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Incidence, Prevalence, and Molecular Identification of *Sclerotium rolfsii* Causing Southern Blight in Tomato Fields of Mysuru District, Karnataka, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Tomato (*Solanum lycopersicum*), a vital crop worldwide, is under significant threat from *Sclerotium rolfsii*, the fungal pathogen responsible for southern blight. This soil-borne pathogen, known for its wide host range and destructive nature, thrives in humid environments, causing severe losses in tomato production.

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Aim: A field survey was conducted between June and September 2024 across 30 villages in six taluks of Mysuru district, Karnataka, India, to assess the incidence, prevalence, and severity of southern blight in tomato fields.
Results: Disease incidence ranged from 10.73% to 69.64%, while prevalence varied between 20% and 83.33%. Mysuru taluk recorded the highest disease intensity, with a mean incidence of 30.79% and a prevalence of 45.53%. The pathogen was isolated from infected tomato plants, and morphological identification revealed the characteristic sclerotia of *S. rolfsii*. Pathogenicity tests confirmed the fungus as the causative agent by reproducing typical symptoms on healthy tomato seedlings. Further molecular characterization of the internal transcribed spacer (ITS) region of the fungus confirmed its identity with high sequence similarity to known *S. rolfsii* strains in GenBank.
Conclusion: These findings underscore the urgent need for targeted disease management strategies to mitigate the spread of southern blight and protect tomato production in the Mysuru region, safeguarding both local and global food security.

Keywords: Tomato; southern blight; Sclerotium rolfsii; disease incidence; prevalence.

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most widely cultivated and consumed vegetables globally, originating from the Andean region of South America. As a member of the Solanaceae family, tomatoes have evolved into a vital crop due to their versatility in culinary applications and their rich nutritional profile, particularly their lycopene content, which has proven health benefits (Yusufe et al., 2017; Saurez et al., 2008). Although botanically classified as a berry, tomatoes are often used as vegetables in various dishes, sauces, and salads. Tomatoes thrive in tropical and temperate climates, growing as perennials in their native habitats but cultivated as annuals in temperate regions (Purseglove, 1988). Adapted to a range of environments, tomato varieties are grown across vast regions, with China, India, and the USA being the top global producers (FAOSTAT, 2014).

In India, tomato cultivation has become prominent, with major producing states like Madhya Pradesh, Andhra Pradesh, Karnataka, and Tamil Nadu contributing significantly to the nation's output (APEDA, 2021). The widespread adaptability of tomatoes across various agro-climatic zones, from Jammu & Kashmir to Tamil Nadu, makes them a crucial crop for enhancing food security and improving the income of small and marginal farmers. Despite tomatoes' importance, their production is often threatened by various pests and diseases, including nematodes, fungi, bacteria, and viruses, which lead to yield losses. Effective crop rotation and integrated pest management strategies are essential to mitigating these risks and ensuring sustainable tomato cultivation worldwide (Bergougnoux, 2014; Gilbertson and Batuman, 2013).

Numerous fungal diseases have been reported on tomatoes from different regions worldwide. Southern blight of tomato is a major fungal disease caused by *Sclerotium rolfsii* and can occur at any stage of growth (Begum et al., 1985). *S. rolfsii* Sacc. (teleomorph: *Agroathelia rolfsii*) is a serious disease-causing fungal pathogen that affects diverse crops worldwide (Dell'Olmo et al*.,* 2024; Pai and Chandra, 2023; Garcia-Gonzalez et al., 2022; Xie, 2014). Because of its broad host range, *S. rolfsii* is considered one of the most destructive pathogens worldwide; indeed, about 500 plant species from 100 families, including tomatoes, potatoes, chilli pepper, cabbage, beans, groundnuts, and sweet potatoes (Aycock, 1996; Farr et al., 1989) are affected by this pathogen. The fungus generally infects the collar region or stems near the soil surface (Mullen, 2001; Mahadevakumar et al., 2016; Huang et al*.,* 2024). *S. rolfsii* produces sclerotia on hosts, survives and overwinters for a long time, and infects the same host or other nearby crops (Aycock, 1996; Smith et al*.,* 1989; Punja, 1985).

This survey aimed to assess the incidence and prevalence of southern blight of tomato caused by *Sclerotium rolfsii*. This soil-borne fungal pathogen is known for its destructive impact on tomato crops, leading to significant yield losses in various tomato-growing regions. The survey aimed to gather comprehensive data from different locations to understand the geographical distribution and intensity of the disease. By evaluating factors such as the percentage of infected plants (incidence), and the extent of affected fields (prevalence), the

survey sought to provide valuable insights into the disease's spread and its potential risks to tomato production.

2. MATERIALS AND METHODS

2.1 Survey for Incidence and Severity of Southern Blight of Tomato

A survey was conducted in tomato fields across various randomly selected localities in six taluks of Mysuru district, Karnataka, India, to assess the incidence and severity of southern blight of tomato caused by *Sclerotium rolfsii* (Table 1). The samples were collected between June and September 2024. In each locality, 3-8 tomato fields were chosen randomly; in each field, 50-100 plants were sampled every 10 steps following a zigzag pattern. The plants were carefully uprooted to a depth of 15-30 cm using a trowel. The infected stems, roots and surrounding soil were placed in labelled polythene bags and transported to the Department of Studies in Botany at Manasagangotri, University of Mysore, for fungal species identifications.

The incidence and prevalence of southern blight in individual fields were determined as follows:

$$\text{Incidence (\%)} = \frac{Total\ number\ of\ infected\ plants}{Total\ number\ of\ observed\ plants} \times 100$$

$$\text{Prevalence (\%)} = \frac{Number\ of\ fields\ infected\ with\ root\ rot}{Total\ number\ of\ fields\ surveyed} \times 100$$

2.2 Isolation and Morphological Identification

Stem and roots of infected tomato plants by southern blight disease were collected and transported to the laboratory. Infected plant samples were cut into 1×1 cm^2 pieces using a sterile knife washed separately with running tap water and dried in a laminar air flow chamber. The segments were surface sterilized with 2% sodium hypochlorite (NaOCl) solution for 2-3 minutes and washed thrice with sterile distilled water. The surface sterilized samples were transferred to Potato Dextrose Agar (PDA) supplemented with Chloramphenicol (2%). Sample inoculated Petri plates were then incubated at room temperature (25 ± 2^0 C) under a 12-hour light/dark cycle for 7 days and monitored regularly for the growth of fungal colonies. The resulting colonies were subsequently sub-cultured on fresh PDA plates, were then assigned identification numbers, and incubated for further cultural studies, pathogenicity, and molecular identification (Mahadevakumar et al., 2016; Paul et al., 2017).

2.3 Pathogenicity Test

Pathogenicity tests were conducted on 21-day-old healthy tomato seedlings grown under greenhouse conditions in a mixture of farmyard manure (FYM), sand, and soil in a 1:1:2 ratio. Whole plant inoculation assays were performed by introducing 12 to 15-day-old *S. rolfsii* cultures grown on PDA. Sclerotia were wrapped around the stem at the stem-soil interface of 10 healthy tomato plants, with three replicates for each. Sterile distilled water was used for inoculation in the control group. The experiment was repeated three times, and the development of infection near the inoculation site was monitored at regular intervals. The pathogen was re-isolated from the infected plants showing typical symptoms, and its identity was confirmed based on morphological and cultural characteristics.

2.4 Molecular Identification and Phylogenetic Analysis

Genomic DNA was extracted from 50 mg of mycelium grown on PDA using the CTAB method (Zhang et al., 2010). The internal transcribed spacer (ITS) region was amplified using the primer pair ITS1/ITS4 (White et al., 1990). PCR was carried out in a 25 µl reaction mixture, which included 1 µl of DNA template, 5 µl of MyTaq DNA buffer (Himedia), 0.3 µl of Taq DNA polymerase (Himedia), 1 µl each of forward and reverse primers, and 16.7 µl of nuclease-free water. The thermal cycler conditions were as follows: initial denaturation at 93°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 70°C for 45 seconds, and a final extension at 72°C for 3 minutes. The amplified PCR products were sequenced by a private company, and the sequences were subjected to a BLASTn search in GenBank. Based on nucleotide sequence homology, the isolate was identified at the species level. The representative sequences were deposited in the GenBank database. Sequences from the fungal isolate, along with related sequences retrieved from GenBank, were aligned using the Muscle program. Phylogenetic analysis was performed using the Neighbor-Joining (NJ) method for the ITS dataset with MEGA11 software (Tamura et al., 2021). For all analyses, 1000 bootstrap

replicates were performed to assess the robustness of the tree nodes.

3. RESULTS

3.1 Incidence and Prevalence of *S. rolfsii* in Mysuru District

A field survey conducted from June to September 2024 to evaluate the incidence and prevalence of southern blight in tomatoes, caused by *Sclerotium rolfsii*, across different villages in Mysuru district, revealed varying levels of disease severity across taluks. The survey covered 30 villages in six taluks (Mysuru, Tirumakudalu Narasipura (T. Narasipura), Nanjanagud, Hunsur, Krishnarajanagara (K.R. Nagara), and Heggadadevana Kote (H.D. Kote)) (Fig. 1). Results showed significant differences in both the incidence and prevalence of the disease. The additional data with GPS coordinates enhances the understanding of the geographic distribution of southern blight across different regions in the Mysuru district (Table 1). By mapping the specific locations of surveyed fields, it becomes possible to identify potential spatial patterns and environmental factors contributing to the varying incidence and prevalence rates. Infected plants displayed cottony white mycelium on the affected areas, which developed numerous sclerotia of varying sizes, colors, and shapes. A white mycelial mat also appeared on the surface of the infected plant stems, with symptoms being particularly severe during periods of high humidity and rainfall (Fig. 2).In Mysuru taluk, the incidence of southern blight varied significantly, ranging from 18.75% in Madahalli to 69.64% in Ranganathapura. Prevalence followed a similar trend, with Ranganathapura reaching 80% and Kiralu recording the lowest at 20%. Shettinayakanahalli and Madagalli had moderate incidences (23.33% and 20%, respectively) but showed relatively high prevalence (40%). Manikyapura exhibited both high incidence (36.11%) and prevalence (50%), indicating a higher disease burden. In T. Narasipura taluk, Ankanahalli recorded an incidence of 29.88% and a prevalence of 37.5%, while Sosale and Krishnapura showed similar patterns with incidences of 34.61% and 27.77%, respectively, and both had a prevalence of 50%. Nanjanagud taluk had the lowest overall incidence, with Hullahalli reporting the district's lowest at 10.73%, but Mallahalli had a higher prevalence (50%) despite a lower incidence (13.29%). In Hunsur taluk, Niluvagilu showed moderate disease intensity (29.62% incidence, 50% prevalence), while Kothegala and Thammadahalli reported lower incidences of 18.75% and 17.39%, respectively. K.R. Nagara taluk exhibited variability, with Haradanahalli having the highest incidence (57.97%) and prevalence (66.66%). H.D. Kote emerged as a hotspot, with Masanakuppe and Chamanahalli recording incidences of 58.13% and 55.34%, and prevalences of over 80%. The district's mean incidence was 30.79%, with a prevalence of 45.53%, calling for urgent disease management in critical areas.

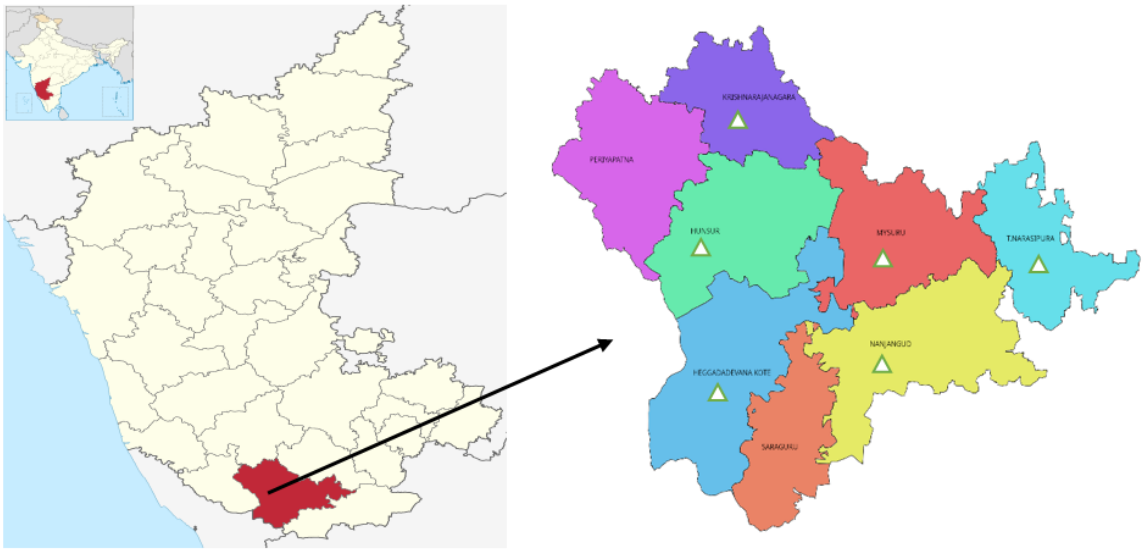

Fig. 1. Map of the surveyed regions of Mysuru district of Karnataka state, India

Table 1. Incidence and Prevalence of *S. rolfsii* in Mysuru District

Taluk	Village	GPS coordinate	No. of fields inspected	% Incidence	% Prevalence
Mysuru	Madagalli	12.3177089, 76.5652623	5	20	40
	Dadadahalli	12.2093058, 76.6456887	7	25	42.85
	Harohalli (J)	12.3208226, 76.7820622	6	38.88	33.33
	Kiralu	12.2325726, 76.7438605	5	19.04	20
	Madahalli	12.2654726, 76.5163516	5	18.75	40
	Manikyapura	12.3097262, 76.5221525	6	36.11	50
	Ranganathapura	12.3263612, 76.8136432	5	**69.64**	**80**
	Shettinayakanahalli	12.2922207, 76.5290438	5	23.33	40
	Siddaramayyana Hundi	12.2139921, 76.8114639	7	36.55	42.85
T. Narasipura	Ankanahalli	12.3316359, 76.4921580	8	29.88	37.5
	Chikkabagilu	12.2609228, 76.9716773	6	16.40	33.33
	Krishnapura	12.2406192, 76.9593811	6	27.77	50
	Naviluru	12.3723988, 75.9946921	6	31.93	28.57
	Sosale	12.2418260, 76.9083621	7	34.61	50
Nanjanagud	Heggadahalli	12.1038724, 76.5969100	6	15.44	33.33
	Hullahalli	12.1008505, 76.5567631	3	10.73	25
	Mallahalli	12.0063241, 76.7074616	4	13.29	50
Hunsur	Kothegala	12.345939, 76.235562	4	18.75	25
	Niluvagilu	12.3012980, 76.2704624	4	29.62	50
	Thammadahalli	12.3315960, 76.2637421	4	17.39	40
K.R. Nagara	Chunchanakatte	12.5014834, 76.2938395	5	23.40	40
	Haradanahalli	12.5838395, 76.2064132	6	**57.97**	**66.66**
	Saaligrama	12.5649649, 76.2628949	8	31.63	37.5
H.D. Kote	Kattemanuganahalli	12.0718474, 76.3149622	7	41.12	**71.42**
	Masanakuppe	12.2421242, 76.4662851	6	**58.13**	**83.33**
	Chamanahalli	12.6106006, 77.0378920	5	**55.34**	**80**
District mean incidence and prevalence				30.79	45.53

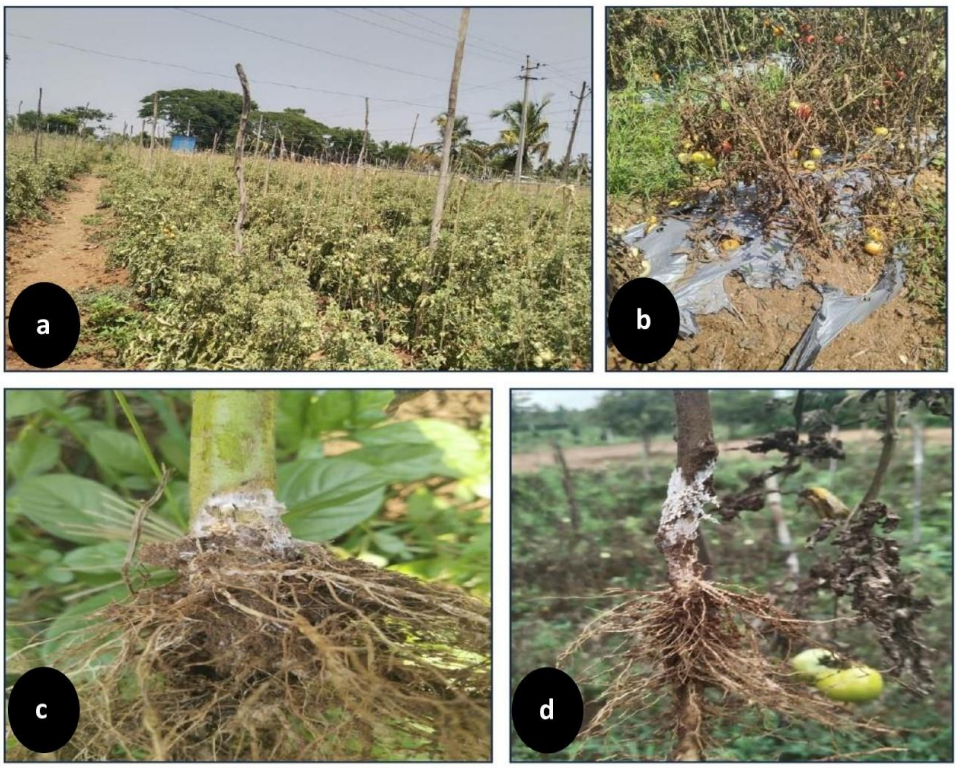

Fig. 2. Southern blight disease on tomato in Mysuru district. (a- Tomato field; b- Death of tomato plant due to southern blight; c & d- White cottony mycelial development on the lower stem adhering to soil during early and later stages of disease development.)

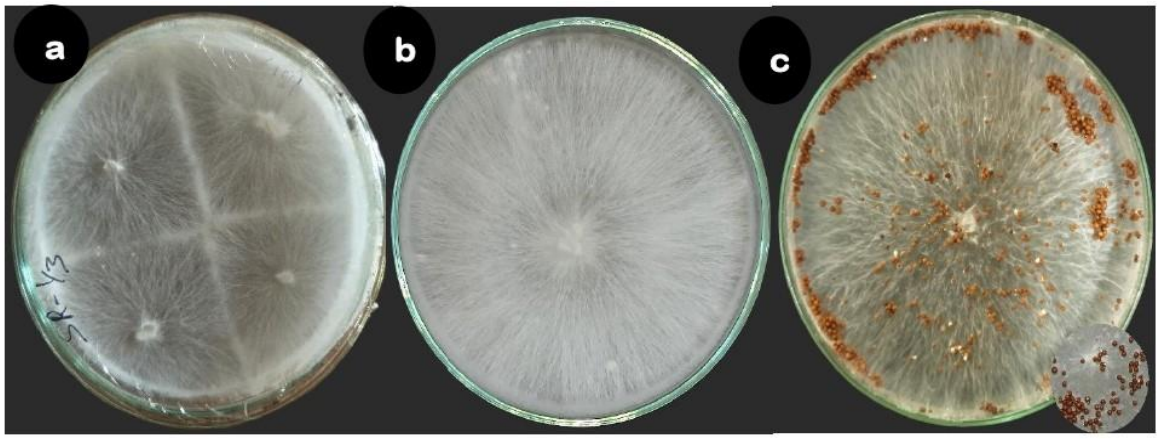

Fig. 3. *S. rolfsii* growth on culture media (PDA). (a & b- White cottony mycelial growth on PDA; c- Formation of matured sclerotial bodies on PDA)

3.2 Morphological Characterization

The fungal colonies on potato dextrose agar (PDA) exhibited dense, aerial, white, cotton-like mycelium with globoid sclerotia forming after 10 days of inoculation. The sclerotia were small, uniformly sized, and present in large quantities (Fig. 3). Sclerotia per plate ranged from 276 to 360 (mean 312 ± 6.14; n=20). The sclerotia measured 1–3 mm in diameter (mean 2.21 ± 0.11; n=20), initially appearing white and turning dark brown upon maturation. Based on the observed cultural and morphological characteristics, the fungal pathogen was identified as *Sclerotium rolfsii* Sacc. (Teleomorph: *Agroathelia rolfsii* (Sacc.) Redhead & Mullineux).

3.3 Pathogenicity Test

Healthy tomato seedlings inoculated with the fungal pathogen began to display typical symptoms 5-7 days post-inoculation. A small, water-soaked lesion formed at the base of the stem near the soil surface, rapidly expanding to wrap the stem. A white mycelial mat developed at the lesion site as the lower stems died, often spreading into the surrounding soil (Fig. 4). No disease symptoms were observed in seedlings inoculated with sterile distilled water. The fungal pathogen was re-isolated from the infected plants, and its identity was reconfirmed through its cultural and morphological characteristics.

3.4 Molecular Characterization

The amplified PCR products were sequenced in both directions. The consensus sequences obtained were aligned with ITS sequences from GenBank. Three amplified DNA sequences were deposited in GenBank under accession numbers PQ136753.1, PQ136831.1, and PQ452721.1. A similarity search using nBLAST revealed 100% similarity between the sequences obtained in this study and representative sequences in GenBank. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method in MEGA 11 software. All characters were analyzed as unordered with equal weighting, and gaps were treated as missing data. Bootstrap analysis with 1000 replicates was conducted to assess node support for the generated trees. This analysis involved 12 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 725 positions in the final dataset. The phylogenetic tree demonstrated that the representative sequence from this study grouped within the same clade as sequences from GenBank, confirming the pathogen's identity (Fig. 5).

4. DISCUSSION

The results of this survey provide a thorough understanding of the incidence and prevalence of southern blight, caused by Sclerotium rolfsii, across various taluks in the Mysuru district. The data reveal substantial variability in disease intensity. Notably, high disease incidence and prevalence were observed in taluks such as Mysuru, H.D. Kote, and K.R. Nagara, underscoring the role of environmental factors, particularly elevated humidity and rainfall, in promoting the spread and development of the pathogen. For example, Ranganathapura and other villages in H.D. Kote taluk recorded some of the highest rates of incidence (up to 69.64%) and prevalence (up to 80%). This can likely be

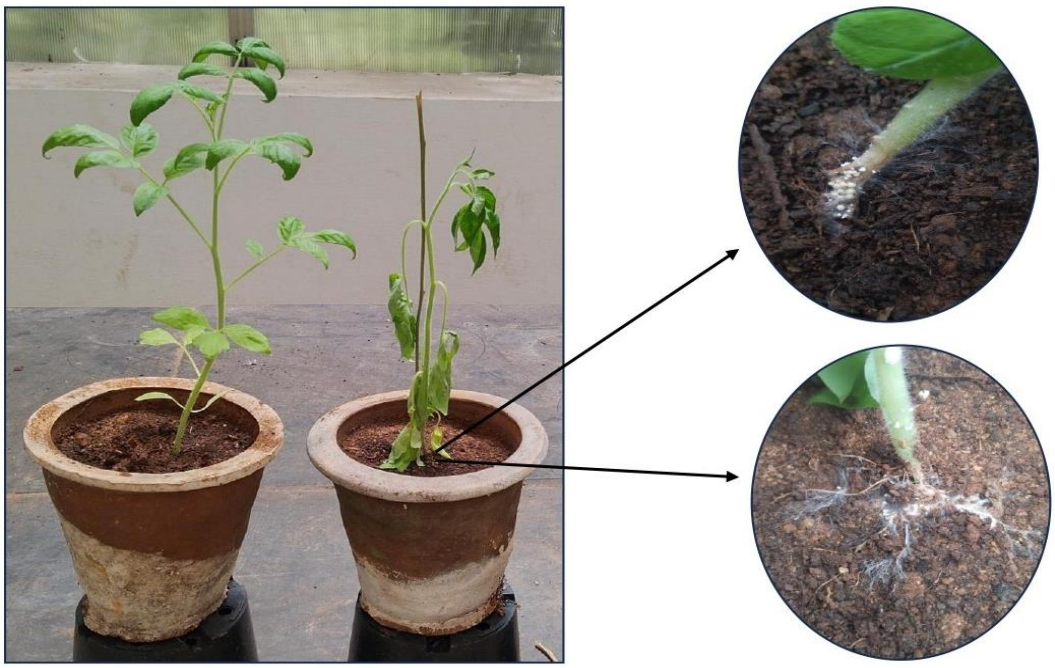

Fig. 4. Pathogenicity test showing typical southern blight symptoms after 3 days of post inoculation of *S. rolfsii*. White mycelial mat development around the lesion site and spreading out to the surrounding soil

attributed to the favorable environmental conditions in these regions, including prolonged moisture, which enhances the pathogen's survival and virulence.

The variation in disease incidence across the district aligns with previous studies that have documented the widespread and destructive impact of *S. rolfsii*. This soil-borne pathogen, capable of infecting over 500 plant species, spreads rapidly due to its production of cell-degrading enzymes (Javaid et al., 2021; Paul et al., 2023). Its persistence in the soil for 2–3 years as sclerotia, as noted by Aycock (1966) and Punja (1985), enables it to initiate new infections in subsequent cropping seasons. In regions with conducive environmental conditions, such as Himachal Pradesh, India, incidences of *S. rolfsii* on tomatoes have been reported between 10% and 45% (Banyal et al., 2008), and crop losses of up to 30% have been documented in Tamil Nadu (Thiribhuvanamala et al., 1999). Similarly, our study found high incidences in taluks like H.D. Kote.

Conversely, the significantly lower incidences recorded in Nanjanagud taluk, with Hullahalli having the district's lowest incidence rate (10.73%), might be attributed to less favorable environmental conditions or differing agricultural practices that limit the pathogen's spread. This variability highlights potential factors that may suppress disease, consistent with findings from other crops such as groundnut, where effective management practices have been implemented (Bera et al., 2014; Iquebal et al., 2017).

Pathogenicity tests further confirmed *S. rolfsii* as the causal agent of southern blight. Infected tomato seedlings exhibited typical symptoms, including water-soaked lesions and a white mycelial mat formation within 5-7 days post-inoculation. These symptoms are characteristic of southern blight and have been described in crops such as groundnut, common bean, cotton, and pumpkin (Mullen, 2001; Mahadevakumar et al., 2015; Aycock, 1966). The pathogen's re-isolation from infected plants reaffirmed its virulence and rapid colonization under favorable conditions.

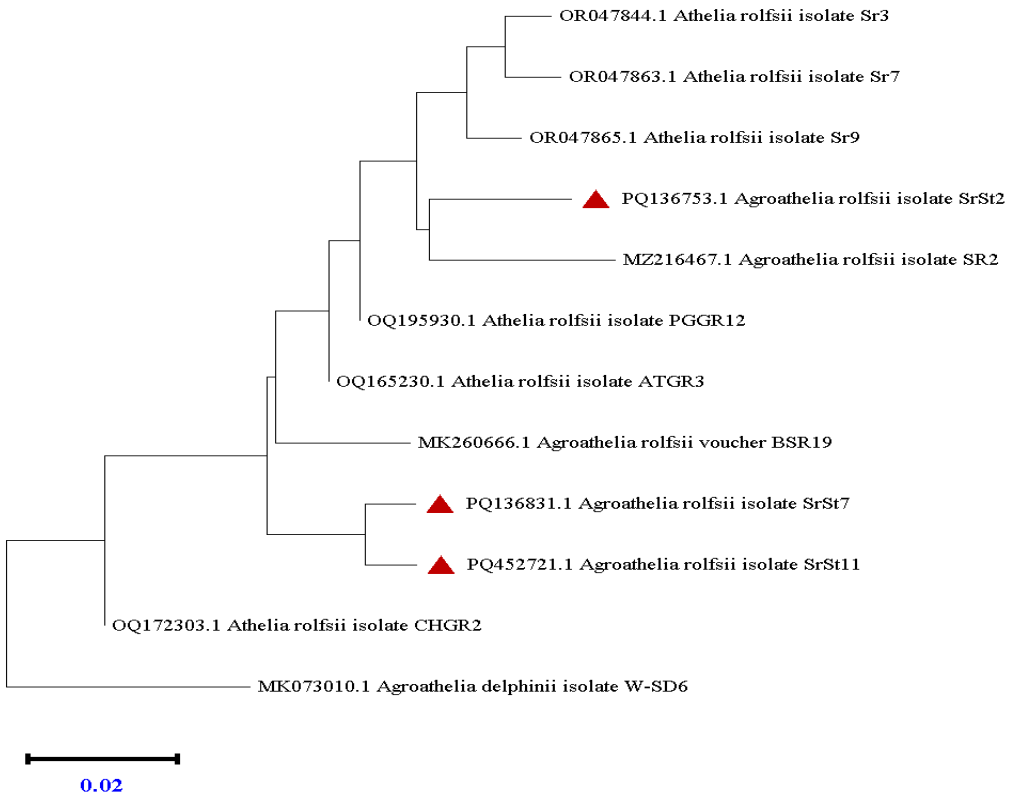

Fig. 5. Phylogenetic tree constructed for ITS region of *S. rolfsii* (*Agroathelia rolfsii*) isolates by Neighbor Joining method and the tree was rooted with *S. delphinii* (*Agroathelia delphinii*). Tamura-Nei substitution model and nearest neighbor-interchange search options with 1000 bootstrap replicates were used

The morphological identification of *Sclerotium rolfsii*, evidenced by the production of small, globoid sclerotia that transition from white to dark brown on PDA plates, corroborates previous descriptions (Mahadevakumar et al., 2015; Punja and Grogan, 1981; Mordue, 1974; Saccardo, 1931). Such morphological features are vital for quick field identification, facilitating early detection and intervention. *S. rolfsii* was differentiated from *Sclerotium delphinii* (*Agroathelia delphinii*) by the number and size of its sclerotia, as *S. delphinii* typically produces 20–30 sclerotia per plate, each measuring 3–5 mm in diameter (Stevens, 1931; Punja and Damiani, 1996; Mahadevakumar and Janardhana, 2016; Mahadevakumar et al., 2018). Molecular techniques, including ITS sequencing, further confirmed the identity of the pathogen, showing 100% sequence similarity with known *S. rolfsii* strains (Bennett et al., 2021; Wei et al., 2021). Phylogenetic analysis using the Neighbor-Joining method (Saitou and Nei, 1987), supported by robust bootstrap values (Felsenstein, 1985), demonstrated that the isolates from Mysuru share a common clade with global strains of *S. rolfsii*, verifying the pathogen's identity. The percentage of replicate trees in which associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Tamura-Nei method (Tamura and Nei, 1993), and the analysis involved 12 nucleotide sequences, with all ambiguous positions removed for each sequence pair. A total of 725 positions were analyzed, and the tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). These findings resonate with global reports of *S. rolfsii* as a prominent pathogen affecting various crops, including common bean, soybean, peanut, and garlic (Mahadevakumar et al., 2015; Parwanayoni et al., 2021; Lukose et al., 2003). The widespread presence of this pathogen, as seen in crops from countries such as the USA, Indonesia, and China (Le et al., 2012; Iquebal et al., 2017), underscores its significance as a soil-borne pathogen capable of causing extensive damage to crops on a global scale. Early detection through both morphological and molecular identification remains crucial for effective management and control of this destructive pathogen.

5. CONCLUSION

In conclusion, this study offers an in-depth analysis of the distribution and severity of southern blight in the Mysuru district, utilizing morphological, pathogenicity, and molecular data. The high incidence rates observed in specific taluks underscore the necessity for targeted disease management strategies, such as adopting resistant tomato varieties, crop rotation, and strategic fungicide applications based on weather conditions. Integrating field surveys with laboratory diagnostics provides a solid framework for future disease control programs aimed at reducing the damaging impact of *Sclerotium rolfsii* on tomato cultivation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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