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Pathogenicity test to identify the virulent isolate of *Sclerotium rolfsii* causing collar rot in brinjal and its impact across different growth stages

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Abstract
Twenty isolates of *Sclerotium rolfsii* were collected from major brinjal-growing districts of Tamil Nadu, including Cuddalore, Salem, Dindigul, and Erode. The isolates were named as Sr1 to Sr20, and were subjected to a pathogenicity test to confirm their virulence and adherence to Koch postulates. The results confirmed the pathogenicity of all isolates, with distinct symptoms of collar rot observed on inoculated plants and the highest disease incidence of 45.59% was recorded in the isolate Sr3 and the least disease incidence of 16.07% was recorded in the isolate Sr17. Further studies were conducted to determine the susceptibility of brinjal plants at different growth stages. The findings revealed that younger plants were significantly more susceptible to *S. rolfsii* infection compared to older plants. This increased susceptibility in the early stage highlights the critical need for early-stage management practices to mitigate losses caused by this pathogen in brinjal cultivation.

Keywords: *Sclerotium rolfsii*, brinjal (*Solanum melongena* L.), pathogenicity test, susceptible stage of crop

Introduction
Brinjal (*Solanum melongena* L.), a significant vegetable crop belonging to the *Solanaceae* family, is highly susceptible to various biotic and abiotic diseases. Among these, collar rot caused by *Sclerotium rolfsii*, is a devastating disease that severely impacts brinjal cultivation [1]. It is a soil-borne, facultative parasitic fungus known for its omnipathogenic nature, infecting over 500 plant species worldwide [2]. The incidence of collar rot range from 60% to 100%, leading to fruit yield losses of 30%-50% [3, 4]. Infestations during the seedling to flowering stages can result in complete crop failure. The disease can manifest at any growth stages of the plant [5] and is characterized by dark brown lesions at the collar region of the stem, just above the soil line, which progress to wilting. Whitish mycelial growth and the development of cream to dark brown sclerotia have been seen on the affected portion. The pathogen persists in the soil through the formation of sclerotia [6].

Materials and Methods
Inoculum Preparation
For the pathogenicity test, the inoculum was prepared using sorghum grains as the substrate. 100 grams of sorghum grains had been soaked overnight and were placed in open-mouthed bottles. These bottles were securely sealed with non-absorbent cotton and autoclaved at 121 °C for 15 minutes to ensure sterilization. Once sterilized, the sorghum substrate was allowed to cool to room temperature. A 5 mm disc of a five-day-old *Sclerotium rolfsii* isolates were inoculated into each bottle separately. The inoculated bottles were incubated at 27±2 °C for 15 days to promote optimal mycelial growth and sclerotial formation. To ensure uniform colonization of *Sclerotium rolfsii* isolates on the sorghum grains, the bottles were shaken every other day throughout the incubation period [7, 8]

Pathogenicity test
The potting mixture was prepared by thoroughly mixing sand, red loam soil, and farmyard manure at the ratio of 1:1:1. The inoculum of each *Sclerotium rolfsii* isolate was added to the soil at a rate of 10 g/kg and filled into earthen pots two weeks prior to transplanting.

Two plants per pot were maintained. Pots without inoculum served as control, with three replications maintained for each treatment. The pathogen *S. rolfsii* was inoculated into the seedbed soil and allowed to proliferate under adequate soil moisture conditions. Fifteen days after transplanting, typical collar rot symptoms were assessed in the brinjal plants. For further confirmation, the pathogen was re-isolated from infected plant tissues and compared with the original isolates to verify pathogenicity [6]. The disease incidence for each isolate at 30 DAT, 60DAT, 90DAT and at harvest stage (105 DAT) was recorded. The percent disease incidence was calculated by using the formula,

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Evaluation of susceptible stage of brinjal plants to *Sclerotium rolfsii*

An experiment was conducted under greenhouse conditions to determine the susceptibility stage of brinjal plants (PLR 1) to collar rot caused by *Sclerotium rolfsii* at different growth stages. Six stages of crop growth viz., 0, 15, 45, 60,75 and 90 days after sowing (DAS) were evaluated. Plants were grown in pots (15 × 25 cm) filled with sterilized soil, with three replications per stage and five pots per replication. At the zero stage, one to two healthy brinjal seed was sown per pot, while in subsequent stages, a single seedling was maintained in each pot. Recommended fertilizer doses were applied uniformly, and untreated control pots were maintained with the same number of replications and pots for all stages.

Inoculation was performed at the collar region of plants for all stages (4 grains per plant) except the zero stage, where inoculum was mixed into the soil at the time of seed sowing. After inoculation, the pots were kept in an open area and irrigated as required [9, 10, 7]. Observations on disease incidence was recorded at 0, 15, 30, 45, 60, 75 and 90 days after inoculation. The percentage of disease incidence was calculated using the following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Results and Discussion

Pathogenicity test

A pathogenicity test was conducted to evaluate the infectivity of 20 *Sclerotium rolfsii* isolates on brinjal through artificial inoculation (Fig 1). Fifteen days post-inoculation, the plants exhibited typical symptoms of collar rot and the control remains healthy. Affected seedlings displayed rotting at the collar region, wilting, and could be easily uprooted. To confirm the causal organism, affected tissue was subjected to re-isolation, yielding a culture identical to the original *Sclerotium rolfsii* isolates, characterized by white radial mycelial growth and sclerotia that transitioned from cream to black in color (Fig 2), thereby all the isolates proved Koch postulates and demonstrating the infectivity of the pathogen. The results revealed that the maximum disease incidence of 45.59% was recorded in the isolate Sr3 and the minimum disease incidence of 16.07% was recorded in the isolate Sr17 (Table 1).

Sivakumar *et al.* [7] reported that the highest disease incidence was recorded in the SR_1 isolate (55.70%) and the lowest disease incidence was recorded in the SR_5 isolate (30.60%) by conducting the pathogenicity test of *S. rolfsii* on groundnut plants. Similarly, Praveen and Kannan [11] reported that the pathogenicity test of twenty isolates of *Scleortium rolfsii* on groundnut plants under pot culture condition revealed that the isolate Sr6 recorded maximum disease incidence of 42.05% and the isolate Sr13 recorded the minimum disease incidence of 16.65%. Sheneka [12] conducted the pathogenicity test of *Sclerotium rolfsii* on brinjal plants and recorded the maximum disease incidence in Sr3 (51.54%) and the minimum disease incidence in Sr1 (16.53%). Similar findings were reported by Ayyandurai *et al*. [13] conducted pathogenicity test of *Sclerotium rolfsii* in groundnut plants and proved Koch postulates and also reported that the virulent isolate SR-8 recorded 89.27% of disease incidence and the isolate SR-10 recorded the least incidence of 18.72%. Similarly, Modi [14] reported that the isolate SrC-17 recorded the highest disease incidence of 56.02% and the isolate SrP-16 recorded the lowest disease incidence of 15.27%. The above reports lend support to the present findings.

Evaluation of susceptible stage of brinjal plants to *Sclerotium rolfsii*

The findings presented in Table 2 indicate a significant variation in percent disease incidence across different growth stages of the crop, with the highest incidence was observed in 15-day-old plants (62.57%) and the lowest in 90-day-old plants (16.54%). These results showed that the young plants were more susceptible than the old plants and also showed that the susceptibility decreased with the age of crop increased.

Similar findings were reported by Singh and Dwivedi [15] where they showed that the 15 days old barely plants were more susceptible to *S. rolfsii*. Twenty days old groundnut plants were more susceptible to *S. rolfsii* and recorded highest disease incidence of 92.66% and lowest incidence was recorded in 120 days old crop [16]. Bekriwala *et al.* [10] reported that *S. rolfsii* cause infection on all growth stages of groundnut plants and more susceptibility was observed in young plants. Similarly, Mahato *et al.* [9] reported that there is significant difference in disease incidence among the different growth stages of tomato plants to *S. rolfsii* and also revealed that the maximum disease incidence was in 30 days old plants (86.25%) and minimum disease incidence in 60 days old plants (42.5%). Modi [14] evaluated the susceptibility stage of brinjal crops to *S. rolfsii* and reported that the 80 days old crop showed highest disease incidence of 52.14% and the 140 days old crops were free of collor rot infection. These earlier reports corroborates with the present observations.

Table 1: Pathogenicity assessment of *Sclerotium rolfsii* Isolates on brinjal under pot culture conditions

Isolates	Collar rot incidence (%)				Mean
	30DAT	60DAT	90DAT	At harvest (105DAT)	
Sr1	30.02^{c} (33.22)	33.26^{e} (35.21)	40.38^{de} (39.45)	49.35^{c} (44.62)	38.25
Sr2	30.86^{bc} (33.74)	34.87^{d} (36.19)	41.09^{d} (39.86)	50.31^{c} (45.17)	39.28
Sr3	34.32^{a} (35.86)	41.25^{a} (39.96)	49.61^{a} (44.77)	57.19^{a} (49.13)	45.59
Sr4	17.32^{i} (24.59)	21.77^{k} (27.81)	26.61^{k} (31.05)	37.02^{h} (37.47)	25.68
Sr5	11.94^{l} (20.21)	17.68^{n} (24.86)	22.19^{n} (28.10)	31.66^{j} (34.24)	20.86
Sr6	31.97^{b} (34.43)	37.66^{c} (37.85)	43.32^{c} (41.16)	54.81^{b} (47.76)	41.94
Sr7	15.09^{j} (22.85)	20.05^{l} (26.60)	24.78^{l} (29.85)	35.92^{hi} (36.82)	23.96
Sr8	28.53^{d} (32.28)	31.59^{f} (34.19)	39.76^{e} (39.09)	48.69^{c} (44.24)	37.14
Sr9	13.28^{k} (21.37)	19.56^{lm} (26.24)	24.07^{lm} (29.38)	34.99^{i} (36.26)	22.97
Sr10	23.42^{f} (28.94)	28.49^{h} (32.25)	34.99^{g} (36.26)	45.43^{de} (42.37)	33.08
Sr11	22.19^{g} (28.10)	27.83^{h} (31.83)	33.26^{h} (35.21)	43.72^{e} (41.39)	31.75
Sr12	18.22^{i} (25.26)	23.19^{j} (28.78)	29.85^{j} (33.11)	39.64^{g} (39.02)	27.72
Sr13	9.82^{m} (18.26)	16.06^{op} (23.62)	20.51^{op} (26.92)	29.64^{k} (32.98)	19.00
Sr14	20.59^{h} (26.98)	24.37^{i} (29.58)	31.08^{i} (33.88)	41.59^{f} (40.15)	29.40
Sr15	9.16^{mn} (17.61)	15.26^{q} (22.99)	20.09^{q} (26.62)	28.41^{k} (32.20)	18.23
Sr16	8.52^{n} (16.97)	14.05^{r} (22.01)	19.77^{r} (26.39)	26.83^{l} (31.19)	17.13
Sr17	7.16^{op} (15.52)	13.63^{r} (21.66)	18.09^{s} (25.17)	25.42^{l} (30.27)	16.07
Sr18	33.71^{a} (35.49)	39.58^{b} (38.98)	46.83^{b} (43.18)	55.69^{ab} (48.26)	43.95
Sr19	25.06^{e} (30.03)	29.63^{g} (32.97)	36.94^{f} (37.42)	46.31^{d} (42.88)	34.48
Sr20	12.68^{kl} (20.86)	18.89^{m} (25.76)	23.78^{m} (29.18)	32.65^{j} (34.84)	22.00

Mean of three replications
Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range (DMRT)

Table 2: Evaluation of susceptible stage of brinjal plants to *Sclerotium rolfsii*

SI. No.	Treatments (Days)	Percent disease incidence (%)
1	Zero stage	0.00
2	15 days old plants	62.57^{a} (52.28)
3	30days old plants	53.64^{b} (47.08)
4	45 days old plants	45.21^{c} (42.25)
5	60 days old plants	24.49^{d} (29.66)
6	75 days old plant	19.33^{e} (26.08)
7	90 days old plant	16.54^{f} (23.99)
8	Control	0.00

Mean of three replications
Values in the column followed by common letters do not differ significantly at 5% level by Duncan's multiple range (DMRT)

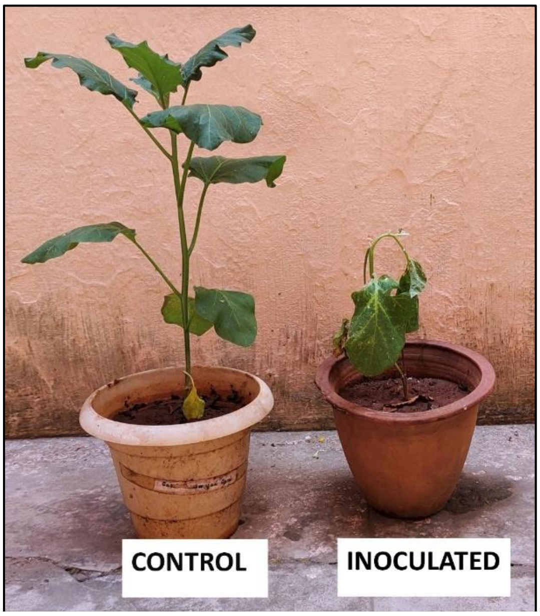

Fig 1: Pathogenicity test of different isolates of *Sclerotium rolfsii* on brinjal under pot culture condition

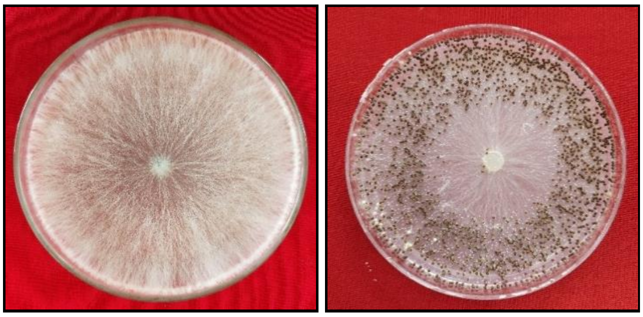

Fig 2: Reisolation and pathogenicity confirmation of *Sclerotium rolfsii* from infected brinjal plant

Conclusion
Pathogenicity test was conducted to test the virulence of the twenty isolates of *S. rolfsii* and the test revealed that all the twenty isolates proved Koch postulates and the maximum disease incidence was recorded in the isolate Sr3 and the minimum disease incidence was recorded in the isolate Sr17. Another experiment was conducted to found out the susceptibility stage of brinjal crops to *Sclerotium rolfsii* and the results revealed that the 15 days old crops (62.57%) are more susceptible to *Sclerotium rolfsii* and the least disease incidence was recorded in 90 days old crops (62.57%). Finally, we concluded that the susceptibility range was decreased, when the age of crops increased.

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