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Isolation, characterization, and pathogenic assessment of *Sclerotium rolfsii* Saac causing collar rot in Brinjal (*Solanum melongena* L.)

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Abstract
Collar rot is an emerging and destructive soil-borne disease in brinjal, significantly affecting yield and quality. The present study was undertaken to isolate and characterize the causal organism associated with collar rot symptoms in brinjal and to assess its pathogenic potential. The pathogen was isolated from infected collar region tissues collected from severely affected brinjal plants. Morphological and microscopic observations revealed typical characteristics of a white, fast-growing fungus producing abundant sclerotia. Pathogenicity testing was conducted on two brinjal cultivars under pot culture conditions. Typical symptoms of collar rot developed in artificially inoculated plants, including yellowing of lower leaves, drooping, and basal stem necrosis. The re-isolation of the pathogen from symptomatic plants confirmed Koch's postulates. The results confirmed the pathogen's identity and its virulence under controlled conditions. This study provides important insights into the identification and disease expression of collar rot in brinjal, serving as a baseline for future integrated management strategies.

Keywords: Collar rot, Brinjal, pathogenicity, *Sclerotium rolfsii*, isolation, characterization

Introduction
Brinjal (*Solanum melongena* L.) is a major solanaceous vegetable grown widely in India and other parts of the world. It is highly susceptible to several biotic stresses, of which collar rot caused by *Sclerotium rolfsii is among the most destructive. The disease has been reported to cause significant yield losses* under warm and humid conditions. It affects the basal stem and root region, leading to rapid plant wilting and death.
The pathogen *Sclerotium rolfsii* is a soil-borne fungus known for producing numerous sclerotia that facilitate its survival and dispersal. Several studies have highlighted its wide host range and adaptability to various agro-climatic zones (Bindu *et al*., 2011; Borah *et al*., 2022) [10, 11]. Early and accurate identification of the pathogen is crucial for effective disease management. Although much has been studied on *S. rolfsii* in legumes and other crops, limited region-specific work exists for brinjal, particularly in central India.
The present study was carried out with the objectives of isolating and identifying the pathogen associated with collar rot symptoms in brinjal and evaluating its pathogenic potential under controlled conditions.

Materials and Methods
Study Location and Sample Collection
The present investigation was carried out in the Department of Plant Pathology, School of Agricultural Sciences, G. H. Raisoni University, Saikheda (Madhya Pradesh), during 2024-25. Field-grown brinjal plants (*Solanum melongena* L.) showing collar rot symptoms were collected to isolate the causal organism. The symptoms included water-soaked lesions near the collar region, white mycelial growth on the stem base, and characteristic sclerotia on the soil surface.

Isolation and Purification
Infected collar and root tissues were cut into small pieces, surface-sterilized using 0.1%

sodium hypochlorite for 1 minute, rinsed in sterile distilled water, and placed aseptically on Potato Dextrose Agar (PDA) medium in Petri plates. Plates were incubated at 25±1°C for 5-7 days. Emerging fungal colonies were purified using the hyphal tip method. The purified culture was maintained on PDA slants at 4°C for further study.

Mass multiplication of Fungi

A mass inoculum of this fungus was prepared using maize grains as the substrate. 500g of maize grains were ground and soaked in distilled water overnight. Any floating grains or debris were removed. The grains were then washed multiple times with tap water. Excess. water was drained, and the\. grains were autoclaved in a bag with a cotton plug to ensure sterility. After cooling, five 8mm diameter discs of *Sclerotium rolfsii* culture were introduced into the bag containing sterile maize grains. This inoculated bag were placed in an incubator for. 15 days. to allow the. fungus to colonize and sporulate on the grains (Plate 2).

(A)

(B)

(C)

Plate 1: sick soil method
A: Infected plant on the brinjal field
B: Colonization of *sclerotium rolfsii* on brinjal root
C: Colonization of *sclerotium rolfsii* in soil through the sick soil method.
D: *Sclerotium rolfsii* started to infecting plants. Brinjal plant showing the early symptoms of collar rot.

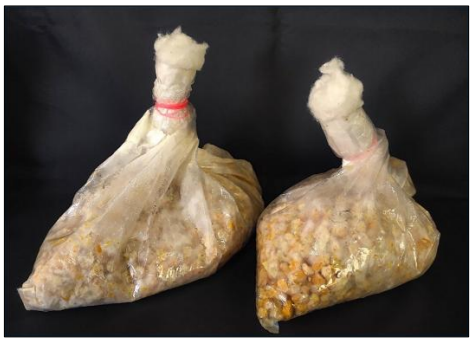


Plate 2: Mass multiplication of *Sclerotium rolfsii* on maize grains

Morphological and Microscopic Identification

The fungal culture was examined for colony morphology, mycelial characteristics, and sclerotial formation on PDA. Microscopic observations were conducted using a compound microscope to examine hyphal structure and size, shape, and color of sclerotia. Morphological characters were compared with standard descriptions reported by Kushwaha *et al*. (2019) [13] and Meena *et al*. (2024) [4] for confirmation.

Cultural Characteristics

Three isolates of *Sclerotium rolfsii* were evaluated on PDA medium for colony growth, pattern, pigmentation, and sclerotia formation. Radial growth was recorded at 24-hour intervals (24, 48, 72, and 96 hours). Cultural variability was documented to confirm typical growth behavior.

Pathogenicity Test

Pathogenicity of the isolated fungus was tested on two brinjal cultivars: Pusa Purple Round and Arka Neelkanth using the pot method. Five pots per variety were filled with sterilized soil inoculated with the pathogen; one pot per variety served as a control. Plants were observed daily for symptom development and severity. Growth parameters such as plant height, fresh weight, and dry weight were recorded post-infection. Re-isolation of the pathogen from infected plants was done to confirm Koch's postulates.

Isolation and Maintenance of the Pathogen

The collar rot pathogen *Sclerotium rolfsii* was isolated from infected brinjal plants using the standard tissue isolation method (Riker & Riker, 1936). Infected stem tissues were

surface-sterilized with 0.1% sodium hypochlorite for 1 minute, rinsed with sterile distilled water, and plated on Potato Dextrose Agar (PDA) medium. The cultures were incubated at 25±2 °C for 5-7 days. Pure cultures were maintained on PDA slants at 4°C for further use.

Percent Inhibition (%) = [(C − T) / C] × 100

Where C = radial growth in control, T = radial growth in treatment.

Instruments and Software Used

- **Autoclave:** Used for media sterilization at 121°C and 15 psi.
- **Laminar Air Flow Chamber:** For aseptic culture transfers.
- **Incubator:** Maintained at 25 ± 2°C.
- **Petri Plates:** Standard 90 mm borosilicate sterile plates.
- **Measurement Tools:** Digital calipers and ruler for radial growth measurement.
- **Statistical Analysis:** Data were analyzed using Microsoft Excel and SPSS.

Results

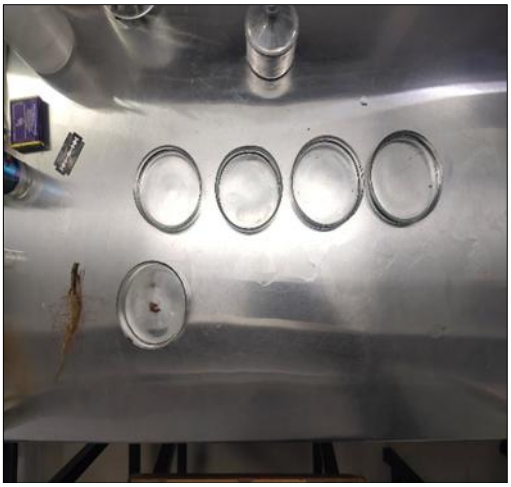
Symptomatology

The disease initially appeared as small, water-soaked lesions on the collar region, which expanded into soft rotting tissue. As the infection progressed, plants exhibited yellowing of older leaves, drooping, and eventual wilting. Profuse white mycelial growth was observed at the stem base, accompanied by sclerotia formation-white initially, turning brown upon maturity. These symptoms were consistent across both cultivars, with *Pusa Purple Round* showing faster and more severe disease progression than *Arka Neelkanth*

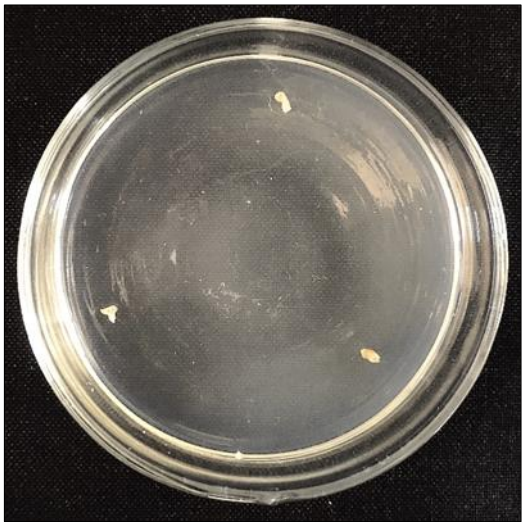
Isolation and Identification

Colonies on PDA were white, fluffy, and fast-growing with dense, radiating mycelial growth. Microscopic examination revealed branched, septate, hyaline hyphae and sclerotia measuring 0.5-2.5 mm in diameter. The sclerotia were round to oval, appearing white and maturing to dark brown. These characteristics confirmed the pathogen as *Sclerotium rolfsii*, aligning with observations by Arya and Kushwaha (2019) [3] and Punja (2020) [1].

(A)



(B)



(C)

Plate 3: Isolation of *Sclerotium rolfsii* from disease sample of Brinjal plants
A: Infected plant part of Brinjal
B: Surface sterilization of diseased sample
C: Inoculation of disease sample of Brinjal on PDA

Morphological and cultural characteristics studies

- **Colony Characteristics:** Initially white with a rapid, radiating growth pattern, later developing dense, cottony mycelium. The underside of the colony showed cream to light brown pigmentation, darkening over time.
- **Mycelium Characteristics:** Profusely branched, septate, and creeping growth. Initially hyaline, turning slightly pigmented with age.
- **Sclerotia Formation:** Small, round sclerotia (0.5-2.5 mm) appeared white initially, maturing to light and dark brown. Embedded within the mycelium or on agar, ensuring pathogen survival.
- **Asexual Reproduction:** *Sclerotium rolfsii* relies on sclerotia for survival and spread.
- **Cultural Variability:** Grows rapidly on PDA, covering the plate within 5-7 days.

These findings align with Punja (2020) [1] and Sharma *et al*. (2023), confirming the characteristic morphology and growth patterns of *Sclerotium rolfsii*.

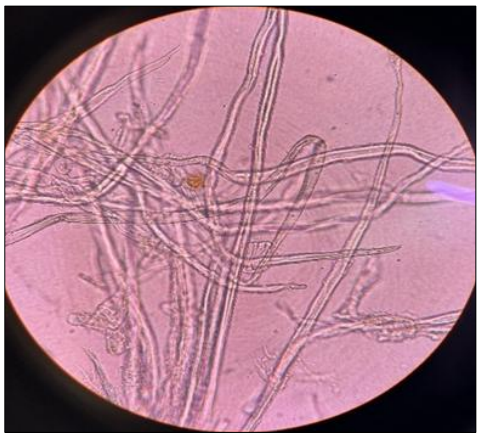
Table 1: Cultural character of *Sclerotium rolfsii.*

Sr. No.	Colony Growth	Colony Pattern	Substrate Colour	Mycelium Colour	Reference
SR1	Fast	Fluffy to cottony	White to light brown	White	Aycock (1966); Punja (1985) [6]
SR2	Rapid	Dense and radiating	Light brown to yellowish	White to off-white	Tariq *et al*. (2018)
SR3	Moderate to fast	Aerial and compact	White with brown margin	White	Singh *et al*. (2020) [8]; Kumar *et al*. (2019) [9]

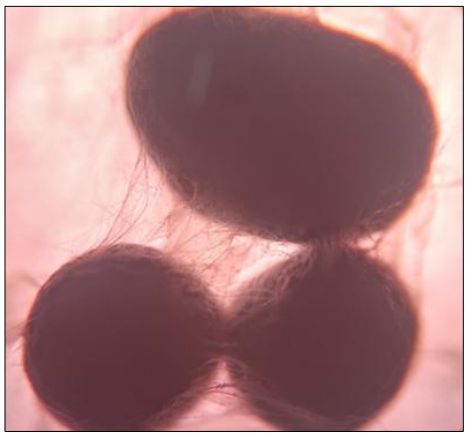
Table 2: Growth of sclerotium rolfsii in interval of 24 hours

Sr. No.	Replication	24hr	48hr	72hr	96hr
1	SR1	14.5	29.6	45.8	70.0
2	SR2	13.8	28.2	44.0	68.5
3	SR3	14.2	28.9	45.1	69.3
	Mean	14.2	28.9	45.0	69.3

*These values are mean of three replications



(A)



(B)

(C)

(D)

Plate 4: Morphological and cultural study of *Sclerotium rolfsii*.
A: Formation of the sclerotia observed in the microscope in 40x
B: Sclerotia of the pathogen observed under the microscope
C: Growth of isolated Pathogen *Sclerotium rolfsii* after 7 days
D: Sclerotia formed after 14 days of subculturing

Pathogenicity

Both brinjal cultivars showed 100% disease incidence under inoculated conditions, while controls remained symptom-free.

Table 3: Disease Incidences of the Pusa Purple Round and Arka Neelkanth

Variety	Total Plants	Infected Plants	Control (Infection)	Disease Incidence (%)
Pusa Purple Round	10	10	0	100%
Arka Neelkanth	10	10	0	100%

In *Pusa Purple Round*, symptoms developed faster with severe stem necrosis and plant collapse within 12-15 days. *Arka Neelkanth* showed delayed and less intense symptoms, indicating relatively better tolerance.

(A)

Plate 5: *In vivo* pathogenicity test of *sclerotium rolfsii* in brinjal
(A) Pathogenicity test of brinjal variety Pusa Purple Round
(B) Pathogenicity test of brinjal variety Arka Neelkanth

Table 4: Suppression of. Brinjal (PUSA PURPLE ROUND) height due *Sclerotium rolfsii sacc.* (cm)

R1	R2	R3	R4	R5	(CONTROL)
14.5	9.6	13.8	12.1	10.5	19.7

Table 4: Suppression of Brinjal (PUSA PURPLE RPUND) plant fresh weight due to *Sclerotium rolfsii sacc.* (shoot) (gm)

R1	R2	R3	R4	R5	(CONTROL)
0.67	0.48	0.78	0.63	0.46	1.79

Table 5: Suppression of Brinjal (PUSA PURPLE ROUND) plant dry weight due to *Sclerotium rolfsii sacc.* (shoot) (gm)

R1.	R2	R3	R4	R5	(CONTROL)
0.29	0.26	0.32	0.29	0.31	0.91

Table 6: Suppression of Brinjal (ARKA NEELKANTH) height due to *Sclerotium rolfsii sacc.* (cm)

R1	R2	R3	R4	R5	(CONTROL)
15.8	13.6	12.2	14.5	11.9	20.2

Table 7: Suppression of Brinjal (ARKA NEELKANTH) plant fresh weight due *to Sclerotium rolfsii sacc.*(shoot) (gm)

R1	R2	R3	R4	R5	(CONTROL)
0.91	0.98	0.82	0.74	0.84	2.23

Table 8: Suppression of Brinjal (ARKA NEELKANTH) plant dry weight due to *Sclerotium rolfsii sacc.* (shoot) (gm)

R1.	R2	R3	R4	R5	(CONTROL)
0.48	0.49	0.38	0.29	0.56	0.92

Discussion

The study confirms *Sclerotium rolfsii* as the causal agent of collar rot in brinjal, exhibiting characteristic symptoms like basal stem decay, wilting, and sclerotia formation. The pathogen demonstrated rapid growth on PDA and developed mature sclerotia within a week. Morphological and cultural traits were consistent with earlier descriptions by Punja (2020) [1] and Meena *et al*. (2024) [4].

Pathogenicity tests revealed significant suppression of plant growth and biomass, especially in the Pusa Purple Round variety, which showed rapid symptom onset and severe necrosis. Arka Neelkanth, although infected, displayed slower disease progression and comparatively less reduction in growth parameters, suggesting moderate resistance. This differential host response aligns with findings by Sharma *et al*. (2023) [2] and highlights the importance of cultivar selection in disease management strategies.

The ability of the fungus to produce resilient sclerotia further complicates disease control, as these structures can persist in soil for extended periods, making repeated crop cycles vulnerable. Therefore, integrated management approaches involving resistant cultivars, soil sanitation, and biocontrol agents are essential for effective disease suppression.

Conclusion

- The collar rot disease in brinjal was conclusively attributed to *Sclerotium rolfsii* (Sacc.), confirmed through isolation, morphological identification, and pathogenicity testing.
- The pathogen showed rapid growth and dense mycelial development on PDA, with abundant sclerotia formation ranging from 0.5-2.5 mm in diameter.
- Microscopic analysis revealed characteristic branched, septate hyphae and absence of conidia, consistent with diagnostic features of *S. rolfsii*.
- Pathogenicity tests on two brinjal cultivars showed 100% disease incidence, with Pusa Purple Round exhibiting higher susceptibility than Arka Neelkanth.
- Significant suppression in plant height, fresh weight, and dry weight was recorded in inoculated plants compared to the control.
- Re-isolation of the fungus from infected tissues fulfilled Koch's postulates, confirming its role as the collar rot pathogen.
- The ability of the pathogen to form long-surviving sclerotia emphasizes its persistence in the soil and the challenge it poses to brinjal cultivation.
- The differential cultivar response indicates potential for varietal resistance screening in future breeding programs.
- Integrated disease management strategies-utilizing resistant cultivars, cultural control, and biocontrol agents-are essential to mitigate collar rot and ensure sustainable brinjal production.

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