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Integrated management strategies against tomato collar rot (*Sclerotium rolfsii*): A characterisation to management study

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ABSTRACT

The study was carried out during winter (*rabi*) season of 2022–2023 at Dharmapuri and Cuddalore districts of Tamil Nadu to formulate an integrated strategy for managing tomato collar rot, a major disease caused by *Sclerotium rolfsii* Sacc. The survey identified Keerapatti village as a hotspot with 47.53% incidence. Among fifteen isolates, Sr_{10} was the most virulent, causing 62.30% disease incidence in pot experiments. Molecular characterisation confirmed the pathogen as *S. rolfsii* and antagonists as *B. subtilis* showing 98.8–99.0% nucleotide identity with reference sequences. A native antagonist, *B. subtilis* isolate Bs_3, exhibited strong antifungal activity with 78.94% inhibition *in vitro*, significantly suppressing mycelial growth (77.41%) and sclerotial formation (84.40%) through secondary metabolites. Molecular docking analysis further demonstrated that key antifungal compounds from *B. subtilis* showed strong binding affinity with pathogen target proteins, suggesting disruption of pathogenicity-related mechanisms. In pot trial, the integrated treatment (T_7), combining *B. subtilis* with neem cake, was most effective, reducing collar rot incidence by 78.29%. Additionally, treated plants exhibited enhanced vigour, with maximum plant height, germination rate, and fruit yield. Collectively, these findings highlighted that integrating *B. subtilis* and neem cake, supported by docking-based mechanistic evidence, offers a sustainable and eco-friendly alternative to chemical fungicides for collar rot management in tomato (*Solanum lycopersicum* L.).

Keywords: *Bacillus subtilis*, Integrated disease management, Neem cake, Soil borne

Tomato (*Solanum lycopersicum* L.) is one of the world's most widely cultivated and economically significant vegetable crops. However, its productivity is severely threatened by *Sclerotium rolfsii* Sacc., the causal agent of collar rot, a pervasive soil-borne fungus that causes wilting, stem lesions, and plant death under warm and humid conditions (Aljabali *et al.* 2025). The control of this pathogen typically relies on chemical fungicides, a practice that is now widely scrutinised due to growing concerns over environmental toxicity, human health risk and rapid selection of fungicide resistant pathogen strains. Consequently, there is an urgent global shift towards sustainable and ecofriendly disease management strategies (Bhagat 2014). Biological control, particularly utilising plant growth promoting rhizobacteria has shown exceptional potential for both disease suppression and growth promotion. *Bacillus* species suppress pathogen through multiple mechanism including the production of antifungal secondary metabolites, direct competition for nutrients and inducing systemic resistance in host plant (Baruzzi *et al.* 2011). In addition, combinations of bioagents with soil amendments and other organic materials have been found to significantly promote plant growth while reducing disease severity (Senthilraja *et al.* 2010, Latha *et al.* 2011).

To accelerate the discovery and validation of these biocontrol mechanism, molecular docking provides critical, cutting-edge dimension. This *in silico* approach enables the computational screening of potential molecular inhibitors that can precisely target and disrupts pathogen proteins or pathways. This capability significantly accelerates the development pipeline for novel biopesticides and reduces subsequent resource intensive *in vitro* and pot trial evaluations. This study aimed to survey, characterise and identify the *Sclerotium rolfsii*, while concurrently evaluating integrated ecofriendly management strategies that are mechanistically supported by molecular docking analysis.

MATERIALS AND METHODS

Survey and sample collection: A roving field survey was conducted during winter (*rabi*) season of 2022–2023 at Dharmapuri and Cuddalore districts of Tamil Nadu to determine the incidence of tomato collar rot disease of

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tomato. Samples were collected from various tomato fields from which fifteen purified pathogen isolates were obtained and subsequently used for pathogen isolation and diversity analysis. Percent disease incidence (PDI) was calculated using the formula suggested by Sunkad (2012):

PDI = Number of plants affected /Total number of plants observed ×100

Isolation, morphological, sclerotial characterisation, and scanning electron microscopy (SEM): The pathogen, *S. rolfsii*, was isolated from the lower stem sections of infected plants using a tissue segmentation method (Rangasami and Mahadevan 1999). 5 mm infected segments were surface sterilised with 1% NaOCl solution, washed thoroughly for 5 min, and placed on potato dextrose agar (PDA) medium amended with 0.1% streptomycin sulphate, plates were then incubated at 28 ± 2°C for seven days. The isolates were purified using single hyphal tip culture and pure cultures were labelled as isolates Sr_1–Sr_{15}. Mycelial growth and colour were recorded at five days after incubation (DAI). Sclerotial characteristics including numbers/plate, size, shape, and colour were observed after 30 DAI. A screw gauge (0–25 mm Caliper Steel, Generic, Mathworld) was used to measure sclerotial size, and a mycological colour chart was used for colour assignment (Rayner 1970). Scanning Electron Microscopy (SEM) was used to capture high-resolution images of the hyphal morphology (Elad *et al.* 1982).

Pathogenicity test: The pathogenicity of the *Sclerotium* isolates was tested using pot culture experiments under greenhouse conditions. A sand maize medium (19:1) was used to mix the inoculum of each isolate into sterilised soil at a rate of 20 g/kg, corresponding to approximately 6×10^3 sclerotia/kg soil based on the average sclerotial density of the culture. The potting mixture comprised of soil, sand and farmyard manure in a 1:1:1 ratio and was sterilised prior to use. Approximately 5 kg of the soil was filled into 30 cm diameter pots 10 days before transplanting tomato varieties PKM1 and Shivam. A control was maintained without the inoculum. One-month old seedlings were transplanted, and observations on collar rot symptoms were recorded at 30 days after transplanting (DAT).

Molecular characterisation and phylogenetic analysis

Fungal isolates: Genomic DNA was extracted from mycelia following protocol of Zolan and Pukkila (1986). The ITS (Internal Transcribed Spacer) region was amplified using universal primers ITS1 and ITS4, and PCR (Polymerase chain reaction) products were resolved on a 2% agarose gel and visualised under UV light. Sequence analysis of all generated nucleotide sequences after sequenced were compared with reference sequences in NCBI GenBank using BLAST, and phylogenetic relationships were inferred using MEGA 12 (v.7.0.20) with the maximum likelihood method and bootstrap analysis (Kumar *et al.* 2018, Tao *et al.* 2020).

Isolation and characterisation of native rhizosphere antagonists: Native antagonists were isolated from rhizosphere of healthy tomato plants (cv. Shivam) using serial dilution up to 10^{-7}. Aseptic plating of 1 mL of 10^{-6} and 10^{-7} dilutions onto nutrient agar medium, followed by incubation at 28 ± 2ºC for 48 h. Colonies exhibiting creamy white and yellow pigmentation were selected and purified by repeated streaking and designated sequentially as Bs_1–Bs_{15}. Well-grown actively growing cultures of *Bacillus* spp. were subjected to biochemical characterisation following standard procedures described in Bergey's manual for determinative bacteriology (Bergey *et al.* 1984).

Dual culture assay: The antagonistic activity of the *Bacillus subtilis* isolates (Bs_1–Bs_{15}) against *S. rolfsii* was evaluated using the dual culture technique (Jeevanantham *et al.* 2025a). A loopful of the *Bacillus* isolate was streaked on a medium alongside a seven days old culture of the pathogen. The radial growth of the pathogen and the width of the inhibition zone were measured at 7 DAI. The percentage of growth inhibition was calculated using the formula described by Datta *et al.* (2004).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where C, Diameter of mycelial growth of the pathogen in control; T, Diameter of mycelial growth of the treatment.

Molecular characterisation of bacterial isolate (Bs_3)

Bacterial isolates: The genomic DNA of the potent bacterial isolate was extracted from broth culture. The *16S rRNA* gene was amplified using universal bacterial primers, and PCR products were sequenced to obtain nucleotide data (Ghosh *et al.* 2014, Sagar *et al.* 2024). Amplicons were checked on a 2% agarose gel under UV illumination and deposited in NCBI database.

GC-MS and in silico *analysis*: Analysis of antifungal compounds produced by *B. subtilis* was performed using Gas Chromatography-Mass Spectrometry (GC-MS). A thermo scientific GC-MS system with an RTX 5Ms capillary column was used, employing helium as the mobile phase. The process, including temperature ramping from 50–280°C, took approximately 40.33 min. GC-MS data were analysed for pathway involvement. The biologically relevant metabolites were annotated with KEGG compound identifiers and subjected to KEGG pathway enrichment analysis using MetaboAnalyst v5.0.

AutoDock Vina within the PyRx virtual screening tool (Trott and Olson 2010) was utilised for docking where drug molecules from the PubChem Database and the crystal structure of the *S. rolfsii* protein 4YLD was retrieved from protein database based on several studies. Molecular docking investigations were conducted using AutoDock 4.2 in the PyRx Virtual Screening Tool (Saddala *et al.* 2013, Saddala and Adi 2018) to generate the docking key files. The interaction was visualised using BIOVIA Discovery Studio.

Pot assay: A pot culture experiment was conducted using a standardised potting mixture prepared from red soil, sand and FYM in a 2:1:1 ratio (v/v) in completely randomised design (CRD) with three replications. The

susceptible tomato cv. PKM 1 was used, and the soil was amended with *S. rolfsii* (20 g/pot) inoculum before transplanting. The experiment included nine treatments, viz. T_1, Seed treatment with *B. subtilis* @10 mL/kg of seeds; T_2, Soil application of *B. subtilis* @40 mL/pot; T_3, soil application of neem oil cake 20 g/pot; T_4, $T_1 + T_2$; T_5, $T_1 + T_3$; T_6, $T_2 + T_3$; T_7, $T_1 + T_2 + T_3$; T_8, Carbendazim 50% WP @4.0 g/kg; T_9, Inoculated control; and T_{10}, Healthy control. Disease incidence was recorded at 40 and 60 days after sowing (DAS) and at harvest.

Statistical analysis and data visualisation: Data from all experiments were analysed using WASP Version 1.0, a web-based statistical package for agricultural research. Disease incidence values were arcsine-transformed and treatment means were separated using Duncan's Multiple Range Test (DMRT) at the 5% significance level ($p<0.05$) (Gomez and Gomez 1984). For data processing and visualisation, Python version 3.7.8 was employed.

RESULTS AND DISCUSSION

Survey: A survey conducted during 2022–2023 across major tomato growing regions of Dharmapuri and Cuddalore districts of Tamil Nadu revealed varying level of collar rot incidence in field (Fig. 1). The highest incidence was recorded in Keerapatti (47.53%) while the lowest was observed in Palacode (17.66%). These findings revealed significant spatial variation, aligning with earlier reports of Tanjila *et al.* (2024) who conducted the survey in northern districts of Bangladesh and concluded the higher foot rot disease incidence up to 54.09% in Bagmara. Similarly, crown and root rot of tomato in Beni Suef County, Egypt recorded the maximum disease incidence of 48.7% and 59.0% and disease severity of 22.3% and 31.8% in 2018 and 2019, respectively (Abd-Elghany *et al.* 2021). Collar rot is one of the major threats in Bangladesh, with incidence ranging from 10–45% (Ahmed and Hossain 1985, Meah 2003).

Morphological characterisation: All *S. rolfsii* isolates exhibited distinct morphological characteristics, with white, fan-like mycelial growth. Isolates Sr_{10} showed the maximum radial growth (90.00 mm), producing 260–295 sclerotia/plate, while Sr_{12} exhibited minimum growth (62.00 mm) (Table 1). Colony colour varied from pale to dark brown and sclerotia differed in oval, spherical and round shape and size varied from 1.10–1.65 mm. SEM analysis of the Sr_{10} isolate revealed profuse hyphal branching and spherical sclerotial structure (Fig. 2). Our observation was consistent with previous studies. Paul *et al.* (2023) reported two isolates $BTCBSr_3$ and $BTCBSr_4$ which produced hyaline mycelium, colony diameters of 4.10–8.00 cm, and sclerotia numbers between 261.7 and 1048.7. Similarly, Tanjila *et al.* (2024) categorised isolates by growth rate, with sclerotial sizes of 1.10–2.10 mm and shapes ranging from spherical to round (Manu *et al.* 2018). Hawaladar *et al.* (2022) also noted that isolates SrD1 and SrB2 produced larger and smaller sclerotia, respectively (0.88–2.33 mm). These morphological differences likely reflect genetic diversity and adaptive responses to environmental conditions, which may influence pathogenicity and survival.

Pathogenicity test: Pathogenicity was confirmed through artificial soil inoculation, where all the *S. rolfsii* isolates caused disease produced similar disease symptoms on both tomato varieties, PKM 1 and Shivam. Among the isolates, Sr_{10} recorded the maximum PDI in both varieties accounting 62.30% in PKM and 56.90% in Shivam (Fig. 3). These results supported the earlier findings of Nofal *et al.* (2021) that all isolates were pathogenic, causing characteristic wilt disease symptoms and (F1) isolate up to incidence of 60%. Similarly, Mahato and Biswas (2017) reported the pathogenicity test of *S. rolfsii* on tomato seedling and isolates i.e. SRPU-1, SRPU-2 and SRPM-1 showed highly disease reaction. All 10 isolates of *S. rolfsii* tested on tomato plants significantly influenced the germination percentage, pre-emergence death, damping off, foot rot and plant stand (Meah 2003). The consistency of

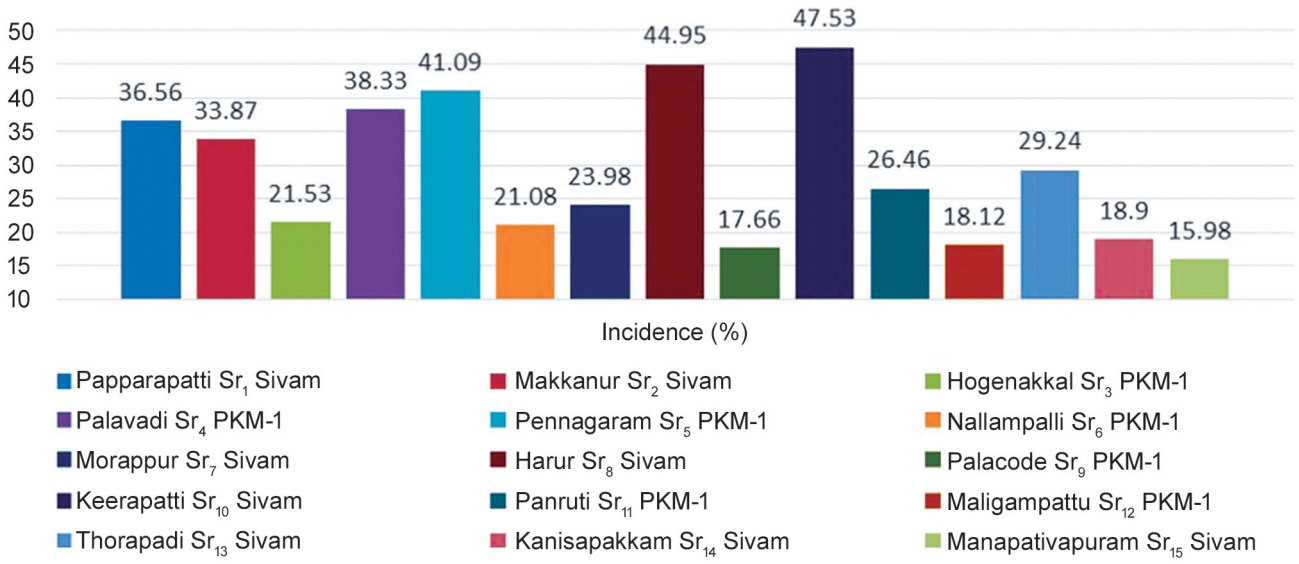

Fig. 1 Survey on the incidence of collar rot of tomato.

Table 1 Cultural and morphological variability of mycelial and sclerotia of *S. rolfsii*

Isolate no.	Colour of mycelium	Mycelial growth at 5 DAI (mm)*	Colour of sclerotia	Shape of sclerotia	Diameter of sclerotia (mm)*	No. of sclerotia/plate (30 DAI)	Sclerotial production
Sr_1	Fluffy white	88.13	Pale brown	Oval	1.50	219	++++
Sr_2	White cottony	86.45	Dark brown	Spherical	1.45	201	++++
Sr_3	White cottony	88.89	Dark brown	Round	1.30	252	++++
Sr_4	Dull white	73.15	Pale brown	Oval	1.25	96	++
Sr_5	Fluffy white	87.53	Brown	Round	1.55	158	+++
Sr_6	White cottony	88.91	Dark brown	Spherical	1.45	260	++++
Sr_7	White cottony	79.09	Dark brown	Spherical	1.10	174	+++
Sr_8	Dull white	90.00	Pale brown	Oval	1.50	278	++++
Sr_9	Dull white	79.53	Brown	Round	1.10	109	+++
Sr_{10}	Milky white	90.00	Dark brown	Oval	1.65	295	++++
Sr_{11}	White cottony	69.21	Pale brown	Spherical	1.20	113	+++
Sr_{12}	White cottony	62.00	Brown	Round	1.10	72	++
Sr_{13}	Dull white	74.33	Dark brown	Spherical	1.45	130	+++
Sr_{14}	Milky white	70.45	Brown	Oval	1.45	121	+++
Sr_{15}	Dull white	63.12	Pale brown	Oval	1.10	98	++

DAI, Days after incubation; ++++, Excellent; +++, Good; ++, Moderate. *Mean of three replications.

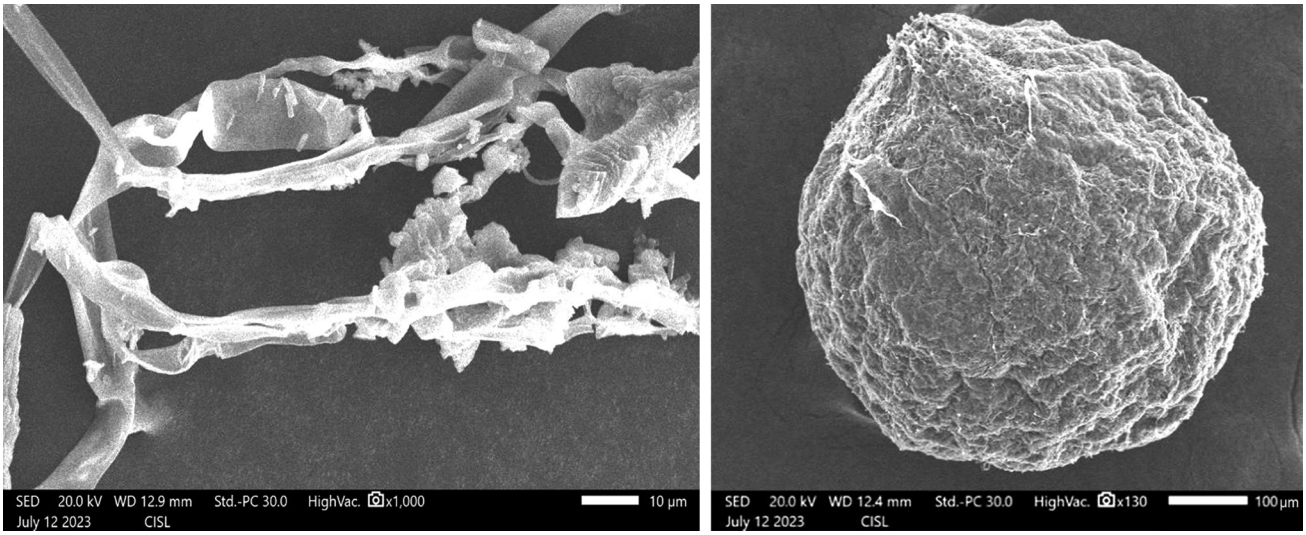

Fig. 2 Scanning electron micrographs showing mycelial aggregation (1000×) and sclerotia (resting structure 130×) of *Sclerotium rolfsii* isolate Sr_{10}.

these results with earlier studies strengthens the evidence that aggressiveness of isolates varies widely, and such variability plays a critical role in disease severity across different tomato varieties.

Molecular confirmation and dendrogram analysis: Molecular identification using ITS1 and ITS4 primers successfully amplified DNA fragments ranging from 464–684 bp in *S. rolfsii* isolates, which were identified as *Agroathelia rolfsii* through BLAST analysis, showed 99% similarity with previously reported strains and sequences were submitted to NCBI (Accession Nos. OR594404, OR594405, OR594403, OR594401, and OR594402). Similar amplicon sizes were reported by Sailaja Rani *et al.* (2024), who obtained ITS amplicons of 650–700 bp. Likewise, Jebaraj *et al.* (2017) also recorded amplification of 22 *S. rolfsii* isolates in range of 650–700 bp. Paul *et al.* (2023) demonstrated that isolates BTCBSr3 and BTCBSr4 showed 98.14% homology with *A. rolfsii* strains, while Hawaladar *et al.* (2022) further confirmed the identity of ten isolates with 90.10–98.80% similarity, where phylogenetic clustering validated their close relationship with known *S. rolfsii* isolates. In present study, dendrogram analysis grouped the isolates into a single clade, indicating a high degree of genetic relatedness, through with bootstrap support

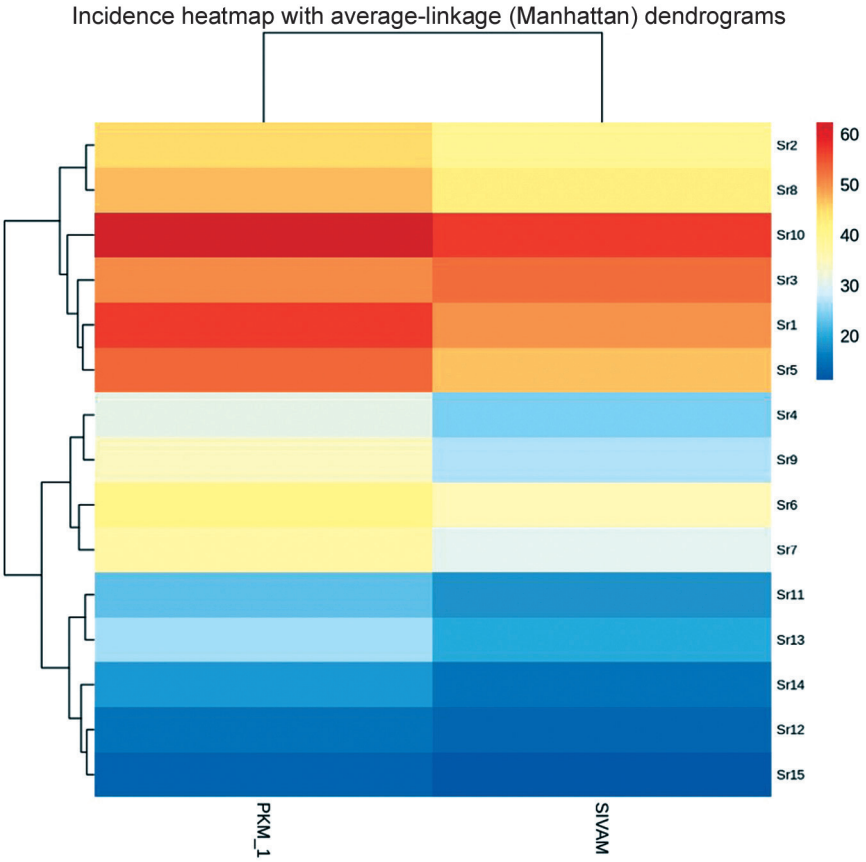

Fig. 3 Heatmap illustrating disease incidence of *Sclerotium rolfsii* isolates clustered using Manhattan distance and average-linkage hierarchical clustering.

values ranging from 34–100 value (Fig. 4). Similarly, molecular confirmation of *Bacillus subtilis* isolate using *16S rRNA* gene amplification yielded 1258 bp amplicons, with BLAST results revealing 99–100% similarity in reference to *B. subtilis* sequences, and the sequences were submitted to NCBI (Accession no PX317253). Phylogenetic analysis of *Bacillus subtilis* isolates clustered the isolates into a single clade, suggesting genetic relatedness, with bootstrap support values ranging from 27–36 (Supplementary Fig. 1). These results were corroborating previous reports of Shi *et al.* (2024) where *B. subtilis* MC4-2 strain using partial genome sequencing in combination with *16s rRNA* gene analysis shared high sequence similarity strain. In a similar trend, phylogenetic analysis of the *16S rRNA* gene sequence of isolate A1S6 revealed that the bacterial isolate belongs to the genus *Bacillus*, as it clustered with reference *Bacillus* species in the phylogenetic tree with a bootstrap support value of 78 (Sadiqi *et al.* 2022).

In vitro *analysis*

Dual culture: The antagonistic potential of fifteen *B. subtilis* isolates against *S. rolfsii* was evaluated under *in vitro* condition. Among them, isolate Bs_3 exhibited the highest efficacy, suppressing mycelial radial growth by 78.94%, followed by Bs_4 (75.30%) (Supplementary Fig. 2). These findings highlighted the strong inhibitory capacity of specific *Bacillus* isolates by producing secondary metabolites against the *S. rolfsii* by shrinkage and wrinkling (Supplementary Fig. 3). The findings of the present study were in agreement with earlier reports showing strong antagonistic activity of *Bacillus* spp. against soil-borne pathogens. In a previous evaluation of bacterial bioagents, all tested isolates significantly suppressed the radial growth of the pathogen, with inhibition levels reaching up to 73% within four days of incubation, indicating rapid and effective antagonism (Vasanthi *et al.* 2025). Similar levels of growth suppression observed in our study suggest that the inhibitory potential of *Bacillus* isolates is largely attributable to their ability to produce extracellular antifungal metabolites. Similar results have been documented in earlier studies. Farhaoui *et al.* (2022) reported that *Bacillus* strains reduced the radial growth of *S. rolfsii* more than 50% within four days, with *B. amyloliquefaciens* showing up to 70.35% inhibition. Likewise, Kumari *et al.* (2021) demonstrated comparable antifungal activity in dual culture assay, where isolates CBK2007 and CBK5001 effectively suppressed *S. rolfsii*, largely attributed to the production of secondary metabolites.

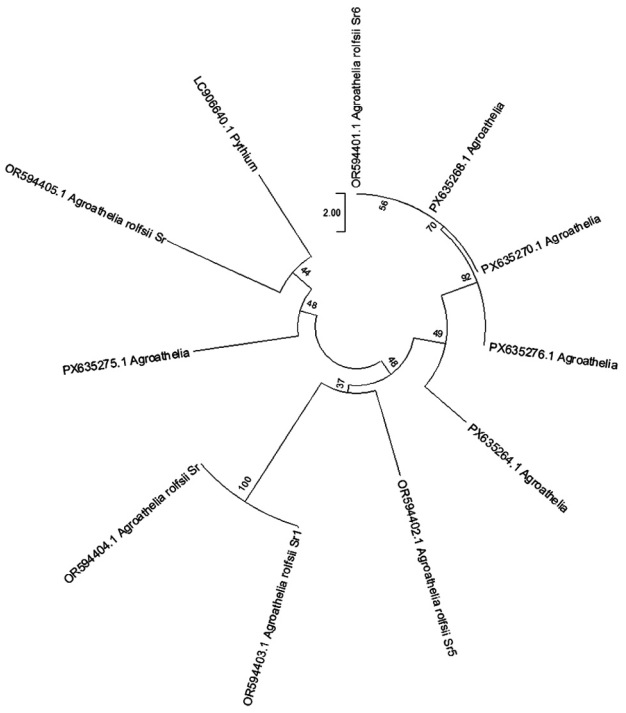

Fig. 4 ITS rDNA–based phylogenetic tree of *Agroathelia rolfsii* (*Sclerotium rolfsii*) constructed using the maximum likelihood method in MEGA 12.
Bootstrap values (1000 replications) are shown at the nodes.

GC-MS and molecular docking: GC-MS identified 30 putative metabolites and most of the metabolites showed predominant enrichment of lipid-associated pathways, with glycosylphosphatidylinositol (GPI)-anchor biosynthesis, biosynthesis of unsaturated fatty acids, and steroid biosynthesis being the most significant. Additional enrichment of fatty acid biosynthesis, elongation, and degradation pathways indicated a central role of lipid and sterol metabolism in antagonist-derived metabolites (Supplementary Fig. 4). The docking analysis successfully identified that three bioactive compounds derived from *B. subtilis* from GC-MS notably, Squalene, cyclohexasiloxane and ergost-5-en-3-ol(beta) successfully interacted with the lectin protein (4YLD) of *A. rolfsii* (Supplementary Table 1). Among the tested metabolites, squalene exhibited the highest binding profile of -6.17 kcal/mol, forming stable complexes through conventional hydrogen bonds (Supplementary Fig. 5). Squalene is a triterpene precursor in the ergosterol biosynthetic pathway. Ergosterol is a cholesterol analog essential for maintain the fluidity, stability and integrity of the fungal cell membrane and its disruption is a known mechanism of action for several commercial antifungal activity. The strong affinity observed in squalene compound may interfere with lection associated functions or mimic sterol interactions critical for *S. rolfsii* virulence. Previous docking studies indicate that metabolites from *Bacillus licheniformis* AUB7 can interact strongly with virulence-associated proteins of *Sclerotium rolfsii*, exhibiting high binding affinities (Jeevanantham *et al.* 2025b). This finding is significant because sterol is a precursor and its accumulation is known to disrupt ergosterol biosynthesis, leading to fungal membrane instability (Ryder 1992). The strong predicted interaction of *Bacillus*-derived secondary metabolites with transferase proteins of *Macrophomina phaseolina* suggests a potential inhibitory effect on key metabolic processes of the pathogen (Dhanabalan *et al.* 2024). Ergosterol is essential for fungal membrane stability, virulence and stress tolerance (Alcazar-Fuoli *et al.* 2016).

Integrated management on disease incidence and biometrics under pot culture: In pot trial, the integrated treatment (T_7) combining *B. subtilis* and neem cake was most effective, reducing collar rot incidence to 9.76% at harvest, outperforming carbendazim (11.45%) and control (44.97%). The treatment T_7 significantly enhanced plant growth and maximised fruit yield (2.13 kg/plant) compared to both carbendazim and control treatments (Table 2). The present result aligned well with earlier studies. Wang *et al.* (2024) demonstrated that combined application of *B. amyloliquefaciens* with organic amendments significantly reduced disease incidence and improved the fruit quality on tomato under pot trail. Similarly, Khalid *et al.* (2023) reported that the integration of organic amendments with phosphate solubilising bacteria enhanced plant traits such as plant height (66%), root length (52%), pods dry

Table 2 Integrated management of *S. rolfsii* using *B. subtilis* and neem oil cake under pot culture

Treatments	Collar rot incidence (%)*				Per cent disease reduction over control			Yield biometrics		
	40 DAT	60 DAT	At harvest	Mean	45 DAT	60 DAT	At harvest	Plant height (cm)	Fruit/ plant	Fruit yield (kg/plant)
T_1	28.34 (32.28)	32.94 (35.15)	34.10 (35.86)	31.12 (34.03)	26.71	20.41	24.17	98.02 ^{g}	15.11 ^{g}	1.06 ^{g}
T_2	21.22 (27.42)	28.10 (32.01)	30.19 (33.32)	26.50 (30.98)	45.12	32.10	32.86	101.20 ^{f}	16.91 ^{f}	1.22 ^{f}
T_3	31.30 (34.01)	35.28 (36.43)	37.13 (37.53)	34.57 (36.00)	19.05	14.76	17.43	97.50 ^{h}	13.54 ^{h}	0.92 ^{h}
T_4	11.20 (19.54)	14.30 (22.21)	15.50 (23.18)	13.66 (21.68)	71.03	65.45	65.53	105.87 ^{d}	20.97 ^{d}	1.62 ^{d}
T_5	14.46 (22.34)	17.29 (24.57)	19.45 (26.16)	17.06 (24.39)	62.60	58.22	56.74	101.78 ^{e}	18.33^{e}	1.40 ^{e}
T_6	7.45 (15.83)	11.67 (19.97)	13.33 (21.41)	10.81 (19.19)	80.73	71.80	70.83	110.93^{c}	22.03 ^{c}	1.80 ^{c}
T_7	3.89 (11.37)	7.22 (15.58)	9.76 (18.20)	06.95 (15.28)	90.01	82.55	78.29	120.21^{a}	26.22 ^{a}	2.13 ^{a}
T_8	4.56 (12.32)	8.90 (17.35)	11.45 (19.77)	08.30 (16.74)	88.20	78.49	74.53	116.88 ^{b}	24.11 ^{b}	1.97 ^{b}
T_9	38.67 (38.44)	41.39 (40.04)	44.97 (42.11)	41.67 (40.20)	--	--	--	71.22 ^{j}	09.12 ^{j}	0.59 ^{j}
T_{10}	33.14 (35.14)	36.91 (37.41)	37.96 (38.03)	36.00 (36.86)	--	--	--	77.21^{i}	10.96^{i}	0.78^{i}

DAT, Days after treatment. *Mean of three replications. *In a column, mean followed by a common letter are not significantly different at 5% level by DMRT. Treatment details are given under Materials and Methods.

biomass (89). Tang *et al.* (2023) also highlighted that the application of bioformulation (organic manure + biocontrol agent) suppressed soil borne disease in tomato more than effectively than chemical fertiliser. Furthermore, Dukare *et al.* (2011) observed the lowest disease incidence (12.5%) in treatments involving compost tea II + *Bacillus* spp. (T1E), while potting mixture supplemented with compost and *Bacillus* spp. yielded significant improvements in plant height and shoot length as compared to untreated control. The novelty of this study lies in integrating bioagents with locally available organic products, which provided dual benefits i.e. disease suppression and soil health improvement. These findings not only corroborated earlier reports on the efficacy of biocontrol agents but also highlighted the added advantage of organic amendments in sustaining their activity.

This study confirmed *A. rolfsii* as the causal agent of tomato collar rot through morphological and molecular characterisation. Integrated eco-friendly strategies, especially biocontrol agents with organic amendments, effectively reduced disease incidence while enhancing plant growth and yield. These results demonstrated that sustainable approaches can serve as promising alternatives to chemical fungicides, ensuring crop productivity and environmental safety. Future research should focus on large-scale field validation and the development of cost-effective formulations for wider adoption.

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