



RESEARCH ARTICLE

Disease prevalence, symptomatology and epidemiology of stem rot of rapeseed-mustard in Bihar, India

Anshuman Raul¹, Chandra Sekhar Choudhary^{2*}, Bhabani Sankar Rout^{1*}, Ashim Kumar Mishra²,
Ram Kumar Choudhary⁴, Md. Minnatullah³, Somanath Joshi⁵ & Rabi Ranjan Sethi⁶

¹Department of Plant Pathology, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India

²Department of Plant Pathology, Tirhut College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa 848 125, Bihar, India

³Department of Plant Pathology, Sugarcane Research Institute, Dr. Rajendra Prasad Central Agricultural University, Pusa 848 125, Bihar, India

⁴Department of Agricultural Statistics, Tirhut College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa 848 125, Bihar, India

⁵Department of Entomology, Odisha University of Agriculture and Technology, Bhubaneswar 751 003, Odisha, India

⁶Department of Plant Pathology and Nematology, Post Graduate College of Agriculture, Dr. Rajendra Prasad Central Agricultural University, Pusa 848 125, Bihar, India

*Correspondence email - cshekhar@rpcau.ac.in; bhabanirout.rs24@ouat.ac.in

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Abstract

Rapeseed-mustard is one of the major oilseed crops known for its high-quality edible oil containing approximately 38–42 % oil. The crop thrives well under cool climatic conditions with an optimum temperature range of 10–25 °C and is widely cultivated across temperate and subtropical regions. In India, the productivity of rapeseed-mustard is increasingly threatened by several fungal diseases, among which stem rot caused by *Sclerotinia sclerotiorum* has emerged as a major constraint to productivity. The present investigation was aimed at studying the natural occurrence, symptomatology and epidemiology of stem rot disease of rapeseed-mustard. A field survey conducted across Samastipur, Muzaffarpur and Darbhanga districts of Central Bihar in rabi, 2023–24 and 2024–25 revealed a high disease incidence. Symptomatic plants exhibited water-soaked stem lesions progressing to necrosis, white mycelial growth and internal sclerotial formation, often leading to stem breakage and plant mortality. Laboratory isolation and pathogenic confirmation based on morphological features along with molecular (ITS1/4 primer pairs) studies identified *S. sclerotiorum* as the causal agent, with characteristic fluffy white mycelium and concentric sclerotia observed on PDA medium. Microscopic analysis confirmed hyaline, branched hyphae. The disease severity was found to be higher during January than November–December due to favourable environmental conditions such as high humidity, low temperature and prolonged moisture. The studies of environmental factors with the help of a regression model explained the disease prediction model up to $R^2 = 89.26$ % of lesion progression variability. Area under disease progress curve (AUDPC) value came out as 456.4, i.e., the variety DRMR-150-35 is under the moderately resistant category. The study underscores the urgent need for resistant cultivar development and effective disease management strategies to mitigate significant yield losses in mustard cultivation. The findings of the present investigation provide valuable insights for disease forecasting, sustainable crop protection and the development of integrated management strategies to enhance productivity and resilience in rapeseed-mustard cultivation.

Keywords: disease incidence; rapeseed-mustard; *Sclerotinia sclerotiorum*; stem rot

Introduction

Rapeseed-mustard (*Brassica campestris* L.) is an important oilseed crop belonging to the family Brassicaceae. The genus *Brassica* consists of amphidiploid species with a chromosome number of $2n = 36$ and includes several species known for producing high-quality edible oils. The crop is believed to have originated in the South-East Asian region and has become one of the major oilseed crops cultivated in India. It plays a significant role in meeting the growing domestic demand for edible oils. At the global level, rapeseed-mustard ranks as the third most important source of vegetable oil after soybean and oil palm. Apart from oil extraction, different parts of the plant have traditionally been used in food preparation, medicinal purposes and as natural preservatives.

In 2023–24, the total oilseed production in India was about 39.59 Mt, of which rapeseed-mustard ranked first with a production of 131.61 lakh tonnes, contributing nearly 33.24 % of the total oilseed output. The major producing states include Rajasthan (45.40 %), followed by Uttar Pradesh (14.24 %), Madhya Pradesh (13.28 %), Haryana (10.78 %) and West Bengal (6 %), with about 74 % of the cultivated area under irrigation. In Bihar, rapeseed-mustard occupies nearly 3 % of the cultivated area. In 2023–24, rapeseed-mustard occupied about 30.3 % of the total area under the nine major oilseed crops in India and contributed nearly 33.2 % to the overall oilseed production. The crop recorded an estimated production of around 13.2 Mt, making it the leading oilseed crop in terms of total output. Its national average productivity was about 1443 kg ha⁻¹, which is higher than the overall average productivity of

oilseeds (1314 kg ha⁻¹) (1). The present study was undertaken with the hypothesis that environmental conditions and regional variability significantly influence the incidence and progression of stem rot disease in rapeseed-mustard under Bihar conditions. Therefore, the study aimed to assess the natural occurrence, symptomatology, pathogen characterisation and epidemiological behaviour of *Sclerotinia sclerotiorum* in major mustard-growing regions of Bihar.

Rapeseed-mustard cultivation faces a significant challenge in the form of fungal diseases, which can significantly reduce crop yields and affect the overall quality of the seeds. Among these, *S. sclerotiorum*, causing stem rot in mustard possess a serious threat in major growing areas of India. It has been reported to infect around 400 different host species. In India, the disease is found to be causing severe losses of 20–60 % in Rajasthan, Bihar, Uttar Pradesh and Madhya Pradesh (2). A significant increase in the sclerotial population in the soil due to monocropping and cultivation of rapeseed-mustard under irrigated conditions has made *Sclerotinia* rot a very serious disease of oilseed *Brassica* crops in states including Rajasthan, Haryana, Punjab, Assam, West Bengal, Madhya Pradesh, Uttar Pradesh and Bihar (3, 4). Up to 80 % disease incidence has been reported in Punjab and Haryana (5). In Rajasthan, 60 % seed yield loss has been reported in severely infected plants (6).

Management of *S. sclerotiorum*, the causal organism of stem rot, is difficult due to the absence of effective host resistance, its wide host range and the complexity of pathogen survival and spread. Moreover, limited information is available on the variability of the pathogen despite its ability to infect a large number of crop species under diverse agro-climatic conditions. Another major limitation in developing suitable management strategies is the limited knowledge about the intraspecific variability of the pathogen. Because it can infect a large number of crop species, traditional practices such as crop rotation and cultural methods often provide limited control. Characterisation of the pathogen is essential for accurate disease diagnosis, understanding pathogen variability, development of resistant cultivars and formulation of effective disease management strategies. Therefore, the present study was undertaken to survey major mustard-growing regions of Bihar to assess the incidence and severity of the disease. 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.

Materials and Methods

The study was conducted at the School of Natural Farming, RPCAU, Pusa, Bihar during the rabi season of 2023–24 and 2024–25. The region experiences a sub-humid climate with an average annual rainfall of 1354.28 mm, primarily occurring from June to September. For the experiment, a well-drained plot with proper labelling was chosen. This site, located approximately at 25.98° N latitude and 85.68° E longitude, is 53.18 m above sea level. The plot had a relatively uniform topography, with silty loam soil and 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. Field experiments were conducted during rabi 2024–25 at the natural farming field, Pusa, to study the influence of environmental factors on stem rot

development in rapeseed-mustard. The experiment was laid out in a randomised complete block design (RBD) with 3 replications. The variety DRMR-150-35 was sown on 26 October with a spacing of 30 × 10 cm and a seed rate of 5 kg ha⁻¹ in a plot measuring 19 × 5 m². Disease development was assessed about 60 days after sowing by measuring stem lesion length from 10 randomly selected and tagged plants using a scale. Disease observations were recorded at weekly intervals from randomly selected plants in each plot. Correlation and regression analyses were performed using R software and KAU GRAPES to determine the relationship between environmental variables and disease development. Weather data, including maximum and minimum temperature, morning and evening relative humidity (RH), evaporation and wind speed, were obtained from the Advanced Centre of Climate Change, RPCAU, Pusa. Stepwise multiple regression analysis was used to evaluate the relationship between these weather parameters and disease progression and a prediction model was developed. Correlation, regression and heatmap analyses were performed using RStudio and KAU GRAPES software, while the area under disease progress curve (AUDPC) was calculated based on lesion length recorded at regular intervals.

Survey and collection of diseased samples

During the current investigation, a survey was conducted across various rapeseed-mustard growing regions in the Central Bihar districts throughout the 2023–24 and 2024–25 cropping season. The aim was to document the stem rot incidence in rapeseed-mustard. The survey encompassed the districts of Samastipur, Muzaffarpur, Darbhanga and their respective blocks. Within each block, the survey was carried out in three different mustard-growing fields on farms, where disease incidence was recorded and plant parts exhibiting distinct symptoms were collected for further analysis.

To evaluate the stem rot incidence, mustard fields in various localities of the districts of Central Bihar were surveyed during the 2023–24 and 2024–25 cropping seasons: once in the mid-week of January.

Disease incidence was recorded during the survey as follows according to the formula:

$$\text{Disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100 \quad (\text{Eqn. 1})$$

Symptomatology

The symptoms of stem rot disease in mustard were analysed across different plant parts, such as the stem, leaves, pedicles and fruits or seeds. *Sclerotinia sclerotiorum*, due to its ability to infect a wide variety of plant hosts, lacks a universally diagnostic symptom. In the initial stage of infection, symptoms of stem rot first appear on the leaves near the base of the stem as water-soaked areas, which within a few days spread and cause the leaves to droop, develop blight-like symptoms and eventually dry out. On the stem, small water-soaked spots first appear during the flowering stage and later enlarge into pale whitish-brown lesions that may extend to branches and inflorescences. As the infection advances, these lesions girdle the stem and are often accompanied by characteristic cottony white mycelial growth on the infected stem, branches and pods. In severe cases, numerous black sclerotia are formed on the surface of infected tissues. Internally, when diseased stems are split

open, the pith becomes hollow and contains sclerotia of different sizes and shapes. Initially, these sclerotia appear whitish but gradually turn dark and develop a smooth texture, with smaller ones being rounded and larger ones flattened and irregular.

Isolation and purification of pathogen

All glassware was initially washed with potassium dichromate sulfuric acid solution, thoroughly rinsed with distilled water and sterilised in a hot air oven at 180 °C for 2 hr. Potato dextrose agar (PDA) medium (39 g L⁻¹) was prepared and the pH was adjusted to 5.6 before sterilisation by autoclaving at 121.5 °C under 15 psi pressure for 20 min. For fungal isolation under *in vitro* conditions, infected stem tissues showing white mycelial growth were selected. Small tissue bits (1–2 mm) were cut using a sterilised blade and surface sterilised with 0.1 % HgCl₂ (mercuric chloride) for 10–20 sec. The pieces were then rinsed 3 times with sterile distilled water and dried on sterile filter paper to remove excess moisture. Finally, the sterilised tissue pieces were transferred onto PDA plates and incubated under aseptic conditions at 19 ± 2 °C in a BOD incubator 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 % HgCl₂ solution for 1 min, followed by three 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 ± 2 °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 isolated fungus was cultured until sporulation occurred. The sporulating culture was then identified using its cultural and morphological features, particularly the formation of sclerotia. For precise confirmation, molecular identification of the pathogen was also performed.

Pathogenicity

Healthy mustard plants were inoculated with actively growing mycelial discs of *S. sclerotiorum*, while uninoculated plants served as controls. The experiment was conducted with 3 replications under controlled conditions and symptom development was recorded periodically. The pathogenic nature of the fungus isolated from diseased plants was confirmed on mustard through artificial inoculation under nursery conditions in pot culture using the cotton mycelium method (Fig 1). Inoculum was prepared from both infected plant tissues and actively growing cultures of the pathogen.



Fig. 1. Pathogenicity testing in pot culture.

Results and Discussion

Survey for natural occurrence of stem rot disease of mustard in districts of Central Bihar

Rapeseed-mustard is an important oilseed crop cultivated in Bihar. However, its productivity is significantly affected by disease infestations, particularly stem rot caused by *Sclerotinia sclerotiorum*. The epidemiology of stem rot disease was strongly influenced by the interaction of temperature, RH and moisture availability. In the present study, a systematic field survey was conducted to assess the prevalence and severity of stem rot disease in major mustard-growing regions of Central Bihar during the rabi season of 2023–24 and 2024–25. The survey was carried out across five blocks in the districts of Samastipur, Muzaffarpur and Darbhanga, with four locations selected per district. Disease symptoms were consistently observed on the stem in all surveyed fields, with initial detection recorded during the first week of January 2023–24. The highest disease incidence of 39.20 % was observed in Vidhyajhap (Moraul block, Muzaffarpur district), followed by Dwarikapur (37.79 %) and Itharasulpur (34.30 %). The mean disease incidence of Muzaffarpur district was 37.09 % indicating that this region is a severe hotspot for sclerotinia rot. Similarly, Bhagwanpur Desua (27.91 %) and Bibhutipur (25.68 %) in Samastipur district also showed a moderate to high level of disease pressure, while Morwa (18.75 %) recorded comparatively lower disease pressure. The mean disease incidence of Samastipur district was 24.11 %. On the contrary, the lowest disease incidence was reported in Ghanashyampur (Korthu block, Darbhanga district) at just 2.85 %, marking it as the least affected site among the surveyed locations. This was followed by Alinagar (3.65 %) and Jaitipur (5.90 %), both situated in Darbhanga district. The mean disease incidence of Darbhanga district was 4.13 % indicating comparatively minimal disease occurrence among the surveyed districts. The comparatively lower disease incidence in Darbhanga district may be attributed to less favourable environmental conditions, reduced irrigation frequency, better air circulation within crop canopy and possible differences in varietal susceptibility and agronomic practice. The variation in disease incidence was likely due to differences in agronomic practices, varietal susceptibility and local microclimates along with the environmental conditions (Tables 1–3 and Fig. 2–5). Statistical analysis revealed significant variation in disease incidence among the surveyed locations and districts. Comparative analysis with previous studies highlights the severity of the disease in rapeseed-mustard. A maximum incidence of 47 % in mustard crops in Samastipur, Muzaffarpur and Vaishali districts of Bihar, with the highest incidence in Marsharbahadur village of Pusa block (7). Similarly, research documented a lower incidence (maximum 11.52 %) in mustard fields across Morena, Bhind, Gwalior, Sheopur and Datia districts of Madhya Pradesh. The disparity in disease incidence may be attributed to differences in agroclimatic conditions and varietal resistance. A similar survey conducted across 11 districts in Rajasthan and Haryana confirmed the widespread occurrence and significance of stem rot disease (8, 9). The highest incidence was reported in Dausa, Rajasthan (29.20 %), followed by Rohtak, Haryana (24.80 %), reinforcing the findings of the present study. Comparable results were also observed for the same pathogen infecting a different host species (10). The elevated disease incidence in the surveyed regions may be attributed to dense crop canopies and frequent irrigation schedules,

Table 1. Disease incidence (%) of stem rot of rapeseed-mustard in Smastipur district

Block	Variety	Disease incidence (%) (2023–24)	Disease incidence (%) (2024–25)	Mean disease incidence (2023–24)	Mean disease incidence (2024–25)	Pooled mean disease incidence
Ujiarpur	Local	24.80	28.50	24.16	27.91	26.04
	Local	23.60	27.20			
	Local	22.95	26.90			
	Local	23.50	29.04			
Bibhutipur	Local	26.60	25.50	26.90	25.68	26.29
	Local	27.20	25.60			
	Local	26.80	26.70			
	Local	27.00	25.82			
Morwa	Local	17.80	17.25	18.88	18.75	18.81
	Local	18.55	18.65			
	Local	19.20	19.25			
	Local	19.95	20.00			

Table 2. Disease incidence (%) of stem rot of rapeseed-mustard in Muzaffarpur district

Block	Variety	Disease incidence (%) (2023–24)	Disease incidence (%) (2024–25)	Mean disease incidence (2023–24)	Mean disease incidence (2024–25)	Pooled mean disease incidence
Dwarikapur	Local	38.90	36.50	39.74	37.79	38.76
	Local	39.40	37.25			
	Local	40.10	38.60			
	Local	40.55	38.81			
Itharasulpur	Local	34.20	33.00	35.88	34.30	35.09
	Local	35.80	34.90			
	Local	36.10	34.60			
	Local	37.40	36.70			
Moraul	Local	38.75	38.10	39.05	39.20	39.52
	Local	39.05	39.00			
	Local	40.20	39.70			
	Local	40.95	40.00			

Table 3. Disease incidence (%) of Stem rot of rapeseed-mustard in Darbhanga district

Block	Variety	Disease incidence (%) (2023–24)	Disease incidence (%) (2024–25)	Mean disease incidence (2023–24)	Mean disease incidence (2024–25)	Pooled mean disease incidence
Darbhanga	Local	7.00	5.40	7.60	5.90	6.75
	Local	7.40	5.70			
	Local	7.81	6.10			
	Local	8.20	6.40			
Alinagar	Local	5.60	3.20	6.20	3.65	4.92
	Local	6.00	3.50			
	Local	6.40	3.90			
	Local	6.80	4.00			
Korthu	Local	3.10	2.50	3.45	2.85	3.15
	Local	3.30	2.70			
	Local	3.60	3.00			
	Local	3.80	3.20			

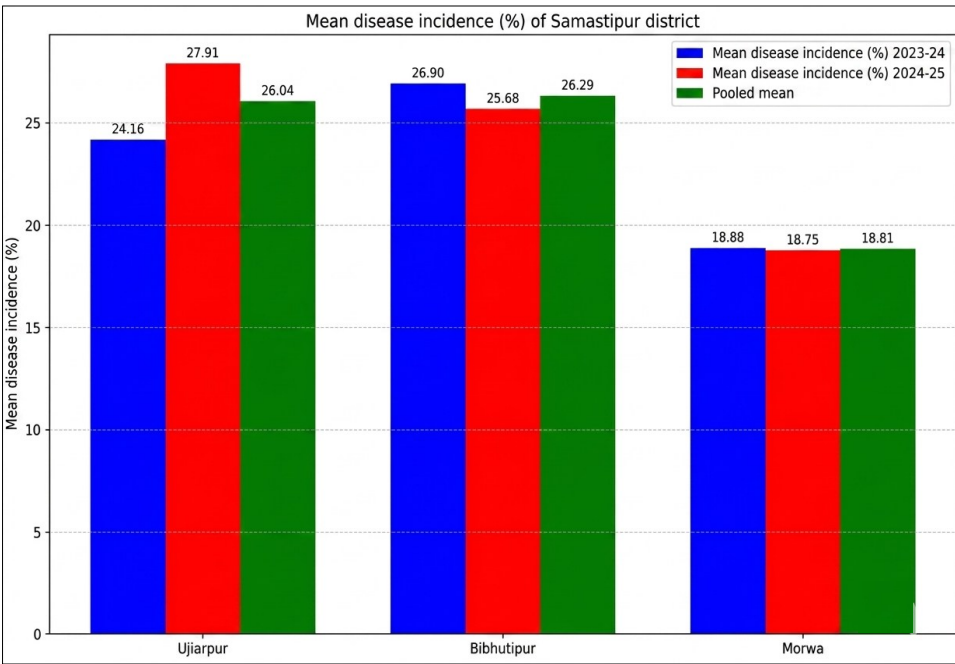


Fig. 2. Mean disease incidence (%) of Samastipur district.

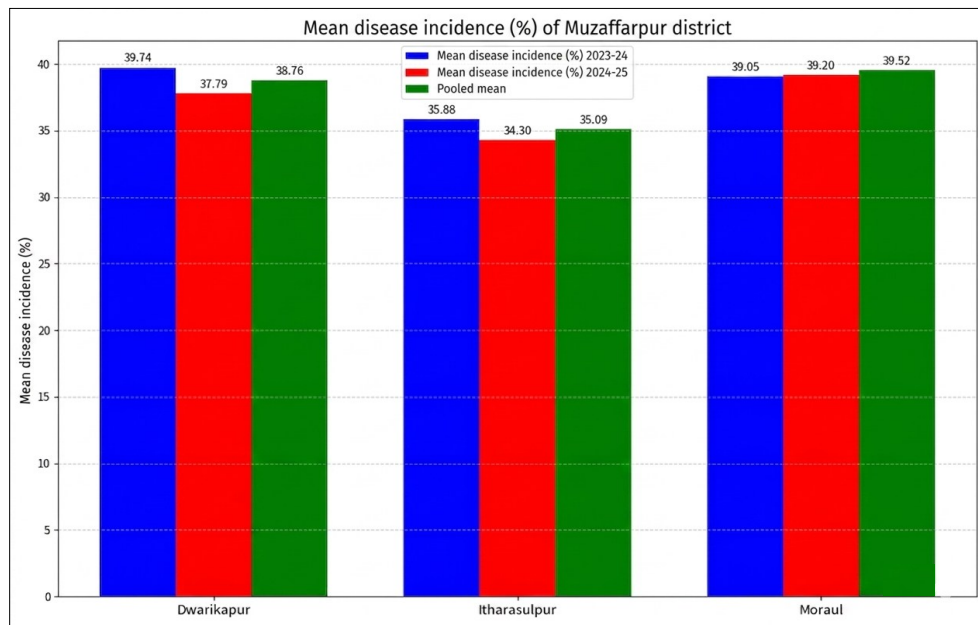


Fig. 3. Mean disease incidence (%) of Muzaffarpur district.

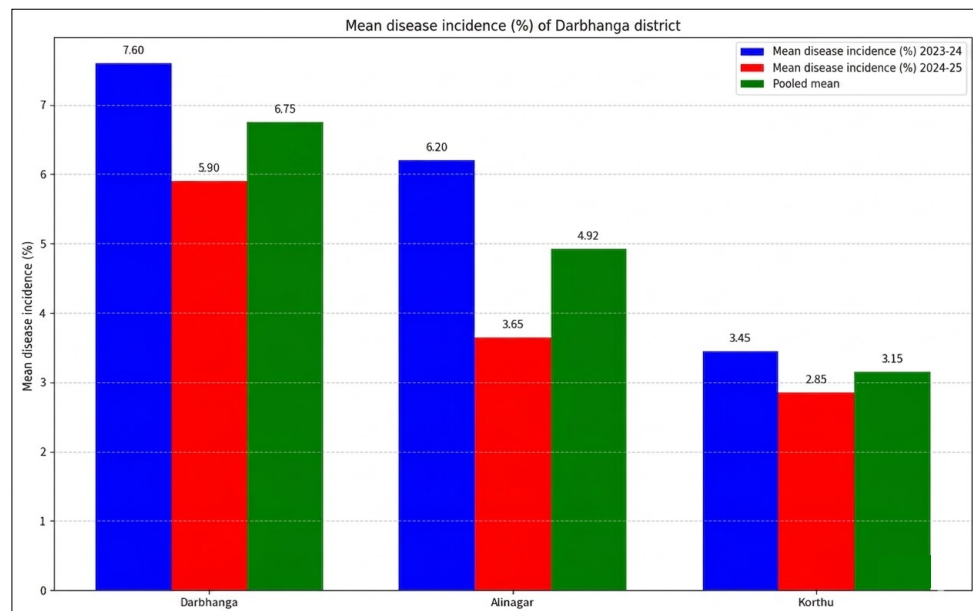


Fig. 4. Mean disease incidence (%) of Darbhanga district.



Fig. 5. Survey during rabi 2024–25 with GPS map.

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 the course of study (2023–24 and 2024–25), the symptoms of the disease on the plants were observed as small water-soaked lesions on the stem portion, which later coalesced to form a big spot (Fig. 6). This observation was marked in basil leaves, in which the stem had brown lesions which rapidly spread both upwards and downwards on the main stem (11). Necrotic tissues with white mycelium covered on the main stem were observed in our study. Research studied the symptoms of sclerotinia rot and found that lesions initially appear bleached and necrotic, eventually developing patches of fluffy white mycelium, which is the most noticeable sign of mycelial infection (12). Research studied *S. sclerotiorum* causing disease in chilli seedlings. Initial symptoms included water-soaked lesions at the stem base, followed by white



Fig. 6. Sclerotinia stem rot symptoms on rapeseed-mustard infected by *Sclerotinia sclerotiorum*.

mould growth, wilting and plant death (13). In an experiment conducted, white mould disease was visually identified by water-soaked lesions initially appearing on stems and pods (14). These lesions expanded, covering entire racemes and pods with white fungal growth. Black coloured hard sclerotial masses were also seen inside the dried stem in the field during the experiment and in a similar experiment conducted, black sclerotial masses were reported inside the infected stem of oilseed brassica (15). As the fungus is devoid of asexual spores, it infects the host through carpogenic germination by producing ascospores released from the apothecium as a fruiting body, which occurs under favourable environmental conditions, as reported, but in current experiment, an apothecium was not observed (16). 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 rapeseed-mustard plants, the diseased plant parts were taken to the laboratory for isolation of the pathogen. Research reported the symptoms of stem rot of berseem as water-soaked lesions at the stem base, which were followed by the development of white fluffy patches of pathogen mycelium, which gradually produced hard sclerotia (17). Similarly, research observed the stem rot symptoms as water-soaked lesions on the stem which caused bleaching and wilting of the infected stem, leading to crown rot (18).

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. 7 and 8). All the samples from different surveyed areas were also isolated (Fig. 9). The fungus isolated from the stem of rapeseed mustard formed white to light brown colonies on PDA (19). Sixty *S. sclerotiorum* isolates from bean were characterised based on morphological traits (20). Small mycelial tufts began to form at the Petri plates' edge and glossy water droplets could be observed surrounding these tufts, proceeding with the formation of sclerotia as concentric circles around the mycelium at the periphery of the plates. White mycelium net was observed to have individual sclerotia embedded in it. Mycelium varied in abundance, regularity and colour (white or beige). These results are similar to the white-coloured mycelium observed in our experiment. Colony shape was round or diverse. The difference in the colour of the colony may be due to the pathogen being isolated from different hosts. Sclerotial masses, when measured in laboratory was $2-10 \times 13-15$ mm. Cultured *S. sclerotiorum* isolated from Indian mustard was found the sclerotial measuring $2.5-8.5 \times 2-5.5$ mm, which is somewhat similar to the findings (21). The difference in the sclerotial sizes may be due to the isolation of the pathogen from different host crops.

Research indicates that the morphological studies on the pathogen isolated from *Brassica juncea* and found the white colour mycelium covering the plates in 9–10 days (22). This research finding somewhat matches current results. The difference in days may be due to differences in the temperature and the season in which the pathogen was isolated. It has been observed that the shape of sclerotia during the winter season was somewhat semi-spherical to



Fig. 7. Diseased samples for isolation.



Fig. 8. Pure culture of *Sclerotinia sclerotiorum*.

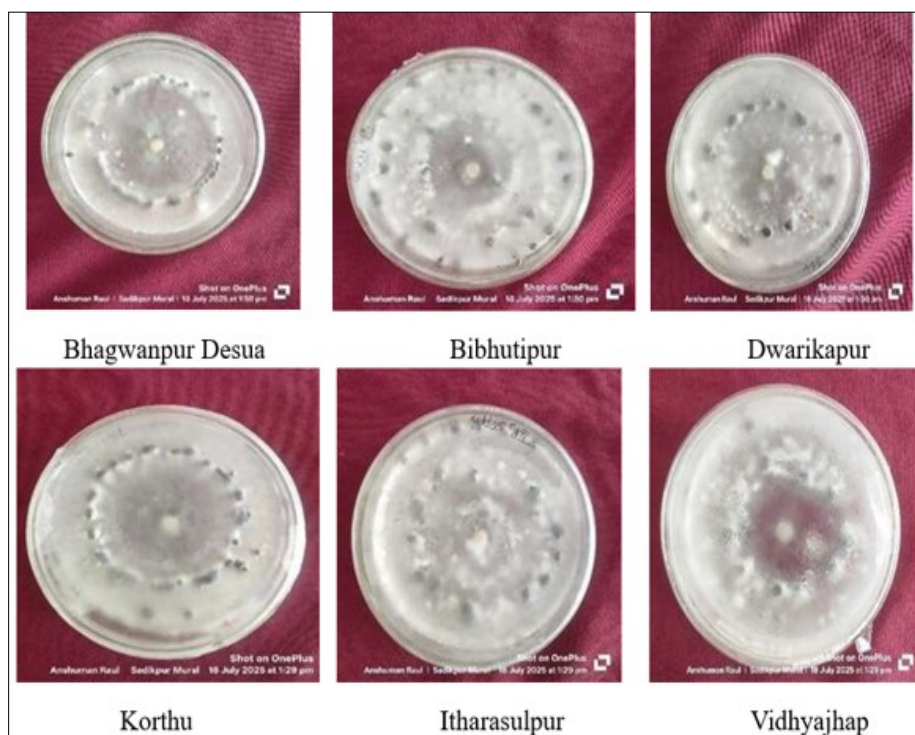


Fig. 9. Pure culture of *Sclerotinia sclerotiorum* isolated from an exact sample of a different survey area.

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. 10). This result was supported by the experiment conducted in which hyaline branched hyphae along with apothecia and ascospores were observed under the microscope (14). Research also observed ascospores during the study (23). In our experiment, apothecia were not observed due to the temperature and RH of Dholi, in accordance with other places. The absence of apothecia may be due to unsuitable environmental conditions such as inadequate soil moisture, temperature fluctuations or lack of prolonged canopy wetness required for carpogenic germination of sclerotia. Ecologically, this suggests that disease spread may have occurred predominantly through myceliogenic rather than carpogenic germination. Formation of apothecia requires specific temperature and favourable RH, which may not be present during the course of the experiment. The detailed morphological study was carried out to confirm the pathogen as *S. sclerotiorum*, the causal agent of stem rot of rapeseed-mustard.

Molecular identification

The pure culture (natural farming isolate mustard) was sent to Barcode Bioscience, Bangalore (ISO 9001:2015 certified) for identification. Morphological and molecular analyses confirmed the pathogen as *S. sclerotiorum*. Molecular identification of the pathogen was carried out by amplifying the ITS region of rDNA using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR amplification was performed with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The amplified products were sequenced and sequence analysis was carried out using BLAST in the NCBI GenBank database. Phylogenetic analysis was performed using MEGA software to confirm the identity of the pathogen. The ITS sequence was subjected to BLAST analysis (NCBI GenBank PX102192), where the top ten hits showed 99.65 % identity with known *S. sclerotiorum* isolates, including SFJ1 (ON656665.1), CN145A2 (OP21023.1), CN145A3, CN144H9 (OP210222.1), CN145A9, CN144H8 and CN145A4 and a phylogenetic tree with accession numbers was also obtained (Fig. 11 and Table 4).

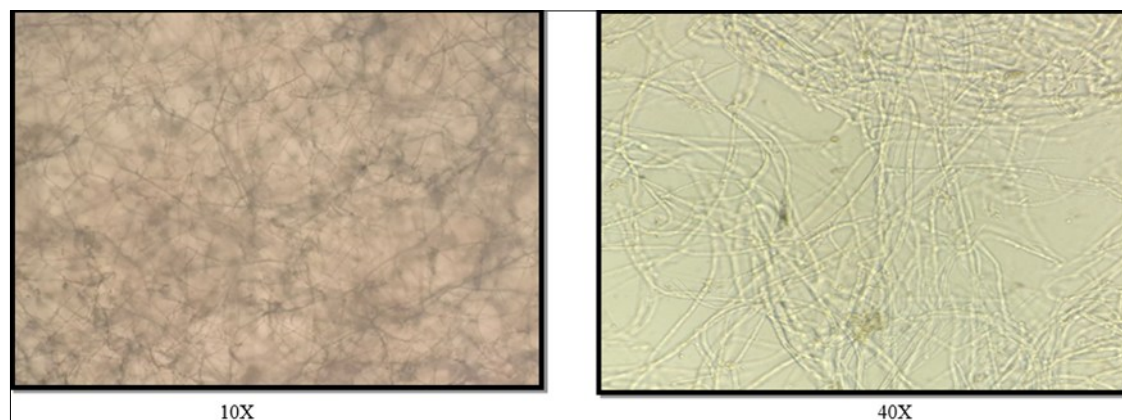


Fig. 10. Microscopic view of mycelium of *Sclerotinia sclerotiorum*.

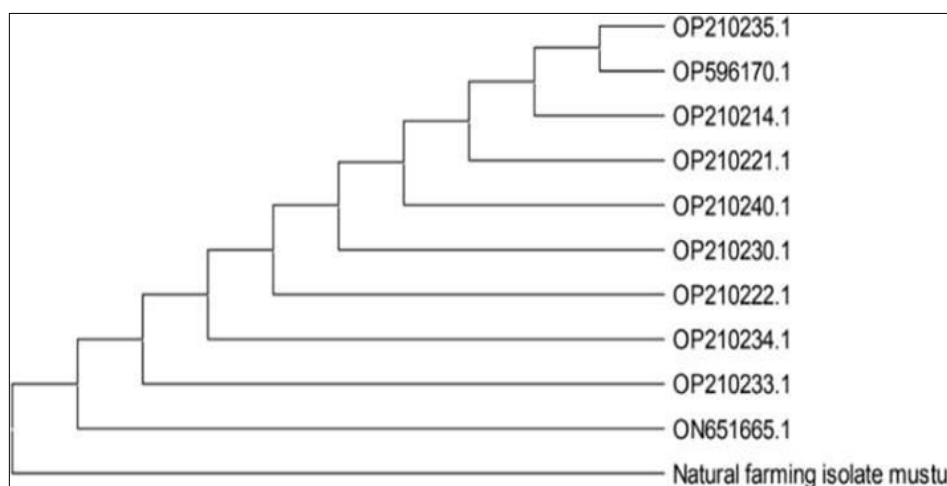


Fig. 11. Phylogenetic tree of natural farming isolate mustard with other isolates.

Table 4. Accession No of natural farming mustard isolate

Description	Max score	Total score	Query cover (%)	E value (%)	Ident (%)	Accession
<i>Sclerotinia sclerotiorum</i> isolate SFJ1	1051	1051	100	0.00	99.83	ON651665.1
<i>Sclerotinia sclerotiorum</i> strain CN145A2	1044	1044	100	0.00	99.65	OP210233.1
<i>Sclerotinia sclerotiorum</i> strain CN145A3	1044	1044	100	0.00	99.65	OP210234.1
<i>Sclerotinia sclerotiorum</i> strain CN144H9	1044	1044	100	0.00	99.65	OP210222.1
<i>Sclerotinia sclerotiorum</i> strain CN144I8	1044	1044	100	0.00	99.65	OP210230.1
<i>Sclerotinia sclerotiorum</i> strain CN145A9	1044	1044	100	0.00	99.65	OP210240.1
<i>Sclerotinia sclerotiorum</i> strain CN144H8	1044	1044	100	0.00	99.65	OP210221.1
<i>Sclerotinia sclerotiorum</i> strain CN144H1	1044	1044	100%	0.00%	99.65%	OP210214.1
<i>Sclerotinia sclerotiorum</i> strain CN145A4	1044	1044	100%	0.00%	99.65%	OP210235.1
<i>Sclerotinia sclerotiorum</i> strain CN144F7	1044	1044	100%	0.00%	99.65%	OP596170.1

Influence of environmental factors on the development of sclerotinia stem rot

Environmental factors influencing stem rot development were studied under School of Natural Farming conditions at Pusa. DRMR-150-3 variety was sown in the field (Fig. 12). The weather data were collected from the Centre of Advanced Climate Change, Pusa Centre, RPCAU (Table 5). Weather parameters such as maximum and minimum temperature, RH and evaporation were analysed in relation to disease severity measured by stem lesion length. A correlation matrix was obtained using the software R and KAU GRAPES (Table 6). Statistical analysis showed that temperature had the greatest influence on disease development and was negatively correlated with lesion progression. The relationship between environmental parameters and disease progression was found to be statistically significant. Regression analysis indicated that climatic variables together explained about 84–89 % of the variation in disease incidence. The regression relationship between morning RH and lesion length ($Y = 2.354X_3 + 8.31$) suggested that disease initiation requires around 78.31 % RH and further increases

in humidity may enhance lesion development. Similarly, the equation for evening RH ($Y = 0.592X_4 - 30.874$) indicated that higher humidity favours disease progression (Table 7). Reduced evaporation also promoted disease severity, as lower evaporation



Fig. 12. Field view of rapeseed-mustard in natural farming during rabi 2024–25.

Table 5. Influence of meteorological parameter on stem lesion length of *Sclerotinia sclerotiorum*

Date of observation (2024–25)	Stem lesion length (Y)	Max temp (X ₁)	Min temp (X ₂)	RH morning (X ₃)	RH evening (X ₄)	Evaporation (X ₅)	Windspeed (X ₆)
28/12/2024	1.50	28.46	12.09	94.29	52.71	3.16	7.41
04/01/2025	3.32	27.06	11.23	94.57	68.00	1.23	7.00
11/01/2025	5.13	25.89	10.00	94.71	62.57	3.29	8.94
18/01/2025	6.71	21.00	10.47	94.00	66.57	2.63	10.53
25/01/2025	8.30	22.93	10.39	96.86	65.00	2.43	10.00
01/02/2025	9.85	21.00	9.25	97.43	70.00	1.51	10.65
08/02/2025	11.20	21.04	9.00	95.00	65.00	1.40	12.20
15/02/2025	12.75	18.60	8.94	97.71	72.86	1.36	14.20
25/02/2025	16.50	18.96	7.93	98.00	73.71	1.07	14.95

Table 6. Correlation matrix for cumulative progression of stem rot disease in relation with weather variables

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇
X ₁	1.000	-0.9181**	-0.8961**	0.7992*	0.7808*	-0.6670**	0.9649
X ₂		1.000	0.8358	-0.6859	-0.7928	0.5794	-0.9298*
X ₃			1.000	-0.7601	-0.7687	0.6183	-0.8173
X ₄				1.000	1.000	-0.5726	0.7627
X ₅					1.000	0.7120*	
X ₆						1.000	-0.5537
X ₇							1.000

X₁: Stem lesion length (PDI); X₂: Maximum temperature (°C); X₃: Minimum temperature (°C); X₄: Morning relative humidity (%); X₅: Evening relative humidity (%); X₆: Evaporation; X₇: Wind speed (km hr⁻¹); (**) significant at 0.01 level; (*) significant at a 0.05 level.

Table 7. Individual regression equation of each weather variable with different models

Date of observation	Si	S(i - 1)	Average (Si + S(i - 1))/2
28/12/2024	1.50	3.32	2.4
4/1/2025	3.32	5.13	4.2
11/1/2025	5.13	6.71	5.9
18/1/2025	6.71	8.30	7.5
25/1/2025	8.30	9.85	9.07
1/2/2025	9.85	11.20	10.52
8/2/2025	11.20	12.75	11.97
15/2/2025	12.75	16.50 (at 25/2/2025) final data	14.62
Total			65.2 (Sum of averages)

Time interval for observation of data is 7 days AUDPC = 65.2 × 7 = 456.4

rates were associated with increased lesion length. The results further showed that stem rot development was more likely when maximum temperatures remained below 22.93 °C and minimum temperatures below 8.00 °C. Significant variation in lesion progression was observed under different environmental conditions. Research demonstrated the occurrence of “polio disease” in rapeseed-mustard at fortnightly intervals and analysed its association with weather parameters such as temperature, humidity and rainfall (24). Their observations showed that the disease increased under cooler environmental conditions, especially when the maximum temperature was about 26.9 °C and the minimum temperature was around 14.6 °C, which ultimately resulted in considerable yield losses. Research noted that infection by *S. sclerotiorum* resulting in wilting, rotting and plant collapse was strongly favoured by moist environments with RH around 95 % and soil temperatures ranging from 4 to 18 °C (25). Research also reported that temperature and RH were important factors for disease development and among the weather factors, temperature was observed as the most important factor in the development of disease, having a significant negative correlation with disease severity (26). The stem lesion length data taken from Table 8 can be referred to based on the 0–4 disease rating scale (27). The analysis

showed that wind speed had the strongest positive association with lesion length ($r = +0.89$), indicating that higher wind speeds may enhance disease spread, possibly by aiding the dispersal of sclerotia or spores and altering the crop canopy microclimate. In contrast, maximum and minimum temperatures showed strong negative correlations with lesion length ($r = -0.92$ and -0.90), suggesting that cooler conditions favour disease development. This supports the known biology of *S. sclerotiorum*, which thrives under cool and moist environments. Both morning and evening RH were positively correlated with lesion length ($r = +0.80$ and $+0.78$), indicating that prolonged humid conditions and extended surface wetness promote sclerotia germination, mycelial growth and subsequent lesion expansion. Stem lesion length increased gradually during the observation period, beginning at 1.5 in the first week and reaching 12.75 by the seventh week, with a final value of 16.50 in the eighth week, indicating steady disease progression. The mean lesion length for successive intervals ranged from 2.4 in the initial stage to 14.62 in the final stage, with a total average value of 65.2. Based on these values, the AUDPC curve was calculated by multiplying the total mean value (65.2) by the 7-day interval, giving an AUDPC of 456.4 as shown in Table 9 (28).

The AUDPC analysis indicated moderate disease development in the tested variety under prevailing environmental conditions. This value represents the cumulative development of stem lesions over time and is useful for comparing disease intensity under different conditions. The gradual rise in lesion length suggests that the disease progressed more rapidly after the early stage, possibly due to favourable environmental conditions such as higher humidity and suitable temperatures. The initial low lesion value indicates a short latent phase, followed by a noticeable increase from the third week onwards. The AUDPC value of 456.4 reflects moderate disease pressure and indicates that the tested variety, DRMR-150-35, can be classified as moderately resistant (Table 10). The results showed a significant association between environmental factors and disease progression. Similar findings assessed the AUDPC curve for 14 groundnut genotypes against late leaf spot disease (29). Similar types of findings were also reported that assessed disease progression in 261 mung bean germplasm by calculating the AUDPC (30–32).

Table 8. Disease rating scale for lesion length

Resistant	Rating	Lesion (cm)
Resistant	0	< 3
Moderately tolerant	1	3–5
Moderately susceptible	2	5–10
Susceptible	3	10–15
Highly susceptible	4	>15

Table 9. Weekly progression and calculation of AUDPC for *Sclerotinia sclerotiorum* for rapeseed-mustard

Regression equation	R ²
$Y = 36.3207 - 1.2282X_1$	0.8429
$Y = 39.4411 - 0.7506X_1 - 1.4307X_2$	0.8539
$Y = -27.0626 - 0.7018X_1 - 0.9806X_2 + 0.6364X_3$	0.8632
$Y = -26.5545 - 0.7221X_1 - 1.0055X_2 + 0.6555X_3 - 0.0247X_4$	0.8759
$Y = -13.808 - 0.7727X_1 - 0.9336X_2 + 0.6248X_3 - 0.0231X_4 - 0.7261X_5$	0.8864
$Y = -18.9598 + 0.0242X_1 - 0.7856X_2 + 0.2609X_3 - 0.0231X_4 - 0.7261X_5 + 1.1694X_6$	0.8926

Table 10. Disease rating scale for AUDPC

AUDPC value	Disease reaction
350	Resistant (R)
351–650	Moderately resistant (MR)
651 and above	Susceptible (S)

Conclusion

Overall, the present study confirmed that stem rot caused by *Sclerotinia sclerotiorum* is a major constraint to rapeseed-mustard production in Central Bihar, significantly affecting crop productivity. Field surveys revealed widespread disease occurrence, emphasising the need for continuous monitoring and integrated disease management strategies. The evaluated variety showed moderate resistance; however, the lack of complete resistance highlights the importance of developing resistant germplasm for sustainable management. Epidemiological studies indicated that temperature and relative humidity strongly influence disease development, suggesting that host resistance should be integrated with proper agronomic practices such as optimum spacing, irrigation and moisture management. The findings provide valuable baseline information for researchers, breeders, extension workers and farmers and support the development of eco-friendly disease management approaches and durable resistant varieties. However, further multi-season and multi-location studies are needed to validate the consistency and wider applicability of the results under diverse agro-climatic conditions.

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Authors' contributions

CSC and AR profound knowledge in plant disease diagnosis, fungal taxonomy and disease management strategies provided the scientific foundation upon which this research was built. BSR guided the initial isolation and microscopic identification of the pathogen, to standardising pathogenicity tests. MM and RKC helped in epidemiological aspects for data evaluation. AKM helped in survey work. RRS helped in field layout experiment. SJ gave moral support throughout the journey. All authors read and approved the final manuscript.

Compliance with ethical standards

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

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