



RESEARCH ARTICLE

Survey, molecular characterisation and *in vitro* management of sugarcane red rot (*Colletotrichum falcatum*)

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Abstract

Red rot of sugarcane, caused by *Colletotrichum falcatum* Went (formerly *Glomerella tucumanensis*), is a highly destructive disease that significantly reduces sugarcane yield and quality. A field survey was conducted from August to October during 2024–2025 in five blocks of Muzaffarnagar district, Uttar Pradesh, to assess disease incidence. The highest incidence was recorded in Jansath block (11.23 %), followed by Sadar (11.13 %), while Bhagra and Shahpur each recorded 6.09 %. The lowest incidence was observed in the Purkaji block (4.71 %). Molecular identification using PCR amplification of the 18S rDNA region with universal primers (ITS1, ITS4) and specific primers (Bt-1, Bt-2) confirmed the presence of *C. falcatum*, producing amplicons of 570 bp and 750 bp. *In vitro* evaluation of biocontrol agents using the dual-culture technique revealed that *Trichoderma harzianum* exhibited the highest inhibition (88.89 %), followed by *Trichoderma viride* (80.00 %), *Bacillus subtilis* (72.22 %) and *Pseudomonas fluorescens* (66.66 %) at 9 days post-incubation. Fungicides tested at 100, 200 and 300 ppm using the food-poisoned technique showed that Carbendazim 50 % WP and Azoxystrobin 23 % SC achieved 100 % inhibition at all concentrations. Propiconazole 25 % EC and hexaconazole 5 % SC showed moderate inhibition. The findings highlight the potential use of effective biocontrol agents and fungicides for integrated management of red rot in sugarcane. These results may assist farmers and researchers in developing sustainable disease management strategies, while further field validation and long-term studies are required to confirm their effectiveness under diverse agro-climatic conditions.

Keywords: biocontrol agents; *Colletotrichum falcatum*; disease incidence; fungicidal evaluation; molecular identification; red rot

Introduction

Sugarcane (*Saccharum officinarum*) is a major commercial crop extensively grown in tropical and subtropical regions. Its significance lies primarily in its capacity to accumulate high levels of sucrose in its stalks, making it valuable for sugar production as well as ethanol, a vital renewable biofuel (1, 2). Sugarcane covers an area of 25.28 lakh hectares, with cane production of 205.65 million tons and a productivity of 107.00 tons ha⁻¹ (3). Globally, around one hundred sugarcane diseases have been identified, with red rot, caused by the fungus *Colletotrichum falcatum* Went, being the most prevalent and destructive. This disease significantly affects both the yield and quality of vulnerable sugarcane varieties across the Indian subcontinent (4). It can reduce cane weight by up to 29 % and cause a 31 % drop in sugar recovery (5). The red rot pathogen secretes the enzyme invertase, which breaks down stored sucrose into glucose and fructose, thereby increasing molasses content. Due to its devastating impact, red rot is often referred to as the "cancer" of sugarcane. The first documented outbreak of red rot in epidemic form occurred in 1901 in the Godawari Delta (Madras). Later, severe

outbreaks were observed in 1906 in Champaran (Bihar) and again in 1922 in Jammu and Kashmir. The movement of cane seeds between different regions facilitated the spread of the disease from south to north. In Uttar Pradesh, red rot was first recorded in 1936 in Gonda district. Since then, it has caused significant damage to sugarcane crops in Rajasthan, Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal and Odisha (6). Eastern Uttar Pradesh and Northern Bihar have become "hot spots" for the disease, experiencing multiple red-rot epiphytotic outbreaks (6). Once the disease appears in the field, it cannot be controlled with existing agrochemicals, resulting in severe losses for the industry. The spores are transparent and falcate (sickle-shaped) with one end slightly blunt and the other pointed. Their size varies between 16–48 µm in length and 4–8 µm in width. The acervuli contain a pink spore mass along with long black setae.

Different strains or biological races of the fungus exist, exhibiting variations in pathogenicity and virulence depending on the sugarcane variety. Additionally, the fungus produces chlamydospores on the stalk surface, inside cane tissues, or in culture media. These spores remain viable for a limited period and

germinate, forming an infection thread that can penetrate intact plant surfaces and tissues (7).

Given the importance of managing this disease, various fungicides have been employed. However, chemical control raises environmental concerns. As a result, biological control methods are increasingly being explored as eco-friendly alternatives for disease management.

Plant diseases can be suppressed through three key strategies: (I) cultivating disease-resistant plant varieties, (II) using chemical fungicides and (III) implementing biological control using antagonistic microorganisms (8). Recently, there has been a noticeable rise in interest in organic fertilisers. Biological approaches to managing plant pathogens rely on methods that disrupt the pathogen's ability to survive and spread. Among these, rhizobacteria have emerged as promising biocontrol agents due to their long-term effectiveness in managing plant diseases (4). Certain rhizobacteria, particularly those from the genus *Bacillus*, have shown significant potential in suppressing soil-borne pathogens affecting various crops (9, 10). Additionally, specific strains of rhizobacteria have demonstrated success in controlling fungal infections in sugarcane. This study focuses on assessing the prevalence of red rot disease, isolating its pathogen and evaluating the use of different rhizobacterial strains for disease suppression.

Materials and Methods

Field survey of red rot disease

To see the status of sugarcane red rot, extensive field surveys were conducted in sugarcane-producing the Jansath, Purkaji, Bhagra, Sadar and Shahpur blocks of Muzaffarnagar district (Fig. 1). Five villages were randomly selected from the sugarcane cultivated area

in selected blocks from five farmers' field was selected from each village and cane samples were collected from farmer's fields during the survey and approximately 20 % sample were collected from 5 % area. The presence of the disease was initially confirmed based on symptomatology. Sampling was carried out using the Z-pattern method (11). All collected specimens were placed in sterilised polybags and carefully carried to the laboratory for subsequent analysis.

Disease incidence

The occurrence of red rot disease was assessed by examining plants for visible symptoms. The characteristic signs of the disease, such as red lesions along the stalk, spread of symptoms through the nodes and white patches, were used to identify the infection (12). The following formula was used to calculate the disease incidence:

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

Isolation of the causal pathogen

Field observations were conducted to identify infected sugarcane samples, which were then collected and carefully cut from the centre with a sterilized blade to confirm typical red rot symptoms. Characteristic signs included reddish discoloration spreading through the stem and small white spots visible in cross-sections of the stalk. The procedure for isolating the fungal pathogen was adapted from the method outlined by (13). Infected tissue samples were cut into small fragments of about 2–3 mm and surface-sterilised using a sodium hypochlorite (NaOCl) 1 % for 30–60 sec. These tissue pieces were placed on potato dextrose agar (PDA),

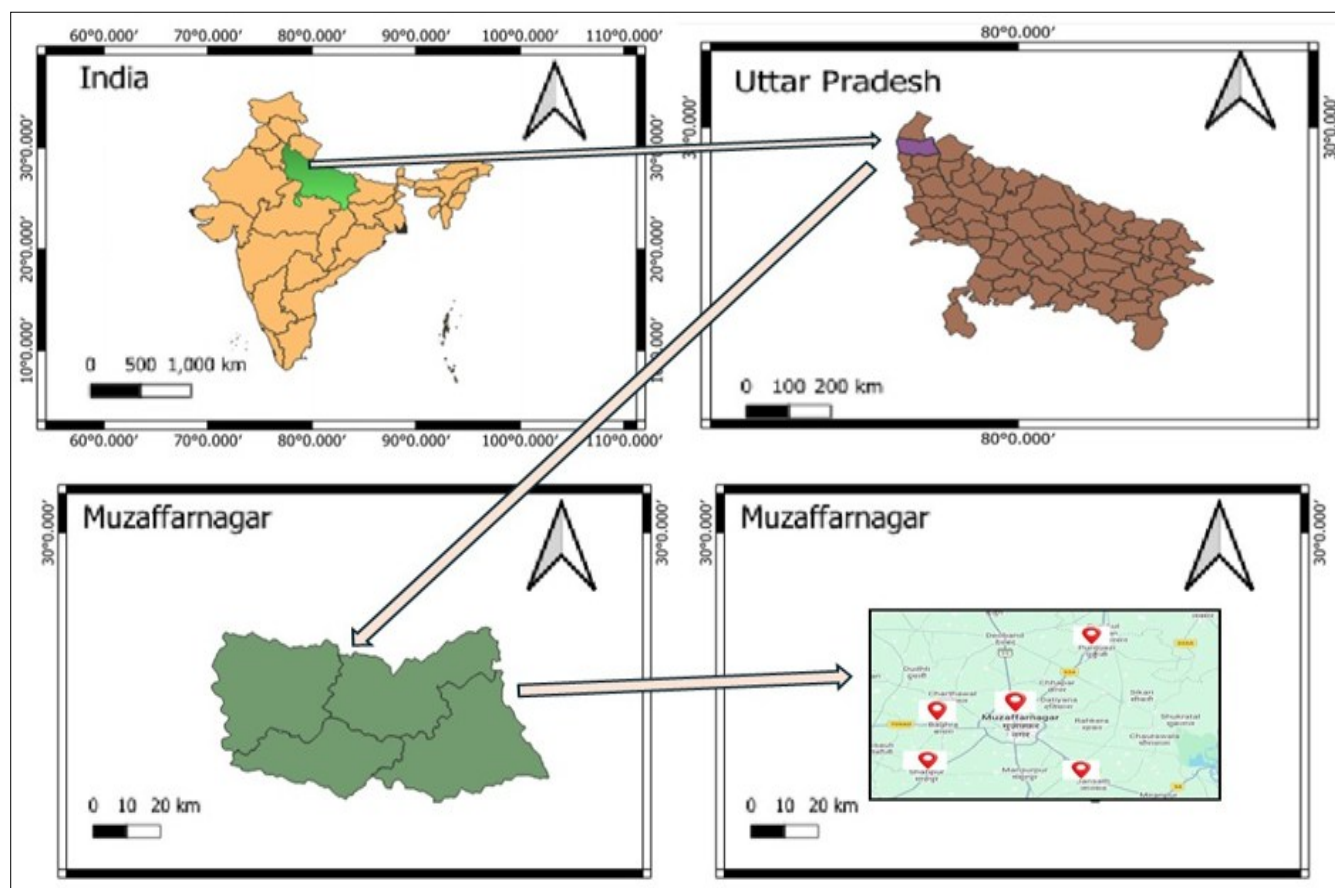


Fig. 1. Blocks where the survey has been conducted.

maintained at 28 ± 2 °C. Subsequently, the fungal pathogen was purified through single-spore isolation on PDA plates.

Morphological and microscopic characterization of the pathogen

Ten days after incubation, observations were made of the fungal colonies to document their physical features, including colour, surface texture and edge structure (11). Using a light microscope, measurements of conidial length and width were taken. Additionally, mycelial structure and growth patterns were examined under 40X magnification.

PCR amplification and phylogenetic analysis

Fungal isolates were obtained through isolation procedures and maintained as pure cultures. Genomic DNA from *C. falcatum* Went isolates was isolated at the Centre of Excellence for Sanitary and Phytosanitary, Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, using the cetyltrimethylammonium bromide (CTAB) method (14). Mycelial mats were collected from a 5–7-day-old culture, which served as the source material. The DNA pellet was washed with 70 % ethanol, air-dried at room temperature, and dissolved in 50 µL of nuclease-free water. DNA quantity and purity were evaluated through agarose gel electrophoresis and spectrophotometric analysis using a Nano-spectrophotometer.

Further confirmation of the isolates was done by cultural characterisation and molecular identification using universal and specific primers.

Universal primers: ITS1 Forward 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 Reverse 5'-TCCTCCGTTATTGATATGC-3' (13)

Specific primers: Bt-1 Forward (AACATGCGTGAGATTGTAAGT) and Bt-2 Reverse (ACCCTCAGTGTAGTGACCTTGCC) (15)

PCR reactions were carried out in a total volume of 40 µL, containing 2 µL of DNA template, 1 µL each of forward and reverse primers (20 pmol), 20 µL of PCR master mix, and 16 µL of nuclease-free water. The thermal cycling conditions included an initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 sec, annealing at 56–58 °C for 30 sec and extension at 72 °C for 45 sec. A final extension step was performed at 72 °C for 10 min (15). Amplified PCR products were visualized on 1.2 % agarose gel stained with ethidium bromide under a UV transilluminator. The PCR product was sent for sequencing (Biokart India Pvt. Ltd., Bangalore). The resulting sequence data were aligned and identified using the NCBI BLAST tool. Phylogenetic and evolutionary relationships of the isolate were analysed using MEGA 12 software.

In vitro efficacy of biocontrol agents and fungicides

Assessment of the *in vitro* antagonistic efficacy of biocontrol agents against *C. falcatum* Went was conducted using the dual-culture method, in which two bacterial (*P. fluorescens*, *B. subtilis*) and two fungal (*T. harzianum* and *T. viride*) biocontrol agents were used. Observations were recorded at 3, 6 and 9 days. *In vitro* efficacy of fungicides (Propiconazole, Hexaconazole, Azoxystrobin and carbendazim) at different concentrations (100, 200 and 300 ppm) was evaluated against *C. falcatum* Went using the poisoned food technique and observations were recorded at 9 days. Mycelial growth was measured and the inhibition percentage was calculated using the following formula:

$$I = \frac{C-T}{C} \times 100 \quad (\text{Eqn. 2})$$

I = Per cent Growth inhibition

C = Radial growth of pathogen in control plate (mm)

T = Radial growth of pathogen in treated plate (mm)

Results

In this study, we conducted the survey from August 2024 to October 2025. The sugarcane fields of five blocks of the Muzaffarnagar District (Jansath, Purkaji, Sadar, Shahpur, Bhagra) were surveyed for the occurrence of Red Rot of Sugarcane. The survey encompassed 25 villages across five blocks. According to Table 1, the highest incidence of red rot disease in sugarcane was observed in Jansath (11.23 %), followed by Sadar (11.13 %), Bhagra (6.09 %) and Shahpur (6.09 %). However, the lowest disease incidence was recorded in Purkaji (4.71 %).

Table 1. Diseases incidence percentage of red rot of sugarcane in Muzaffarnagar

Sr. No.	Block Name	Disease incidence (%)
1.	Sadar	11.13
2.	Jansath	11.23
3.	Bhagra	6.09
4.	Purkaji	4.71
5.	Shahpur	6.09

Morphological characterisation of *Colletotrichum falcatum*

The morphological characteristics of *C. falcatum* were examined on Potato Dextrose Agar (PDA). The colonies displayed moderate to rapid growth, reaching 85–90 mm in diameter within 9 days of incubation at 27 ± 2 °C. Initially, colonies appeared white; gradually, they aged. The aerial mycelium was cottony and fluffy in texture, with the reverse side of the culture plates showing pigmentation ranging from light red to pinkish colour. Under microscopic observation, conidiophores were found. The conidia were sickle-shaped or falcate (Fig. 2).

Molecular characterisation using PCR amplification

A total of 3 isolates suspected of being *C. falcatum* were tested using PCR amplification of the 18S rDNA gene with universal and specific primers, i.e., ITS1/ITS4 and Bt-1/Bt-2. PCR amplification of the 18S rDNA gene resulted in a 570 bp fragment with universal primers (ITS1 and ITS4) (Fig. 3) and a 750 bp fragment with custom-designed specific primers (Bt-1 and Bt-2) (Fig. 4) in all three isolates. Later, PCR products were sent for sequencing (Biokart India Pvt. Ltd., Bangalore); no variability was found among samples from different blocks.

The phylogenetic tree constructed using ITS region sequences clearly places the SVPUAT, Meerut isolate within the *C. falcatum* clade (Fig. 5), showing close relatedness with reference isolates such as KU290263.1, KP265041.1 and MK681011.1. The clustering distinctly separates *C. falcatum* from other species like *C. tucumanensis* (JN390936.1, HQ836351.1) and *Monilochaetes pteridophytophila* (MW262218.1), which was used as an outgroup. This confirms that the test isolate is molecularly identified as

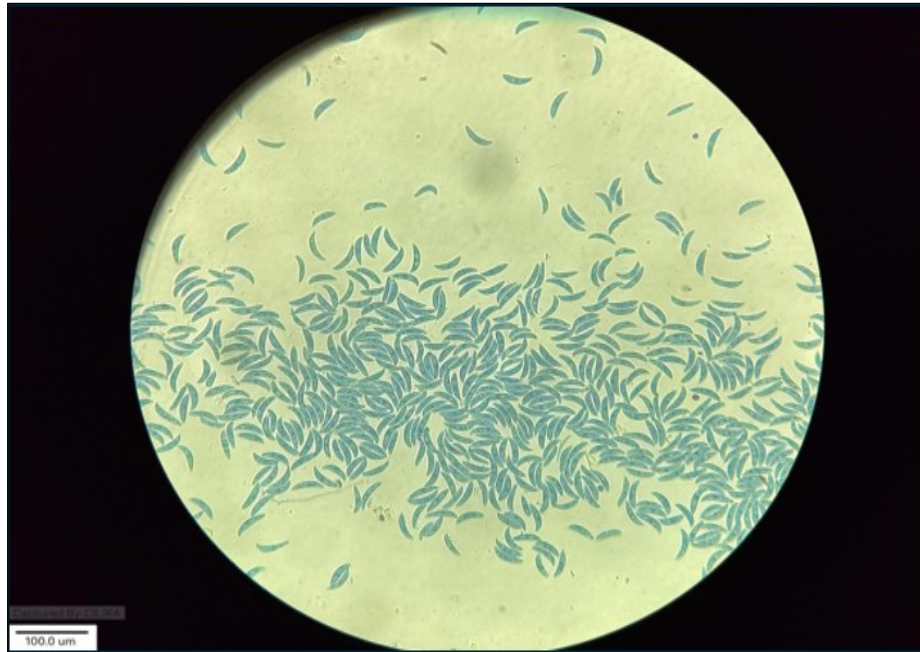


Fig. 2. Microscopic view of conidia of *Colletotrichum falcatum*.

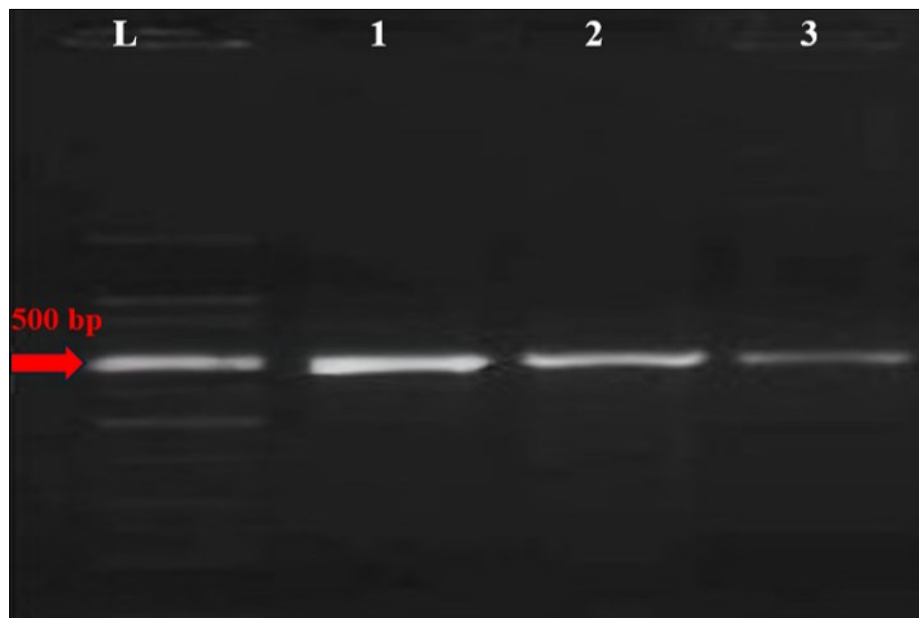


Fig. 3. Agarose gel electrophoresis showing PCR amplification of the ITS region of *Colletotrichum falcatum* (~570 bp).

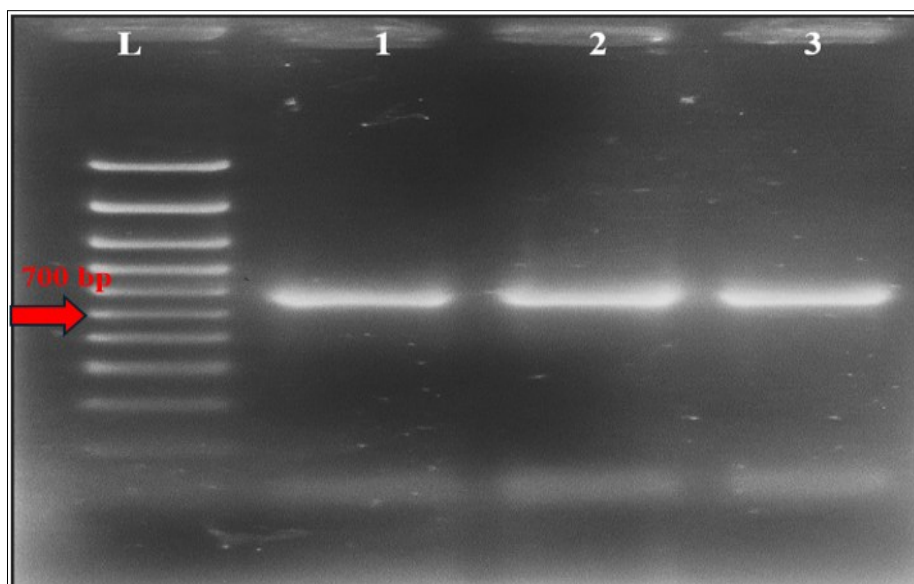


Fig. 4. Agarose gel showing the DNA fragment of *Colletotrichum falcatum* at ~750 bp, three isolates with β -tubulin primer (Bt-1 and Bt-2).

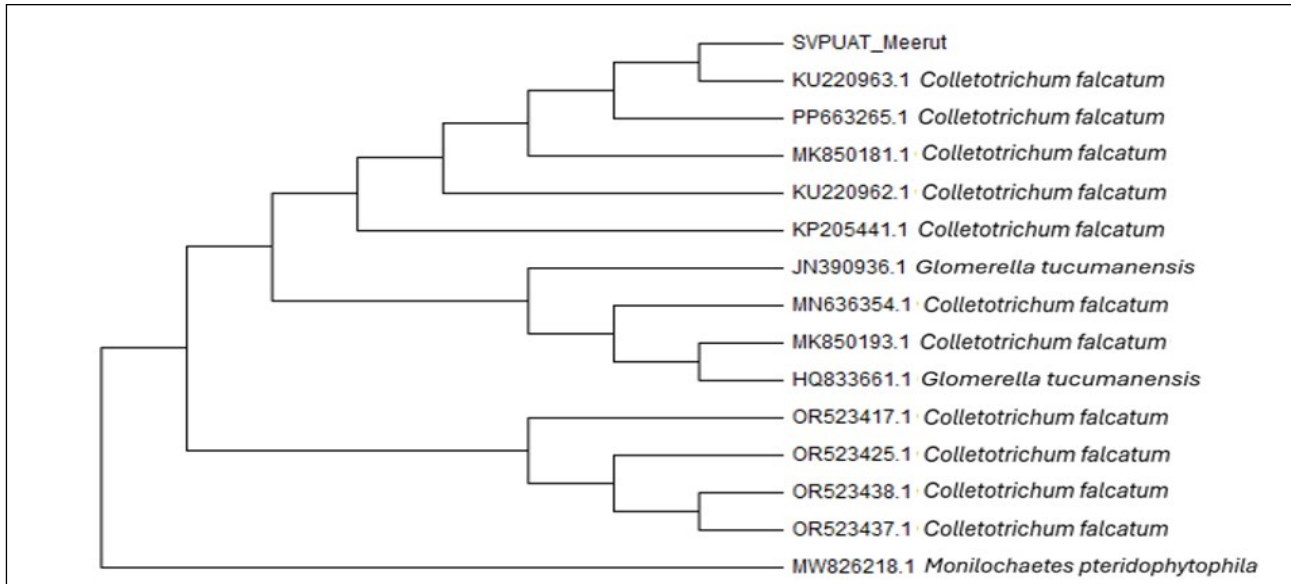


Fig. 5. Phylogenetic tree based on ITS region sequences showing the relationship of the SVPUAT_Meerut isolate of *Colletotrichum falcatum* with reference isolates retrieved from NCBI.

C. falcatum, the causal organism of sugarcane red rot. The pairwise identity heatmap further validates these findings, showing very high nucleotide similarity (97–100 %) among *C. falcatum* isolates, represented by red shades, while distant taxa such as *C. tucumanensis* and *M. pteridophytophila* exhibit significantly lower identity values (81–87 %), reflected in blue-green colours (Fig. 6). Together, these analyses demonstrate the strong genetic uniformity among *C. falcatum* isolates and their clear distinction from related species, providing a reliable basis for molecular identification and subsequent pathogenicity studies.

Efficacy of biocontrol agents and fungicides against the pathogen

According to Table 2, all tested bio-agents significantly reduced the pathogen's radial growth compared to the untreated control. Among them, *T. harzianum* exhibited the highest level of inhibition, suppressing fungal growth by 88.89 % and demonstrated strong microparasitic behaviour; it was the next most effective, inhibiting growth by approximately 80.00 %, followed by *B. subtilis*, with 72.22 % suppression. *P. fluorescens* showed the least antagonistic effect by reducing fungal growth by 66.66 %. Both bacterial agents formed visible inhibition zones, suggesting the secretion of antifungal substances. This experiment showed the superior efficacy

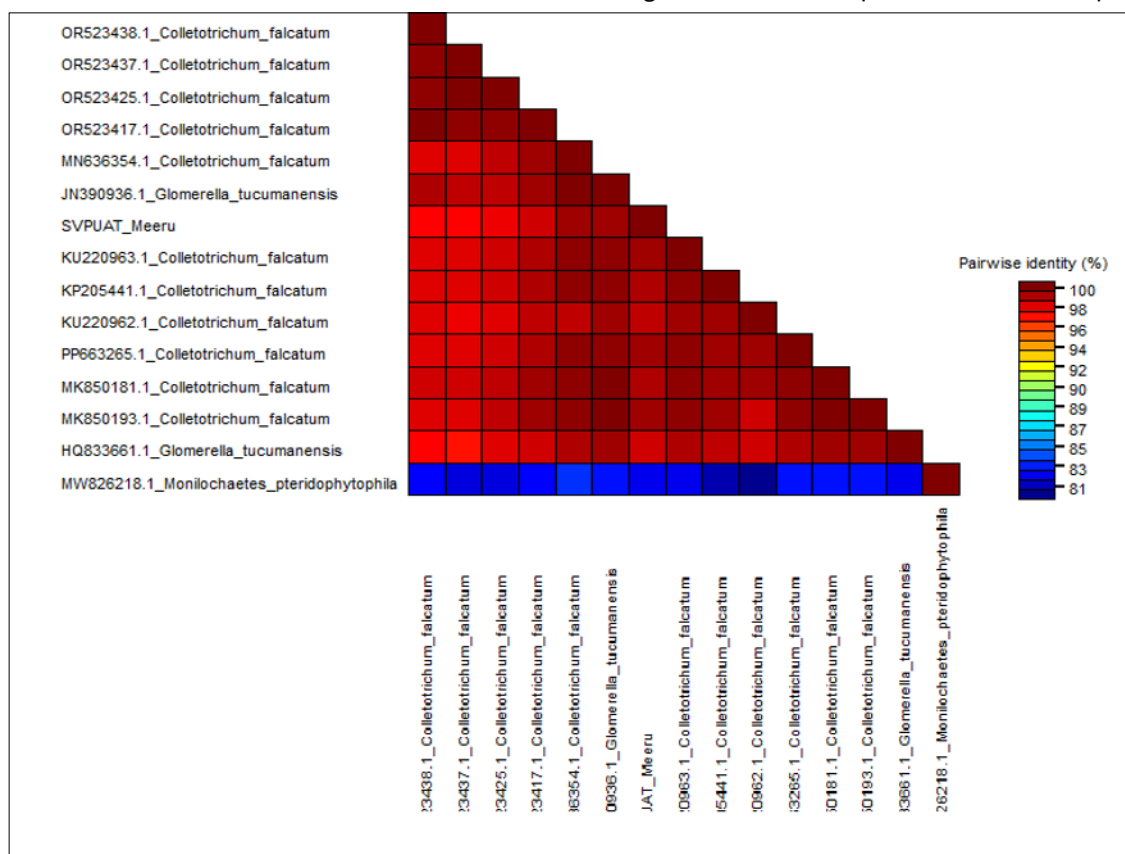


Fig. 6. Heatmap showing pairwise sequence identity (%) of *Colletotrichum falcatum* isolates with reference sequences from the NCBI database.

Table 2. *In vitro* efficacy of biocontrol agents against *Colletotrichum falcatum*

Tr. No.	Bio-Control agents	Percentage of Growth Inhibition of <i>Colletotrichum falcatum</i>		
		3 Days	6 Days	9 Days
T ₁	<i>T. harzianum</i> Isolate-TS004	47.53	64.83	88.89
T ₂	<i>T. viride</i> Isolate-TS006	41.67	58.35	80.00
T ₃	<i>P. fluorescens</i> Isolate-PF008	32.66	44.00	66.66
T ₄	<i>B. subtilis</i> Isolate-B005	34.53	48.23	72.22
T ₅	Control	0.00	00.00	00.00
	CD at 1 %	0.678	1.490	2.202
	CV	1.190	2.140	2.420

CD: critical difference; CV: coefficient of variation.

of *T. harzianum* and *T. viride* and supports their potential use in environmentally friendly disease management strategies for sugarcane.

Based on the data in Table 3, Carbendazim 50 % WP and Azoxystrobin 23 % WS were the most effective fungicides, completely inhibiting the radial growth of *C. falcatum* (100 % inhibition) at all tested concentrations (100, 200 and 300 ppm). Propiconazole 25 % EC showed moderate efficacy, with percent inhibition increasing from 35.55 % at 100 ppm to 64.44 % at 300 ppm. Hexaconazole 5 % SC exhibited the least antifungal activity, with inhibition percentages of 42.22 %, 52.59 % and 59.33 % at 100, 200 and 300 ppm, respectively. The untreated control showed maximum radial growth (90 mm), confirming the effectiveness of the tested fungicides. These results emphasise the superior efficacy of Carbendazim and Azoxystrobin in managing red rot disease under *in vitro* conditions.

Discussion

Disease incidence and field survey

Red rot is recognised as one of the most destructive diseases affecting sugarcane. In the current study, field assessments were conducted to evaluate the prevalence of red rot in Muzaffarnagar District. The fungal pathogen responsible for the disease was identified as *C. falcatum*, a species known to infect multiple parts of the sugarcane plant, including leaves, stalks, roots and buds. The disease becomes more severe when the stem is attacked, leading to significant crop damage. Additionally, infected plants can serve as a source of inoculum, facilitating the pathogen's spread across different areas. The incidence of red rot disease in sugarcane was observed in Jansath block, followed by Purkaji, Bhagra and

Muzaffarnagar. However, the lowest disease incidence was recorded in Shahpur. A similar study conducted in the sugarcane-growing regions of Punjab during 2019–2020 reported severe red rot outbreaks in the variety Co 0238, with incidence levels ranging from 70 % to 100 %, resulting in substantial crop losses across northern India (16, 17). It was reported that severe red rot incidence in Bihar, particularly in the sugarcane variety CoS 8436, resulted in an estimated yield loss of about 147084 quintals during 2015 to 2018 (12). This is largely due to the widespread cultivation of resistant sugarcane varieties in commercial fields to minimise disease occurrence.

Morphological and molecular identification of the pathogen

Isolates of *C. falcatum* exhibited noticeable morphological diversity. Historically, pathogenic variability in this species was recognised with isolates categorised as either light or dark based on cultural traits (18). Colony coloration ranged from pale grey to deep grey, with most strains showing a flat and relatively smooth surface, while only a few appeared fluffy and elevated. Similar morphological and cultural variability in *C. falcatum* has been reported in earlier studies (19). However, relying solely on morphological and colony characteristics for identification is unreliable, as environmental factors can significantly influence fungal appearance. Therefore, molecular methods were employed to support the findings. Molecular techniques not only enable rapid identification of fungal isolates but also provide insights into their genetic relationships (2, 13). The *C. falcatum* isolates were tested for genetic variability using random amplified polymorphic DNA (RAPD) primers. Out of seven amplified primers, only two primers showed the polymorphism among 11 isolates (seven reference pathotypes and four new isolates) of *C. falcatum*. Analysis of the genetic coefficient matrix

Table 3. *In-vitro* efficacy of fungicide agents against *Colletotrichum falcatum*

Tr. No.	Treatment Details	Concentration (ppm)	Radial growth pathogen (mm)	% Inhibition
T1	Carbendazim 50 % WP	100	00.00	100.00
T2	Carbendazim 50 % WP	200	00.00	100.00
T3	Carbendazim 50 % WP	300	00.00	100.00
T4	Propiconazole 25 % EC	100	58.00	35.55
T5	Propiconazole 25 % EC	200	44.00	51.11
T6	Propiconazole 25 % EC	300	32.00	64.44
T7	Azoxystrobin 25 % WS	100	00.00	100.00
T8	Azoxystrobin 25 % WS	200	00.00	100.00
T9	Azoxystrobin 25 % WS	300	00.00	100.00
T10	Hexaconazole 5 % SC	100	52.00	42.22
T11	Hexaconazole 5 % SC	200	42.00	52.59
T12	Hexaconazole 5 % SC	300	36.00	59.33
T13	Control	Untreated	90.00	100.00
	CD	-	0.71	0.18
	CV	-	1.70	1.32

CD: critical difference; CV: Coefficient of variation.

derived from RAPD profile scores showed that the minimum and maximum per cent similarities among the tested *C. falcatum* isolates ranged from 11.11 to 87.5.

Evaluation of biocontrol agents and fungicides

Biological control agents showed considerable antagonistic activity against the pathogen. Among the tested biocontrol agents, *T. harzianum* exhibited the highest mycelial growth inhibition, followed by *T. viride*, *B. subtilis* and *P. fluorescens*. Similarly, another study found that *B. subtilis* exhibited the greatest inhibition (71 %), followed by *T. harzianum* (20 %). In line with these results, *T. harzianum* was reported to be more effective than other biocontrol agents, particularly in the states of Uttar Pradesh and Bihar (20, 21). Similar findings were also reported in other studies, in which the bacterial antagonist *B. subtilis* showed the highest inhibition percentage (60 %) (22, 23). Systemic fungicides were more effective in controlling the pathogen than non-systemic fungicides. Multiple studies have reported similar results, with azoxystrobin and carbendazim exhibiting 100 % inhibition (24–26).

Conclusion

The present study highlights the significant prevalence of red rot disease in sugarcane across various blocks of Muzaffarnagar, with the highest incidence recorded in Jansath. Molecular characterisation confirmed the identity of *C. falcatum* using both universal and specific primers. Among biocontrol agents, *T. harzianum* exhibited the highest antagonistic activity *in vitro*. Chemical control using Carbendazim 50 % WP and Azoxystrobin 23 % SC was highly effective, achieving complete inhibition at all tested concentrations. These findings underscore the importance of integrated disease management strategies for effective control of red rot in sugarcane.

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Authors' contributions

VC conducted the field survey, laboratory work and data collection with assistance from AK and prepared the manuscript. RS conceptualised the study, supervised the research and critically reviewed the manuscript. SS contributed to molecular detection, *in vitro* management and referencing. RB assisted with molecular detection and characterization, performed the statistical analysis and prepared the figures and tables. SM supported molecular detection and *in vitro* management. S provided technical inputs and contributed to the final editing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of Interest: The authors do not have any conflicts of interest to declare.

Ethical Issues: None

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process: During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to enhance clarity and improve overall readability. After using this tool, the authors carefully reviewed and edited the content as needed and took full responsibility for the content of the publication.

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