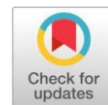


Original Research Article

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Overview on occurrence and prevalence of damping-off pathogens impacting summer season okra (*Abelmoschus esculentus* L.) in Himachal Pradesh, India



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ABSTRACT

Damping-off, caused by a range of fungal pathogens, is widely recognized as a major bottleneck in the global production of okra, including in India. In India, it is known to cause substantial and sometimes catastrophic losses, particularly during the summer season. This study aimed to assess the current status of damping-off in okra and to analyze the frequency distribution of its associated pathogens in major okra-growing regions of Himachal Pradesh. A systematic survey conducted during the summer season of 2023–24 in three districts—Kangra, Mandi, and Hamirpur—revealed a wide range of disease incidence, from 31.67% to 84.33%. The highest mean disease incidence was recorded in Hamirpur district (65.46%), followed by Mandi (59.04%), while the lowest incidence (53.33%) was observed in Kangra. A total of 44 fungal isolates associated with damping-off in okra were recovered, among which *Rhizoctonia solani* isolate JPO1 was identified as the predominant pathogen based on morpho-molecular characterization. The highest colonization frequencies were recorded for *Rhizoctonia solani* (16.67%) and *Fusarium solani* (9.37%). Overall, eight fungal genera were found to be associated with okra damping-off in Himachal Pradesh. The findings from this study underscore the prevalence and severity of damping-off in summer-grown okra and highlight the dominant pathogenic fungi responsible. This information lays the groundwork for devising effective disease management strategies against this destructive malady.

Keywords: Damping off; summer season; prevalence; colonization frequency; morpho-molecular characterization; predominant pathogen; effective management

Introduction

Okra [*Abelmoschus esculentus* L. (Moench)] is an important multipurpose indigenous vegetable cultivated primarily in tropical and sub-tropical regions across the world, including India (11). It is referred to by several names, including gumbo, ladyfinger, okra and bamia (17). In most Indian states, okra is cultivated throughout the year, including during the summer and rainy seasons (29). The crop is cherished worldwide for its exceptional nutritional value and the diverse uses of its various plant parts—leaves, buds, flowers, pods, stems, and seeds (28). The green tender fruits of okra offer an affordable source of carbohydrates, proteins, vitamins (A, B and C), calcium, potassium and other minerals (25), (19). In recent years, the seeds of okra have also attracted considerable attention as a potential source of oil (30–40%) and protein (15–20%) (6). Furthermore, the bioactive components of okra underscore its expanding applications in pharmaceutical industries for

combating various human diseases (7).

Despite of multifaceted benefits of okra, its cultivation is hampered by various biotic and abiotic stressors. Among biotic factors, damping off incited by a multitude of fungal pathogens is recognized as a major production bottleneck (16). Although the disease is reported to inflict serious losses during both summer and rainy seasons, okra is particularly susceptible to damping-off pathogens during the summer (27). Moreover, due to the destructive nature of disease, most commercial cultivars remain highly vulnerable to its causal agents. Damping-off typically leads to the decay of germinating seeds and young seedlings, which represents yield constraints both in nurseries and field conditions (22).

Given the limited information available on the colonization frequency and diversity of pathogens associated with damping-off in okra, particularly during the summer season, this study was undertaken to survey major okra-growing regions of Himachal Pradesh. There exists a significant knowledge gap regarding the colonization dynamics of damping-off pathogens in summer-grown okra crops. Considering the economic importance of the disease, the present investigation was undertaken to shed light on the prevalence of damping off across major okra growing districts of Himachal Pradesh.

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This study is significant as it provides an updated overview of the current status of damping-off in okra, with special emphasis on the fungal pathogens active during the summer season. The findings will aid in the development and deployment of more effective disease management strategies against okra damping-off.

Materials and Methods

Disease survey

To ascertain the prevalence of damping-off of okra, periodic surveys were systematically conducted in three districts (Kangra, Mandi and Hamirpur) of Himachal Pradesh during the summer season (April-June) of 2023 (Fig. 1). Data on the incidence of damping-off in okra were recorded from farmers' fields across various locations in the aforementioned districts of Himachal Pradesh. The altitude of the surveyed sites ranged from 350 to 2251 meters above sea level (m.a.s.l.). Field studies and plant material collections were conducted following local legislation, and appropriate permissions were obtained wherever required.

Eight locations were selected from each district, and three fields were chosen randomly within each location. A 1.0 m² area in each field, comprising approximately 20–25 plants, was randomly selected to assess the incidence of damping-off. The per cent disease incidence was calculated using the following formula:

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

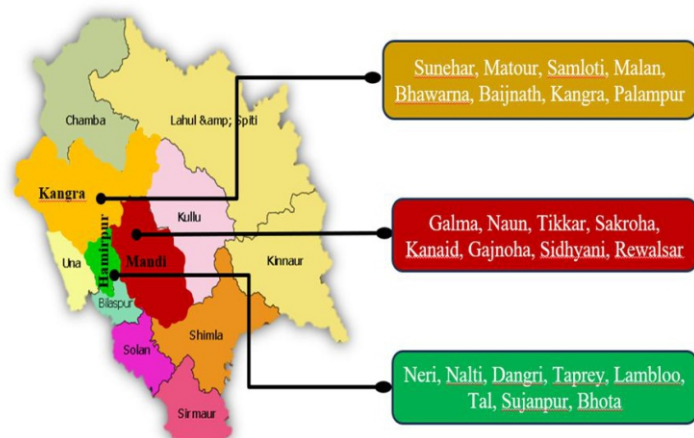


Fig 1. List of areas surveyed for recording incidence of damping-off disease of okra in different districts of Himachal Pradesh

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

During survey, healthy and symptomatic roots of okra plants displaying typical damping-off symptoms (notably water-soaked and brown lesions on the stem at the collar region) were collected for the isolation of fungal endophytes and associated pathogens, respectively. The roots of healthy and diseased plants were carefully uprooted with the help of a spade, placed in sterile polythene zip-lock bags, and immediately brought to the laboratory. Samples were processed within 24 to 48 hours of collection for the enumeration of endophytic fungi and identification of causative fungal pathogens prevalent in the major okra-growing regions of Himachal Pradesh.

Isolation and purification of pathogen(s) associated with damping-off disease of okra

To isolate the causative pathogen(s) associated with damping-

off disease, infected samples were collected and washed thoroughly under running water to remove all adhering soil particles. The samples were then air-dried for a short period on Whatman no.1 filter paper. Using a sharp, sterilized blade, small sections (about 0.5-1.0 cm) were excised longitudinally from the interface of diseased and healthy tissue, particularly from the basal stem regions exhibiting characteristic brown discoloration.

The tissue fragments were surface sterilized with 70% ethanol for 1 minute, followed by disinfection in 1% sodium hypochlorite (NaOCl) solution for another minute. The segments were then rinsed four times with autoclaved distilled water to eliminate residual disinfectants. Subsequently, the surface-dried tissues were placed on sterile filter paper to remove excess moisture and aseptically transferred to the Petri plates (four segments per plate) containing Potato Dextrose Agar (PDA) medium, amended with antibiotics to suppress bacterial contamination.

The inoculated Petri plates were incubated at 25±2°C in a BOD incubator for 4 days and observed periodically for fungal growth. Within 2 days, visible fungal mycelial outgrowth was observed from the tissue segments. Pure cultures of pathogen (s) were obtained using the hyphal tip method, in which single hyphae were transferred to fresh PDA plates and monitored under a microscope (8).

Additionally, the colonization rate (CR) or frequency of pathogen (s) associated with damping off disease of okra during the summer season was also assessed using formula (20):

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

The pure cultures were examined under a compound microscope (Olympus BX50) and identified based on cultural and morphological characteristics using standard descriptions and relevant taxonomic literature (36), (26), (3), (12), (24), (2), (30). The isolates were maintained on PDA slants at 4°C for storage and sub-cultured as required for further experimentation. Among the various isolates obtained, the predominant fungal species was selected for pathogenicity testing and morpho-molecular identification.

Pathogenicity

Preparation of the test pathogen inoculum

The inoculum of *Rhizoctonia solani* was prepared following the protocol described by Sharma (2011). Briefly, a sand-wheat meal in the ratio of 8:2 (w/w) was added to a 500 ml Erlenmeyer flask, sealed with a non-absorbent cotton plug, and autoclaved at 121 °C for 20 minutes. Ten mycelial discs each 5 mm in diameter, were cut from the actively growing margin of a three-day-old *R. solani* culture and transferred aseptically into each flask. The mycelial discs were then thoroughly mixed with the sterilized sand-wheat meal medium.

Subsequently, the flasks were incubated in a BOD at 25 ± 2 °C for 12 days. At 2–3-day intervals, the flasks were shaken to ensure uniform distribution of fungal mycelium. After the incubation period, the colonized sand-wheat meal medium was air-dried under sterile conditions and stored at 4 °C for further experimental use.

Raising of okra in pots

In order to evaluate the pathogenicity of the test fungus, a susceptible okra (cv. Palam Komal) was selected, and the sick soil method was employed under controlled glasshouse conditions.

A soil mixture comprising fine clay soil, sand, and farmyard manure (FYM) in a 2:1:1 ratio was prepared and sterilized by treatment with 5% formalin solution for ten days in airtight polythene bags. To eliminate residual formalin fumes, the sterilized soil was aerated by exposing it to open air for one week. Surface sterilization of the pots was also conducted using 5% formalin solution 24 hours before sowing.

Subsequently, the sterilized plastic pots (20 cm in diameter) were filled with the prepared soil mixture and thoroughly moistened with water. Healthy and surface-sterilized seeds of the susceptible okra variety were then sown into the sterilized soil-filled pots.

Pathogenicity test

The pathogenicity test of the isolated pathogen (*Rhizoctonia solani*) was conducted by mixing 12-day-old inoculum, prepared on sterilized sand–wheat meal (8:2) medium, at the rate of 100 g per kg in sterilized soil (31). Six visibly healthy, surface-sterilized seeds of the susceptible okra variety 'Palam Komal' were sown in each inoculated (sick) pot. Seeds sown in pots containing only sterilized soil served as the control. The pots, after inoculation, were placed in a glasshouse and monitored regularly for disease symptoms, with consistent watering.

The pathogen was re-isolated from symptomatic plants and cultured following the procedure described above. The symptoms developed upon reinoculation were compared with the originally observed symptoms to fulfill Koch's postulates, thereby confirming the pathogenicity of the test isolate.

The most virulent *R. solani* isolate, obtained from Palampur, was selected for subsequent experiments. The pathogen was identified based on its mycelial characteristics (colour, diameter, and branching pattern with constrictions), and sclerotial size and colour on potato dextrose agar (PDA).

Identification and characterization of pathogen (s)

Morpho-cultural identification

The cultural and morphological characterization (after 7 days of incubation) of the isolated pathogens was carried out on potato dextrose agar (PDA) media under *in vitro* conditions. The isolated fungal species were identified based on morpho-cultural and microscopic characteristics, including colony and hyphal features (4).

Characteristic morpho-cultural traits such as colony colour, septation of hyphae and conidia, spore shape, constriction at the branching point, pigmentation, chlamydospore formation and sclerotia development were considered. The observed morphological and cultural characteristics were compared with the standard identification keys for *Rhizoctonia* spp. (26), *Fusarium* spp. (3), (24), *Pythium* spp. (36), *Didymella* spp. (2), (30) and *Phytophthora* spp. (12).

Pure cultures of the isolated pathogens were grown on PDA and were evaluated further for their colony pigmentation, mycelial growth pattern, sporulation and growth rate. For microscopic observations, the slide culture technique was used to examine the morpho-cultural characters. The mycelium of the fungal pathogens was stained with lactophenol cotton blue for 10 minutes. Stained slides were then observed at 40X magnification using a compound microscope (Olympus CX51, Japan).

The morphological observations were compared with established taxonomic keys provided by earlier researchers (24), (21), (30), (13), (33), (38), (32).

Molecular characterization of predominant causative fungal pathogen

The morpho-cultural identity of the most predominant pathogen was confirmed further by molecular identification. For this, pure cultures of the most predominant pathogen (*Rhizoctonia solani*) were maintained on sterile, autoclavable plastic Petri plates containing potato dextrose agar (PDA) medium. The plates were then dispatched to Eurofins Genomics India Pvt. Ltd., for fungal DNA extraction and sequencing the Internal Transcribed Spacer (ITS) region using universal primers ITS1 and ITS4.

Phylogenetic analysis

The fungal pathogen sequences retrieved after sequencing were subjected to BLASTn analysis using the Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov) to identify their closest homologous sequences. All ambiguous regions were excluded from the alignments. A phylogenetic tree was constructed using the Maximum Likelihood method implemented in MEGA v.11.0 software (35). The resultant consensus sequences were submitted to the National Center for Biotechnology Information (NCBI) GenBank database for accession numbers to obtain accession numbers.

Results and Discussion

Disease survey

To assess the prevalence and distribution of damping-off disease in okra across different districts (Kangra, Mandi and Hamirpur) of Himachal Pradesh, comprehensive surveys were conducted during the summer season (April-June) in the year 2023 (Fig. 1). It is evident from the data presented in Table 1 that the damping-off was widely prevalent in all the okra growing areas, exhibiting moderate to severe intensity, particularly at the post-emergence stage.

The highest disease incidence (84.33%) was observed in Tal area of Hamirpur district, followed by the Bhota (81.67%) area of the same district and the Kanaid (81.00%) area of Mandi district. In contrast, the lowest incidence (31.67%) was recorded in the Naun area of Mandi district. Overall, survey locations in Hamirpur district exhibited the highest mean incidence (65.46%), followed by district of Mandi (59.04%). Kangra district reported the lowest mean incidence at 53.33%. The disease incidence ranged from 31.67% to 84.33% across surveyed sites, and none of the fields assessed were free from damping-off. This highlights the serious nature of the disease and its potential to adversely affect successful okra cultivation in Himachal Pradesh.

During surveys, it was also observed that early-sown okra crops (April-May) were much more susceptible to damping-off compared to the late-sown crop (May-June), likely due to environmental conditions conducive to disease development. The alarming emergence of damping-off across okra-growing regions in the state underscores the need for effective and sustainable management strategies.

The elevated incidence observed in the Tal area of Hamirpur district may be attributed to prolonged warm and dry conditions, in contrast to Kangra and Mandi districts. Additionally, okra is a warm-season vegetable crop and the damping-off pathogens thrive under elevated soil temperatures, this combination likely contributed to the severe economic losses observed. The widespread occurrence of damping-off in okra has also been reported from various regions of India by previous researchers (15), (27).

Table 1. Status of damping-off disease of okra in different districts of Himachal Pradesh during 2023

District	Location	Latitude (°N)	Longitude (°E)	Disease incidence (%)
Kangra	Sunehar	32.0911	76.3370	62.33
	Matour	32.1357	76.2928	74.67
	Samloti	32.0842	76.3500	55.33
	Malan	32.1134	76.4202	58.67
	Bhawarna	32.0398	76.4997	41.00
	Bajjnath	32.0521	76.6493	38.33
	Kangra	32.1015	76.2731	51.33
	Palampur	32.1109	76.5363	45.00
	Mean			53.33
Mandi	Galma	31.6003	76.8764	53.67
	Naun	31.5597	77.0262	31.67
	Tikkar	31.1906	77.6268	63.00
	Sakroha	31.6207	76.9787	75.33
	Kanaid	31.5789	76.9139	81.00
	Gajnoha	31.7081	76.9313	65.33
	Sidhyani	31.5998	76.8447	47.33
	Rewalsar	31.6322	76.8332	55.00
	Mean			59.04
Hamirpur	Neri	31.6789	76.4860	51.33
	Nalti	31.6686	76.4635	65.00
	Dangri	31.7512	76.3739	73.33
	Taprey	31.6967	76.6126	54.67
	Lambloo	31.6689	76.5858	45.33
	Tal	26.8188	76.5811	84.33
	Sujanpur	31.8339	76.5055	68.00
	Bhota	31.6098	76.5676	81.67
	Mean			65.46

Isolation of pathogen (s) associated with damping-off of okra

In the present study, a total of 44 pathogenic isolates were recovered from samples showing symptoms of damping-off in okra. The highest colonization frequency was exhibited by *Rhizoctonia solani* (16.67%) followed by *Fusarium solani* (9.37%). In total, eight fungal genera were found to be associated with damping-off of okra in Himachal Pradesh (Table 2).

Following preliminary morpho-cultural identification, the isolates were identified as *Rhizoctonia solani* (16 isolates), *Fusarium solani* (8 isolates), *Pythium* spp. (6 isolates), *Fusarium oxysporum* (4 isolates), *Phytophthora* spp. (4 isolates), *Aspergillus* spp. (3 isolates), *Didymella* spp. (2 isolates) and *Sclerotium rolfsii* (1 isolates). The study indicated that *Rhizoctonia solani* was the most predominant pathogen associated with damping-off disease in okra.

The findings are in agreement with those of Patel et al. (2019), who reported *R. solani* as the dominant pathogen associated with okra damping-off in the Kangra district. Similarly, Sharma (2011) also documented the association of *R. solani* and *F. solani* with damping-off in okra in Himachal Pradesh. Further, Jandaik et al. (2015) observed that *F. oxysporum*, *R. solani*, *Sclerotium rolfsii* were key pathogens affecting okra plants in the Solan region of the state. In a recent study from Iraq, Jalal et al. (2019) reported the association of *R. solani*, *F. solani* and *Macrophomina phaseolina* with damping-off in okra.

Table 2. Frequency percentage of pathogenic fungal isolates associated with okra damping-off disease in Himachal Pradesh

S. No.	Pathogenic fungal isolate	Colonization rate (%)
1	<i>Rhizoctonia solani</i>	16.67
2	<i>Fusarium solani</i>	9.37
3	<i>Pythium</i> spp.	6.25
4	<i>Fusarium oxysporum</i>	5.21
5	<i>Phytophthora</i> spp.	5.21
6	<i>Aspergillus</i> spp.	4.17
7	<i>Didymella</i> spp.	3.12
8	<i>Sclerotium rolfsii</i>	2.08

The colonization frequency results from our study are also consistent with those reported by Madhi et al. (2020), who observed the highest colonization frequency for *R. solani* (14.6, 16%) followed by *F. solani* (13.1, 14%) and *M. phaseolina* (11, 1.10%). Although other fungal isolates were detected, their frequencies were comparatively low.

More recently, Ghoneem et al. (2023) isolated thirty-five isolates of *Rhizoctonia solani* from damping-off infected samples of *Phaseolus vulgaris* collected from twenty-one field locations across six governorates of the East Delta region of Egypt, namely Ismailia, Sharkia, Port Said, South Sinai, and Suez. The highest percentage of *R. solani* was recorded in Sharkia, followed by Ismailia, South Sinai, Suez, North Sinai, and Port Said (49.7%, 36.7%, 33%, 29.2%, and 27.8%, respectively). Pathogenicity assays revealed that the seven most virulent strains of *R. solani* caused pre-emergence damping off ranging from 17 to 30 per cent and post-emergence damping off ranging from 11 to 20 per cent. Additionally, the study noted that soil type and pH had the greatest influence on the diversity of *R. solani*, followed by soil moisture retention and porosity.

Pathogenicity test and symptomatology

Out of the 44 pathogenic isolates, *Rhizoctonia solani* was identified as the predominant fungal genus associated with okra and was therefore further selected for *in vitro* and *in vivo* experiments. The pathogenic potential of *R. solani* was assessed under controlled pot conditions using a susceptible okra cultivar (cv. *Palam Komal*) wherein the soil was artificially infested by incorporating fungal inoculum.

Characteristic early symptoms of the disease were observed on the seedlings (predominantly at 2-3 leaf stage) seven days post-inoculation. The basal portion of the infected plant stems developed irregular, water soaked, brown and sunken lesions. These symptoms led to wilting and drooping of leaves followed by seedling collapse (Fig. 2 a & b).

Koch's postulates were fulfilled by re-isolating *R. solani* from the infected stem tissue. A comparison of morphological characteristics between the original and re-isolated strains confirmed the causal role of *R. solani*, thus validating the pathogenicity results.

The symptoms documented in this study are consistent with those reported by El-Mohamedy (2004), who demonstrated pathogenicity of *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*—all causal agents of damping-off and root rot in okra— and found *F. solani* and *R. solani* to be the most aggressive. Similarly, Sharma (2011) demonstrated the pathogenicity of *R. solani* and *F. solani* by incorporating 12 days old inoculum prepared on as and: wheat meal (8:2) into pot soil, which resulted in typical damping-off symptoms.

Pathogenicity of other common soil-borne fungi, including *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani*, *Pythium* sp. and *Sclerotium rolfsii* from rotted tomato root samples has also been extensively documented (10), (18).

**Fig 2. Pathogenicity test and symptomatology [a] Pathogenicity test (Water soaked brown sunken lesions on basal portion of stem) (b) Control]**

Morpho-cultural identification of the predominant okra damping-off pathogen (s)

A pure culture of the most predominant pathogen isolate (*Rhizoctonia solani*) was obtained on Potato Dextrose Agar (PDA) within 5-6 days after incubation at 25 ±1°C. On PDA, colonies of the pathogen typically showed a cream to light

brown color, accompanied by floccose aerial hyphae (Fig. 3a). After 20 days of incubation, colonies became dark brown and developed dark brown sclerotia. The majority of sclerotia (0.2–1.0mm) appeared dark-brown and were small, spherical structures with a diameter ≤ 1.0 mm (Fig. 3b). Microscopic examination revealed that the pathogen's mycelium exhibited characteristic right-angled hyphal branching with constriction and a septum near the point of branching. The width of pathogen's hyphae ranged from 6.7 to 7.9 μm (mean = 7.3 μm) (Fig. 3c).

On the basis of morpho-cultural characters, the predominantly associated pathogen causing damping-off in okra in Himachal Pradesh was tentatively identified as *Rhizoctonia solani*, which accounted for 16.66 per cent of the total pathogenic isolates recovered from the different sites during the course of survey. The morphological characteristics recorded in the present study followed the descriptions provided by earlier researchers (26), (1), (10). They reported a wide range of mycelial colours including brown, light brown, dark brown and yellowish brown. In another study, Neeraja et al. (2003) reported the significance of the mycelial and sclerotial characteristics in categorizing *R. solani* strains into distinct groups. Similarly, Sumalatha et al. (2017) observed greyish white initial mycelia colour in that later turned to black in colour with cottony aerial mycelial growth. The hyphae produced frequent right-angled branches with constriction at the origin, often accompanied by a septum near the branch origin. They also observed microsclerotia ranging from 0.8 - 1.0 mm in size in sixty-day-old cultures.

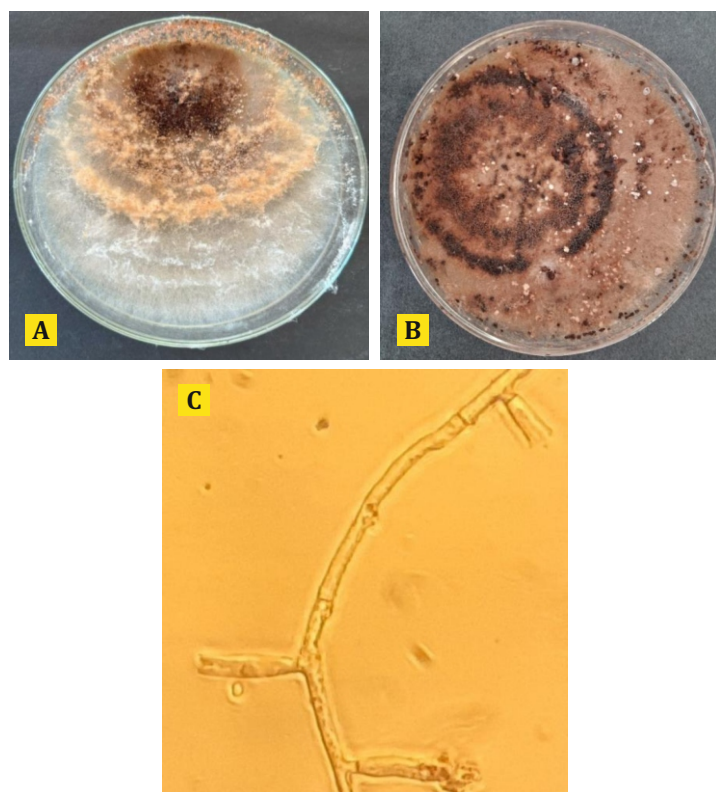


Fig 3. Morpho-cultural characteristics of most predominant okra damping-off pathogen [(a) Pure culture of *Rhizoctonia solani* on Potato Dextrose Agar medium (b) Formation of sclerotia (c) Characteristic right-angled hyphal branching with constriction and a septum near the ramification]

Molecular characterization of predominant pathogen

The predominant pathogen was molecularly characterized using the Internal Transcribed Spacer (ITS) region-based sequence analysis a universally accepted fungal barcode marker. The ITS region was amplified from genomic DNA and subsequently sequenced using primer pairs ITS1 and ITS4.

The BLAST (Basic Local Alignment Search Tool) analysis of the consensus ITS sequence of the isolate revealed a high similarity with *Rhizoctonia solani* (99.36 %) and thereby confirming its identity as *Rhizoctonia solani* isolate JPO1. The assembled sequence of the isolate was also submitted to the GenBank database of NCBI (accession number PQ605661).

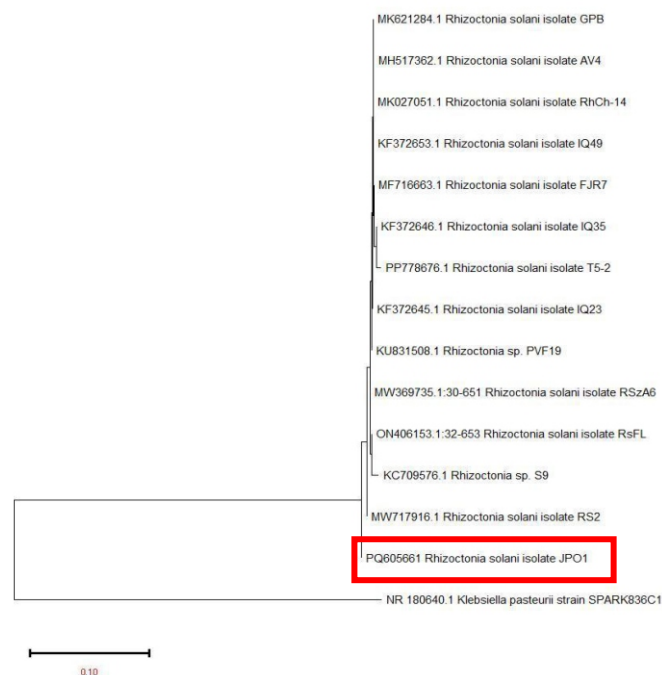


Fig 4. Phylogenetic tree drawn using the Maximum Likelihood method showing relationship between *Rhizoctonia solani* and similar other sequences deposited in the NCBI Genbank (Evolutionary analyses were conducted in MEGA vs. 11)

The evolutionary relationship was further validated through phylogenetic tree analysis based on Maximum Likelihood (ML) method using MEGA version 11 software (Fig. 4). The phylogenetic tree analysis showed a strong clustering of the test isolate, *Rhizoctonia solani* JPO1, with other *R. solani* isolates already deposited in the NCBI database. The isolate *Rhizoctonia solani* was found to be most closely related to *Rhizoctonia solani* isolate GPB (accession number MK621284.1).

Conclusion

The present investigation provides valuable insights into the status and frequency distribution of predominant damping-off pathogens affecting summer-grown okra in Himachal Pradesh. The findings can assist farmers, particularly those cultivating okra during the summer season, in implementing targeted disease management strategies. This, in turn, will contribute to enhanced crop protection, increased okra productivity, and improved food security. The data retrieved from this study underscore the current status of damping-off prevalent during summer-grown okra in Himachal Pradesh, India and highlight the pre-dominant pathogenic fungi responsible. This information lays the groundwork for devising effective disease management strategies against this destructive disease.

Conflict of Interest

The authors declare no competing interests

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