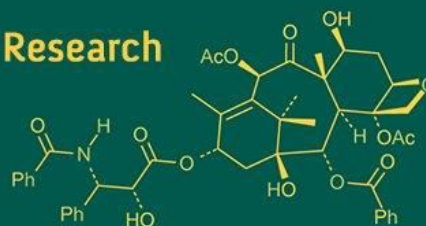


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(4): 01-10
www.biochemjournal.com
Received: 01-01-2026
Accepted: 04-02-2026

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Cultural, morphological and biochemical variability of *Sclerotium rolfii* isolates causing stem rot of cowpea in Kerala

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i4Sa.8045>

Abstract

Cowpea is an important legume crop cultivated widely in tropical and subtropical regions, and its productivity is significantly affected by stem rot caused by *Sclerotium rolfii*. The present study was undertaken to assess the cultural, morphological and biochemical variability among 19 isolates of the pathogen collected from three districts of Kerala, India. Considerable variability was observed among the isolates in colony characteristics, growth rate, sclerotial formation and pathogenicity. Pathogenicity tests on cowpea varieties Bhagyalakshmi and Anaswara revealed variation in lesion length ranging from 1.2 to 13.8 cm and days required for complete wilting from 5 to 13 days, with differences observed between the varieties. The growth rate of the isolates ranged from 20.98 to 32.13 mm per day. The isolates produced dark brown sclerotia with variation in size and in the time required for initiation and maturation. Enzyme assays indicated that all isolates produced cellulase and pectinase, with clear variation in activity among isolates and across days after inoculation. Cellulase activity ranged from 12.33 to 87.67 mm, while pectinase activity ranged from 12.33 to 88.33 mm. Correlation analysis revealed a positive relationship between enzyme activity and lesion development, with pectinase showing a stronger and significant association compared to cellulase. Molecular characterisation of representative isolates confirmed their identity as *Athelia rolfii*. The study highlights significant variability among *Sclerotium rolfii* isolates in Kerala and emphasises the role of extracellular enzymes, particularly pectinase, in pathogenicity. These findings are useful for understanding the pathogen variability and for developing effective management strategies for stem rot of cowpea.

Keywords: Cowpea, *Sclerotium rolfii*, variability, pathogenicity, cellulase, pectinase

1. Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important annual legume crop belonging to the family Fabaceae and widely cultivated in tropical and subtropical regions, with its origin in West Africa (Harlan, 1971) [17]. It is utilized in various forms such as dry seeds, green pods, fodder, green manure, and as a cover crop (Metwally *et al.*, 2021) [26]. Cowpea serves as a major source of dietary protein, vitamins, minerals, and micronutrients, particularly in developing countries, and is often referred to as “meat for the poor” (Baoua *et al.*, 2021; Foyer *et al.*, 2016; Phillips *et al.*, 2003) [8, 15, 35]. The crop is well adapted to drought and low-fertility soils and contributes to soil fertility through biological nitrogen fixation (Carsky *et al.*, 2001; Timko *et al.*, 2007) [10, 48]. Globally, cowpea is cultivated over about 15.78 million hectares, with a production of 10.10 million metric tonnes (FAO, 2025) [13]. India is a major producer and a secondary centre of genetic diversity, where cowpea is grown for grain, vegetable, and fodder purposes (Jeswani and Baldev, 1997; Pant *et al.*, 1982) [21, 32]. In Kerala, cowpea is cultivated as a pure crop in rice fallows, as a floor crop in coconut gardens and as an intercrop in tapioca (KAU, 2024) [24].

Despite its importance, cowpea production is significantly constrained by various biotic stresses, among which basal stem rot or Southern blight caused by *Sclerotium rolfii* Sacc. is one of the most destructive diseases (Adegbite and Amusa, 2008; Tanimu *et al.*, 2018) [2, 46]. The disease, favoured by warm and humid climatic conditions, occurs throughout the crop growth period and is manifested as yellowing, wilting, stem and collar rot, and eventual plant death (Vasanthrai, 2013; Vijayakumar and Appusami, 2025) [51, 53].

Yield losses up to 53.4 per cent have been reported, with significant impacts on plant vigour and productivity (Fery and Dukes, 2002) [14]. Under controlled conditions, Vijayakumar and Appusami (2025) [53] reported 80 per cent disease incidence and yield losses ranging from 12 to 34 per cent. In India, the disease is a major constraint to cowpea production, particularly in Kerala, where its significance has increased in recent years (Sajeena *et al.*, 2014; Sunil and Thara, 2022) [40, 45].

S. rolfii is a soil-borne fungus with a wide host range and global distribution. Its ability to survive in soil through sclerotia and its high virulence contribute to its persistence and frequent occurrence in diverse agro-ecological conditions. (Aycock, 1966; Punja, 1988) [6, 36]. The pathogen exhibits considerable variability among isolates in cultural, morphological, pathogenic and biochemical traits, which is closely associated with differences in virulence. Extracellular enzymes such as cellulase and pectinase facilitate the degradation of host cell walls and contribute to the pathogenicity of the pathogen (Awasthi and Dasgupta, 2011; Taubenhaus, 1919) [5, 47]. Variations in the production of pathogenicity factors such as oxalic acid, cellulase and polygalacturonase, influence disease severity (Vaniya *et al.*, 2022; Yu *et al.*, 2023) [50, 55]. Such variability enhances its adaptability and survival under diverse ecological conditions.

Owing to its high variability, wide host range and facultative parasitic nature, understanding the variability of *S. rolfii* is crucial for developing effective and sustainable disease management strategies. The present study was undertaken to isolate and characterise the pathogen and to evaluate the variability among its isolates based on cultural, morphological and biochemical traits.

2. Materials and Methods

The present study on “Cultural, morphological and biochemical variability of *Sclerotium rolfii* isolates causing stem rot of cowpea in Kerala” was conducted during 2024-2025 at the Department of Plant Pathology, College of Agriculture, Vellanikkara, Thrissur.

2.1 Collection of samples and isolation of pathogen

Purposive sampling was carried out at six locations across Ernakulam (Agro-ecological unit 9: South Central Laterites) and Thrissur and Palakkad districts (Agro-ecological unit 10: North Central Laterites) of Kerala (KAU, 2024) [24]. The survey was conducted from October to January, during the northeast monsoon followed by the *Rabi* season. Cowpea plants showing symptoms of stem rot and collar rot were collected. The disease incidence of stem rot at the survey locations was calculated according to the formula given by Wheeler (1969) [54].

$$\text{Percent disease incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Number of plants observed}} \times 100$$

The infected cowpea samples collected from different locations were washed under tap water and cut into small bits containing both diseased and healthy tissues. The bits were surface sterilized with 0.1 per cent mercuric chloride for 30-60 s, rinsed thrice with sterile distilled water, blotted dry, and transferred onto potato dextrose agar (PDA) medium. The plates were incubated at 26±2 °C and observed for fungal growth. The pathogen isolates were purified by the hyphal tip method, and maintained with periodic subculturing.

2.2 Pathogenicity test

Pathogenicity of different isolates was assessed by soil inoculation on two cowpea varieties, Bhagyalakshmi (bush type) and Anaswara (semi-trailing type), following Pande *et al.* (1994) [30] with slight modifications. Seeds were sown in pots containing sterilised soil:sand (3:1) mixture, and 10-day-old seedlings were used for inoculation. The pathogen was mass multiplied on sorghum grain medium (SGM) and applied at 5 g kg⁻¹ of potting mixture around the collar region of seedlings, while uninoculated plants served as control. The pots were maintained under greenhouse conditions and observed for symptom development. The pathogen was reisolated from infected tissues to confirm Koch's postulates.

2.3 Cultural characterisation of the isolates

The isolates were subcultured on PDA by placing a 5 mm mycelial disc at the centre of Petri plates for cultural characterisation. Observations on colony colour, mycelial texture, growth pattern, reverse colony colour, growth rate (mm day⁻¹), and sclerotial production, including days to initiation and maturation, were recorded. Growth rate was calculated based on three replications per isolate. Sclerotial characteristics such as colour, shape and diameter were recorded under a stereomicroscope (Zeiss SteREO Discovery.V8) coupled with ZEN 3.0 microscopy software.

2.4 Morphological characterisation of the isolates

Morphological characterisation of the isolates was carried out using the slide culture technique (Riddell, 1950) [39]. A small agar block (1 cm²) of water agar was placed on a sterile slide, inoculated on all sides with the fungus, covered with a coverslip, and incubated at 26±2 °C for 3-5 days. The slides were stained with lactophenol cotton blue (LPCB) for microscopic observation. Morphological characters were examined under a compound light microscope (Leica DM750) coupled with Leica Application Suite (LAS) EZ imaging software. Observations on hyphal colour, branching pattern, septation, presence of clamp connections and hyphal width were recorded. The isolates were identified based on their cultural and morphological characteristics.

2.5 Qualitative assessment of cellulase and pectinase production

Cellulase and pectinase activities of the isolates were assessed on carboxy methyl cellulose (CMC) agar and pectin agar medium (pH 5.0), respectively following Gawade *et al.* (2017) [16] with modifications. Plates were inoculated with a 5 mm mycelial disc from a seven-day-old culture and incubated at 26±2 °C, with uninoculated plates as control. After incubation, plates were flooded with Gram's iodine for 3-5 min (Kasana *et al.*, 2008) [23], and the diameter of the clear zone (mm) was recorded. Observations were taken at 1, 2 and 3 days after inoculation, with three replications per isolate.

2.6 Molecular identification

Based on variability in cultural, morphological and biochemical characteristics, two representative isolates were selected for molecular characterisation. The pure cultures of these isolates were sent to C Six Labs Pvt. Ltd., Palakkad for fungal DNA isolation and sequencing of the internal transcribed spacer (ITS) region using universal primers ITS-1F (TCCGTAGGTGAACCTGCGG) and ITS-4R

(TCCTCCGCTTATTGATATGC). The obtained sequences were analysed using the BLASTn program of the National Center for Biotechnology Information (NCBI) to determine the sequence homology.

2.7 Statistical analysis

Experimental data on growth rate of pathogen isolates and cellulase and pectinase activity at 3 DAI were subjected to analysis of variance (ANOVA) under a completely randomized design (CRD) using the RAISINS package (Hisham, 2025) [18]. The data were transformed as required, and treatment means were compared using Duncan's Multiple Range Test (DMRT) at the five per cent level of significance ($p = 0.05$). The data were tested for normality and homogeneity of variance prior to analysis (Statscalculators, 2025) [42]. Correlation analysis was performed to assess the relationship between the pathogenicity parameter (mean lesion length of two varieties) and enzyme activity (cellulase and pectinase zonation at 3 days after inoculation) using Pearson's correlation coefficient.

3. Results and Discussion

The present study was carried out on cowpea stem rot caused by *Sclerotium rolfii* to assess the cultural, morphological and biochemical variability among the isolates of the pathogen. The study included isolation of the

pathogen, pathogenicity test, cultural and morphological characterisation, assessment of cellulase and pectinase activity, and molecular identification. The results of the experiments are presented hereunder.

3.1 Collection of samples and isolation of pathogen

Stem rot samples were collected from six locations representing different cowpea varieties, and per cent disease incidence was calculated (Table 1). All growth stages of the crop were susceptible to the disease, which was characterized by the presence of water-soaked lesions, rotting of the stem and collar region, yellowing of leaves and wilting of plants. These symptoms were in accordance with those described by Agrios (2006) [3]. However, foliar blight symptoms were not observed in the present study, as reported earlier by Ramiah *et al.* (1976) [38].

The PDI was 10 per cent in October, and varied from 5 to 13.33 per cent in December and 3.14 to 11.33 per cent in January. Overall, it ranged from 3.14 to 13.33 per cent during the survey period, which was lower than the 20 per cent incidence reported in Coimbatore, Tamil Nadu (Ramiah *et al.*, 1976) [38], and 15-30 per cent reported in Karamana, Kerala (Sajeena *et al.*, 2021) [41], but comparable with an incidence of 10 per cent in Hunan, China (Huang *et al.*, 2024) [19]. A total of 19 isolates were obtained from the collected samples, purified on PDA by the single hyphal tip method, and used for further studies (Table 1).

Table 1: Survey locations, disease incidence and isolates of *Sclerotium rolfii* from cowpea

Sl. No.	District	Location coordinates	Date of survey	Cowpea variety/accession code	Per cent disease incidence (%)	Isolates
1.	Thrissur	10.5467020°N/ 76.2792289°E	06.12.2024	VU13	10.00	VCS1 VCS2
				VU17	13.33	VCS3 VCS4
				VU25	6.67	VCS5 VCS6
		10.5468965°N/ 76.2694539°E	09.12.2024	Lola	5.00	VCS7 VCS8
		10.5523268°N/ 76.2821675°E	04.01.2025	PGCP6	3.14	VCS9 VCS10 VCS11
2.	Palakkad	10.812202°N/ 76.192836°E	23.01.2025	PGCP63	7.67	PCS1
		10.811453°N/ 76.192422°E	23.01.2025	PGCP63	11.33	PCS2 PCS3 PCS4 PCS5
3.	Ernakulam	10.138588°N/76.420685°E	21.10.2025	Lalita	10.00	KCS1 KCS2 KCS3

3.2 Pathogenicity test

All the pathogen isolates produced stem rot symptoms in cowpea varieties, Bhagyalakshmi and Anaswara. The symptoms included lesions on the stem and collar region, yellowing and drying of leaves, lodging and wilting of plants. The isolates showed variation in lesion length and days taken for complete wilting of plants. Variation was observed among the isolates as well as between the two varieties (Table 2, Plate 1). Plants maintained as control did not show any stem rot symptoms. The lesion length ranged from 7.8 to 12.7 cm in Bhagyalakshmi and 1.2 to 13.8 cm in Anaswara, whereas complete wilting occurred within 5 to 9 days in Bhagyalakshmi and 5 to 13 days in Anaswara. Overall, Anaswara appeared more susceptible than Bhagyalakshmi, as indicated by greater lesion length and

wider variation in wilting period among the isolates.

Soil inoculation using fungus-colonised sorghum grain medium was followed to prove pathogenicity and found effective in inducing stem rot symptoms, with infection at the collar region in all plants, in accordance with Pande *et al.* (1994). Previous works have also reported the effectiveness of other media such as sand-corn meal (Mishra and Bais, 1987) [27], millet seeds (Adandonon *et al.*, 2004) [1] and paddy seeds (Sunil, 2020) [44] for artificial inoculation. Variability in pathogenicity among *S. rolfii* isolates has been reported in several studies, with isolates differing in disease severity and host response across crops such as chickpea and sweet potato (Azhar *et al.*, 2009; Paul *et al.*, 2017) [7, 33].

Table 2: Symptom development by *S. rolfsii* isolates in two cowpea varieties

Sl. No.	Isolate	Bhagyalakshmi*		Anaswara*	
		No. of days taken for complete wilting	Length of lesion on stem and collar region (cm)	No. of days taken for complete wilting	Length of lesion on stem and collar region (cm)
1.	VCS1	8	8.8	6	9.0
2.	VCS2	7	10.9	7	11.5
3.	VCS3	6	9.5	6	9.7
4.	VCS4	8	9.8	8	10.0
5.	VCS5	9	9.6	8	9.8
6.	VCS6	7	9.9	8	10.0
7.	VCS7	9	12.7	6	13.8
8.	VCS8	8	10.7	7	11.4
9.	VCS9	5	10.3	7	10.8
10.	VCS10	8	9.1	6	9.2
11.	VCS11	9	9.4	6	10.2
12.	PCS1	9	8.3	8	8.5
13.	PCS2	7	8.8	7	9.0
14.	PCS3	8	9.2	8	9.6
15.	PCS4	9	11.0	6	11.9
16.	PCS5	8	7.8	13	1.2
17.	KCS1	6	10.4	7	11.0
18.	KCS2	7	11.6	6	12.5
19.	KCS3	6	11.2	5	12.0

*-Mean of two replications

3.3 Cultural characterisation

The pure cultures of all isolates on PDA were white, with either smooth or rough, and fluffy or compact mycelium showing radial growth. The colour of the reverse side of the plates was pale cream to pale yellow. The isolates showed variation in mean growth rate, ranging from 20.98 to 32.13 mm day⁻¹. The isolate PCS1 exhibited a significantly higher growth rate, whereas PCS2 exhibited a significantly lower growth rate. The sclerotia produced by the isolates were dark brown, rugose, and sub-spherical to irregular in shape, and were distributed both at the centre and periphery of the plates, with exudate formation. However, the isolate KCS3 did not produce sclerotia under culture conditions, but dense, white mycelial aggregates with exudate droplets were observed. The days taken for the formation of sclerotial initials and mature sclerotia also varied, ranging from 8 to 33 days, and 11 to 36 days, respectively. The mean diameter

of sclerotia ranged from 0.88 to 1.71 mm (Table 3, Plate 1).

In the present study, variability in cultural characteristics among *S. rolfsii* isolates was observed. Similar variability has been widely reported, with differences observed in growth rate, sclerotial production, size and colour across isolates. Such variation has been attributed to differences in host source and geographical origin, with isolates showing distinct growth patterns and sclerotial traits (Akram *et al.*, 2008; Boopathi, 2023; Sulladmath *et al.*, 1977) [4, 9, 43]. The observed variability may also be influenced by environmental factors such as growth medium and temperature, as earlier studies have shown that PDA supports maximum growth and sclerotial production, and that optimum growth occurs within specific temperature and pH range (Kushwaha *et al.*, 2019; Sajeena *et al.*, 2014) [25, 40].

Table 3: Cultural characteristics of *S. rolfsii* isolates

Sl. No.	Isolate	Growth rate* (mm day ⁻¹)	Sclerotial initiation (DAI)	Sclerotial maturation (DAI)	Diameter of sclerotia** (mm)
1.	VCS1	28.44±0.62 ^{cd}	24	28	1.34
2.	VCS2	27.61±0.61 ^{def}	14	16	1.58
3.	VCS3	28.72±0.61 ^c	33	36	1.26
4.	VCS4	27.11±0.61 ^{efg}	14	17	1.58
5.	VCS5	25.78±0.61 ^h	25	28	1.41
6.	VCS6	23.56±0.62 ⁱ	12	16	1.32
7.	VCS7	28.41±0.70 ^{cd}	13	15	1.69
8.	VCS8	26.96±0.56 ^{fg}	12	15	1.36
9.	VCS9	26.22±0.61 ^{gh}	8	11	1.71
10.	VCS10	28.06±0.62 ^{cde}	11	14	1.56
11.	VCS11	26.22±0.61 ^{gh}	14	17	1.39
12.	PCS1	32.13±0.56 ^a	9	12	0.88
13.	PCS2	20.98±0.49 ^j	13	16	1.41
14.	PCS3	25.22±0.61 ^h	15	19	1.12
15.	PCS4	28.13±0.56 ^{cde}	10	13	1.68
16.	PCS5	28.13±0.56 ^{cde}	19	22	1.17
17.	KCS1	31.67±0.50 ^{ab}	10	13	1.14
18.	KCS2	30.67±0.50 ^b	11	14	1.22
19.	KCS3	31.50±0.50 ^{ab}	-	-	-
SE(m)		0.34			
SE(d)		0.48			
CV(%)		2.11			

DAI-Days after inoculation, *-Mean of three replications. Values represent mean of three replications±SD; Means followed by the same letter are not significantly different at the five per cent level according to DMRT, **-Mean of 10 observations

3.4 Morphological characterisation

The hyphae of all the isolates were hyaline and septate with branching at acute angles. The cells of secondary hyphae and their subsequent branches were shorter than those of the primary hyphae. Clamp connections were observed at the septa of the primary hyphae, and none of the isolates produced spores under the slide culture technique at room temperature. The average hyphal width among the isolates varied, ranging from 3.29 to 5.63 μm (Table 4, Plate 2).

Morphological characteristics of *S. rolfsii* isolates in the present study are comparable with earlier reports. The isolates exhibited hyaline, septate hyphae with distinct clamp connections, which are typical features of the pathogen (Kamalakannan *et al.*, 2007) [22]. Variation in hyphal width was also observed among the isolates, which is in agreement with previous studies that reported hyphal

diameter ranging from 3.0 to 6.25 μm (Nandi *et al.*, 2017) [29] and 1.5 to 3.5 μm (Paul *et al.*, 2021) [34].

In the present study, sporulation of *Sclerotium rolfsii* was not observed on PDA. This is in agreement with earlier reports indicating that the sexual stage of the pathogen is rarely produced under normal culture conditions and requires specific media for induction. Previous studies have reported sporulation on specialised media such as onion extract-based medium and *Cyperus rotundus* rhizome meal agar, where basidia and basidiospores were formed (Pandey *et al.*, 2005; Venkatakrishnaiya, 1946) [31, 52].

The cultural and morphological characters of the isolates were compared with the CMI Descriptions of Pathogenic Fungi and Bacteria No. 410 (Mordue, 1974) [28], which were comparable with the isolates obtained in the present study. Based on this description, all the 19 isolates associated with cowpea stem rot were identified as *Sclerotium rolfsii*.

Table 4: Hyphal width of *S. rolfsii* isolates

Sl. No.	Isolate	Hyphal width* (μm)
1.	VCS1	3.29
2.	VCS2	4.10
3.	VCS3	3.36
4.	VCS4	3.88
5.	VCS5	4.94
6.	VCS6	3.73
7.	VCS7	3.79
8.	VCS8	3.74
9.	VCS9	5.06
10.	VCS10	5.63
11.	VCS11	4.33
12.	PCS1	3.97
13.	PCS2	3.81
14.	PCS3	4.58
15.	PCS4	3.45
16.	PCS5	4.99
17.	KCS1	4.08
18.	KCS2	3.84
19.	KCS3	4.42

*-Mean of ten observations

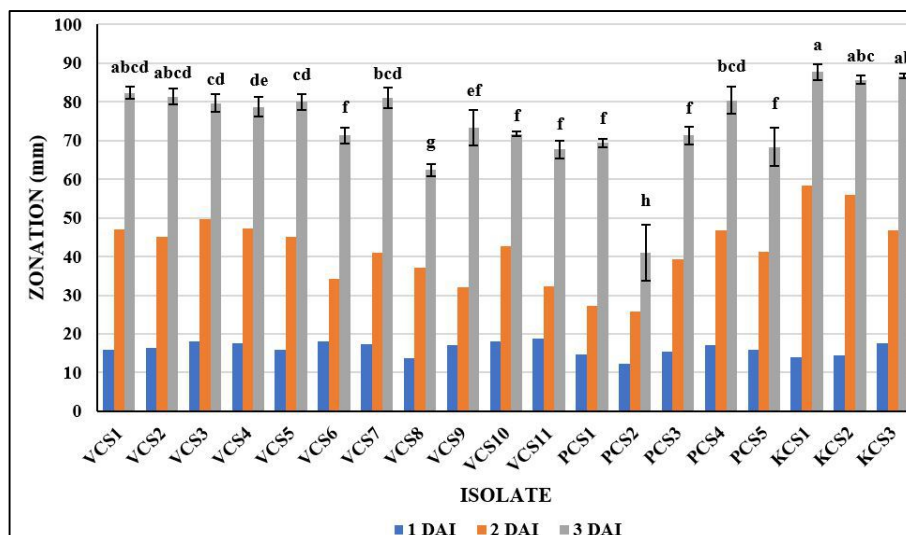
3.5 Qualitative assessment of cellulase and pectinase production

Development of golden yellow clear hydrolytic zones around the fungal colonies on CMC agar and pectin agar plates indicated cellulase and pectinase activity of the isolates. The control plates did not develop clear zones and remained purplish black. However, variation in zonation among the isolates and across different days after inoculation was observed (Plate 2).

The cellulase activity of the isolates, measured as the diameter of hydrolytic zones, ranged from 12.33 to 18.67 mm at 1 DAI, 25.67 to 58.33 mm at 2 DAI, and 41.00 to 87.67 mm at 3 DAI. At 3 DAI, the isolate KCS1 (87.67 mm) exhibited significantly higher cellulase activity, whereas the lowest activity was observed in PCS2 (41.00 mm) (Fig. 1). Similarly, pectinase activity of the isolates ranged from 12.33 to 18.67 mm at 1 DAI, 23.67 to 53.33 mm at 2 DAI, and 41.00 to 88.33 mm at 3 DAI. At 3 DAI, the isolate

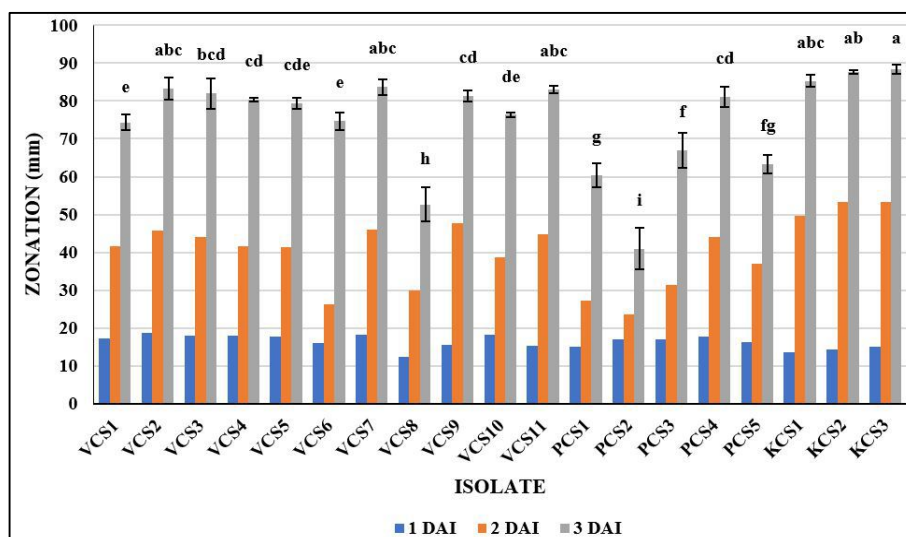
KCS3 (88.33 mm) exhibited significantly higher pectinase activity, whereas PCS2 (41.00 mm) showed the least activity (Fig. 2).

In the present study, enzyme activity was successfully detected using Gram's iodine, whereas earlier studies have reported the use of CTAB solution for visualising clear zones of cellulase and pectinase activity (Gawade *et al.*, 2017) [16], indicating that different staining methods can effectively reveal enzyme production. Production of cellulase and pectinase by *S. rolfsii* isolates showed considerable variation among isolates. Similar variability has been reported earlier, with differences in enzyme production among isolates and across stages of infection (Awasthi and Dasgupta, 2011; Husain, 1958) [5, 20]. These enzymes play a crucial role in host cell wall degradation, and higher enzyme activity has been associated with increased virulence of the pathogen (Yu *et al.*, 2023) [55].



Values represent mean zonation (mm) of three replications. Errors bars indicate standard deviation. Bars sharing the same letter are not significantly different at $p < 0.05$ according to DMRT.

Fig 1: Variability in cellulase activity of *S. rolfsii* isolates at different days after inoculation



Values represent mean zonation (mm) of three replications. Errors bars indicate standard deviation. Bars sharing the same letter are not significantly different at $p < 0.05$ according to DMRT.

Fig 2: Variability in pectinase activity of *S. rolfsii* isolates at different days after inoculation

Correlation analysis revealed a positive relationship between extracellular enzyme activity and lesion length (Table 5). Pectinase activity showed a significant positive correlation with lesion length ($r = 0.49$, $p < 0.05$), indicating its role in disease development. In contrast, cellulase activity exhibited

a moderate positive correlation ($r = 0.42$) and showed marginal significance at the 10 per cent level ($p < 0.10$). These results suggest that pectinase plays a more prominent role in pathogenicity, while cellulase may contribute to virulence to a lesser extent (Fig. 3).

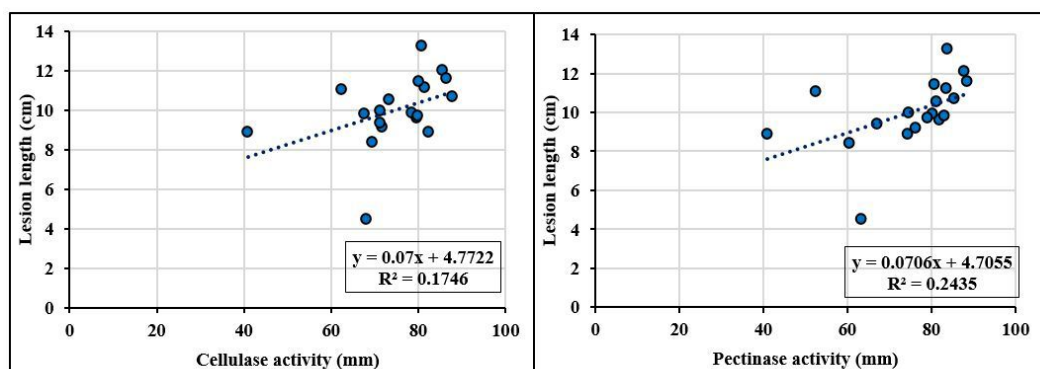


Fig 3: Relationship between lesion length and extracellular enzyme activity (cellulase and pectinase) in *S. rolfsii* isolates

Table 5: Correlation between extracellular enzyme activity and lesion length caused by *S. rolfsii* isolates from cowpea

Pathogenicity parameter	Cellulase activity (mm)	Pectinase activity (mm)
Length of lesion (cm)	0.42*	0.49**
p value	0.075	0.032

Values represent Pearson’s correlation coefficient (r); * significant at $p<0.10$; ** significant at $p<0.05$

An earlier study by Punja *et al.* (1985) [37] in *S. rolfsii* reported a strong positive association of endo-polygalacturonase activity with virulence and poor correlation between cellulase activity and virulence, indicating its secondary role in pathogenesis. Similar observations have been reported in *Rhizoctonia bataticola* infecting soybean by Gawade *et al.* (2017) [16], where cellulase and pectinase production showed a positive association with pathogen virulence, with pectinase exhibiting a comparatively stronger relationship. These findings are in accordance with the present study, where pectinase exhibited a stronger relationship with lesion development compared to cellulase, highlighting the primary role of pectinolytic enzymes in disease progression.

3.6 Molecular identification

The isolates KCS2 and KCS3 were selected based on variability in cultural, morphological and biochemical characteristics for molecular identification. The ITS region sequence of isolates KCS2 and KCS3 yielded amplicon sizes of 615 bp and 584 bp, respectively. Sequence analysis using the NCBI BLASTn database revealed their identity as *Athelia rolfsii*, with 99.51 per cent similarity and 100 per cent query coverage (Accession No. OQ134754.1) for KCS2, and 99.82 per cent similarity with 96 per cent query coverage (Accession No. KC867344.1) for KCS3. The sequences were deposited in GenBank under the accession numbers PZ127017 (KCS2) and PZ148206 (KCS3). *Athelia rolfsii* (Curzi) Tu and Kimbrough is the perfect stage of the anamorph *Sclerotium rolfsii* Sacc. (Tu and Kimbrough, 1978) [49]. The molecular identification of *S. rolfsii* isolates in the present study using ITS1/ITS4 primers was in agreement with earlier reports. Previous studies have successfully confirmed the identity of *S. rolfsii* (syn. *A. rolfsii*) through amplification of the ITS region, producing amplicons of approximately 650-700 bp with high sequence similarity to reference sequences in GenBank (Cer and Morca, 2020; Durgaprasad *et al.*, 2008; Huang *et al.*, 2024) [11, 12, 19].

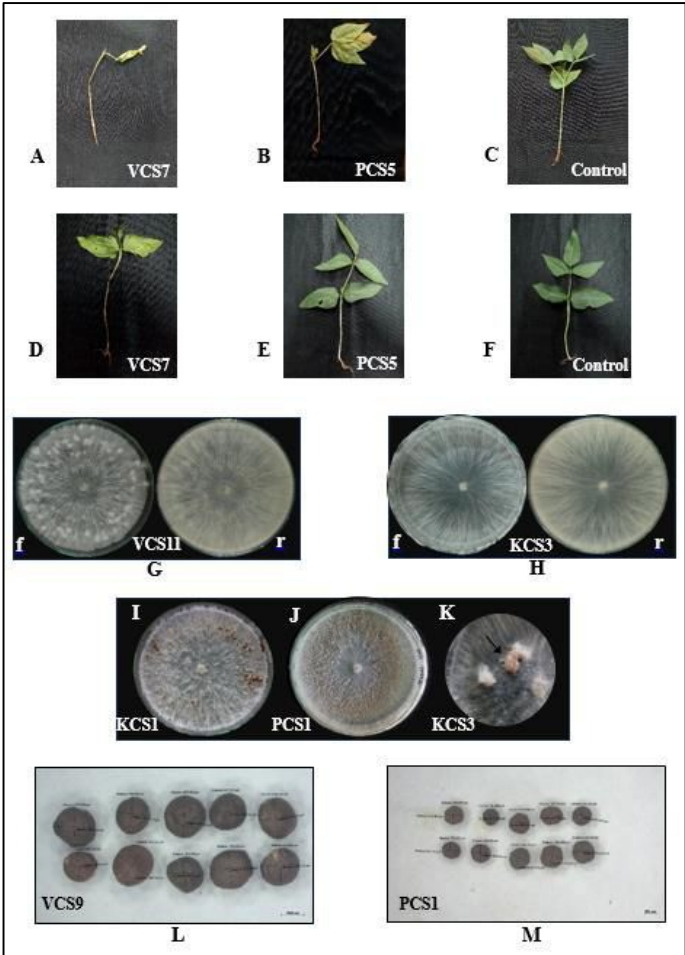
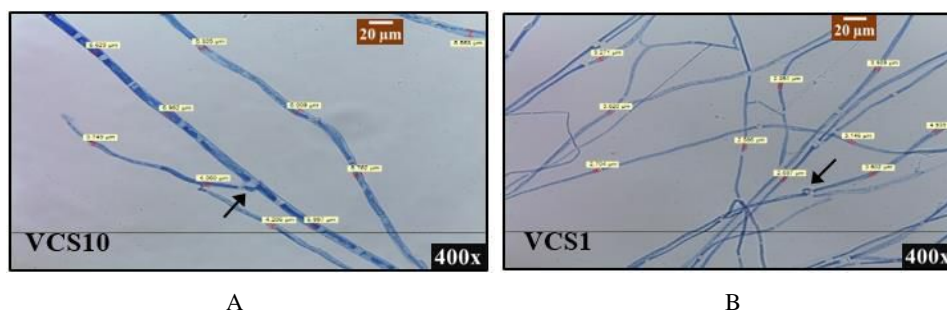


Plate 1: Pathogenicity and cultural characteristics of *Sclerotium rolfsii* isolates
f-Front view; r-Reverse view

A-C: Artificially inoculated cowpea plants (Bhagalakshmi); D-F: Artificially inoculated cowpea plants (Anaswara); G: White, smooth, fluffy mycelium; H: White, rough, compact mycelium on PDA; I-J: Formation of sclerotia on PDA; K: Dense white aggregates of mycelium with exudates; L-M: Sclerotial morphology



Isolate	1 DAI	2 DAI	3 DAI	Control
KCS1				
PCS2				

Cellulase activity of *S. rolfii* isolates on CMC agar

Isolate	1 DAI	2 DAI	3 DAI	Control
KCS3				
PCS2				

Pectinase activity of *S. rolfii* isolates on pectin agar

Plate 2: Hyphal morphology and extracellular enzyme activity *Sclerotium rolfii* isolates
A-B: Hyphal morphology (arrows indicate clamp connections)

4. Conclusion

The study revealed significant variability among *Sclerotium rolfii* isolates infecting cowpea with respect to cultural, morphological, biochemical and pathogenic traits. Enzyme assays confirmed cellulase and pectinase production, with pectinase showing a stronger association with lesion development than cellulase, indicating its major role in pathogenicity. Molecular characterisation confirmed the identity of the pathogen as *Athelia rolfii*. The variability observed among isolates emphasises the importance of developing effective and region-specific disease management strategies.

5. Acknowledgement

The authors thank Kerala Agricultural University for providing facilities and support for the research work.

6. References

- Adandonon A. Damping-off and stem rot of cowpea in Benin caused by *Sclerotium rolfii*. PhD thesis. Pretoria: University of Pretoria; 2004. p. 180.
- Adegbite AA, Amusa NA. The major economic field diseases of cowpea in the humid agro-ecologies of south-western Nigeria. *Afr J Biotechnol*. 2008;7(25):4706-4712.
- Agrios GN. Plant pathology. 5th ed. New Delhi: Elsevier; 2006. 922 p.
- Akram A, Iqbal SM, Qureshi RA, Rauf CA. Variability among isolates of *Sclerotium rolfii* associated with collar rot disease of chickpea in Pakistan. *Pak J Bot*. 2008;40(1):453-460.
- Awasthi DP, Dasgupta B. Role of hydrolytic enzymes in stem rot of groundnut (*Arachis hypogaea* L.) caused by *Sclerotium rolfii* Sacc. *J Mycopathol*. 2011;49(1):135-138.
- Aycock R. Stem rot and other diseases caused by *Sclerotium rolfii*. North Carolina Agricultural

- Experiment Station Technical Bulletin No. 174. Raleigh, North Carolina: North Carolina State University; 1966. 202 p.
7. Azhar H, Iqbal SM, Abdul G, Najma A. Biodiversity among the isolates of *Sclerotium rolfsii* of chickpea in Pakistan. Pak J Phytopathol. 2009;21(2):98-103.
 8. Baoua I, Rabe MM, Murdock LL, Baributsa D. Cowpea production constraints on smallholders' farms in Maradi and Zinder regions, Niger. Crop Prot. 2021;142:105533.
 9. Boopathi P. Morphological and physiological characteristics of *Sclerotium rolfsii* causing stem rot or rhizome rot disease in tuberose (*Polianthes tuberosa*). J Crop Weed. 2023;19(1):276-284.
 10. Carsky RJ, Singh BB, Oyewole B. Contribution of early season cowpea to late season maize in the savanna zone of West Africa. Biol Agric Hortic. 2001;18(4):303-315.
 11. Cer C, Morca AU. First report of *Athelia rolfsii* (*Sclerotium rolfsii* Sacc.) causing collar rot disease on sunflower in Turkey. J Plant Pathol. 2020;102(3):931.
 12. Durgaprasad S, Reddy NPE, Kishore C, Reddy BVB, Sudhakar P, Rao SVRK. Variability among the isolates of *Sclerotium rolfsii* (Sacc.) causing stem rot of peanut (*Arachis hypogaea* L.). In: Gupta VVSR, Ryder M, Radcliffe J, editors. The Rovira Rhizosphere Symposium: Celebrating 50 Years of Rhizosphere Research. Adelaide, Australia: The Crawford Fund; 2008. p. 72-80.
 13. FAO. FAOSTAT: Production: Crops and livestock products [Internet]. 2025 [cited 2025 Dec 22]. Available from: <https://www.fao.org/faostat/en/#data/QCL>
 14. Fery RL, Dukes PD. Southern blight (*Sclerotium rolfsii* Sacc.) of cowpea: yield-loss estimates and sources of resistance. Crop Prot. 2002;21(5):403-408.
 15. Foyer CH, Lam HM, Nguyen HT, Siddique KHM, Varshney RK, Colmer TD, et al. Neglecting legumes has compromised human health and sustainable food production. Nat Plants. 2016;2:16112.
 16. Gawade DB, Perane RR, Suryawanshi AP, Deokar CD. Extracellular enzymes activity determining the virulence of *Rhizoctonia bataticola*, causing root rot in soybean. Physiol Mol Plant Pathol. 2017;100:49-56.
 17. Harlan JR. Agricultural origins: centers and noncenters. Science. 1971;174:468-474.
 18. Hisham MM, Chandran J, Gopinath PP. RAISINS: integrating R and AI for agricultural data analysis. J Sustain Technol Agric. 2025;1(1):1-11.
 19. Huang B, Du J, Huang J, Zhang C, He H, Guo Z, et al. First report of *Agroathelia rolfsii* causing southern blight on cowpea in China. Plant Dis. 2024;108(3):811.
 20. Husain A. Production of cellulolytic enzymes by *Sclerotium rolfsii*. Phytopathology. 1958;48(6):338-340.
 21. Jeswani LM, Baldev B. Advances in pulse production technology. New Delhi: Indian Agricultural Research Institute; 1997. 190 p.
 22. Kamalakannan A, Valluvaparidasan V, Chitra K, Rajeswari E, Salahuddin K, Ladhakshmi D, et al. First report of root rot of stevia caused by *Sclerotium rolfsii* in India. Plant Pathol. 2007;56(2):350.
 23. Kasana RC, Salwan R, Dhar H, Dutt S, Gulati A. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr Microbiol. 2008;57(5):503-507.
 24. KAU. Package of practices recommendations: crops. 16th ed. Thrissur: Kerala Agricultural University; 2024. 435 p.
 25. Kushwaha SK, Kumar S, Chaudhary B, Sahu R. Effect of different media, pH and temperature on growth and sclerotia formation of *Sclerotium rolfsii* Sacc. causing collar rot of lentil. Chem Sci Rev Lett. 2019;8(29):1-5.
 26. Metwally E, Sharshar M, Masoud A, Kilian B, Sharma S, Masry A, et al. Development of high yielding cowpea [*Vigna unguiculata* (L.) Walp.] lines with improved quality seeds through mutation and pedigree selection methods. Horticulturae. 2021;7(9):271.
 27. Mishra BK, Bais BS. Studies on seedling blight and foot rot of barley caused by *Sclerotium rolfsii*. Indian Phytopathol. 1987;40(3):161-167.
 28. Mordue JEM. *Corticium rolfsii*. CMI Descriptions of Pathogenic Fungi and Bacteria No. 41. Kew, Great Britain: Commonwealth Mycological Institute; 1974. p. 1-2.
 29. Nandi S, Hembaram S, Adhikari A, Tiwari BK, Dutta S. Host infection beyond the traditional range of *Sclerotium* (*Athelia*) *rolfsii* with *Physalis minima*. Bioinformation. 2017;13(10):333-338.
 30. Pande S, Rao JN, Reddy MV, McDonald D. A technique to screen for resistance to stem rot caused by *Sclerotium rolfsii* in groundnut under greenhouse conditions. Indian J Plant Prot. 1994;22:151-158.
 31. Pandey MK, Sarma BK, Singh UP. Induction of sexual stage and colony morphology of some isolates of *Sclerotium rolfsii* causing spotted leaf rot in plants. Mycobiology. 2005;33(1):7-11.
 32. Pant KC, Chandel KPS, Joshi BS. Analysis of diversity in Indian cowpea genetic resources. SABRAO J. 1982;14(2):103-111.
 33. Paul NC, Hwang EJ, Nam SS, Lee HU, Lee JS, Yu GD, et al. Phylogenetic placement and morphological characterization of *Sclerotium rolfsii* (Teleomorph: *Athelia rolfsii*) associated with blight disease of *Ipomoea batatas* in Korea. Mycobiology. 2017;45(3):129-138.
 34. Paul SK, Mahmud NU, Gupta DR, Surovy MZ, Rahman M, Islam MT. Characterization of *Sclerotium rolfsii* causing root rot of sugar beet in Bangladesh. Sugar Tech. 2021;23(5):1199-1205.
 35. Phillips RD, McWatters KH, Chinnan MS, Hung YC, Beuchat LR, Sefa-Dedeh S, et al. Utilization of cowpeas for human food. Field Crops Res. 2003;82(2-3):193-213.
 36. Punja ZK. *Sclerotium* (*Athelia*) *rolfsii*, a pathogen of many plant species. Adv Plant Pathol. 1988;6:523-534.
 37. Punja ZK, Huang JS, Jenkins SF. Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence in *Sclerotium rolfsii*. Can J Plant Pathol. 1985;7(2):109-117.
 38. Ramiah M, Narayanasamy P, Subramaniam CL. Leaf blight of cowpea (*Vigna sinensis* Endl.) caused by *Sclerotium rolfsii* Sacc. Curr Sci. 1976;45(9):352.
 39. Riddell RW. Permanent stained mycological preparations obtained by slide culture. Mycologia. 1950;42(2):265-270.
 40. Sajeena A, Kuriakose JM, Jeena TR, Shajan VR, Cherian S. Identification and isolation of *Sclerotium*

- rolfsii*, an emerging and destructive soil borne fungus affecting cowpea. In: Proceedings of the Twenty-sixth Kerala Science Congress. Pookode, Wayanad: Kerala State Council for Science, Technology and Environment, Government of Kerala; 2014. p. 417-422.
41. Sajeena A, Nair DS, Peteti TS, John J, Sudha B, Meera AV. First report of basal stem rot and blight of yardlong bean (*Vigna unguiculata* subsp. *sesquipedalis*) caused by *Athelia rolfsii* in India. J Plant Pathol. 2021;103(1):337.
 42. StatsCalculators Team. Normality test calculator, StatsCalculators [Internet]. 2025 [cited 2025 Nov 18]. Available from: <https://statscalculators.com/calculators/hypothesis-testing/normality-test-calculator>
 43. Sulladmath VV, Hiremath PC, Anilkumar TB. Studies on the variation in *Sclerotium rolfsii* Sacc. Mysore J Agric Sci. 1977;11(3):374-380.
 44. Sunil MVN. Management of stem rot and foliar blight of cowpea (*Vigna unguiculata* (L.) Walp.). MSc (Ag) thesis. Thrissur: Kerala Agricultural University; 2020. 177 p.
 45. Sunil N, Thara SS. Evaluation of biocontrol agents and chemical fungicides against *Sclerotium rolfsii* causing stem rot and foliar blight of cowpea. J Krishi Vigyan. 2022;10(2):141-145.
 46. Tanimu MU, Mohammed IU, Muhammad A, Kwaifa NM. Response of cowpea varieties to basal stem rot (*Sclerotium rolfsii*) disease in Southern Guinea Savanna, Nigeria. Agric Res Technol. 2018;15(4):106-110.
 47. Taubenhaus JJ. Recent studies on *Sclerotium rolfsii* Sacc. J Agric Res. 1919;18(3):127-138.
 48. Timko MP, Ehlers JD, Roberts PA. Cowpea. In: Kole C, editor. Genome mapping and molecular breeding in plants: Vol. 2. Pulses, sugar and tuber crops. Berlin, Heidelberg: Springer; 2007. p. 49-67.
 49. Tu CC, Kimbrough JW. Systematics and phylogeny of fungi in the *Rhizoctonia* complex. Bot Gaz. 1978;139(4):454-466.
 50. Vaniya RG, Singh P, Deshmukh AJ. Variability among the different isolates of *Sclerotium rolfsii* Sacc. causing stem rot of Indian bean. Biol Forum. 2022;14(2):757-765.
 51. Vasantry KM. Symptomatology, morphology and management of stem rot of cowpea (*Vigna unguiculata* (L.) Walp) caused by *Sclerotium rolfsii* Sacc. MSc (Ag) thesis. Navsari: Navsari Agricultural University; 2013. 97 p.
 52. Venkatakrishnaiya NS. Perfect stage of *Sclerotium rolfsii* Sacc. causing pseudostem-rot of plantain (*Musa sapientum*). Curr Sci. 1946;15(9):259.
 53. Vijayakumar P, Appusami S. Functional role of *Trichoderma asperellum* in inducing systemic resistance and suppressing southern blight (*Agroathelia rolfsii*) in cowpea. Physiol Mol Plant Pathol. 2025;140:102909.
 54. Wheeler BEJ. An introduction to plant diseases. London: John Wiley and Sons Ltd; 1969. p. 374.
 55. Yu D, Song W, Wang Q, Kang Y, Lei Y, Wang Z, et al. The quantity of OA and activity of cellulase and polygalacturonase are involved in variation of virulence in *Sclerotium rolfsii*. Oil Crop Sci. 2023;8:121-126.