



RESEARCH ARTICLE

Pathogenicity test of fungal pathogen causing foliar diseases in major cultivars (*Vigna* sp.) of Rajasthan

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Abstract

Leguminous crops are crucial to sustainable agriculture and the second most important crop group after grasses (Poaceae). Greengram (*Vigna radiata* (L.) R.Wilczek) and black gram (*Vigna mungo* (L.) Hepper) are widely cultivated pulse crops across Asia during kharif, rabi and summer. Despite their agronomic and nutritional importance, productivity is often constrained by abiotic and biotic stresses. Continuous monocropping and intensive cultivation increase legume vulnerability to pathogens. Fungal diseases are a major constraint, causing approximately 10–25 % yield losses in legume crops. Foliar diseases also significantly reduce yield depending on crop growth stage, pathogen virulence, disease severity and prevailing environmental conditions. Important foliar diseases affecting *Vigna* species include anthracnose, *Cercospora* leaf spot and *Corynespora* leaf spot. The present study aimed to evaluate the pathogenicity of two important fungal pathogens, *Colletotrichum truncatum* (MTCC 2110) and *Cercospora canescens* (MTCC 10835), responsible for anthracnose and *Cercospora* leaf spot diseases, respectively. A pot experiment was conducted using commonly cultivated varieties of *Vigna* in Rajasthan, including *V. radiata* (MH1142 and IPM-205-7) and *V. mungo* (Pant Urad 31 and Kota Urad 3). Inoculation with the respective pathogens was performed and disease severity was assessed using the percent disease index (PDI). The results revealed variability in disease susceptibility among the tested cultivars. *Vigna mungo* cv. KU-3 exhibited comparatively lower disease severity against *C. canescens* with a percent disease index (PDI) of 24.50 ± 1.42 , while *Vigna radiata* cv. IPM-205-7 showed lower susceptibility to *Colletotrichum truncatum* with a PDI of 48.77 ± 1.23 . The fungal pathogens were re-isolated and molecular characterisation was done using universal primer (ITS 1F and ITS 4R) and the obtained sequences were submitted to National Centre for Biotechnology Information (NCBI). These findings suggest that these cultivars may be suitable for cultivation in regions of Rajasthan where the respective foliar diseases are prevalent. The use of resistant cultivars provides an effective and ecofriendly sustainable strategy for disease management and reducing dependence on chemical fungicides.

Keywords: anthracnose; *Cercospora* leaf spot; disease resistance; fungal pathogen; pathogenicity; Rajasthan; *Vigna* sp.

Introduction

Leguminous crops belonging to the genus *Vigna*, such as green gram (*Vigna radiata* (L.) R.Wilczek) and black gram (*Vigna mungo* (L.) Hepper), are crucial pulse crops grown in India, especially in the arid and semi-arid regions of Rajasthan. These are short-duration crops and contribute to increasing soil fertility by fixing atmospheric nitrogen. The seeds of these crops are an essential source of dietary protein, especially for the vegetarian diet (1).

The productivity of *Vigna* species is notably constrained by their susceptibility to various diseases, particularly those induced by fungal pathogens, which significantly impair yield and seed quality (2). Among these diseases, anthracnose, caused by *Colletotrichum* sp. and leaf spot, caused by *Cercospora* sp., are recognised as the most prevalent and destructive (1, 3, 4).

Green gram is susceptible to infection by *Colletotrichum truncatum* at all stages of growth, the most prominent symptoms being dark, sunken lesions on leaves, stems and pods. High levels of humidity coupled with moderate temperatures intensify the severity of diseases, resulting in

defoliation and the formation of shrivelled seeds, with yield losses estimated between 30 % to 60 % (1, 3). The pathogen is seed-borne and survives in crop residues, allowing it to persist between growing seasons. Its ability to infect a wide range of legume hosts complicates disease management.

Cercospora leaf spot disease, primarily caused by *Cercospora canescens* and *Cercospora cruenta*, poses a significant challenge in the cultivation of black gram. The disease manifests as small, circular to irregular brown lesions on leaves, which may coalesce and lead to early defoliation and inadequate pod development and filling (4). The disease thrives in the warm, humid weather typical of the kharif season in Rajasthan. Severe infections can result in yield reductions up to 70 %, depending on the susceptibility of the cultivar and prevailing environmental conditions (5).

While chemical methods exist to manage these foliar fungal diseases to a certain extent, beyond the immediate expenses primarily to small-scale farmers, the use of chemical fungicides raises broader issues, including environmental pollution and health hazards for humans and animals, which are significant concerns (6).

Consequently, developing and utilising resistant cultivars remains the most efficient, cost-effective and sustainable approach.

However, the success of resistance breeding is dependent on accurately identifying pathogenic strains and measuring host resistance using systematic pathogenicity testing (1). Pathogenicity plays a critical role in confirming the virulence of a pathogen and quantifying host resistance under controlled laboratory conditions. It provides essential information about the interaction between the pathogen and host cultivars, also helps to identify resistant genotypes and understanding the variability in disease response. Such testing is particularly important where variable climatic conditions influence pathogen aggressiveness and disease severity (7).

There is a lack of systematic research assessing the pathogenicity of *C. canescens* and *C. truncatum* in various cultivars of *V. mungo* (Kota Urad 3 and Pant Urad 31) and *V. radiata* (MH1142 and IPM-205-7), which are extensively grown in Rajasthan, India.

The Kota Urad 3 (KU 3) variety, a short-duration crop, suitable for kharif and spring season in Rajasthan, was released by the Agricultural University, Kota, Rajasthan in 2020. It matures in 68–74 days and is moderately resistant to mungbean yellow mosaic virus (MYMV), leaf spot and anthracnose disease (8).

The Pant Urad 31 (PU 31) variety, a photo-thermo-insensitive cultivar of black gram was developed from a cross involving two elite lines; UPU 97-10 (as female) developed at Pantnagar, Uttarakhand and DPU 88-31 (as male) from IIPR, Kanpur and was released in year 2005 and is cultivated in kharif, rabi and zaid across the country (9). It matures in 75–80 days, shows resistance to MYMV and is susceptible (17.45–53.23 %) to *Cercospora* leaf spot (10).

The MH1142 variety is a short-duration crop, suitable for the kharif season in North-eastern and Western Plain Zone and developed by Chaudhary Charan Singh Haryana Agricultural University (HAU), Hisar and released in the year 2020. It matures in 60–65 days and is highly resistant to Yellow Mosaic Virus (YMV) and moderately resistant to powdery mildew and anthracnose (11).

The IPM-205-7 variety is a short duration crop, suitable for summer season in south Zone, Central Zone and North-western Plain Zone, developed by Indian Institute of Pulses Research (IIPR), Kanpur and released in year 2016. It matures in 52–56 days and highly resistant to yellow mosaic virus (YMV) and moderately resistant to powdery mildew and anthracnose (11).

As the information above indicates, all the cultivars, except for the Pant Urad 31 (PU 31) variety which is prone to leaf spot, were originally bred to be resistant to leaf spot and/or anthracnose diseases. However, they have been cultivated consistently for approximately 5 to 20 years, making it highly probable that some pathogenic strains have emerged over time, capable of bypassing the plants' resistance mechanisms.

The present study aims to investigate pathogen virulence and host responses in these *Vigna* cultivars, providing insights into host-pathogen interactions and supporting the identification and future development of disease-tolerant cultivars for sustainable pulse production in Rajasthan.

Materials and Methods

Microbial culture

The fungal cultures of *C. truncatum* (MTCC 2110) and *C. canescens* (MTCC 10835) were obtained from the Microbial Type Culture

Collection (MTCC), Chandigarh, India. The cultures were revived and maintained on Potato Dextrose Agar (PDA) medium at $25 \pm 2^\circ\text{C}$.

Plant material

Vigna radiata cultivars MH1142, IPM 205-7 (Virat) and *V. mungo* cultivars PU 31, KU 3 were collected from Krishi Vigyan Kendra, Banasthali Vidyapith, Rajasthan, India.

Inoculum (*Cercospora canescens*) preparation for pathogenicity test in *Vigna mungo*

A sample of 100 g of sorghum was boiled in a glass beaker until the grains softened without rupturing the seed coat. The excess liquid was subsequently filtered and the remaining grains were transferred to a 250 mL reagent bottle, sealed with non-absorbent cotton and autoclaved at 121°C and 15 psi for 15 min. Once cooled, the bottles were opened under a laminar airflow and inoculated by 10 mycelia discs (7 mm diameter) of 15 days old fungal culture (*C. canescens*). The inoculated bottles were then incubated at 25°C for 20 days. To prepare the inoculum (conidial suspension) 50–100 mL of sterile distilled water was added to the inoculated bottle and it was shaken to dislodge the conidia. The suspension was filtered through two-fold muslin cloth and the process repeated 2–3 times. The conidial concentration was adjusted to $1 \times 10^6 \text{ mL}^{-1}$ in sterile distilled water using a haemocytometer (12).

Inoculum (*Colletotrichum truncatum*) preparation for pathogenicity test in *Vigna radiata*

Fungal conidia were obtained from a 15-day-old fungal culture (*C. truncatum*) grown on potato dextrose agar by adding sterile distilled water and gently scraping the colony surface with an autoclaved spatula. The resulting suspension was filtered through a two-fold sterile muslin cloth to remove mycelial fragments. Conidia concentration was adjusted to $1 \times 10^6 \text{ mL}^{-1}$ in sterile distilled water using a haemocytometer (13).

Pot preparation

Plastic pots with a height 12 cm, top and bottom diameter 12 cm and 10 cm, respectively were used. The pots were washed thoroughly with tap water, sprayed with a 5 % formalin solution, thoroughly washed with sterilised distilled water and air-dried.

Soil sterilisation

The soil utilised in the experiment was sourced from Krishi Vigyan Kendra, Banasthali Vidyapith, Rajasthan, India. Initially, the soil was crushed into fine particles and sieved to ensure uniform particle size. Subsequently, it was autoclaved at 121°C and 15 psi for 15 min.

Seeds sterilisation

Mature seeds of *V. radiata* cultivars MH1142, IPM 205-7 (Virat) and *V. mungo* cultivars PU 31, KU 3, were taken separately in glass beakers and rinsed thoroughly under running tap water for 2–3 times to remove unwanted dust particles or impurities and then surface sterilised with 95 % ethanol for 10–20 sec and then with 2 % sodium hypochlorite solution for 1–2 min, followed by washing with sterile distilled water thrice. The seeds were then soaked separately for 24 hr in sterilised distilled water.

Pot experiment

Pathogenicity of the *Vigna* species against plant pathogens was conducted in plastic pots (Table 1) in triplicate. About 4–5 seeds of each cultivar of *Vigna* sp. was sown in separate pots at a depth of 1–2 cm in the soil and allowed to germinate at 25°C in a plant growth chamber under a 16/8-hr photoperiod. Germinated seedlings were

Table 1. Experimental design for pathogenicity test in cultivars of *Vigna* sp.

Experimental sets	<i>Vigna</i> sp.	Cultivars	Treatment	Pathogen
Set 1	<i>Vigna mungo</i>	KU 3	Control	-
Set 2			Infected	<i>Cercospora canescens</i>
Set 3		PU 31	Control	-
Set 4			Infected	<i>C. canescens</i>
Set 5	<i>V. radiata</i>	MH1142	Control	-
Set 6			Infected	<i>Colletotrichum truncatum</i>
Set 7		IPM 205-7	Control	-
Set 8			Infected	<i>C. truncatum</i>

grown at 30 ± 2 °C under a 16/8-hr photoperiod in the plant growth chamber. After 21 days of sowing, to enhance spore adherence and infection, the plants were lightly misted with sterile distilled water before inoculation. The prepared inoculum suspension was then sprayed uniformly onto the foliage until runoff and control plants were sprayed with sterile distilled water only. The plants were immediately covered with transparent polythene bags for 24 hr to promote maximum adherence under high humidity (14, 15). After the incubation period, the polythene bags were removed and plants were allowed to grow at 30 ± 2 °C with a 16/8-hr light-dark photoperiod. The disease symptoms were observed and confirmed microscopically before assessment of disease intensity.

Microscopic study of leaf showing disease symptoms

The infected leaf part was collected aseptically and was pre-dipped in water and excised into thin slices. A drop of mounting fluid (glycerol) was put on a glass slide and the leaf slices were put on the glycerol. The cover slip was put on it carefully with the help of a needle, followed by slight heating to avoid any air bubble and examined under a microscope (16).

Plant disease intensity assessment

The leaf area and percent leaf area affected was calculated for all leaves showing disease symptom in a plant.

$$\text{Leaf area} = l \times b \quad \text{Eq. (1)}$$

where l = length of leaf, b = breadth of length

$$\text{Percent leaf area affected} = \frac{\text{Infected leaf area}}{\text{Total area of leaf}} \times 100 \quad \text{Eq. (2)}$$

The disease symptom on foliage was monitored through scale given in Table 2 (17, 18).

The percent disease intensity (PDI) was calculated on 45 days after sowing (DAS) and 60 DAS, using the formula given below (19).

$$\text{Percent disease intensity (\%)} = \left(\frac{\text{Sum of total rating}}{\text{Total number of observations}} \times \text{highest grade in the scale} \right) \times 100 \quad \text{Eq. (3)}$$

Re-isolation and molecular characterisation fungal pathogen from diseased spots in leaves of *Vigna* sp.

Re-isolation of fungal pathogen

The fungal pathogens were re-isolated from each cultivar of *Vigna* sp. separately. For re-isolation, the symptomatic leaf parts were cut

into small slices, surface sterilised by dipping in 1 % sodium hypochlorite solution for 1–2 min, followed by washing with sterile distilled water. After that, 1–2 leaf slices were transferred aseptically on petriplates containing potato dextrose agar (PDA) and incubated at 25 °C and sub-cultured repeatedly to obtain a pure fungal isolate (16). After 15 days of incubation, colonies were compared for their diameters, overall colour, texture, form, margin, elevation, opacity, zonation, etc.

Molecular characterisation

The fungal genomic DNA was extracted from each of the re-isolated fungal pathogen using a previously reported protocol (20) with slight modifications. The fungal mycelia (about 100 mg) of 10-day old culture of *C. truncatum*, re-isolated from MH 1142 and IPM 205-7; and 15-day old culture of *C. canescens*, re-isolated from KU 3 and PU 31 were scraped from the surface of the colony separately. The mycelia were grinded with liquid nitrogen in a mortar pestle and 1.5 mL lysis buffer [100 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 10 mM ethylenediaminetetraacetic acid (EDTA), 1 M sodium chloride (NaCl) and 1 % sodium dodecyl sulfate (SDS) adjusted to pH 8] was added to it. The homogenate was transferred into 2 mL eppendorf tube and 5 μ L β -mercaptoethanol, 0.25 g polyvinylpyrrolidone and 50 μ L Proteinase K (10 mg/mL) added to it. The sample was incubated at 37 °C for 15 min and then at 65 °C for another 20 min. After incubation, tubes were centrifuged at 10000 g for 15 min and to the supernatant, 200 μ L of 3M guanidine hydrochloride was added. The content was vortexed 15–20 times and incubated at -20 °C for 10 min. The tubes were centrifuged at 10000 rpm for 10 min and to the supernatant, 100 μ L of 3 M sodium acetate was added, mixed well by inverting 15–20 times and incubated at -20 °C for 10 min. About 150 μ L of chilled isopropanol was added into tubes and left overnight for at 4 °C. The aliquotes were centrifuged at 10000 rpm for 10 min followed by washing of obtained pellet with 70 % ethanol by centrifugation for 5 min at 7500 rpm, air dried and dissolved in 50 μ L of TE buffer.

The extracted DNA was quantified using a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, USA). The quality of isolated DNA was analysed using 1 % agarose gel containing ethidium bromide (10 μ L/100 mL). A molecular weight marker of low range DNA ruler plus 3kb–100bp (GeNei 265277050A) was run as a

Table 2. Standard disease score scale for disease severity measurement

Scale/ Disease rating	Percent leaf area affected	Description	Resistance level
0	No visible symptoms	No infection	Highly resistant
1	< 1 % infection	Necrotic lesion or large soaked lesion	Resistant
3	1–10 % infection	acervuli present	Moderately resistant
5	11–25 % infection	Necrotic lesion, surface covered covered 25 % of leaf surface, acervuli may present	Susceptible
7	26–50 % infection	> 25 % of leaves showing necrosis	Moderately susceptible
9	> 50 % infection	leaves showing necrosis, acervuli present in large number	Highly susceptible

standard to determine the size of dsDNA. The DNA bands were visualised under the Gel Doc system (Gel Doc XR+, Bio-Rad, US)

Polymerase chain reaction (PCR) amplification of isolated DNA template was done using 18S rRNA gene primers (21, 22), namely, ITS 1F (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4R (5' TCCTCCGCTTATTGATATGC 3'). The reaction mixture was prepared in 25 μ L of volume containing 2.5 μ L of Taq buffer (1X), 1.25 μ L of dNTPs (2.5 mM), 0.5 μ L of ITS1F primer (pM/ μ L), 0.5 μ L of ITS4R primer (pM/ μ L), 0.5 μ L of Taq polymerase (0.5 U/ μ L), 5 μ L of DNA

for Biotechnology Information (NCBI) database using BLAST to determine sequence similarity and confirm the identity of the pathogen.

Statistical analysis

Data were analysed using repeated measures two-way analysis of variance (ANOVA) with cultivar and observation day as factors using IBM SPSS Statistics v 20.0. Differences were considered significant at $p \leq 0.05$.

Results and Discussion

Pathogenicity test in cultivars of *Vigna mungo* against *Cercospora canescens*

In the *V. mungo* cultivars PU 31, noticeable disease symptoms emerged four days after *C. canescens* was inoculated, as shown in Fig. 1. In contrast, the KU 3 cultivar exhibited comparable disease symptoms, but these appeared 7–8 days later following pathogen inoculation.

Initially, minute specks emerged on the leaves, which developed into irregular, reddish-brown spots after 6 to 7 days. Over a period of 12 to 16 days, these spots expanded into whitish areas with narrow reddish-brown margins. The symptoms initially appeared on mature leaves before progressing to younger ones. Similar symptoms and disease progression have been documented by other researchers in various *V. mungo* cultivars (23–26).

Microscopic study of symptomatic leaves of *Vigna mungo* infected with *Cercospora canescens*

Microscopic examination of symptomatic leaves from both cultivars revealed that the mycelia were pale yellow in colour, cylindrical and exhibited irregular branching. The microscopic images that were used to show the diseases symptoms belong to cultivar PU 31 (Fig. 2) because it exhibits the maximum disease symptoms. Therefore, based on microscopic characteristics, the pathogen from both cultivars appeared to be identical to *C. canescens*. The identification was grounded on significant features such as intercalary scars of conidia, which are prominent and hyaline. The conidia were straight to sub-straight or slightly curved, obclavate, cylindrical and borne singly. The conidia were observed to germinate through basal, apical and intercalary cells. The apex of the conidia was slightly pointed, while the basal end appeared truncate. The morphological

Table 3. Polymerase chain reaction (PCR) conditions for the thermocycler

Steps	Temperature	Time
Initial denaturation	94 °C	2 min
1. Denaturation	94 °C	30 sec
2. Annealing (for <i>C. canescens</i>)	55 °C	1 min
(for <i>C. truncatum</i>)	57 °C	
3. Extension	72 °C	1 min
Steps 1 to 3 were repeated for 35 cycles		
Final extension	72 °C	7 min

template, 12.75 μ L of nuclease free water. The PCR reaction was performed in a Thermocycler (Verity Thermal Cycler, Applied Biosystems) at PCR condition (Table 3).

The PCR products was run on a 1.5 % agarose gel and visualised under the Gel Doc system (Gel Doc XR+, Bio-Rad, US)

The amplified PCR products were purified using an ExoSAP-IT™ Express PCR Product Cleanup (Thermo Fisher Scientific, USA). The PCR amplicons were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) and the extension products were purified using the BigDye X Terminator Purification Kit (Thermo Fisher Scientific, USA) according to the manufacturer's protocol before capillary electrophoresis analysis. The purified PCR products were directly sequenced using Sanger sequencing (3500 Series Genetic Analyzers, Applied Biosystems) at the Department of Bioscience and Biotechnology, Banasthali Vidyapith.

The obtained nucleotide sequences were analysed and compared with reference sequences available in the National Center

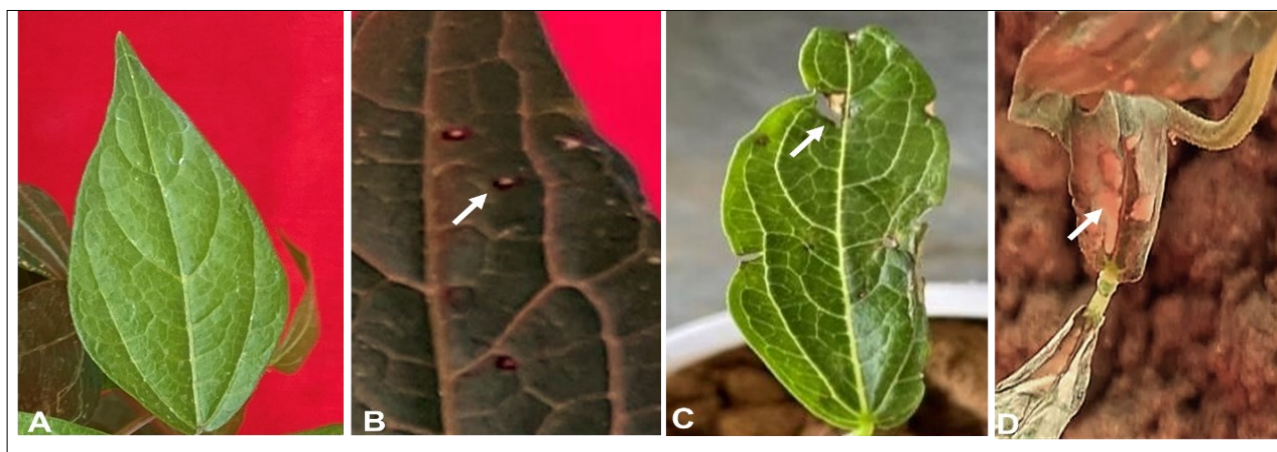


Fig. 1. Pathogenicity test on *Vigna mungo* (PU 31) showing disease symptoms at different time intervals after *Cercospora canescens* inoculation. (A) Control leaf; (B) appearance of silver spots surrounded by a reddish-brown margin 16 days after inoculation (arrow indicates silver spot); (C) crinkled leaf 30–31 days after inoculation (arrow indicates crinkled leaf); (D) necrotic leaf 36 days after inoculation (arrow indicates the dry leaf area associated with severe *C. canescens* infection).

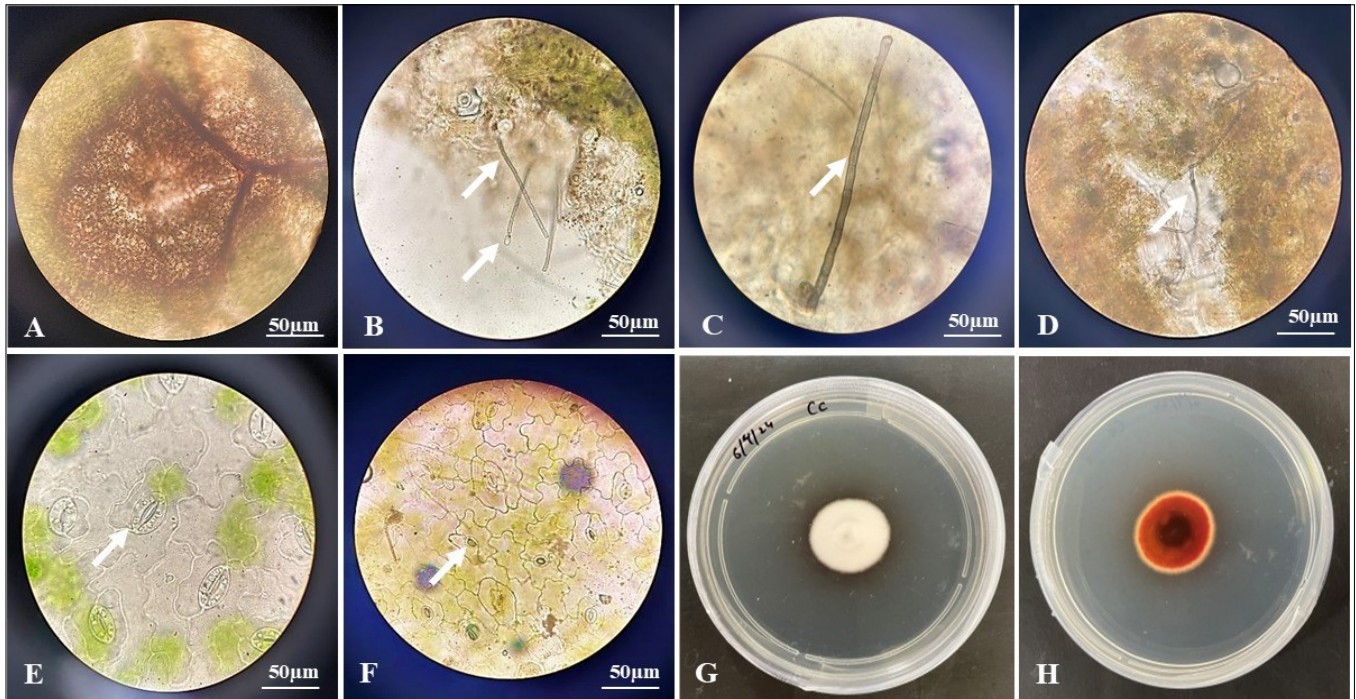


Fig. 2. Microscopic study and re-isolation of the fungal pathogen from leaves showing symptoms of *Cercospora* leaf spot disease in *Vigna mungo* cultivar PU 31. (A) Infected leaf tissue; (B) conidia and appressorium formation (arrow indicates appressorium); (C) conidia of *Cercospora canescens*; (D) infection through the germ tube; (E) stomata of the control plant; (F) stomata of the infected plant (arrow indicates damaged stomata); (G) colony morphology of the re-isolated fungal pathogen (front view of the culture plate); (H) colony morphology of the re-isolated fungal pathogen (reverse view of the culture plate).

characteristics observed in this study, including elongated multiseptate hyaline conidia and fasciculate conidiophores, align with the descriptions reported for *C. canescens* (25).

Disease assessment in cultivars of *Vigna mungo* infected with *Cercospora canescens*

The cultivar KU 3 shows low disease intensity as compared to the

Table 4. Plant disease intensity of *Vigna mungo* against *Cercospora canescens*

Experimental sets	Cultivars	Treatment	PDI	
			45 DAS	60 DAS
Set 1	KU 3	Control	-	-
Set 2		Infected	17.85 ± 1.78	24.50 ± 1.42
Set 3	PU 31	Control	-	-
Set 4		Infected	51.244 ± 7.19	64.10 ± 8.54

DAS: days after sowing; PDI: plant disease intensity.

cultivar PU 31 against *Cercospora* leaf spot disease, as shown in Table 4. It was found that the *V. mungo* KU 3 was a high-yielding and moderately resistant variety against *C. canescens* for growing in the Rajasthan region.

Repeated measures two-way ANOVA revealed a highly significant effect of cultivar on PDI, whereas the effects of observation day ($p = 0.154$) and the cultivar × day interaction ($p = 0.607$) were not significant. The results of the present study indicate variation in susceptibility among the tested cultivars of *V. mungo* against *C. canescens*.

Morphological characterisation of re-isolated *Cercospora canescens* from cultivars of *Vigna mungo*

The growth of the re-isolated pathogen from both the cultivars (KU 3 and PU 31) of *V. mungo* was slow growing on PDA plates, with the mycelial mat expanding in a circular to subcircular pattern (Fig. 2).

The colony exhibited a velvety texture, characterised by a densely intertwined thick mycelial mat. The mycelia displayed a uniform coloration, ranging from creamy-white to light pink, with a darker pink hue on the lower surface of the mycelial mat as illustrated in Fig. 2. G and H. This coloration is attributed to the release of cercosporin toxin (25).

The reisolated pathogen exhibited colony and mycelial characteristics consistent with those of the original strain and aligned with the morphological features of *C. canescens* as documented by various researchers (25, 27–29).

Molecular identification of fungal pathogen re-isolated from cultivars of *Vigna mungo* infected with *Cercospora canescens*

The genomic DNA of the re-isolated pathogen from both cultivars in pure culture exhibits an A_{260}/A_{280} ratio of 1.73 (PU-31) and 1.71 (KU-3), indicating DNA purity (30). Agarose gel electrophoresis demonstrated a distinct genomic DNA band (Supplementary Fig. 1A) and a single PCR amplicon of the expected size (Supplementary Fig. 1B), thereby confirming the successful extraction and amplification of DNA.

The PCR products were sequenced utilising the Sanger's sequencing technique. The resulting nucleotide sequence was analysed through BLAST and compared with sequences available in the NCBI database. The sequences were submitted to the NCBI repository as *C. canescens* BVMF31 (cv. PU 31) and BVMF03 (cv. KU 03), with the accession number PZ298338 and PZ343917 respectively.

The molecular findings corroborated the morphological identification of *C. canescens* and confirmed that the original isolate was successfully re-isolated from *V. mungo* cultivars, thereby satisfying the requirements of Koch's Postulates for pathogen confirmation.

The cultivar KU 3 exhibited comparatively lower disease intensity than PU 31, suggesting a higher level of resistance to

Cercospora leaf spot. The study result aligns with a previous study, which shows PU 31 was a moderately susceptible cultivar against *C. canescens* (31).

Such variability among cultivars has also been reported in earlier studies, where certain black gram genotypes showed reduced disease severity due to inherent genetic resistance (32, 33). The comparatively lower disease incidence in KU 3 may be associated with host defence responses that limit pathogen establishment and spread within leaf tissues. These responses may include structural barriers, production of antifungal compounds, or faster activation of plant defence mechanisms (34). Therefore, the performance of KU 3 in the present study suggests that it could be a suitable cultivar for cultivation in Rajasthan, India.

Pathogenicity test in cultivars of *Vigna radiata* against *Colletotrichum truncatum*

The characteristic disease symptoms appeared 9 days after inoculation with *C. truncatum* in *V. radiata* cultivar MH1142, as depicted in Fig. 3. While similar but delayed disease symptoms by 5 days were observed in *V. radiata* cultivar IPM 205-7. The initial symptom was characterised by the appearance of small, irregular water-soaked spots on the leaf, brown spots started appearing on the lower surface of the leaves midrib, which gradually turned into irregular, reddish brown spots after 11–13 days of inoculation and later spots increased in area into whitish spots with a black dot in it and reddish margin. The symptoms appeared mainly on mature leaves, rather than on young leaves. The observation of the pathogenicity test of the present study aligns with earlier reports of studies, where they reported pathogen as *C. lindemuthianum*, *C. truncatum* and *C. gloeosporioides*, respectively, on green gram plants (35–37).

Microscopic study of symptomatic leaves of *Vigna radiata* against *Colletotrichum truncatum*

Microscopic examination of symptomatic leaf from both cultivars revealed scattered, irregularly shaped, black colored acervuli (Fig. 4. A and B). The microscopic images that were used to show the diseases symptoms belong to cultivar MH1142 (Fig. 4) because it exhibits maximum diseases symptoms. Both the fungus produced characteristic asexual spores (conidia) that were hyaline, single-celled and crescent-shaped with rounded ends as shown in Fig. 4H (38). The conidiophores are hyaline, cylindrical with a truncate top,

pale brown near the base and branched. The setae were commonly septate, smooth and light brown to dark brown in colour and sometimes slightly inflated, with a roundish tip as shown in Fig. 4A–E. Based on morphological and microscopic characters of symptomatic leaves, the pathogen was reisolated and identified as *C. truncatum*. The morphological characteristics observed in this study, including acervuli and truncate top conidiophores, align with the descriptions reported for *C. truncatum* in previous studies (39).

Disease assessment in cultivars of *Vigna radiata* against *Colletotrichum truncatum*

The cultivar IPM 205-7 shows low disease severity as compared to cultivar MH-1142 against anthracnose caused by *C. truncatum*, as shown in Table 5. Repeated measures two-way ANOVA revealed a significant effect of cultivar ($p < 0.05$) and observation day ($p < 0.005$) on PDI, whereas the cultivar $\times$ day interaction was not significant

Table 5. Plant disease intensity of *Vigna radiata* against *Colletotrichum truncatum*

Experimental sets	Cultivars	Treatment	PDI	
			45 DAS	60 DAS
Set 5	IPM 205-7	Control	-	-
Set 6		Infected	23.15 $\pm$ 2	48.77 $\pm$ 1.23
Set 7	MH1142	Control	-	-
Set 8		Infected	52.22 $\pm$ 6	71.76 $\pm$ 6.02

DAS: days after sowing; PDI: plant disease intensity.

($p = 0.500$). The significant cultivar effect indicates that disease severity differed between the two cultivars, with IPM 205-7 exhibiting consistently lower PDI values than MH1142 across observation days. Based on the significantly lower PDI values, IPM 205-7 shows lower susceptibility as compared to MH1142 cultivar against *C. truncatum*.

Morphological characteristics of fungal pathogen reisolated from *Vigna radiata* against *Colletotrichum truncatum*

The growth of the re-isolated pathogen from both *V. radiata* cultivars (MH1142 and IPM 205-7) was slow growing on PDA plates. Both the fungus was identified based on colony characters. Both the re-isolated fungus showed circular to subcircular growth in morphology. The colony appears sticky, furry, with a thin mycelial mat, which is difficult to separate. The colonies growing were creamy - whitish in colour and blackish white on the reverse side of PDA, as

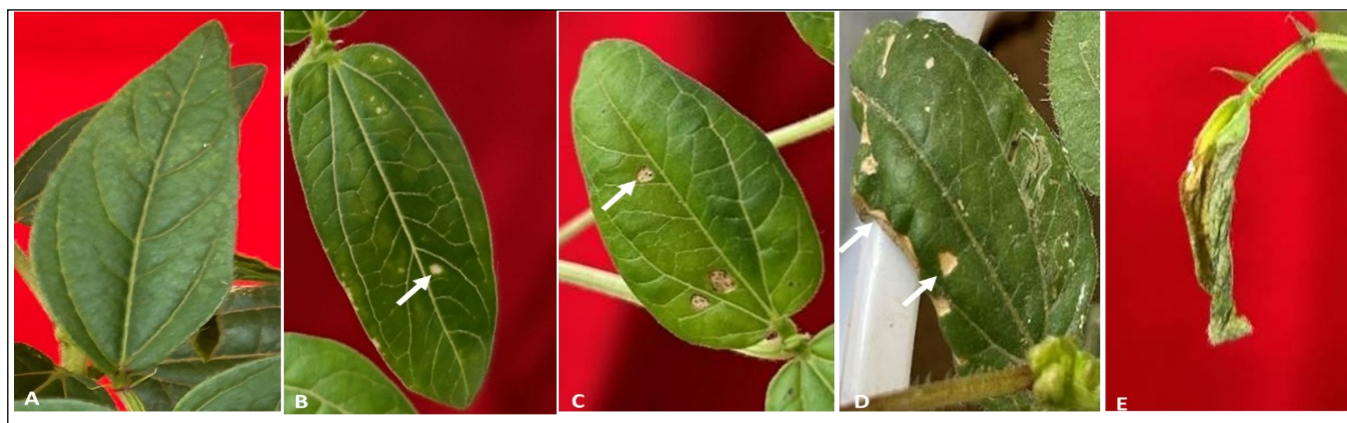


Fig.3. Pathogenicity test on *Vigna radiata* (MH1142); disease symptoms at different time interval after pathogen infestation. (A) control leaf; (B) appearance of water-soaked spots after 9 days of inoculation; (C) after 12 days of inoculation, whitish spots with a black dot in it and reddish margin; arrow indicating the whitish spot; (D) after 25 days of inoculation, dark enlarged spots and shothole due to disease infestation; arrow indicating large spots; (E) Severe symptoms of anthracnose disease.

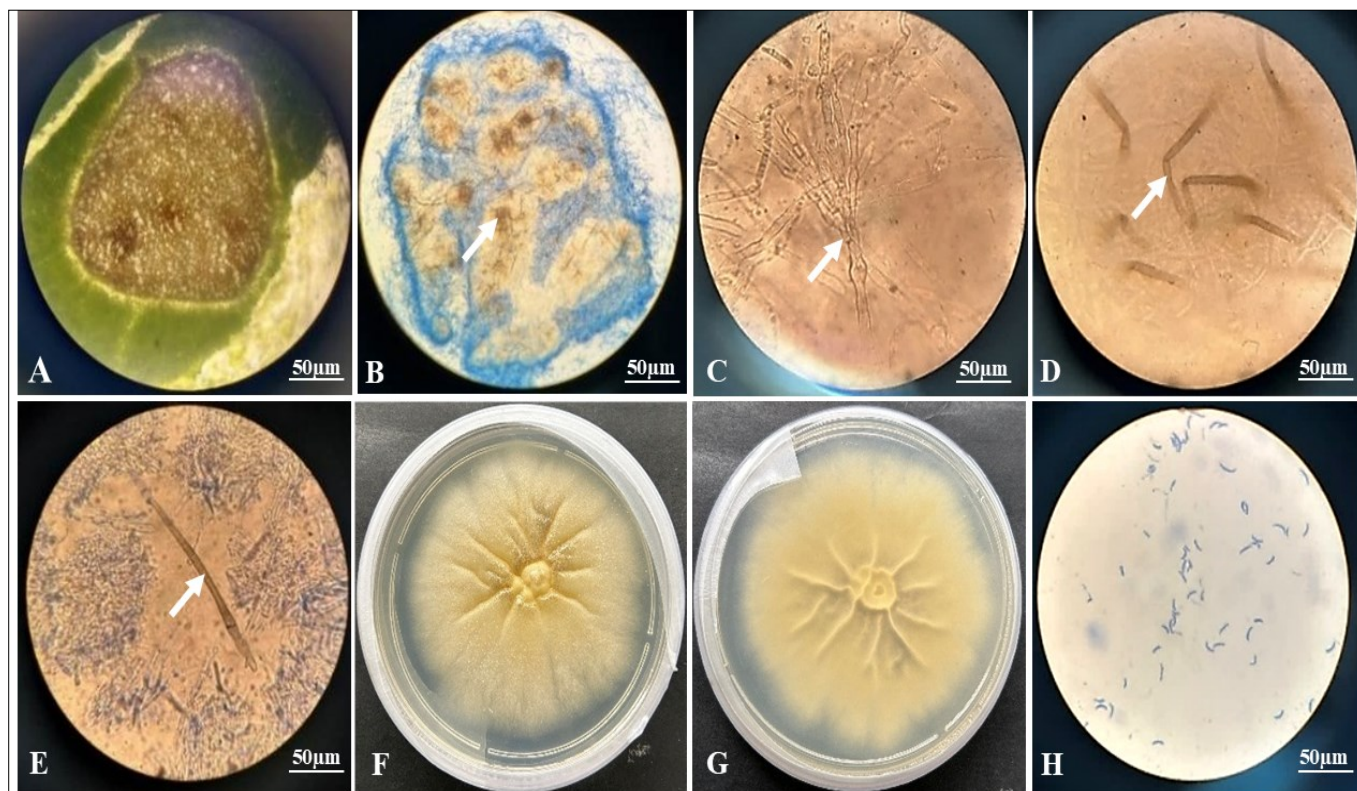


Fig. 4. Microscopic study and re-isolation of the fungal pathogen from leaves showing anthracnose symptoms in *Vigna radiata* cv. MH1142. (A) Microscopic view of the infected leaf tissue; (B) asci of *Colletotrichum truncatum* (arrow indicates a cluster of asci); (C) conidiophore of *C. truncatum*; (D–E) setae (arrow indicates black-coloured setae); (F) colony morphology of the re-isolated fungal pathogen (front view); (G) colony morphology of the re-isolated fungal pathogen (reverse view); (H) microscopic view showing crescent-shaped conidia of the re-isolated fungal pathogen.

shown in Fig. 4F–G. The pathogen was successfully reisolated from the infected leaves of both the cultivars and its identity was confirmed by comparing it with the original culture of *C. truncatum*.

Molecular identification of fungal pathogen reisolated from *Vigna radiata* against *Colletotrichum truncatum*

The reisolated genomic DNA of *C. truncatum* from both cultivars of *V. radiata* shows A_{260}/A_{280} ratio value of 1.83 (MH1142) and 1.90 (IPM-205-7) indicates purity of DNA (30). Agarose gel electrophoresis revealed a distinct genomic DNA band (Supplementary Fig. 2A) and a single PCR amplicon of the expected size, confirming successful DNA extraction and amplification (Supplementary Fig. 2B). The PCR products were sequenced using the Sanger's sequencing method. The obtained nucleotide sequence was analysed using BLAST and compared with sequences available in the NCBI database. The sequences were submitted to the NCBI library as *C. truncatum* BWR18 (cv. MH1142) and BWR19 (cv. IPM 205-7) with the accession no. PZ298345 and PZ343911, respectively.

The molecular characterisation supported the morphological identification of *C. truncatum* and verified that the same isolate was re-isolated from symptomatic leaf tissues of *V. radiata* cv. (MH1142) and *V. radiata* cv. (IPM 205-7), thus fulfilling the requirements of Koch's postulates for confirmation of pathogenicity.

The findings show significant variation in the response of *V. radiata* cultivars to anthracnose caused by *C. truncatum*. The cultivar IPM 205-7 exhibited lower disease severity compared to MH-1142, indicating a comparatively higher level of resistance to the pathogen. Differences in susceptibility among *V. radiata* cultivars have also been reported in earlier studies, where certain genotypes showed reduced disease incidence due to inherent genetic resistance and effective host defence responses (40). Previous

reports suggest that *Colletotrichum* isolates originating from different hosts can infect multiple bean species, although the level of pathogenicity may vary depending on the host genotype (41–43). In the present study, the higher PDI observed in cultivar MH-1142 indicates its greater susceptibility, whereas the comparatively lower disease intensity in IPM 205-7 may be associated with better structural or biochemical defence mechanisms that restrict pathogen development. Therefore, the comparatively resistant performance of IPM 205-7 suggests that it may be a suitable cultivar for *V. radiata* cultivation in Rajasthan and could also serve as a potential source of resistance in future breeding programs aimed at improving disease resistance in *V. radiata*.

Conclusion

The present study shows pathogenicity of *Colletotrichum truncatum* in *Vigna radiata* and *Cercospora canescens* in *Vigna mungo* and provides valuable insight into the variability of host responses among different *Vigna* cultivars under Rajasthan agro-climatic conditions. The comparatively better performance of KU-3 and IPM 205-7 suggests that these cultivars may be suitable for cultivation under the agro-climatic conditions of Rajasthan, where foliar fungal diseases often limit pulse productivity. These cultivars may serve as potential sources of resistance in future breeding programs aimed at developing improved pulse varieties with enhanced disease resistance.

Host-plant resistance is an eco-friendly and cost-effective management strategy for plant diseases. Further studies focusing on the physiological, biochemical and molecular mechanisms of resistance, along with multi-location field evaluations, will help to

confirm the stability of resistance and support the development of improved disease-resistant pulse cultivars suitable for Rajasthan. The results confirm that disease severity varies considerably with different cultivars, indicating differential levels of resistance and susceptibility within the tested cultivars of *Vigna* sp. of Rajasthan.

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Authors' contributions

AB and JS carried out the experiments, collected the data, prepared the manuscript with the collection of references and edited the manuscript and DC conceptualised the work and edited the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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