



RESEARCH ARTICLE

Survey, symptomatology and screening of ajwain genotypes for resistance to *Sclerotinia sclerotiorum* (Lib.) de Bary in Bihar, India

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Abstract

Ajwain (*Trachyspermum ammi* (L.) Sprague) is an important medicinal and spice crop widely cultivated in India. In recent years, its production has been affected by several fungal diseases, among which stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has become a serious problem. A field survey carried out during the rabi seasons of 2022–23 and 2023–24 in the districts of Rohtas, Buxar and Nalanda in Southern part of Bihar revealed that the disease was widely prevalent, with incidence ranging from 39.03–61.89 %. Infected plants initially showed small water-soaked lesions on the stem which gradually enlarged and turned necrotic. As the disease progressed, abundant white cottony mycelial growth appeared on the affected tissues, along with the formation of sclerotia inside the stem. Severe infection often resulted in stem breakage and eventual death of the plant. The pathogen was isolated from diseased tissues and identified based on cultural and morphological characteristics, which were further supported by molecular analysis. On potato dextrose agar (PDA), the fungus grew a large quantity of white, fluffy mycelium, then colonies developed black, irregular sclerotia in concentric circles. Under the microscope, typical hyaline, branched hyphae (mycelium) of *S. sclerotiorum* were seen. Twenty ajwain germplasm lines were tested for resistance to the pathogen; none of the genotypes had complete resistance, but 2 lines (Rajendra Ajwain (RA)-14 and RA-16) showed moderate resistance. These lines may be considered for future breeding purposes.

Keywords: ajwain; disease incidence; *Sclerotinia sclerotiorum*; stem rot

Introduction

India is the world's leading producer and supplier of seed spices and it has a long history of agriculture and a rich spice-growing and spice-trading tradition. India not only meets its domestic demand for spices but also plays an important role in developing international spice markets and the international food industry. Ajwain (*Trachyspermum ammi* (L.) Sprague) or carom seed is a prominent seed spice grown in India. It is also called as Bishop's weed, classified botanically as an annual herbaceous plant belonging to the Apiaceae family and is often recognised by its pleasant aroma and medicinal properties. Cytologically, ajwain is classified as a diploid species with a chromosome count of $2n = 18$. Ajwain is popular in culinary preparations because of its strong taste and its uses in traditional medicine. The major production areas in India are Rajasthan, Gujarat Andhra Pradesh, Madhya Pradesh, Telangana, West Bengal and parts of Bihar. Out of these states, around 90 % of

all ajwains grown in India is produced in Rajasthan. It's high productivity in Rajasthan is attributed to the climate and because of the land, which is described as well-drained consequently it is a suitable crop for these regions. Ajwain can grow best in southern Rajasthan due to these environmental conditions. In 2022, ajwain was grown on approximately 38182 ha, resulting in approximately 39.763 t of production and an average yield of approximately 1041 kg ha⁻¹ (1).

Cultivation of ajwain (*T. ammi*) is often affected by several fungal diseases that can lead to considerable yield losses and deterioration in seed quality. Among these, stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has emerged as a major threat in the principal ajwain-growing regions of India. According to reports, *S. sclerotiorum* is known to infect approximately 400 different host organisms. The disease has caused significant crop losses in several Indian states, including Assam, Rajasthan, Bihar, Uttar Pradesh,

Uttarakhand and Haryana (2). Reported reductions in crop yields, especially in predominantly temperate areas of the country, can be as high as 50 %. In severe infestations, losses can be as high as 80–100 % (3). The fungus *S. sclerotiorum* is recognised as one of the most destructive pathogens affecting spice crops in India, and the disease it causes, commonly known as Sclerotinia rot or white mould, has been associated with significant crop losses across multiple spice crops throughout the country (4–6). Ajwain crop getting affected by this pathogen has not been reported before, so it has a major limitation. These findings indicate that the *S. sclerotiorum* pathogen poses a substantial threat to seed spice production. Management of stem rot in seed spices caused by *S. sclerotiorum* is complicated due to several factors, including limited numbers of resistant cultivars, many susceptible host species and the complexity of managing stem rot. A major barrier to developing effective management strategies for *S. sclerotiorum* is a lack of information regarding the intraspecific variability of the pathogen. Due to the large number of crops that the *S. sclerotiorum* fungus can infect, conventional cultural management approaches such as crop rotation typically do not provide adequate disease management.

Despite the economic importance of ajwain (*T. ammi*), systematic scientific studies on the occurrence and impact of stem rot in Bihar are still lacking. Therefore, the present investigation was undertaken to survey the major ajwain-growing areas of Bihar to determine the incidence and severity of the disease and to generate baseline information for developing suitable management strategies. Diseased plant samples showing typical symptoms were collected from different locations and brought to the laboratory for isolation and characterisation of the pathogen under controlled conditions.

In addition, field evaluation of 20 diverse ajwain genotypes was conducted at the Muraul farm of Tirhut College of Agriculture (TCA), Dholi, under Dr. Rajendra Prasad Central Agricultural University (RPCAU), Pusa. The genotypes were screened under field conditions using artificial inoculation to identify resistant and susceptible lines against stem rot caused by *S. sclerotiorum*. Artificial inoculation was carried out using *S. sclerotiorum*. The pathogen was cultured on potato dextrose agar (PDA) at $25 \pm 2^\circ\text{C}$ for 5–7 days. A 5 mm mycelial disc from the actively growing culture was placed on the stem of plants at 30–35 days after sowing. The inoculated area was covered with moist cotton and wrapped with polythene to maintain high humidity. After inoculation, relative humidity (RH) of 85–90 % and temperature of 20–25 °C were maintained to facilitate disease development.

Materials and Methods

The study was conducted at the Muraul farm of TCA, Dholi, Muzaffarpur (RPCAU, Pusa, Bihar) during the rabi season of 2022–23 and 2023–24. The region is characterised by a sub-humid climate with an average annual rainfall of about 1354.28 mm, most of which is received between June and September. A properly labelled and well-drained field plot was selected for conducting the experiment. The field had uniform topography and consisted of silty loam soil with a pH of 8.5. All recommended agronomic practices for crop cultivation were diligently followed. The following section provides a detailed account of the techniques

employed and materials used throughout the course of the experiment survey and collection of diseased samples.

Survey and collection of diseased samples

During the current investigation, survey was conducted across various ajwain- growing regions in the South Bihar districts throughout the 2022–23 and 2023–24 cropping season. The aim was to document the stem rot incidence in ajwain. The survey was conducted in the districts of Rohtas, Buxar and Nalanda along with their respective blocks. In each block, four different ajwain-growing fields were selected for the survey. Disease incidence was recorded in these fields and plant samples showing typical symptoms were collected from farmers' fields for further examination and analysis. A field survey was conducted in different locations to assess the incidence of stem rot disease. In each location, a minimum of 3–5 fields were surveyed. Within each field, 5 random spots were selected and observations were recorded on a total of 50 plants.

To evaluate the stem rot incidence, ajwain fields in various localities of districts of South Bihar were surveyed during the 2023–24 cropping season: once in the mid-week of January.

Disease incidence was recorded during the survey using the following formula (7):

$$\text{Disease incidence} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants observed}} \times 100$$

Symptomatology

The symptoms associated with stem rot in ajwain were examined on different plant parts including the stem, leaves, pedicels and fruits or seeds. The pathogen *S. sclerotiorum* can infect a wide range of host plants; therefore, it does not produce a single symptom that is unique or diagnostic across all hosts. A characteristic symptom observed in infected plants is the appearance of white, cotton-like fungal growth (mycelium) on the above-ground parts of the plant. This mycelial growth is commonly seen on stems, leaves, petioles and reproductive organs. It is common for fungal infections to cause lesions that appear water soaked and have well defined margins on infected tissue. The area where lesions appear gradually becomes limp and discoloured until it disintegrates; this will create a cottony appearance of multiple tiny clusters of hyphae that cluster together into a single structure that is like a pea. Eventually, sclerotia develop from the cottony appearance of the hyphae and can be found either at the surface of the infected plant tissue or inside the infected plant tissue and cavities.

Isolation and purification of pathogen

Initially, all the glassware used for the experiment was cleaned with a potassium dichromate sulphuric acid solution before being thoroughly washed with sterile water. Subsequently, after cleaning, all the glassware was fitted into a hot air oven and sterilised at 180 °C for approximately 2 hr. The culture medium of PDA (39 g L^{-1}) was autoclaved at 121.5 °C and 15 psi for 20 min. To perform *in vitro* isolation of the fungus under investigation, the isolated fungus from the test fungus was isolated using stem tissues that displayed mycelial growth of *S. sclerotiorum*. A tiny portion of leaf measuring 1–2 mm in size was taken from the infected area using a sterilised blade. These tissue segments were then surface sterilised in 0.1 % mercuric chloride (HgCl_2) solution for approximately 10–20 sec. After sterilisation, the pieces were

washed 3 times with sterile distilled water to remove any residual disinfectant and were placed on sterile filter paper to blot off excess moisture before culturing. The Petri plates were sealed to prevent contamination and incubated in a biological oxygen demand (BOD) incubator at $19 \pm 2^\circ\text{C}$ for 5–7 days.

Isolation was also performed using black sclerotia found within the infected tissues. After surface sterilisation, the sclerotia were cut into small pieces using a sterilised blade. Each piece was then immersed in a 0.1 % HgCl_2 solution for 1 min, followed by 3 successive washes with sterilised water and dried on sterilised blotter paper. One piece was aseptically placed in a 2 % PDA slant culture tube and incubated for 4–7 days at $19 \pm 2^\circ\text{C}$. The fungus was purified using the hyphal tip culture technique and maintained on slants with the same medium. The stock culture was sub-cultured every 7–10 days throughout the study period.

Identification of the pathogen

After purification, the fungal isolate was incubated under suitable conditions to induce sporulation for 5–7 days. The resulting sporulating culture was identified based on the cultural and morphological characteristics (sclerotia). Further, molecular identification of the pathogen was also carried out for specific identification of the pathogen.

Pathogenicity

The pathogenicity of the fungus isolated from infected plants was demonstrated on ajwain plants through artificial inoculation in a pot culture under nursery conditions using cotton mycelium method. The cotton mycelium (non-injury inoculation) method was used for artificial inoculation (8). Both the infected plant parts and cultures of the pathogen were used as sources of inoculum.

Screening of ajwain genotypes under artificial inoculated condition

The experimental plot measured 2×1.5 m and the experiment was laid out in an augmented randomised block design (ARBD). Genotypes, RA-85, RA-16, RA-14, RA-9, RA-2, RA-48, RA-92, RA-74, RA-80, RA-59, RA-7, RA-15, RA-79, RA-88, RA-1, RA-4, RA-82 were the source from Rajendra Agricultural University Pusa and HAJ-4, HAJ-1/12, HAJ-8B were the source from Choudhary Charan Singh Haryana Agricultural University, Haryana. The experiment for screening of varieties against disease was conducted in a randomised block design (RBD) and observations on disease incidence and percent disease index (PDI) were recorded. The data were subjected to analysis of variance (ANOVA) and the significance of differences among varieties was tested using the *F*-test at 5 % level of probability. The standard error of mean (SEM) and critical difference (CD) at $p = 0.05$ were calculated to compare the varietal means. Total of 20 genotypes of ajwain were planted to evaluate its resistance to stem rot disease. Rajendra Ajwain-85 (RA-85) was taken as resistant check for the disease. Two rows of each susceptible variety RA-22 were planted after 5 germplasm to increase disease pressure (Table 1). The experimental details are given in Table 2 and the field layout in Table 3.

Table 1. Ajwain germplasm for screening under artificial field condition

Sl. No.	Germplasm
1	RA-16
2	RA-14
3	HAJ-1/12
4	RA-9
5	RA-2
6	RA-48
7	RA-92
8	RA-74
9	RA-80
10	RA-82
11	HAJ-10
12	RA-59
13	HAJ-4
14	RA-7
15	RA-15
16	RA-79
17	RA-88
18	RA-1
19	RA-4
20	RA-85

Table 2. Experimental details for screening against stem rot disease

No. of test germplasm	20
Row length (paired)	5 m
Spacing	30×10 cm
Replication	2
Susceptible check	RA-22
Resistant check	RA-85
Experimental site	Muraul Farm, Tirhut College of Agriculture (TCA), Dholi

Recording of observation

The crop was monitored closely to record the initial appearance of the disease following germination. Final observations on PDI were conducted 110 days after sowing by randomly selecting 20 plants from each germplasm. Disease data were recorded using a 0–4 scale discussed (Table 4) and then converted into PDI to evaluate the disease reaction of each germplasm, such as R: resistant; MR: moderately resistant; MS: moderately susceptible; S: susceptible; HS: highly susceptible. Finally, the ajwain

Table 4. Disease rating scale for stem rot disease of ajwain

Disease score	Lesion length (cm)	Disease reaction
0	< 3	R
1	3–5	MR
2	5–10	MS
3	10–15	S
4	> 15	HS

R: resistant; MR: moderately resistant; MS: moderately susceptible; S: susceptible; HS: highly susceptible.

Table 3. Field layout of screening field

RA-22 1 × 2 lines	1-5 5 × 2 lines	RA-22 1 × 2 lines	6-10 5 × 2 lines	RA-22 1 × 2 lines	10-15 5 × 2 lines	RA-22 1 × 2 lines	15-20 5 × 2 lines
R ₁							
RA-22 1 × 2 lines	1-5 5 × 2 lines	RA-22 1 × 2 lines	6-10 5 × 2 lines	RA-22 1 × 2 lines	10-15 5 × 2 lines	RA-22 1 × 2 lines	15-20 5 × 2 lines

germplasm tested were classified into various categories of resistance or susceptibility based on the 0–4 disease scoring scale (9) (Table 4).

Percent disease index was calculated using the following the formula:

$$\text{PDI} = \frac{\text{Sum of all disease rating} \times 100}{\text{Total no. of plants observed} \times \text{Maximum disease score}}$$

Results and Discussion

Survey for natural occurrence of stem rot disease of ajwain in districts of South Bihar

Ajwain is an important medicinal and seed spice crop cultivated in several parts of Bihar. Its production, however, is often constrained by various diseases, among which stem rot caused by *S. sclerotiorum* is considered particularly destructive. To understand the distribution and intensity of this disease, a systematic field survey was undertaken in the major ajwain-growing areas of South Bihar during the rabi seasons of 2022–23 and 2023–24. In the districts of Rohtas, Buxar and Nalanda, selected blocks were used for the survey. Examples of farmers' fields that had ajwain growing in them were used to capture the disease present at four different locations per district. During the survey for the January to April season of 2023–24, the earliest detection of the disease occurred in the first week of January 2023. The disease incidence level varied significantly from the surveyed localities, with incidence levels ranging from 31.03–61.89 %. The highest incidence of the disease was seen in Nokha block of Rohtas which had 61.89 % of plants affected. The lowest incidence was within the Nawanagar block of Buxar at 31.03 %. When averaged for all districts, the mean disease incidence level was estimated to be 51.14 % for Rohtas, 49.26 % for Buxar and 49.33 % for Nalanda (Tables 5–7; Fig. 1–3). The response by variety also differed across districts.

'Rajendra Mani' exhibited lower disease susceptibility compared to local varieties. Comparable observations have been reported in earlier studies. Screening of 47 tomato genotypes against *S. sclerotiorum* found that none of the entries were completely resistant. However, 5 genotypes (NDT-3, NDT-38, NDT-17, NDT-56 and NDT-6) exhibited moderate resistance to the pathogen (10). A total of 50 rapeseed-mustard genotypes

(*Brassica juncea* (L.) Czern. & Coss. and related species) were evaluated against *S. sclerotiorum* among them nine genotypes, including Rohini, RL-1359, TERI M-21 and Varuna, showed high susceptibility, with disease incidence greater than 30 % under controlled conditions (11). A similar study on *Phaseolus vulgaris* L. evaluated 32 varieties against *S. sclerotiorum*. Among these, only one variety, Utkarsh, exhibited complete resistance, while 5 varieties showed moderate resistance. In contrast, 24 varieties were found to be highly susceptible to the pathogen (12). These findings indicate that the development of stable and effective resistance against *S. sclerotiorum* remains challenging, highlighting the need for continued breeding efforts to develop resistant cultivars. A survey conducted in 11 districts of Rajasthan and Haryana showed that stem rot caused by *S. sclerotiorum* was widespread. The highest disease incidence was recorded in Dausa, Rajasthan (29.20 %), followed by Rohtak, Haryana (24.80 %) (13). Similar observations have also been reported in other host crops infected by *S. sclerotiorum* (11).

The elevated disease incidence in the surveyed regions may be attributed to dense crop canopies and frequent irrigation schedules, which create favourable microclimatic conditions for *S. sclerotiorum*. Specifically, high soil moisture and reduced air circulation promote apothecia formation and sclerotia germination. To mitigate disease spread, it is recommended that farmers maintain adequate plant spacing and adopt timely, need-based irrigation practices.

Symptomatology

During study (2022–23 and 2023–24), the symptoms of the disease on the plants were observed as small water-soaked lesion on the stem portion which later coalesced to form large spots (Fig. 4). Similar symptoms were observed in basil, where brown lesions developed on the stem and rapidly extended both upward and downward along the main stem. In the present study, necrotic tissues covered with abundant white cottony mycelial growth were observed on the main stem, closely resembling the symptoms described in earlier study (14). Similar symptoms like lesions caused by Sclerotinia rot initially appeared as bleached and necrotic areas which later developed patches of fluffy white mycelium, a characteristic symptom of mycelial infection by *S. sclerotiorum* was studied in an earlier study (15). Initial symptoms of *S. sclerotiorum*

Table 5. Disease incidence (%) of stem rot of ajwain in Rohtas district

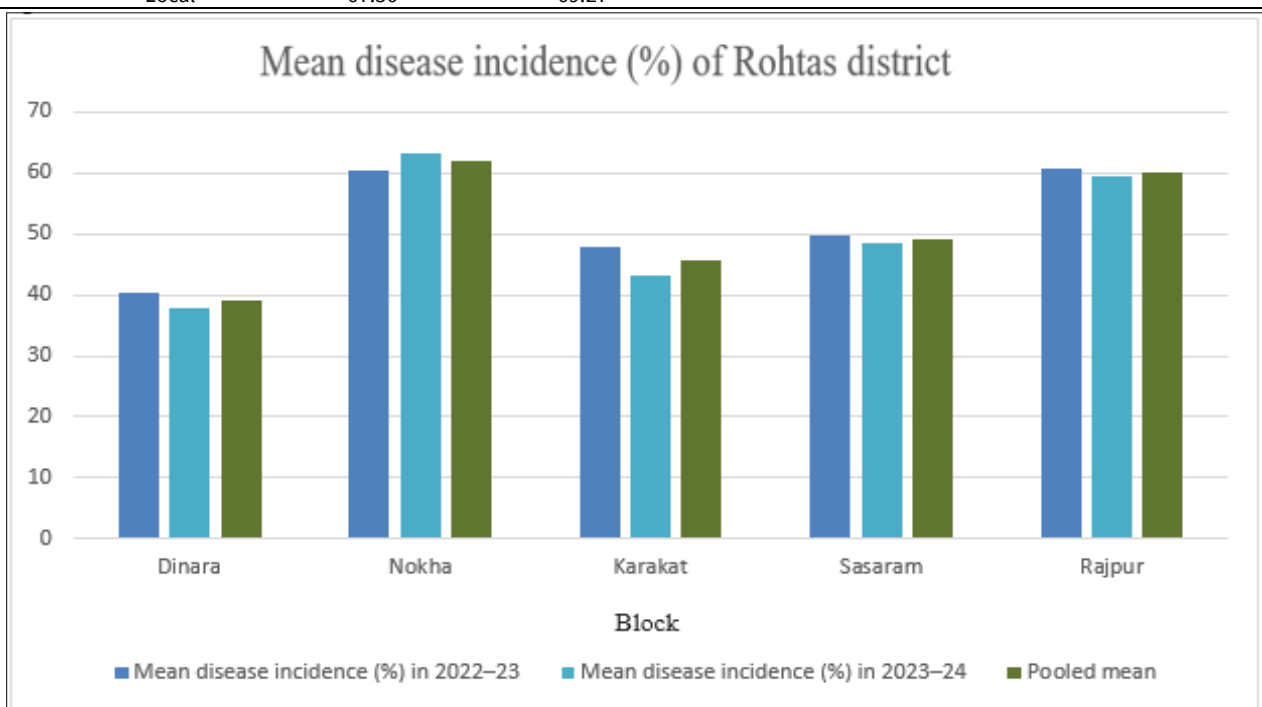
Block	Variety	Disease incidence (%) (2022–23)	Disease incidence (%) (2023–24)	Mean disease incidence (2022–23)	Mean disease incidence (2023–24)	Pooled mean disease incidence
Dinara	Local	37.34	35.49	40.28	37.91	39.10
	Rajendra Mani	40.85	38.53			
	Local	41.65	41.25			
	Rajendra Mani	42.30	36.39			
Nokha	Local	66.48	69.48	60.50	63.28	61.89
	Local	62.45	65.45			
	Local	52.20	55.32			
	Local	60.87	62.89			
Karakat	Local	47.23	40.23	47.75	43.32	45.54
	Local	48.89	45.89			
	Local	40.24	36.54			
	Local	54.64	50.64			
Sasaram	Local	51.59	51.59	49.62	48.57	49.10
	Local	46.84	46.84			
	Local	44.74	40.54			
	Local	55.31	55.31			
Rajpur	Local	60.72	60.72	60.61	59.61	60.11
	Local	68.95	68.95			
	Local	58.42	58.42			
	Local	51.35	50.37			

Table 6. Disease incidence (%) of stem rot of ajwain in Buxar district

Block	Variety	Disease incidence (%) (2022–23)	Disease incidence (%) (2023–24)	Mean disease incidence (2022–23)	Mean disease incidence (2023–24)	Pooled mean disease incidence
Buxar	Local	42.54	45.42	50.25	49.25	49.75
	Local	55.26	54.39			
	Local	59.15	52.54			
	Local	44.05	44.66			
Dumraon	Local	51.34	63.39	58.64	59.24	58.94
	Local	63.21	58.47			
	Local	70.12	60.81			
	Local	49.89	54.29			
Rajpur	Local	53.87	62.49	60.70	62.27	61.49
	Local	67.42	57.54			
	Local	71.15	69.74			
	Local	50.36	59.31			
Itarhi	Rajendra Mani	41.28	39.87	46.64	43.64	45.14
	Local	54.12	42.76			
	Local	48.35	48.42			
	Local	42.81	43.53			
Nawanagar	Local	27.45	35.23	32.25	29.81	31.03
	Local	39.36	30.64			
	Rajendra Mani	35.12	25.51			
	Rajendra Mani	27.07	27.89			

Table 7. Disease incidence (%) of stem rot of ajwain in Nalanda district

Block	Variety	Disease incidence (%) (2022–23)	Disease incidence (%) (2023–24)	Mean disease incidence (2022–23)	Mean disease incidence (2023–24)	Pooled mean disease incidence
Ben	Local	47.98	45.98	48.6	45.57	47.09
	Local	54.24	50.24			
	Local	46.71	42.71			
	Rajendra Mani	45.47	43.36			
Islampur	Local	48.33	52.36	55.11	56.12	55.62
	Local	54.85	54.85			
	Local	60.35	60.35			
	Local	56.94	56.94			
Parbalpur	Local	52.14	52.14	45.1	47.09	46.10
	Rajendra Mani	50.54	50.54			
	Local	34.8	36.77			
	Rajendra Mani	42.92	48.92			
Biharsharif	Local	42.24	40.24	43.27	41.27	42.27
	Rajendra Mani	36.45	35.42			
	Local	45.89	45.89			
	Rajendra Mani	43.56	43.56			
Rajpur	Local	48.54	46.78	56.17	55.02	55.60
	Rajendra Mani	42.35	40.31			
	Local	66.25	63.74			
	Local	67.56	69.27			

**Fig. 1.** Mean disease incidence (%) of Rohtas district.

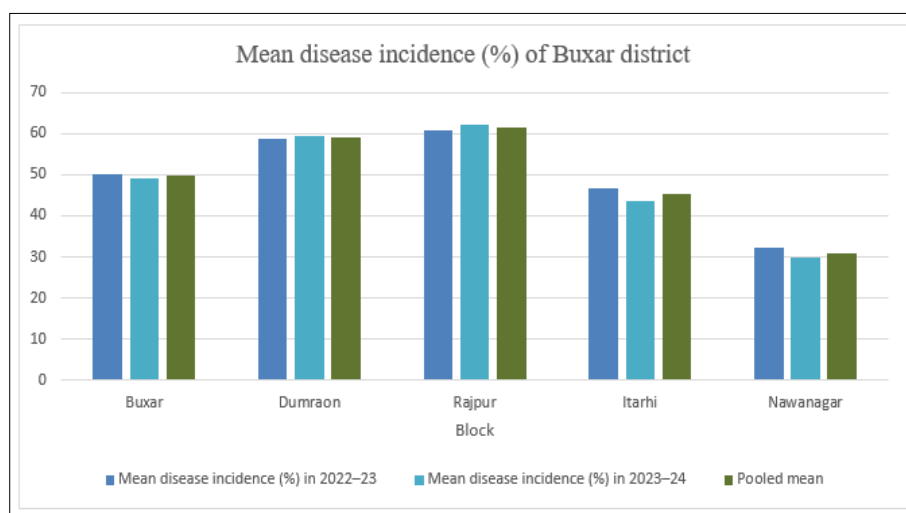


Fig. 2. Mean disease incidence (%) of Buxar district.

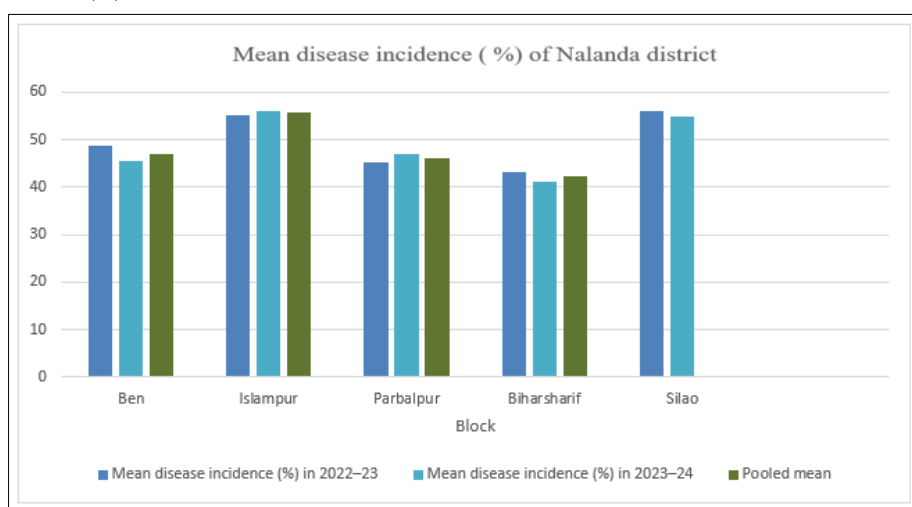


Fig. 3. Mean disease incidence (%) of Nalanda district.

infection in chilli seedlings were characterised by water-soaked lesions at the stem base, which were subsequently followed by the development of white mould growth, wilting and eventual plant death (16). Similar observations have been reported for white mould disease, where water-soaked lesions initially developed on stems and pods and later expanded extensively, covering entire racemes and pods with profuse white fungal growth (17). Black, hard sclerotial masses were observed inside the dried stems of infected plants under field conditions during the present study. Similar observations were reported in oilseed *Brassica*, where black sclerotial bodies were found within the infected stem tissues (16). *Sclerotinia sclerotiorum* is devoid of asexual spores and primarily infects its host through carpogenic germination during which ascospores are produced and released from apothecia under favourable environmental conditions (18). However, no apothecia were observed in the field during the present study. The infection in the stem may be initiated by myceliogenic germination of the sclerotia in which the hyphae infect the stem of the host plant. Thus, after confirming the disease symptoms of *Sclerotinia* stem rot in ajwain plants the diseased plant parts were taken to the laboratory for isolation of the pathogen.

Isolation and identification

The fungus on modified culture media, specifically PDA supplemented with streptomycin sulphate, was identified using morphological descriptions provided by previous researchers. On this enriched medium, the pathogen grew moderately, achieving a

diameter of 90 mm after 12 days at $19 \pm 2^\circ\text{C}$. White mycelial growth was observed which totally covered the plates in 12 days (Fig. 5 and 6). All the samples from different surveyed areas were also isolated (Fig. 7). The fungus isolated from rapeseed-mustard stems exhibited white to light brown colonies on PDA, as observed in an earlier study (19). Altogether sixty isolates of *S. sclerotiorum* obtained from bean were characterised based on their morphological traits in earlier study (20). Small mycelial tufts began to form at the Petri plate's edge and glossy water droplets could be observed surrounding these tufts proceeding with formation of sclerotia as concentric circles around the mycelium at the periphery of the plates. White mycelium net was observed to have individual sclerotium embedded in it. Mycelium varied in abundance, regularity and colour (white or beige). These results are like with the white colour mycelium observed in our experiment. Colony shape was round or diverse. The difference in the colour of the colony may be due the pathogen isolated from different hosts sclerotial masses when measured in laboratory was $2-10 \times 13-15$ mm. *Sclerotinia sclerotiorum* isolated from Indian mustard produced sclerotia measuring $2.5-8.5 \times 2.0-5.5$ mm, which were somewhat similar to those observed in an earlier study (21). The variation in sclerotial size may be attributed to differences in the host crops from which the pathogen isolates were obtained.

Morphological studies of *S. sclerotiorum* isolated from *B. juncea* revealed the development of white mycelium that completely covered the Petri plates within 9-10 days, which was



Fig. 4. Sclerotinia stem rot symptoms on ajwain infected by *Sclerotinia sclerotiorum*.

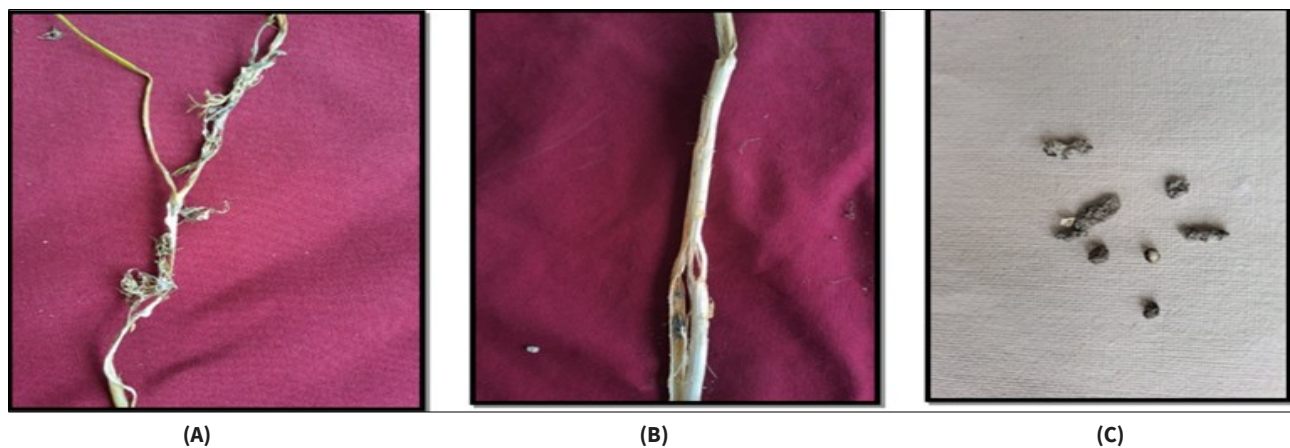


Fig. 5. Diseased samples for isolation. (A) completely dried plant; (B) dried stem with sclerotia; (C) sclerotia.

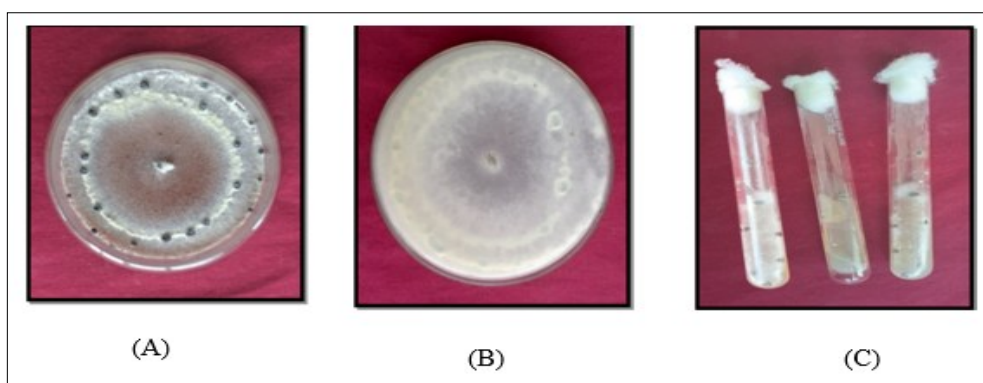


Fig. 6. Pure culture of *Sclerotinia sclerotiorum*. (A) front view; (B) back view; (C) slant.

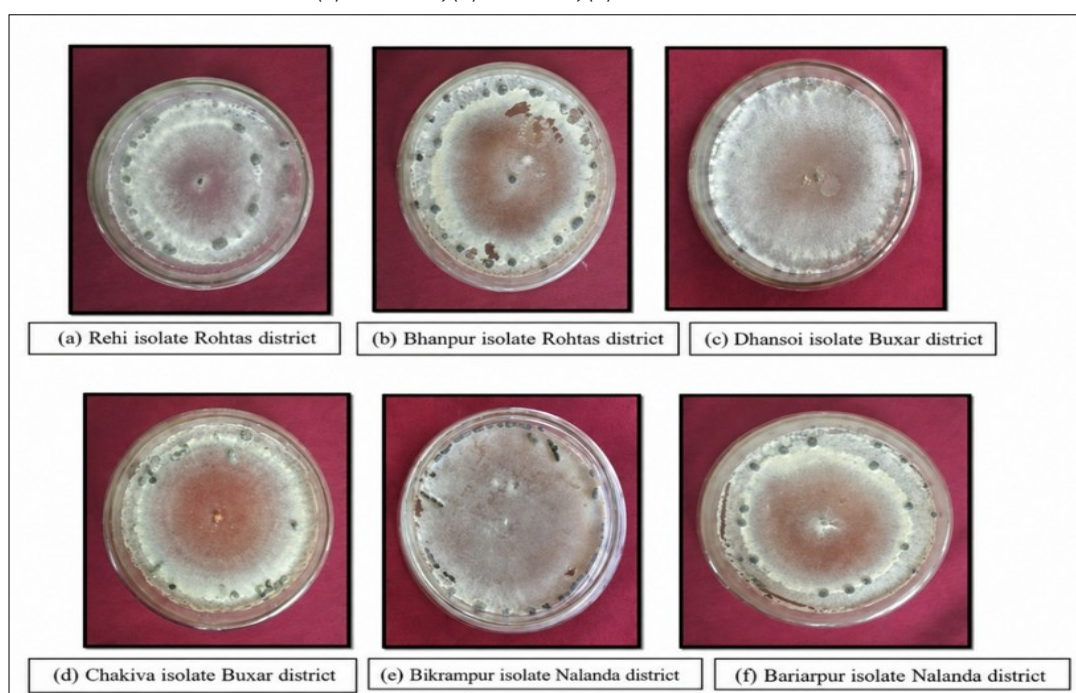


Fig. 7. Isolation of *Sclerotinia sclerotiorum* from different surveyed areas.

comparable to the observations recorded in an earlier study (22). The slight variation in the number of days required for complete mycelial growth may be attributed to differences in temperature and seasonal conditions prevailing during pathogen isolation and culturing. The circular shape of the sclerotia observed in the present study was also in agreement with previous findings reported for *S. sclerotiorum* isolated from rapeseed-mustard (23).

It has been observed that the shape of sclerotia during the winter season was somewhat semi spherical to circular and the shape slightly changed during subculturing the same pathogen in the summer season. This change in shape and size of sclerotia may be due to the temperature difference. After this the pathogen was observed under the microscope. The pathogen had hyaline, branched and multinucleated hyphae (Fig. 8). The observation of hyaline branched hyphae was similar with previous reports in which hyaline branched hyphae, apothecia and ascospores were recorded under microscopic examination (14). Similar observations of ascospores were also reported previously (24). In our experiment apothecia were not observed due to the temperature and RH of Dholi in accordance with other places. Formation of apothecia requires specific temperature and favourable RH which may not be present during experiment. The detailed morphological study was carried out to confirm the pathogen as *S. sclerotiorum*, the causal agent of stem rot of ajwain.

Molecular identification

Genomic DNA was extracted from the pure culture (SSR of ajwain 2) following the cetyltrimethylammonium bromide (CTAB) method. The internal transcribed spacer (ITS) region of rDNA was amplified using universal primers ITS1 and ITS4. Polymerase chain reaction (PCR) was performed in a 25 µL reaction volume containing template DNA, primers and master mix. The amplification conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. The amplified PCR products were resolved on 1.2 % agarose gel and visualised under ultraviolet (UV) light to confirm amplification. The pure culture (SSR of ajwain 2) was sent to Barcode Bioscience, Bangalore (ISO 9001:2015 certified) for identification. Morphological and molecular analyses confirmed the pathogen as *S. sclerotiorum*. The ITS sequence was subjected to

BLAST analysis (NCBI GenBank), where the top ten hits showed 100 % identity with known *S. sclerotiorum* isolates, including YKY211 (OR206374.1), TH9 (MW375454.1), MK074848.1, YKY212 (OR206375.1), TH7 (MW3375455.1), TH4 (M375458.1), CN145A4, CN144H6 and CN145A7 and phylogenetic tree with accession number were also obtained (Fig. 9 and 10). Ajwain is known to suffer from Sclerotinia collar rot (25), but no resistant variety exists. Based on United States Department of Agriculture (USDA) fungal collections and earlier literature (26, 27) no previous report documents *S. sclerotiorum* causing stem rot of ajwain in Bihar. This study reports *S. sclerotiorum* as the causal agent of ajwain stem rot in this region for the first time.

Screening of ajwain germplasm against stem rot disease

In the current study, an effort was made to identify resistant sources in ajwain germplasm by screening 20 accessions for stem rot resistance under field conditions during the rabi 2022–23 and 2023–24 season. The PDI ranged from 23.80–71.32 %. The susceptible check, RA-22, recorded the highest PDI (71.32 %) with a disease score of 4, while the resistant check, RA-85, exhibited the lowest PDI (23.80 %) with a score of 1. Among the test entries, only 2 germplasm lines RA-14 and RA-16 demonstrated moderate resistance on the rating scale (Table 8 and 9; Fig. 11). These findings are consistent with previous reports in which 47 tomato varieties were screened against *S. sclerotiorum* and none exhibited complete resistance; however, 5 genotypes, namely NDT-3, NDT-38, NDT-17, NDT-56 and NDT-6, were found to be moderately resistant (10). A comparable study evaluating 50 rapeseed and mustard genotypes identified Rohini, RL-1359, TERI M-21 and Varuna as exhibiting varying degrees of susceptibility to *S. sclerotiorum* (28). These varieties exhibited greater than 30 % incidence of disease in a controlled environment. Similar screening studies have reported the occurrence of resistant and moderately resistant genotypes, with Utkarsh exhibiting complete resistance and 5 other rajmash varieties showing moderate resistance (29). However, most of the experiment's 24 tested varieties were reported to be highly susceptible to disease, indicating that breeding for a higher level of resistance is needed. The low level of resistance to Sclerotinia disease present in the current study may be due to the low levels of genetically inherited resistance found in the germplasm tested or the high levels of virulence of the pathogen present in the environment

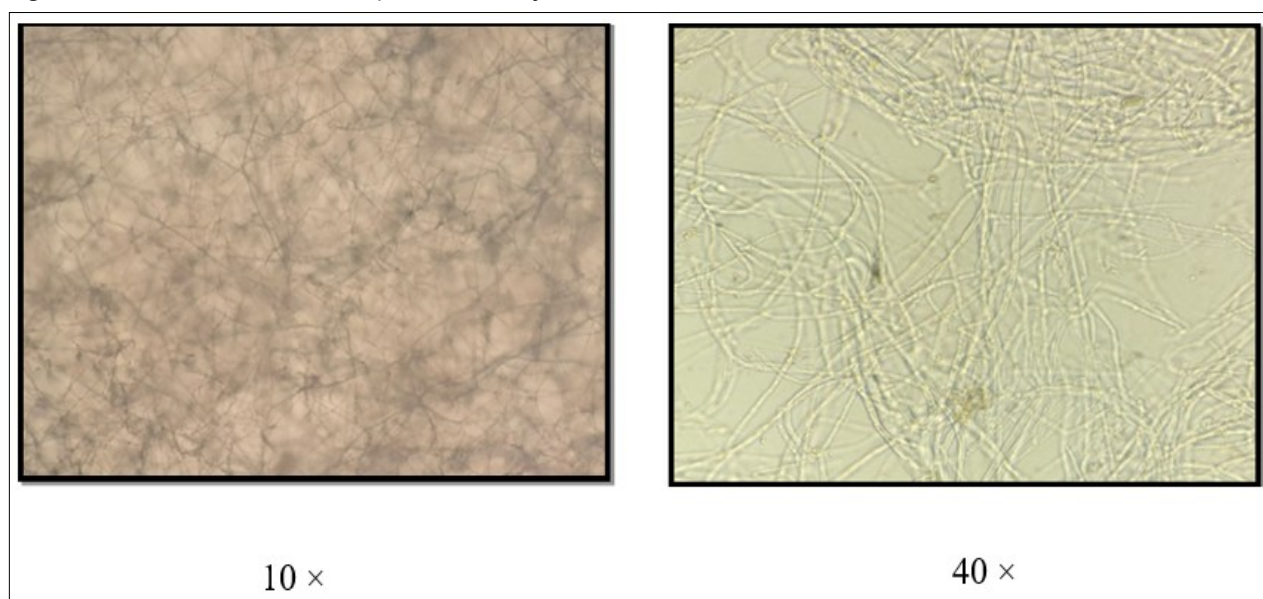


Fig. 8. Microscopic view of mycelium of *Sclerotinia sclerotiorum*.

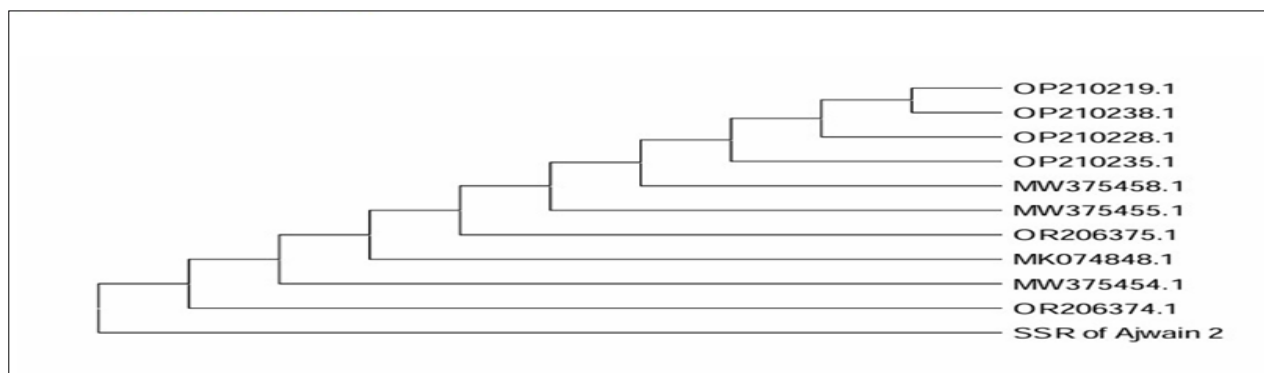


Fig. 9. Phylogenetic tree of *Sclerotinia* stem rot ajwain 2 with other isolates.

Description	Max. score	Total score	Query cover (%)	E value (%)	% ident	Accession
<i>Sclerotinia sclerotiorum</i> isolate YKY211	1003	1003	100	0.00	100.00	OR206374.1
<i>Sclerotinia sclerotiorum</i> strain TH9	1003	1003	100	0.00	100.00	MW375454.1
<i>Sclerotinia sclerotiorum</i>	1003	1003	100	0.00	100.00	MK074848.1
<i>Sclerotinia sclerotiorum</i> isolate YKY212	1003	1003	100	0.00	100.00	OR206375.1
<i>Sclerotinia sclerotiorum</i> strain TH7	1003	1003	100	0.00	100.00	MW375455.1
<i>Sclerotinia sclerotiorum</i> strain TH4	1003	1003	100	0.00	100.00	MW375458.1
<i>Sclerotinia sclerotiorum</i> strain CN145A4	1002	1002	100	0.00	100.00	OP210235.1
<i>Sclerotinia sclerotiorum</i> strain CN144I6	1002	1002	100	0.00	100.00	OP210228.1
<i>Sclerotinia sclerotiorum</i> strain CN144H6	1002	1002	100	0.00	100.00	OP210219.1
<i>Sclerotinia sclerotiorum</i> strain CN145A7	1002	1002	100	0.00	100.00	OP210238.1

Fig. 10. Accession no. of *Sclerotinia* stem rot ajwain 2 isolate.

of Muraul farm, TCA, Dholi, as compared to earlier reports (28, 29). In an additional study, 516 accessions of common bean collected from 22 countries were evaluated for their resistance to *S. sclerotiorum* using germplasm obtained from the National Bureau of Plant Genetic Resources (NBPGR) (30). Based on previous investigations of *Brassica* crops affected by *S. sclerotiorum*, 12 accessions were identified as exhibiting a high level of resistance. In contrast, 121 accessions displayed moderate levels of resistance to the same pathogen. The findings indicate the challenges associated with identifying stable and durable resistance to this pathogen within diverse germplasm collections. A screening study involving 90 mustard (*B. juncea*) genotypes revealed that RRN 505, RLM 619 and RGN 13 exhibited a high level of resistance to stem rot, whereas 12 other genotypes showed moderate resistance to *S. sclerotiorum* infection (31). The remaining entries exhibited varying levels of susceptibility ranging from moderate to high. Observations from this study emphasise the importance of identifying and utilising resistant germplasm for crop improvement programme that are designed to enhance tolerance to stem rot. The resistant ajwain lines identified in this study follow similar trends as those reported for the resistant *Brassica* crops; however, this study represents the first known attempt to systematically evaluate ajwain germplasm for their resistance to stem rot at TCA in Dholi, with no prior work documented. Moreover, the data generated from this research will serve as baseline data for future spice crop improvement programme.

The findings present valuable starting information for all programme that are aimed at improving upon the crops of spices. The resistant lines determined as RA-14, RA-16 and RA-85 have the potential to be used in breeding efforts going forward and it can be recommended that farmers in Bihar grow these varieties in order to reduce the incidence of disease in areas where these diseases occur at high levels. These results will be of major benefit to breeders and agronomists who have an interest in producing ajwain on a sustainable basis.

Conclusion

The findings of the present investigation showed that the stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major limiting factor affecting the production of ajwain in the surveyed regions of South Bihar. The prevalence of the disease is widespread and the incidence observed at multiple sites provides clear evidence of the potential for crop yield and production loss for producers in these areas. Evaluation of available germplasm did not identify any genotypes with complete resistance to the disease. This indicates that the available germplasm has very low resistance to this pathogen. This observation highlights the necessity for more intensive and well-planned breeding programmes aimed at developing varieties with improved resistance to stem rot. However, the identification of a few moderately resistant genotypes offers a promising base for future improvement efforts. Strengthening

Table 8. Screening of ajwain germplasm against stem rot disease during rabi, 2022–23 and 2023–24 under field conditions

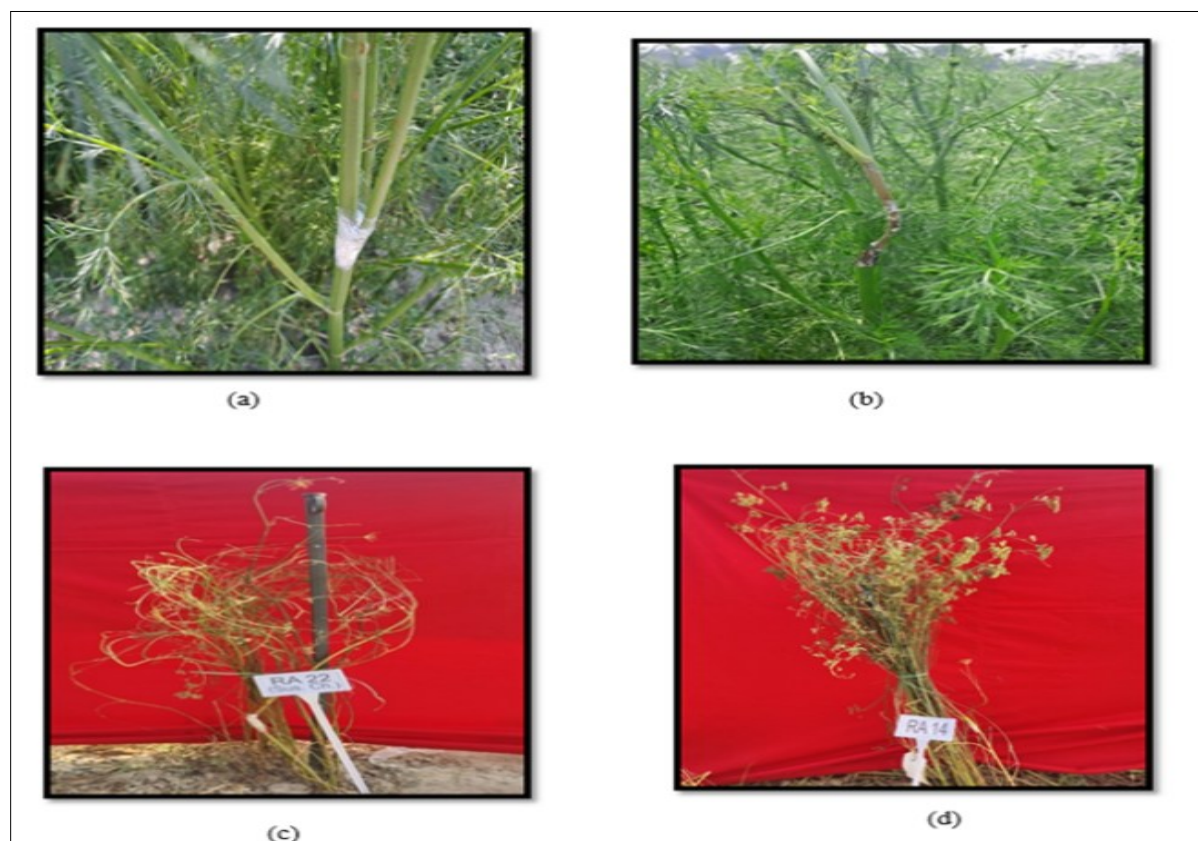
Sl. No.	Germplasm	PDI (2022–23)	PDI (2023–24)	Mean PDI	Disease score (0–4)	Disease reaction
1	RA-16	28.12	25.93	27.03	1	MR
2	RA-14	30.05	27.46	28.76	1	MR
3	HAI-1/12	33.47	31.13	32.30	2	MS
4	RA-9	47.28	45.90	46.59	3	S
5	RA-2	49.11	46.73	47.92	3	S
6	RA-48	46.9	45.84	46.37	3	S
7	RA-92	38.75	37.16	37.96	3	S
8	RA-74	36.8	35.25	36.03	3	S
9	RA-80	44.95	43.69	44.32	3	S
10	HAI-8	50.12	49.42	49.77	3	S
11	HAI-10	42.36	40.69	41.53	3	S
12	RA-59	45.8	44.67	45.24	3	S
13	HAI-4	46.05	45.32	45.69	3	S
14	RA-7	48.2	47.29	47.75	3	S
15	RA-15	47.05	46.34	46.70	3	S
16	RA-79	51.1	49.90	50.50	3	S
17	RA-88	41.95	40.32	41.14	3	S
18	RA-1	50.25	49.15	49.70	3	S
19	RA-4	66.75	65.59	66.17	4	HS
20	RA-82	70.05	69.82	69.94	4	HS
Susceptible check	RA-22	72.1	70.54	71.32	4	HS
Resistant Check	RA-85	25.05	22.54	23.80	1	R
SEm		2.08	3.10	2.93		
CD ($p = 0.05$)		5.76	6.35	5.21		
CV		12.75	11.45	9.08		

PDI: percent disease index; RA: Rajendra Ajwain; HAI: Hisar Ajwain; R: resistant; MR: moderately resistant; MS: moderately susceptible; S: susceptible; HS: highly susceptible; CD: critical difference; SEm: standard error of mean; CV: coefficient of variation.

Table 9. Categorisation of germplasm screened against stem rot disease of ajwain

Disease score (0–4)	Disease reaction	Germplasm
0	R	RA-85
1	MR	RA-16, RA-14
2	MS	HAI-1/12
3	S	RA-1, RA-4, RA-88, RA-79, RA-15, RA-7, RA-59, RA-80, RA-74, RA-92, RA-48, RA-92
4	HS	RA-4, RA-82, RA-22 (susceptible check)

R: resistant; MR: moderately resistant; MS: moderately susceptible; S: susceptible; HS: highly susceptible.

**Fig. 11.** Screening of ajwain germplasm. (A) artificial inoculation of pathogen; (B) symptom occurrence; (C) susceptible check; (D) moderately resistant germplasm.

resistance in ajwain will require the continued exploration of diverse germplasm resources, planned hybridisation programmes and the use of advanced breeding techniques along with detailed pathological screening. Furthermore, since the occurrence and severity of the disease are greatly influenced by environmental factors and crop management practices, the integration of improved agronomic practices and proper irrigation management with resistance breeding will be important for the effective management of stem rot.

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Authors' contributions

BSR wrote the manuscript draft and did all the experiment. AKM's profound knowledge of plant disease diagnosis, fungal taxonomy and disease management strategies provided the scientific foundation upon which this research was built. CSC guided the initial isolation and microscopic identification of the pathogen and assisted in standardising pathogenicity tests. AR¹ and RKC formulated effective management strategies under *in vitro* conditions. AR² and RP contributed to the survey work and carried out the design and layout of the experimental field. SS and SJ provided moral support throughout the research journey. All authors read and approved the final manuscript. (AR¹ stands for Ankur Raj and AR² stands for Anshuman Raul).

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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