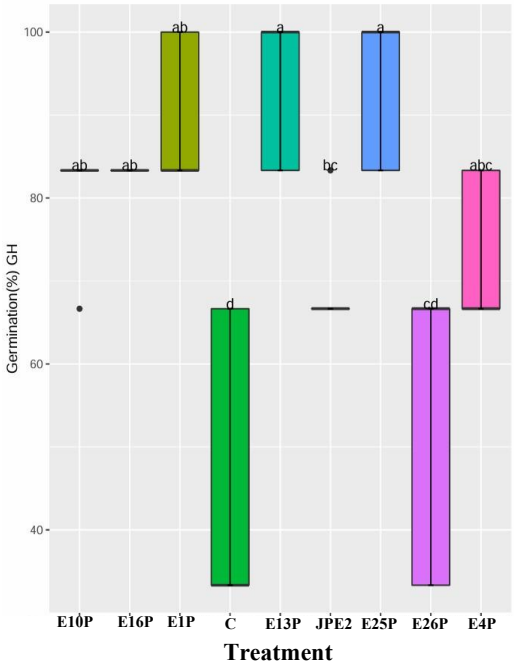
Treatment	Per cent incidence of damping -off disease			
	Pre emergence (%)*	Disease control (%)	Post emergence (%)*	Disease control (%)
Coniothyrium sp. isolate E1P	20.00[a] (26.44)	71.43	18.33[b] (25.26)	71.80
Xylaria juruensis isolate E4P	25.00[ab] (29.73)	64.29	20.00[b] (26.44)	69.23
Aspergillus terreus isolate E10P	33.33[c] (35.25)	52.38	28.33[c] (32.09)	56.41
Epicoccum sp. isolate E13P	21.66[a] (27.63)	69.05	16.66[b] (24.08)	74.37
Aspergillus welwitschiae isolate E16P	26.67[b] (31.04)	61.90	25.00[c] (29.99)	61.53
Fusarium proliferatum isolate E25P	16.66[a] (24.08)	76.20	11.66[a] (19.69)	82.06
Fusarium solani isolate E26P	45.00[d] (42.10)	35.72	38.33[d] (38.21)	41.03
Epicoccum sp. isolate JPE2	36.66[c] (37.22)	47.62	26.67[c] (31.04)	58.97
Control	70.00[e] (56.82)		64.99[e] (53.72)	
CD (p=0.05)	3.85		3.31	
SE(m)	1.33		1.15	
SE(d)	1.89		1.63	
C.V.	8.66		8.25	

**Figures in parentheses are arc sine transformed value*

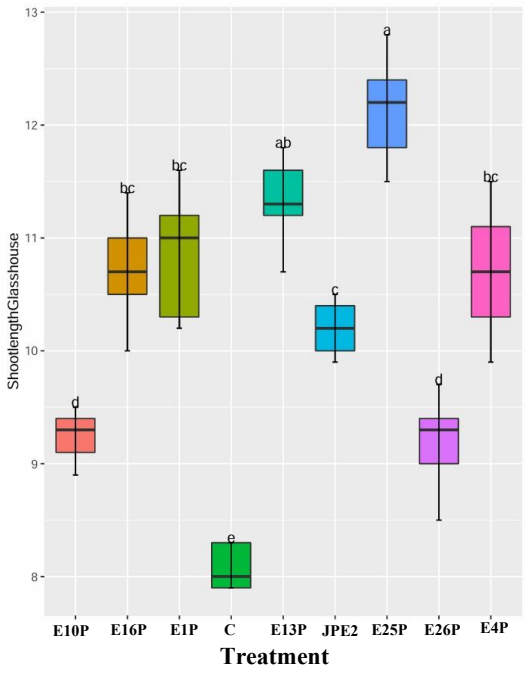
Figures denoted by same letter do not differ significantly

Evaluation of Endophytic Fungi on Plant Growth Parameters Under Glasshouse Conditions

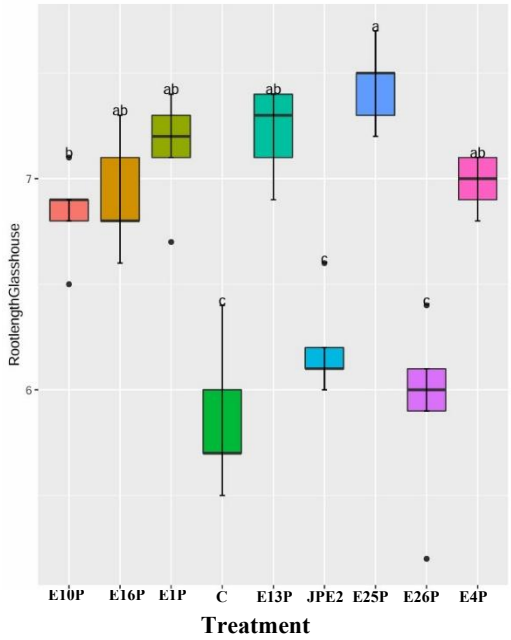
Eight promising fungal endophytes were evaluated under glasshouse conditions for their plant growth–promoting potential in okra. Endophyte-treated seeds showed significant improvements in germination, shoot length, root length, and vigour index compared to the control (Fig. 5).



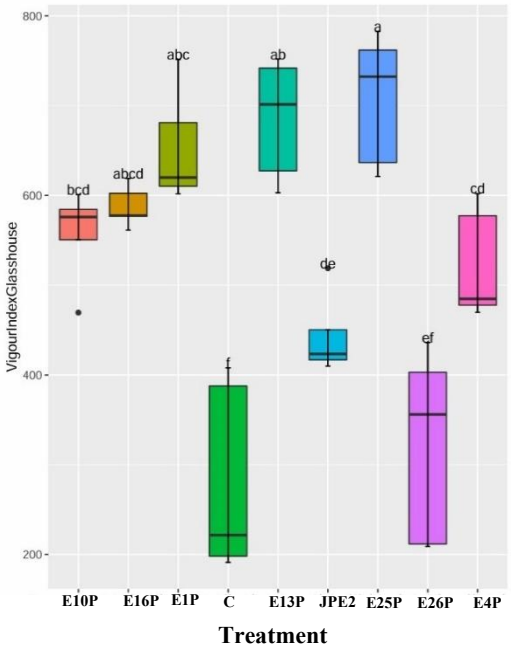
Germination (%)



Shoot length (cm)



Root length (cm)



Vigour Index

Fig. 5 Effect of fungal endophytic formulations on plant growth parameters of okra under glasshouse conditions [E10P: *Aspergillus terreus* isolate, E16P: *Aspergillus welwitschiae* isolate, E1P: *Coniothyrium* sp. isolate, C: Control, E13P: *Epicoccum* sp. isolate, JPE2: *Epicoccum* sp. Isolate, E25P: *Fusarium proliferatum* isolate, E26P: *Fusarium solani* isolate, E4P: *Xylaria juruensis* isolate]

E25P recorded the highest germination (83.33%), shoot length (12.14 cm), root length (7.44 cm), and vigour index (706.80). E1P, E13P, and E10P were statistically similar for germination and early growth promotion. E26P exhibited the lowest growth performance (germination 66.66%, shoot 9.18 cm, root 5.92 cm, vigour index 323.15), statistically comparable to E4P, JPE2, and E16P. Tukey's post hoc test ($P \leq 0.05$) indicated E25P and E13P consistently formed the superior group across parameters, with moderate responses for E1P, E10P, and E16P, while E26P and control ranked lowest. These results highlight the selective and parameter-specific nature of plant–endophyte interactions, identifying E25P and E13P as the most reliable isolates for enhancing early seedling growth under controlled conditions.

Biocontrol and Growth Promotion Under Field Conditions

The most efficient glasshouse isolates were evaluated under field conditions at the CSKHPKV Experimental Research Farm.

Damping-off incidence: E25P minimized pre- and post-emergence damping-off (23.33% and 16.66%), followed by E1P (28.33% pre-emergence) and E13P (30.00% pre-emergence; 18.33% post-emergence). E26P was least effective (30.44% and 39.04%, respectively).

Disease control: E25P showed the highest suppression (69.57% pre-emergence, 75.62% post-emergence), followed by E1P (63.04%) and E13P (73.18%) (Table 5).

Table 5 Efficacy of fungal endophytic formulations against damping-off of okra under field conditions

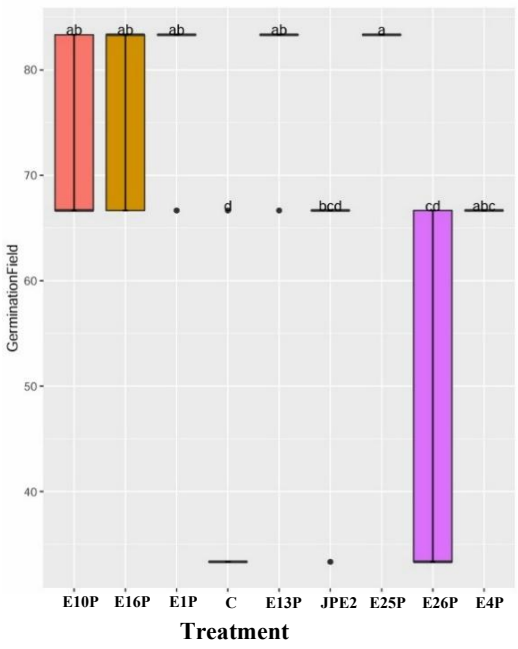
Treatment	**Per cent incidence of damping-off disease**			
	Pre emergence (%)*	**Disease control (%)**	**Post emergence (%)***	**Disease control (%)**
Coniothyrium sp. isolate E1P	28.33^{b} (32.09)	63.04	21.66^{b} (27.63)	68.29
Xylaria juruensis isolate E4P	31.66^{cd} (34.20)	58.70	31.66^{d} (34.20)	53.66
Aspergillus terreus isolate E10P	41.53^{e} (40.08)	45.83	35.00^{e} (36.24)	48.78
Epicoccum sp. isolate E13P	30.00^{bc} (33.14)	60.87	18.33^{ab} (25.26)	73.18
Aspergillus welwitschiae isolate E16P	35.00^{d} (36.24)	54.35	26.67^{c} (31.04)	60.97
Fusarium proliferatum isolate E25P	23.33^{a}	69.57	16.66^{a}	75.62

	(28.81)		(24.08)	
Fusarium solani isolate E26P	53.33^{e} (46.90)	30.44	41.66^{f} (40.16)	39.03
Epicoccum sp. isolate JPE2	43.33^{e} (41.14)	43.48	28.33^{cd} (32.09)	58.54
Control	76.67^{g} (61.16)	0.00	68.33^{g} (55.76)	0.00
CD (p=0.05)	3.47		3.33	
SE(m)	1.198		1.152	
SE(d)	1.694		1.629	
C.V.	6.814		7.566	

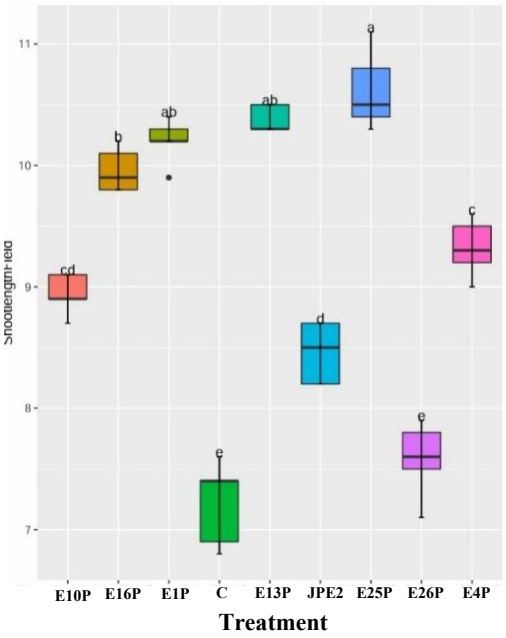
**Figures in parentheses are arc sine transformed value*

Figures denoted by same letter do not differ significantly

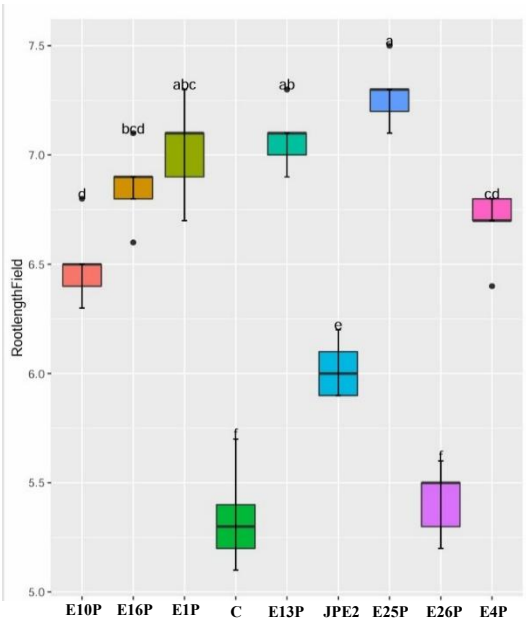
Plant growth promotion: Field evaluation demonstrated significant improvements in germination, shoot length, root length, and vigour index (Fig. 6). E25P showed the highest performance (germination 83.33%, shoot 10.62 cm, root 7.28 cm, vigour index 617.26), followed by E13P (80%, 10.38 cm, 7.08 cm, 576.69) and E1P (statistically comparable). Isolates E10P and E16P showed moderate improvements, while E4P, JPE2, E26P, and control exhibited the lowest values. Comparative analysis indicated E25P and E13P maintained superior and stable performance in both glasshouse and field trials.



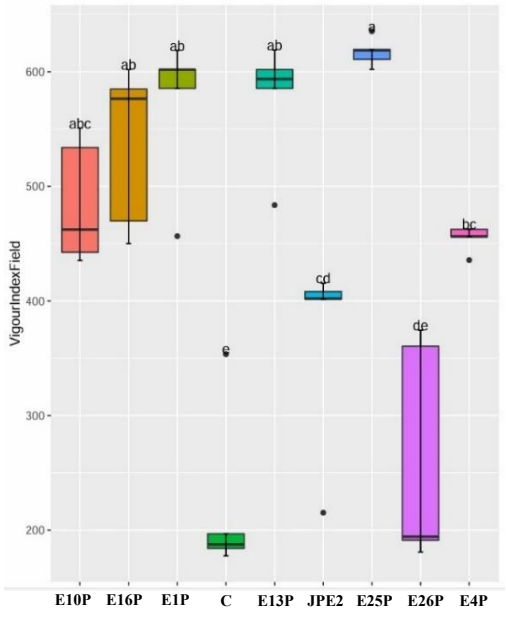
Germination (%)



Shoot length (cm)



Root length (cm)



Vigour Index

Fig. 6 Effect of fungal endophytic formulations on plant growth parameters of okra under field conditions [E10P: *Aspergillus terreus* isolate, E16P: *Aspergillus welwitschiae* isolate, E1P: *Coniothyrium* sp. isolate, C: Control, E13P: *Epicoccum* sp. isolate, JPE2: *Epicoccum* sp. Isolate, E25P: *Fusarium proliferatum* isolate, E26P: *Fusarium solani* isolate, E4P: *Xylaria juruensis* isolate]

Root Colonization by Fungal Endophytes

The colonization potential of selected fungal endophytes in okra roots was assessed using both plate count techniques and confocal laser scanning microscopy (CLSM). Plate counts revealed that all inoculated endophytes successfully established within the root tissues, with significantly higher populations compared to uninoculated controls. Colonization increased over time, being higher at 14 days after inoculation (DAI) than at 7 DAI (Fig.7). Among the tested isolates, *Epicoccum* sp. E13P and *Fusarium proliferatum* E25P exhibited the highest population densities (7.67 × 10^2 and 7.33 × 10^2 cfu/g, respectively), while *Fusarium solani* E26P showed the lowest colonization (2.33 × 10^2 cfu/g). Re-isolation confirmed the persistence of these endophytes, with E25P, E1P (*Coniothyrium* sp.), and E13P showing the strongest establishment within root tissues. CLSM analysis further validated these results, revealing that the endophytes predominantly colonized the intercellular spaces of the epidermis and the outer cortex of okra roots. The colonization pattern varied among isolates, with treated roots showing dense and uniform colonization, whereas control roots displayed no fungal presence (Fig. 8). These findings collectively demonstrate that the selected fungal endophytes not only establish effectively in okra roots but also persist over time, supporting their role as potential biocontrol and plant growth-promoting agents.

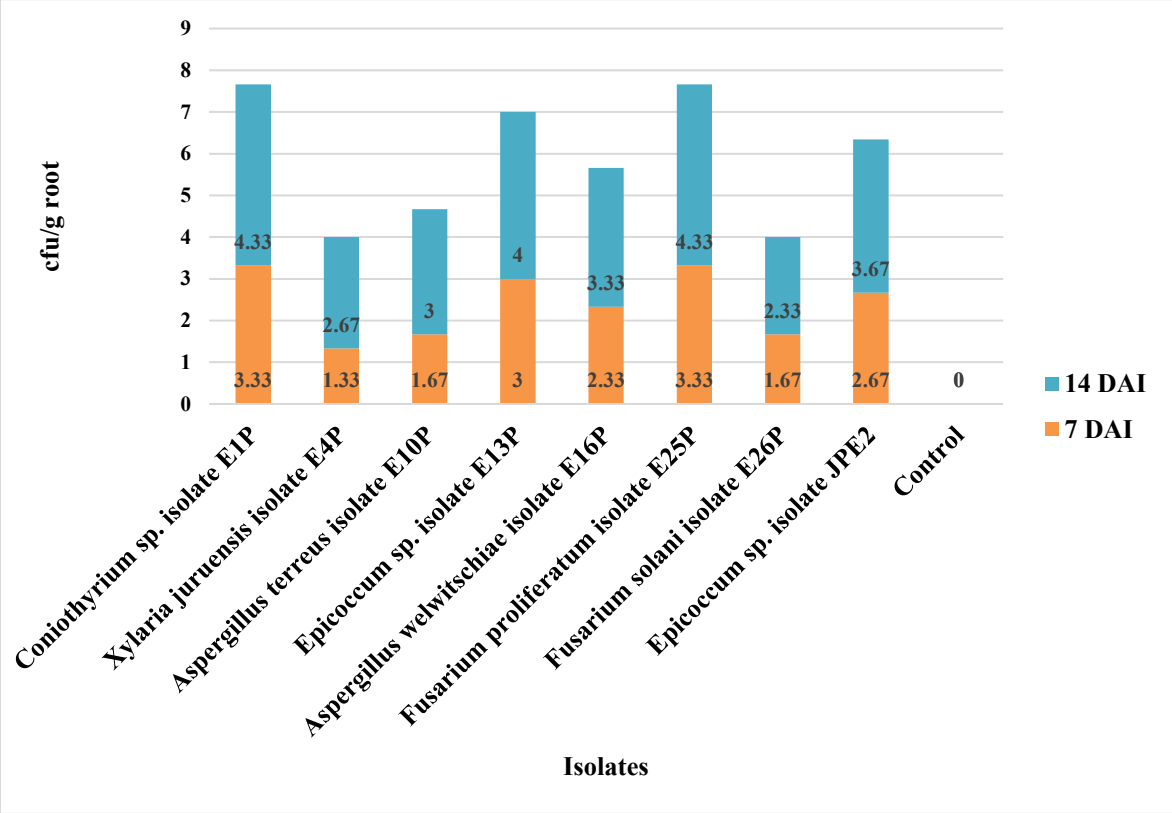
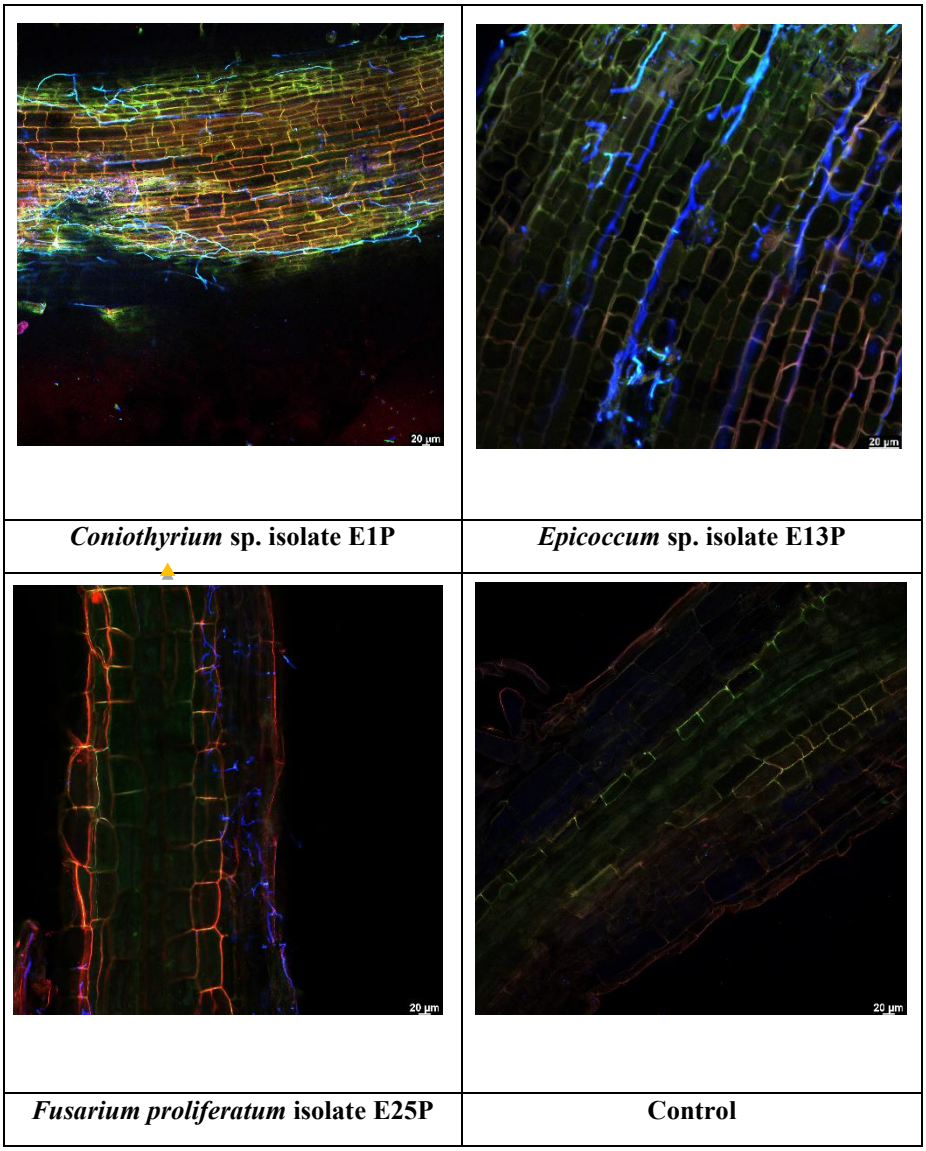


Fig. 7 Re-isolation frequency and colonization confirmation of inoculated endophytic fungi by plate count technique



***Coniothyrium* sp. isolate E1P**	***Epicoccum* sp. isolate E13P**
***Fusarium proliferatum* isolate E25P**	**Control**

Fig. 8 Confocal microscopic analysis of colonized and established fungal endophytes. Blue colored fungal hyphae (yellow arrows) showing colonization in the roots of okra (a, b & c). No fungal colonization observed in control images (d). Images were captured at 10X & 20X objective. (Scale bar- 20 µm)

Discussion

Okra (*Abelmoschus esculentus* L.) is a nutritionally rich and economically important vegetable cultivated widely across tropical and subtropical regions of the world (De Rosa et al. 2010; Doymaz 2005; Gemede 2015; Wu et al. 2022). Its remarkable nutritional profile and adaptability have contributed to its high market demand, particularly during the summer season in regions such as Himachal Pradesh (Tikoo et al. 2025). However, okra productivity is constrained by multiple soil-borne pathogens, among which damping-off disease is a major bottleneck, particularly affecting early crop establishment. The opportunistic fungal pathogen *Rhizoctonia solani* has emerged as a principal causative agent responsible for considerable losses in okra-growing regions of Himachal Pradesh (Sharma 2011; Patel et al. 2019). Damping-off can lead to yield reductions of 10–30%, and under severe conditions may result in

555 complete crop failure, depending on varietal susceptibility and environmental factors (Patil et al. 2015; Mohankumar
556 et al. 2016; Wu et al. 2022; Mimma et al. 2023). Traditional strategies, including crop rotation, seed treatments, soil
557 fumigation, and chemical fungicide applications, have shown limited success due to the emergence of resistant
558 pathogen strains, environmental contamination, and negative impacts on non-target microflora (Lamichhane et al.
559 2017; Pandey et al. 2018; Bhattarai et al. 2022; Akber and Fang 2024). Consequently, there is a pressing need for
560 sustainable, eco-friendly strategies for disease management, especially in high-demand summer crops (Sharma et al.
561 2015; Abdelaziz et al. 2022).

562 In this context, fungal endophytes represent a promising avenue for integrated disease management. These
563 microorganisms colonize plant tissues internally, establishing mutualistic associations that confer multiple benefits,
564 including disease suppression, growth promotion, and enhanced resilience against abiotic stresses (Attia et al. 2022a,
565 b; Hashem et al. 2023; Pandian et al. 2024; Zotchev 2024). Unlike rhizospheric microbes, endophytes inhabit the
566 same ecological niche as vascular pathogens, experiencing less antagonism and greater stability under biotic and
567 abiotic stresses (Dutta et al. 2014; de Lamo and Takken 2020; Jiajia et al. 2023). Additionally, their ability to
568 produce diverse bioactive secondary metabolites—such as alkaloids, steroids, terpenoids, flavonoids, quinones, and
569 phenols—enhances their biocontrol and plant growth-promoting potential (El Enshasy et al. 2020; Hazra et al.
570 2024). To the best of our knowledge, the present study provides the first comprehensive report on endophytic fungi-
571 mediated management of okra damping-off, offering a novel, sustainable approach for mitigating crop losses while
572 improving plant growth and resilience.

573 Field surveys conducted across Kangra, Mandi, and Hamirpur districts revealed widespread incidence of damping-
574 off, ranging from 31.67% to 84.33%, with early-sown okra (April–May) exhibiting greater susceptibility compared
575 to late-sown crops (May–June). These findings underscore the urgency of effective management strategies and are
576 consistent with earlier reports of widespread damping-off of okra in Himachal Pradesh (Jandaik et al. 2015; Patel et
577 al. 2019). A total of 44 fungal isolates were recovered from symptomatic samples, with *R. solani* emerging as the
578 predominant pathogen (16 isolates). Detailed morpho-molecular characterization confirmed the causal agent as *R.*
579 *solani* isolate JPO1 (GenBank accession MK621284.1), corroborating previous studies from India and Iraq where *R.*
580 *solani* was identified as the primary pathogen responsible for okra damping-off (Sharma 2011; Jandaik et al. 2015;
581 Patel et al. 2019; Madhi et al. 2020).

582 Pathogenicity assays conducted on the susceptible okra cultivar '*Palam Komal*' confirmed the virulence of *R. solani*,
583 with characteristic water-soaked sunken lesions appearing seven days' post-inoculation, followed by wilting and
584 seedling collapse. These observations align with previous reports of damping-off symptoms caused by *R. solani*,
585 *Fusarium spp.*, and *Macrophomina phaseolina* (El-Mohamedy 2004; Sharma 2011; Gondal et al. 2019; Kushwaha
586 et al. 2024).

587 Quantitative assessment of root endophytic fungi revealed significant spatial variation across surveyed districts, with
588 41 isolates recovered. *Fusarium* and *Aspergillus* spp. were the most frequent endophytes, consistent with reports
589 indicating their widespread ecological distribution and dominance across diverse host plants (Maciá-Vicente et al.

2008; Fontana et al. 2021; Singh et al. 2021; Gupta et al. 2023; Zhang et al. 2024; Kushwaha et al. 2024). Diversity was influenced by host genotype, age, soil properties, altitude, and geographic location (Arnold 2007; Katoch et al. 2014; Adnan et al. 2018; Jin et al. 2021).

Eight isolates exhibiting ≥40% in vitro antagonism were selected for further characterization. ITS-based molecular analysis validated morpho-cultural identifications: E1P as *Coniothyrium* sp., E4P as *Xylaria juruensis*, E10P as *Aspergillus terreus*, E13P as *Epicoccum* sp., E16P as *Aspergillus welwitschiae*, E25P as *Fusarium proliferatum*, E26P as *Fusarium solani*, and JPE2 as *Epicoccum* sp. Phylogenetic analysis confirmed these identities.

Dual-culture assays demonstrated pronounced antagonistic activity, with microscopic examination revealing marked deformation of pathogen hyphae, likely mediated by endophyte-produced secondary metabolites. This supports the multifunctional biocontrol potential of fungal endophytes through mechanisms including nutrient competition, antibiosis, and mycoparasitism, consistent with earlier reports (Lahlali and Hijri 2010; Shentu et al. 2014; Hadimani and Naik 2018; Pal et al. 2020; Singh et al. 2021; Alves et al. 2021; Kushwaha et al. 2024; Zhang et al. 2024).

PGP assays confirmed the ability of endophytes to solubilize phosphate, produce siderophores and HCN, and express lytic enzymes. Stress tolerance studies revealed resilience to a range of pH, salinity, and temperature conditions, while phytochemical profiling identified alkaloids, phenols, tannins, triterpenoids, saponins, and flavonoids. These properties are associated with enhanced plant growth and pathogen suppression, consistent with prior findings on multifunctional endophytes (Waqas et al. 2015; Ripa et al. 2019; Husna et al. 2021; Gopane et al. 2024; Narayanan et al. 2024).

Glasshouse and field trials confirmed *Fusarium proliferatum* E25P and *Epicoccum* sp. E13P as the most effective isolates in reducing both pre- and post-emergence damping-off, enhancing germination, shoot/root length, and vigour index. *Coniothyrium* sp. E1P demonstrated moderate but consistent performance. Root colonization, assessed through plate counts and confocal microscopy, revealed extensive establishment within epidermal and cortical tissues, supporting both biocontrol and growth promotion functions. These results are in line with studies demonstrating successful colonization and functional impact of endophytes in crops like sorghum, tomato, pea, and legumes (Favaro et al. 2012; Rajini et al. 2020; Ebrahimi et al. 2023; Kushwaha et al. 2024).

Collectively, this study demonstrates that selected endophytic fungi can simultaneously suppress *R. solani*-induced damping-off and enhance early growth and vigour of okra under glasshouse and field conditions. The isolate-specific efficacy and stable performance of E25P and E13P highlight their potential as bioformulations for sustainable okra cultivation. This research not only expands the knowledge base on endophyte-mediated disease management but also provides a practical, environmentally sustainable strategy to mitigate a major constraint in summer okra production.

This study demonstrates the high potential of endophytic fungi as sustainable biocontrol agents against *Rhizoctonia solani*-induced damping-off in okra. Among the 42 isolates screened, *Fusarium proliferatum* E25P and *Epicoccum* sp. E13P consistently exhibited superior antagonistic activity, enhanced seed germination, and improved early

seedling growth under both glasshouse and field conditions. The isolates successfully colonized okra root tissues, as confirmed by plate counts and confocal microscopy, establishing stable and persistent endophytic populations.

In addition to disease suppression, selected endophytes displayed multiple plant growth-promoting traits, stress tolerance, and production of bioactive secondary metabolites, highlighting their multifunctional role in enhancing crop health and resilience. These findings underscore the feasibility of developing fungal endophytic formulations as eco-friendly, effective, and practical alternatives to chemical fungicides for damping-off management in okra, particularly under summer cultivation.

Overall, this research provides novel insights into endophyte-mediated disease control and growth promotion, offering a promising strategy for sustainable okra production while contributing to the broader understanding of plant–endophyte interactions in agroecosystems.

References

Abbas A, Jiang D, Fu Y (2017) *Trichoderma* spp. as antagonist of *Rhizoctonia solani*. J Plant Pathol and Microbiol 8:1-9. https:// doi.org/ 10.4172/2157-7471.1000402

Abd Alhakim A, Hashem A, Abdelaziz AM, Attia MS (2022). Impact of plant growth promoting fungi on biochemical defence performance of tomato under fusarial infection. Egyptian Journal of Chemistry 65:291-301. https://doi.org/10.21608/ejchem.2022.124008.5532

Abdelaziz AM, Attia MS, Salem MS, Refaay DA, Alhoqail WA, Senousy HH (2022) Cyanobacteria-mediated immune responses in pepper plants against fusarium wilt. Plants 11:2049. https://doi.org/10.3390/plants11152049

Adnan M, Alshammari E, Ashraf SA, Patel K, Lad K, Patel M (2018) Physiological and molecular characterization of biosurfactant producing endophytic fungi *Xylaria regalis* from the cones of *Thuja plicata* as a potent plant growth promoter with its potential application. BioMed Research International 7:21-48. https://doi.org/10.1155/2018/7362148

Agarwal GP, Hasija SK (1986) Microorganisms in Laboratory-a laboratory guide of mycology, microbiology and plant pathology. Print House India Ltd., Lucknow, Inc p. 155

Akber MA, Fang X (2024) Research progress on diseases caused by the soil-borne fungal pathogen *Rhizoctonia solani* in Alfalfa. Agronomy 14:1483. https://doi.org/10.3390/agronomy14071483

Alam B, Lǐ J, Gě Q, Khan MA, Gōng J, Mehmood S, Gǒng W (2021) Endophytic fungi: from symbiosis to secondary metabolite communications or vice versa. Frontiers in Plant Science 12:791033. https://doi.org/10.3389/fpls.2021.791033

Alves NM, Guimaraes RA, Guimaraes SSC, de Faria AF, Santos ÍAFM, de Medeiros FHV, Cardoso PG (2021) A Trojan horse approach for white mold biocontrol: Paraconiothyrium endophytes promotes grass growth and inhibits *Sclerotinia sclerotiorum*. Biological Control 160:104685. https://doi.org/10.1016/j.biocontrol.2021.104685

Arnold AE, Henk DA, Eells RL, Lutzoni F, Vilgalys R (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia 99:185-206. https://doi.org/10.1080/15572536.2007.11832578

Attia MS, Abdelaziz AM, Al-Askar AA, Arishi AA, Abdelhakim AM, Hashem AH (2022) Plant growth-promoting fungi as biocontrol tool against Fusarium wilt disease of tomato plant. Journal of Fungi 8:775. https://doi.org/10.3390/jof8080775

Attia MS, El-Wakil DA, Hashem AH, Abdelaziz AM (2022) Antagonistic effect of plant growth-promoting fungi against Fusarium wilt disease in tomato: in vitro and in vivo study. Applied Biochemistry and Biotechnology 194:5100-5118. https://doi.org/10.1007/s12010-022-03975-9

Barretti PB, Souza RM, Pozza AAA, Pozza EA, Carvalho JG, Souza JT (2008) Aumento da eficiência nutricional de tomateiros inoculados com bactérias endofíticas promotoras de crescimento. Revista Brasileira de Ciencia do Solo 32:1541-1548. https://doi.org/10.1590/S0100-06832008000400018

Benchasri S (2012) Okra (*Abelmoschus esculentus* (L.) Moench) as a valuable vegetable of the world. Field & Vegetable Crops Research/Ratarstvo i povrtarstvo 49:105-112. https://doi.org/ 10.5937/ratpov49-1172

Bhardwaj A, Sharma D, Jadon N, Agrawal PK (2015) Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinus roxburghii*. Archives of Clinical Microbiology 6:56-59

Bhardwaj P, Jain R, Rawat N, Joshi R, Kumar A, Pandey SS, Kumar S (2025) *Naganishia liquefaciens* ARY7, a Psychrophilic Yeast Endophyte Improves Plant Low Temperature Acclimation Through Auxin and Salicylic Acid Signaling. Physiologia Plantarum 177:e70267. http://doi.org/10.1007/s00299-024-03216-4

Bhattarai A, Sharma A, Yadav RK, Wagle P (2022) Interacting effects of botanicals, biocontrol agents, and potting media on *Rhizoctonia solani* led damping-off of okra seedlings. Journal of Agriculture and Food Research 10:100410. http://doi.org/10.1016/j.jafr.2022.100410

Boerema GH, Gruyter J, Noordeloos ME, Hamers MEC (2004) Phoma identification manual differentiation of specific and infra-specific taxa in culture. CABI Publisher, Inc. p 1-7

Booth C (1971) Methods in Microbiology. Academic press, Inc. p 324-330

Campos RPC, Jacob JKS (2021) Biocontrol potential of endophytic *Aspergillus* spp. against *Fusarium verticillioides*. Biotropia 28:141-148. http://doi.org/10.1007/s00253-019-09746-1

683 Christensen M and Raber KB (1978) Synoptic key to *Aspergillus nidulans* group species and related *Emericella*
684 species. Transactions of the British Mycological Society 71:177-191. http://doi.org/10.1016/S0261-2194(78)80029-
685 8

686 Constantin ME, De Lamo FJ, Vliege BV, Rep M, Takken FL (2019) Endophyte-mediated resistance in tomato to
687 *Fusarium oxysporum* is independent of ET, JA, and SA. Frontiers in Plant Science 10:979.
688 https://doi.org/10.3389/fpls.2019.00979

689 Coskuntuna A, Ozer N (2008) Biological control of onion basal rot disease using *Trichoderma harzianum* and
690 induction of antifungal compounds in onion set following seed treatment. Crop Protection 27:330–336.
691 https://doi.org/10.1016/j.cropro.2007.06.002

692 Daroodi Z, Taheri P, Tarighi S (2021) Direct antagonistic activity and tomato resistance induction of the endophytic
693 fungus *Acrophialophora jodhpurensis* against *Rhizoctonia solani*. Biological Control 160:104696.
694 http://doi.org/10.1016/j.cropro.2007.08.001

695 de Lamo FJ, Constantin ME, Fresno DH, Boeren S, Rep M, Takken FL (2018) Xylem sap proteomics reveals
696 distinct differences between R gene-and endophyte-mediated resistance against Fusarium wilt disease in
697 tomato. Frontiers in Microbiology 9:2977. https://doi.org/10.3389/fmicb.2018.02977

698 de Lamo FJ, Takken FL (2020) Using Endophyte-Mediated Resistance: Role of Endophytes in Plant Health and
699 Defense Against Pathogens. http://doi.org/10.1016/j.biocontrol.2021.104696

700 De Rosa IM, Kenny JM, Puglia D, Santulli C, Sarasini F (2010) Morphological, thermal and mechanical
701 characterization of okra (*Abelmoschus esculentus*) fibres as potential reinforcement in polymer
702 composites. Composites Science and Technology 70:116-122. http://doi.org/10.1016/j.compscitech.2009.09.006

703 Diwakar MP, Rajput JC, D'Souza TF (1986) Diseases of okra in Konkan region of Maharashtra. Pesticides 20:3-34

704 Doymaz İ (2005) Drying characteristics and kinetics of okra. Journal of food Engineering 69:275-279.
705 http://doi.org/10.1016/j.jfoodeng.2004.11.003

706 Dutta D, Puzari KC, Gogoi R, Dutta P (2014) Endophytes: exploitation as a tool in plant protection. Brazilian
707 archives of Biology and Technology 57:621-629. http://doi.org/10.1590/S1516-89132014005000001

708 Ebrahimi L, Tadayon RF, Lotfi M (2023) Antagonism of endophytic fungi depends on pathogen and host plant. Bio
709 Control 68:655-668. http://doi.org/10.1007/s10526-023-10192-1

710 El Enshasy HA, Hanapi SZ, Malek RA, Abdelgalil SA, Leng OM (2019) Endophytic Fungi: The Desired
711 Biostimulants for Essential Oil Production. Springer International Publishing, Inc. p 211-232

El Enshasy HA, Ambehabati KK, El Baz AF, Ramchuran S, Sayyed RZ, Amalin D, Hanapi SZ (2020) Trichoderma: biocontrol agents for promoting plant growth and soil health. In Agriculturally important fungi for sustainable agriculture: Volume 2: Functional annotation for crop protection (pp. 239-259). Cham: Springer International Publishing

El-Mohamedy RSR (2004) Bio-priming of okra seeds to control damping off and root rot diseases. Annals of Agricultural Science 49:339-356

El-Sharkawy HH, Rashad YM, Elazab NT (2022) Synergism between *Streptomyces viridosporus* HH1 and *Rhizophagus irregularis* effectively induces defense responses to fusarium wilt of pea and improves plant growth and yield. Journal of Fungi 8:683. http://doi.org/10.3390/jof8070683

Favaro LCDL, Sebastianes FLDS, Araujo WL (2012) *Epicoccum nigrum* P16, a sugarcane endophyte, produces antifungal compounds and induces root growth. PloS One 7:e36826. http://doi.org/10.1371/journal.pone.0036826

Fontana DC, de Paula S, Torres AG, de Souza VHM, Pascholati SF, Schmidt D, Dourado Neto D (2021) Endophytic fungi: Biological control and induced resistance to phytopathogens and abiotic stresses. Pathogens 10:570. http://doi.org/10.3390/pathogens10050570

Gaigole AH, Wagh GN, Khadse AC (2011) Antifungal activity of Trichoderma species against soil borne pathogen. Asiatic Journal of Biotechnology Resources 2:461-465

Gaur SS, Raveendran A, Athulya A, Narayanan SA, Shams R, Dash KK, Béla K (2025) Exploring the nutritional, pharmacological, and industrial potential of okra and its byproducts: a comprehensive review. Discover Food 5:251. https://doi.org/10.1007/s44187-025-00492-6

Gemede HF, Ratta N, Haki GD, Woldegiorgis AZ, Beyene F (2015) Nutritional quality and health benefits of okra (*Abelmoschus esculentus*): A review. Journal of Food Processing and Technology 6:2

Ghaffar I, Imtiaz A, Hussain A, Javid A, Jabeen F, Akmal M, Qazi JI (2018) Microbial production and industrial applications of keratinases: an overview. International Microbiology 21:163-174. https://doi.org/10.1007/s10123-018-0022-1

Gondal AS, Rauf A, Naz F (2019) Anastomosis groups of *Rhizoctonia solani* associated with tomato foot rot in Pothohar region of Pakistan. Scientific Reports 9:3910. http://doi.org/10.1038/s41598-019-40325-8

Gopane B, Tchatchouang C-DK, Regnier T, Ateba CN, Manganyi M (2024) Bioactivity and enzymatic properties of culturable endophytic fungi associated with black seeds (*Nigella sativa* L.). Journal of Applied Pharmaceutical Science 14:102-110. https://dx.doi.org/10.7324/JAPS.2024.160668

Gupta A, Meshram V, Gupta M, Goyal S, Qureshi KA, Jaremko M, Shukla KK (2023) Fungal endophytes: Microfactories of novel bioactive compounds with therapeutic interventions; A comprehensive review on the

743 biotechnological developments in the field of fungal endophytic biology over the last
744 decade. Biomolecules 13:1038. https://doi.org/10.3390/biom13071038

745 Hadimani B, Naik ST (2018) Hydrolytic enzyme and plant growth promotion activity of fungal and bacterial
746 endophytes of tomato. Journal of Pharmacognosy and Phytochemistry 7:766-768

747 Hankin L, Anagnostakis S (1975) The use of solid media for detection of enzyme production by fungi. Mycologyia
748 67:597-607. https://doi.org/10.1080/00275514.1975.12019782

749 Hashem AH, Al-Askar AA, Abd Elgawad H, Abdelaziz AM (2023) Bacterial endophytes from *Moringa oleifera*
750 leaves as a promising source for bioactive compounds. Separations 10:395.
751 https://doi.org/10.3390/separations10070395

752 Hazra S, Das D, Moulick D, Hossain A (2024) Endophytes: the treasure house of bioactive compounds with
753 potential applications in sustainable agriculture and other sectors. In Biocontrol agents for improved agriculture (pp.
754 477-506). Academic Press. https://doi.org/10.1016/B978-0-443-15199-6.00001-4

755 Ho HH (1981) Synoptic keys to the species of Phytophthora. Mycologia 73:704-714.
756 https://doi.org/10.1080/00275514.1981.12021398

757 Husna HA, Shah M, Hamayun M, Qadir M, Iqbal A (2021) Heavy metal tolerant endophytic fungi *Aspergillus*
758 *welwitschiae* improves growth, ceasing metal uptake and strengthening antioxidant system in *Glycine max* L.
759 Environmental Science and Pollution Research 29:15501-15515. https://doi.org/10.1007/s11356-021-16640-1

760 Hussain F, Abid M, Shaukat SS (2014) Ethnobotanical study of Mirpurkhas region in Sindh-Pakistan. Scholar's
761 Press, Germany, Inc. p 224

762 Jagadevi Shivaputrappa VGM (2018) Phytochemical and antimicrobial evaluation of endophytic *Alternaria*
763 *alternata*. Asian Journal of Pharmacy and Pharmacology 4:456-461. https://doi.org/10.31024/ajpp.2018.4.4.13

764 Jandaik S, Thakur P, Kumar V (2015) Efficacy of cow urine as plant growth enhancer and antifungal agent.
765 Advances in Agriculture 12:122. https://doi.org/10.1155/2015/620368

766 Jiajia L, Lijuan L, Arif M, Dongdong D, Xin H, Changxiao L (2023) Newly formed riparian microhabitats simplify
767 bacterial community structure and diversity. Journal of Soils and Sediments 23:1927-1943.
768 https://doi.org/10.1007/s11368-023-03454-6

769 Jin L, Yang L, Li W, Xu D, Yang N, Li G, Wan P (2021) Diversity and biocontrol potential of culturable endophytic
770 fungi in cotton. Frontiers in Microbiology 12:698930. https://doi.org/10.3389/fmicb.2021.698930

771 Jiskani MM, Pathan MA, Wagan KH, Imran M, Abro H (2007) Studies on the control of tomato damping-off
772 disease caused by *Rhizoctonia solani* Kuhn. Pakistan Journal of Botany 39:2749-2754

Jukte S, Badgujar SL, Suryawanshi AP (2016) Efficacy of plant extracts against *Pythium aphanidermatum* causing damping off in Okra. International Journal of Plant Protection 11:116-118

Karmakar P, Maurya BK, Sagar V, Singh S (2025) Okra breeding for export and processing. Training manual 4:51

Katoch M, Singh G, Sharma S, Gupta N, Sangwan PL, Saxena AK (2014) Cytotoxic and antimicrobial activities of endophytic fungi isolated from *Bacopa monnieri* (L.) Pennell (Scrophulariaceae). BMC Complementary and Alternative Medicine 14:1-8. http://doi.org/10.1186/1472-6882-14-87

Kumar A, Singh J, Singh A, Pratap D (2025) Study of genetic variability and emerging strains of Okra enation leaf curl virus: increasing risks to okra production in India. Physiology and Molecular Biology of Plants 31:767-783. https://doi.org/10.1007/s12298-025-01578-2

Kumar R, Karmakar P, Bahadur A, Sreekanth D, Kumar Verma R, Krishna H (2025) Exploring drought tolerance in okra through morpho-physiological, biochemical characteristics and yield based indices. Journal of Crop Health 77:91. http://doi.org/10.1007/s10343-025-01091-5

Kushwaha K, Pal J, Banyal DK, Paul S, Katoch G (2024) Unlocking the biocontrol and plant growth potential of endophytic fungi against pea root rot complex in Himachal Pradesh, India. European Journal of Plant Pathology 170:1-18. http://doi.org/10.1007/s10658-024-02867-5

Lahlali R, Hijri M (2010) Screening, identification and evaluation of potential biocontrol fungal endophytes against *Rhizoctonia solani* AG3 on potato plants. FEMS Microbiology Letters 311:152-159. http://doi.org/10.1111/j.1574-6968.2010.02076.x

Lamichhane JR, Durr C, Schwanck AA, Robin MH, Sarthou JP, Cellier V, Aubertot JN (2017) Integrated management of damping-off diseases, A review. Agronomy for Sustainable Development 37:1-25. http://doi.org/10.1007/s13593-017-0417-y

Llorens E, García-Agustín P, Lapeña L (2017) Advances in induced resistance by natural compounds: towards new options for woody crop protection. Scientia Agricola 74:90-100. http://doi.org/10.1590/1678-992X-2016-0101

Lorck H (1948) Production of hydrocyanic acid by bacteria. Plant Physiology 1:142-146. http://doi.org/10.1111/j.1399-3054.1948.tb07118.x

Macia-Vicente JG, Jansson HB, Samir K, Abdullah SK, Descals E, Salinas J, Lopez-Llorca LV (2008) Fungal root endophytes from natural vegetation in Mediterranean environments with special reference to *Fusarium* spp. FEMS Microbiology Ecology 64:90-105. http://doi.org/10.1111/j.1574-6941.2008.00491.x

Madhi QH, Daghir GM, Jumaah AM (2020) Investigation of the causes of root rot and damping-off diseases of okra and testing the effectiveness of *Pseudomonas fluorescens* in disease control. Plant Archives 20:1361-1366

804 Mathur SB, Olga K (2003) Common laboratory seed health testing methods for detecting Fungi. DK-1871
805 Frederiksberg C, Copenhagen, p 425

806 Matny ON (2013) First report of damping-off of okra caused by *Phytophthora nicotianae* in Iraq. Plant Disease
807 97:558-558. http://doi.org/10.1094/PDIS-09-12-0863-PDN

808 Miles LA, Lopera CA, González S, de García MC, Franco AE, Restrepo S (2012) Exploring the biocontrol potential
809 of fungal endophytes from an Andean Colombian Paramo ecosystem. Bio Control 57:697-710.
810 http://doi.org/10.1007/s10526-011-9427-2

811 Mimma AA, Akter T, Haque MA, Bhuiyan MAB, Chowdhury MZH, Sultana S, Islam SMN (2023) Effect of
812 *Metarhizium anisopliae* (MetA1) on growth enhancement and antioxidative defense mechanism against Rhizoctonia
813 root rot in okra. Heliyon 9:18978. https://doi.org/10.1016/j.heliyon.2023.e18978

814 Mishra GP, Singh B, Seth T, Singh AK, Halder J, Krishnan N, Singh PM (2017) Biotechnological advancements
815 and begomovirus management in okra (*Abelmoschus esculentus* L.): status and perspectives. Frontiers in plant
816 science 8:360. https://doi.org/10.3389/fpls.2017.00360

817 Mohankumar S, Karthikeyan G, Durairaj C, Ramakrishnan S, Preetha B, Sambathkumar S (2016) Integrated pest
818 management of okra in India. Integrated Pest Management of Tropical Vegetable Crops. Springer, Inc. p 167-177.
819 https://doi.org/10.1007/978-94-024-0924-6_7

820 Muimba-Kankolongo A (2018) Root and Tuber Crops. Springer, Inc. p 120

821 Murthy HN, Lee EJ, Paek KY (2014) Production of secondary metabolites from cell and organ cultures: strategies
822 and approaches for biomass improvement and metabolite accumulation. Plant Cell, Tissue and Organ Culture 118:1-
823 16. http://doi.org/10.1007/s11240-014-0467-7

824 Muthusamy Pandian T, Esakkimuthu R, Rangasamy A, Rengasamy K, Alagesan S, Devasahayam JSS (2024)
825 Exploring the potential of bacterial endophytes in plant disease management. Current Microbiology 81:403.
826 http://doi.org/10.1007/s00284-024-03663-5

827 Narayanan MM, Metali F, Shivanand P, Ahmad N (2024) Mangrove endophytic fungi: Biocontrol potential against
828 *Rhizoctonia solani* and biofertilizers for fragrant rice cultivation. Heliyon 10:e32310.
829 http://doi.org/10.1016/j.heliyon.2024.e32310

830 Nasimi Z, Barriuso J, Keshavarz T, Zheng A. 2024. Molecular, physiological, and biochemical properties of
831 sclerotia metamorphosis in *Rhizoctonia solani*. Fungal Biology Reviews 48:100351.
832 http://doi.org/10.1016/j.fbr.2024.100351

833 Nelson PE, Toussoun TA, Marasas WFO (1983) Fusarium Species: An Illustrated Manual for Identification.
834 Pennsylvania State University Press, University. Park P. 193

Pal J, Sharma SK, Devi S, Sharma R, Raj H, Karn M, Sharma A (2020) Screening, identification, and colonization of fungal root endophytes against *Dematophora necatrix*: a ubiquitous pathogen of fruit trees. Egyptian Journal of Biological Pest Control 30:1-14. http://doi.org/10.1186/s41938-020-00273-4

Pandey AK, Burlakoti RR, Kenyon L, Nair RM (2018) Perspectives and challenges for sustainable management of fungal diseases of mungbean [*Vigna radiata* (L.) R. Wilczek var. *radiata*]: A review. Frontiers in Environmental Science 6:53. http://doi.org/10.3389/fenvs.2018.00053

Parmeter J (1970) *Rhizoctonia Solani*, Biology and Pathology: Based on an american phytopathological society symposium on *Rhizoctonia solani* held at the Miami meeting of the Society. Berkeley, University of California Press

Patel JV, Singh A, Kaur K, Bhandhari D, Banyal DK (2019) Biological control of damping off of okra caused by *Rhizoctonia solani*. International Journal of Current Microbiology and Applied Sciences 8:2277-2292. https://doi.org/10.20546/ijcmas.2019.812.270

Potshangbam M, Devi SI, Sahoo D, Strobel GA (2017) Functional characterization of endophytic fungal community associated with *Oryza sativa* L. and *Zea mays* L. Frontiers in Microbiology 8:325. http://doi.org/10.3389/fmicb.2017.00325

Rajini SB, Nandhini M, Udayashankar AC, Niranjana SR, Lund OS, Prakash HS (2020) Diversity, plant growth promoting traits, and biocontrol potential of fungal endophytes of *Sorghum bicolor*. Plant pathology 69:642-654. http://doi.org/10.1111/ppa.13150

Rashad Y, Aseel D, Hammad S, Elkelish A (2020) *Rhizophagus irregularis* and *Rhizoctonia solani* differentially elicit systemic transcriptional expression of polyphenol biosynthetic pathways genes in sunflower. Biomolecules 10:379. http://doi.org/10.3390/biom10030379

Ripa FA, Cao WD, Tong S, Sun JG (2019) Assessment of plant growth promoting and abiotic stress tolerance properties of wheat endophytic fungi. BioMed Research International 6:12. http://doi.org/10.1155/2019/6105865

Rubatzky VE, Yamaguchi M (1997) World vegetables principles, production and nutritive values. Fruits 5:381

Samson RA, Varga J, Frisvad JC (2011) Taxonomic studies on the genus *Aspergillus*. Studies in Mycology 69:91-97. http://doi.org/10.3114/sim.2011.69.06

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160:47-56. http://doi.org/10.1016/0003-2697(87)90612-9

Sharaf MH, Abdelaziz AM, Kalaba MH, Radwan AA, Hashem AH (2022) Antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis of fungal endophytes isolated from *Ocimum basilicum*. Applied Biochemistry and Biotechnology 194:1-19. http://doi.org/10.1007/s12010-022-03909-0

Sharma N (2011) Etiology and eco-friendly management of damping off of okra. M.Sc. Thesis, p 27. CSK Himachal Pradesh Krishi Vishavavidalaya, Palampur, Himachal Pradesh, India

Sharma S, Roy S (2015) Isolation and Identification of a novel endophyte from a plant *Amaranthus spinosus*. Int J Curr Microbiol Appl Sci 4:785-798

Sharma IJ, Samnotra RK, Kumar V, Rai, AP, Dhotra B (2015) Effect of organic and inorganic fertilizers on the growth and yield of okra under sub-tropical region. Annals of Plant and Soil Research 17:215-218

Shehabeldine AM, Abdelaziz AM, Abdel-Maksoud MA, El-Tayeb MA, Kiani BH, Hussein AS (2024) Antimicrobial characteristics of endophytic *Aspergillus terreus* and acute oral toxicity analysis. Electronic Journal of Biotechnology 72:1-11. http://doi.org/10.1016/j.ejbt.2024.01.002

Shentu X, Zhan X, Ma Z, Yu X, Zhang C (2014) Antifungal activity of metabolites of the endophytic fungus *Trichoderma brevicompactum* from garlic. Brazilian Journal of Microbiology 45:248-254. http://doi.org/10.1016/j.bjm.2013.11.012

Singh A, Kumar J, Sharma VK, Singh DK, Kumari P, Nishad JH, Gautam VS, Kharwar RN (2021) Phytochemical analysis and antimicrobial activity of an endophytic *Fusarium proliferatum* (ACQR8), isolated from a folk medicinal plant *Cissus quadrangularis* L. South. African Journal of Botany 140:87-94. https://doi.org/10.1016/j.sajb.2021.03.004

Song JJ, Pongnak W, Soytong K (2016) Isolation and identification of endophytic fungi from 10 species palm trees. International Journal of Agricultural Technology 12:349-363

Tamura K, Stecher G and Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Molecular Biology and Evolution 38:3022-3027. http://doi.org/10.1093/molbev/msab120

Thorati M, Mishra JK (2017) Antibacterial activity of crude extract from *Aspergillus niger* isolated from the stilt roots of *Rhizophora apiculata* along South Andaman coast, India. Journal of Pharmacognosy and Phytochemistry 6:1635-1638

Tikoo SK, Angadi S, Tiwari A, Yadav RK, Tomar BS, Adeniyi AH (Eds.) (2025) Okra: Status, Challenges and Opportunities. https://doi.org/10.1007/978-981-97-9963-3

Toghueo RMK, Zabalgogeazcoa I, Vázquez de Aldana BR, Boyom FF (2017) Enzymatic activity of endophytic fungi from the medicinal plants *Terminalia catappa*, *Terminalia mantaly* and *Cananga odorata.* South African Journal of Botany 109:146-153. http://doi.org/10.1016/j.sajb.2016.12.009

Waghunde RR, Shelake RM, Shinde MS, Hayashi H (2017) Endophyte microbes: a weapon for plant health management. Microorganisms for Green Revolution: Microbes for Sustainable Crop Production 1:303-325. https://doi.org/10.1007/978-981-10-6241-4_16

Waqas M, Khan AL, Hamayun M, Shahzad R, Kang SM, Kim JG, Lee IJ (2015). Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: an example of *Penicillium citrinum* and *Aspergillus terreus*. Journal of plant interactions 10:280-287. http://doi.org/10.1080/17429145.2015.1079743

Waterhouse GM (1967) Key to *Pythium pringsheim*. CMI Mycology Paper 109:1-15

Wu DT, He Y, Fu MX, Gan RY, Hu YC, Peng LX, Zou L (2022) Structural characteristics and biological activities of a pectic-polysaccharide from okra affected by ultrasound assisted metal-free Fenton reaction. Food Hydrocolloids 122:107085. http://doi.org/10.1016/j.foodhyd.2021.107085

Wu DT, He Y, Yuan Q, Wang S, Gan RY, Hu YC, Zou L (2022) Effects of molecular weight and degree of branching on microbial fermentation characteristics of okra pectic-polysaccharide and its selective impact on gut microbial composition. Food Hydrocolloids 132:107897. http://doi.org/10.1016/j.foodhyd.2022.107897

Ying LIU, Zhong DING, Peng DL, Liu SM, Kong LA, Huan PENG, Huang WK (2019) Evaluation of the biocontrol potential of *Aspergillus welwitschiae* against the root-knot nematode *Meloidogyne graminicola* in rice (*Oryza sativa* L.). Journal of Integrative Agriculture 18:2561-2570. http://doi.org/10.1016/S2095-3119(19)62669-5

Yurnaliza AINP, Esyanthi RR, Susanto A (2014) Antagonistic activity assessment of fungal endophytes from oil palm tissue against *Ganoderma boninense* Pat. Plant Pathology Journal 13:257-267

Zhang JQ, Wang D, Wei T, Lai X, Tang G, Wang LH, Liang J (2024) First report of *Epicoccum nigrum* causing brown leaf spot of sweet cherry (*Prunus avium*) in China. Plant Disease 108:2217. http://doi.org/10.1094/PDIS-11-23-2521-PDN

Zotchev SB (2024) Unlocking the potential of bacterial endophytes from medicinal plants for drug discovery. Microbial Biotechnology 17:e14382. http://doi.org/10.1111/1751-7915.14382

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplimentryFile.pptx