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## Isolation, morphological characterization and integrated management of *Sclerotium rolsfii* through chemical and biological control agents under *in vitro* and pot culture conditions

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**Abstract**

Collar rot incited by *Sclerotium rolsfii* Sacc. is a major constraint to groundnut production due to its persistent soil-borne nature and ability to cause substantial plant mortality under favourable environmental conditions. The present investigation was conducted to isolate and characterize *S. rolsfii* associated with collar rot of groundnut and to assess the effectiveness of selected fungicides and biological control agents under laboratory and pot culture conditions. A field survey carried out in major groundnut-growing areas of Namakkal district led to the collection of four pathogen isolates, namely SR-1, SR-2, SR-3 and SR-4. Considerable variation was observed among the isolates with respect to colony appearance, growth behaviour, mycelial biomass and sclerotial production. Among the isolates, SR-3 was found to be the most vigorous, recording the highest disease incidence (65.84%), fresh mycelial weight (5.75 g), dry mycelial weight (0.78 g) and sclerotial weight (35.76 mg per 100 sclerotia). Evaluation of fungicides using the poisoned food technique revealed complete suppression of mycelial growth by Nativo (Tebuconazole 50% + Trifloxystrobin 25%), whereas Custodia and UPL Saaf recorded 83.0 and 66.0 per cent inhibition, respectively. In dual culture studies, *Trichoderma viride* showed the strongest antagonistic activity with 77.8 per cent inhibition of pathogen growth, followed by *Pseudomonas fluorescens* (61.1%). Pot culture experiments demonstrated significant improvement in plant growth and reduction in disease incidence under integrated disease management treatments. T<sub>2</sub> recorded the highest shoot length (24.33 cm), root length (15.67 cm), fresh weight (6.84 g) and dry weight (0.855 g), whereas T<sub>1</sub> recorded the lowest disease incidence (12.6%). The findings suggest that combining effective fungicides with compatible biological control agents can provide reliable and environmentally sustainable management of groundnut collar rot.

**Keywords:** Groundnut, *Sclerotium rolsfii*, Collar rot, *Trichoderma viride*, Nativo, Biological control, Integrated disease management

**Introduction**

Groundnut (*Arachis hypogaea* L.) is one of the most widely cultivated legume-oilseed crops and serves as an important source of edible oil, dietary protein and income for farming communities. The crop contributes significantly to nutritional security and agricultural sustainability, particularly in semi-arid and tropical regions. Despite its economic importance, groundnut productivity remains below its potential owing to several biotic constraints, among which collar rot caused by *Sclerotium rolsfii* Sacc. is a major concern Kumar *et al.* (2013)<sup>[9]</sup> and Mayee and Datar (1988)<sup>[12]</sup>.

*Sclerotium rolsfii* is a destructive soil-inhabiting fungus capable of infecting a broad spectrum of cultivated plant species. The pathogen survives in soil and crop residues through the production of sclerotia, which function as persistent survival structures and serve as a primary source of inoculum during subsequent cropping seasons. Under favourable environmental conditions, especially high temperature and soil moisture, the fungus rapidly colonizes host tissues around the collar region, resulting in tissue maceration and plant death Agrios (2005) and Patra *et al.* (2023)<sup>[1, 14]</sup>.

Infected groundnut plants initially exhibit lesions near the soil line, followed by development of characteristic white mycelial growth and formation of spherical brown sclerotia.

Advanced infection leads to chlorosis, wilting and drying of the entire plant. The disease often occurs in patches within the field and may cause substantial economic losses depending on cultivar susceptibility, pathogen load and prevailing environmental conditions Mayee and Datar (1988)<sup>[12]</sup> and Kumar *et al.* (2013)<sup>[9]</sup>.

Previous investigations have demonstrated considerable variability among *S. rolfii* isolates with respect to colony morphology, growth behaviour, sclerotial production and virulence. Such variability may influence disease development and the effectiveness of management practices. Therefore, characterization of pathogen populations occurring in different geographical regions is essential for designing effective disease management programmes Paul *et al.* (2017)<sup>[15]</sup> and Chandra Sekhar *et al.* (2017)<sup>[3]</sup>.

Although fungicides remain an important component of disease control, sole dependence on chemical management is increasingly discouraged because of environmental concerns, residue issues and the potential development of fungicide resistance. Biological control agents such as *Trichoderma viride* and *Pseudomonas fluorescens* have received considerable attention due to their ability to suppress pathogens through mechanisms including competition, antibiosis, mycoparasitism and induction of host resistance. Integration of chemical and biological approaches is therefore regarded as a sustainable strategy for long-term management of soil-borne diseases Harman *et al.* (2004)<sup>[5]</sup> and Hoitink *et al.* (2006)<sup>[6]</sup> and Singh *et al.* (2017)<sup>[17]</sup>.

Considering the economic importance of collar rot in groundnut and the need for environmentally sound management practices, the present investigation was undertaken to isolate and characterize *Sclerotium rolfii* from different locations, evaluate the efficacy of selected fungicides and bio-control agents under *In vitro* conditions and assess integrated disease management approaches under pot culture conditions.

## Materials and Methods

The study on the " Isolation, Morphological Characterization and Integrated Management of *Sclerotium rolfii* Through Chemical and Biological Control Agents Under *In vitro* and Pot Culture Conditions" was conducted at PGP College of Agricultural Sciences, Namakkal, from March to July 2026. The location sits at 11.229545° latitude and 78.200957° longitude, at 218 meters above sea level.

## Survey and Collection of Diseased Samples

A roving survey was conducted during the cropping season in major groundnut-growing areas of Namakkal district, Tamil Nadu, to assess the occurrence of collar rot disease. Diseased plants exhibiting typical symptoms such as wilting, collar region rotting and white mycelial growth with sclerotial formation were collected from Erumapatty, Pudhuchatram, Karavalli and Mohanur. Disease incidence was recorded using the formula:

Disease incidence (%) = (Number of infected plants / Total number of plants observed) × 100

James, W.C. (1974)<sup>[7]</sup>

The collected samples were placed in sterile polythene bags, labelled appropriately and transported to the laboratory for further studies.

## Isolation and Purification of the Pathogen

Small pieces (5-10 mm) of infected collar tissue were washed thoroughly under running tap water to remove adhering soil particles. The tissue bits were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds and subsequently rinsed three times with sterile distilled water. Excess moisture was removed using sterile blotting paper, and the tissue pieces were aseptically transferred onto Potato Dextrose Agar (PDA) medium in Petri plates. The plates were incubated at 28 ± 2 °C for 5-7 days. Emerging fungal colonies were purified by the hyphal tip technique and maintained on PDA slants for further experimentation.

## Morphological Characterization of Isolates

The purified isolates of *Sclerotium rolfii* were cultured on PDA medium and incubated at 28 ± 2 °C. Observations were recorded on colony colour, texture, radial growth rate, fresh mycelial weight, dry mycelial weight, time required for sclerotial initiation, sclerotial colour, shape and distribution pattern. The weight of 100 sclerotia was also determined using a digital balance to assess variation among isolates.

## In vitro Evaluation of Fungicides

The efficacy of selected fungicides against *S. rolfii* was assessed by the poisoned food technique. Required quantities of fungicides were incorporated into sterilized and cooled PDA medium to obtain a final concentration of 0.1 per cent. Approximately 20 ml of amended medium was poured into sterile Petri plates. After solidification, a 9 mm diameter mycelial disc obtained from the actively growing margin of a seven-day-old culture was placed at the centre of each plate. Plates without fungicide served as the untreated control. The plates were incubated at 28 ± 2 °C and radial growth of the pathogen was measured after incubation.

Per cent inhibition of mycelial growth was calculated using the formula:

$$I = [(C - T) / C] \times 100$$

Vincent, J.M. (1947)<sup>[19]</sup>

Where,

I = Per cent inhibition of mycelial growth

C = Radial growth in control

T = Radial growth in treatment

## In vitro Evaluation of Bio-control Agents

Antagonistic activity of bio-control agents against *S. rolfii* was evaluated using the dual culture technique. A 9 mm mycelial disc of the pathogen was placed on one side of a PDA plate, while the antagonist culture was inoculated on the opposite side. The plates were incubated at 28 ± 2 °C and observations on pathogen growth inhibition were recorded after incubation. Per cent inhibition was calculated using the standard formula described for fungicide evaluation.

## Pot Culture Experiment

Pot culture experiments were conducted to evaluate integrated disease management treatments against collar rot of groundnut. Sterilized soil was filled into earthen pots and

groundnut seeds subjected to different treatment combinations were sown.

The experiment was maintained under suitable environmental conditions and observations were recorded at the appropriate growth stage. Parameters such as shoot length (cm), root length (cm), fresh weight (g) and dry weight (g) were measured to assess treatment effects on plant growth and disease suppression.

## Results and Discussion

### Survey and Disease Incidence

A field survey conducted in major groundnut-growing areas of Namakkal district (Table-1) revealed the occurrence of collar rot disease with varying levels of incidence across locations. Four isolates of *Sclerotium rolfsii* were obtained from infected plants collected from Erumapatty, Pudhuchatram, Karavalli and Mohanur. Disease incidence ranged from 18.52 to 65.84 per cent, indicating considerable variation in pathogen prevalence among the surveyed areas. The highest incidence was recorded in Karavalli (65.84%), followed by Mohanur (33.75%), Erumapatty (23.58%) and Pudhuchatram (18.52%).

The observed variation in disease incidence may be associated with differences in environmental conditions, soil moisture status, cropping pattern and inoculum density prevailing in each location. Warm temperatures and high soil moisture are known to favour the germination of sclerotia and subsequent infection by *S. rolfsii*. Similar variability in disease incidence and distribution of collar rot has been reported by Chandra Sekhar *et al.* (2017)<sup>[13]</sup>, Paparu *et al.* (2020)<sup>[13]</sup> and Patra *et al.* (2023)<sup>[14]</sup>.

### Morphological Characterization

The four isolates of *S. rolfsii* exhibited noticeable differences in cultural and morphological characteristics when grown on PDA medium (Table-2). Variations were observed in colony colour, texture, growth pattern and biomass production. Among the isolates, SR-3 produced dense cottony mycelial growth with a pure white colony appearance and exhibited rapid radial growth. This isolate also recorded the highest fresh mycelial weight (5.75 g) and dry mycelial weight (0.78 g). In contrast, SR-2 showed sparse and comparatively thin mycelial growth with lower biomass production.

The differences observed among isolates suggest the existence of substantial variability within the pathogen population. Such variability may influence pathogenic behaviour, adaptability and response to management practices. Similar observations on cultural and morphological diversity among *S. rolfsii* isolates infecting groundnut and other crops have been reported by Kumar *et al.* (2014)<sup>[10]</sup>, Akram *et al.* (2015)<sup>[12]</sup> and Manu *et al.* (2018)<sup>[11]</sup>.

### In vitro Evaluation of Bio-control Agents

Among the bio-control agents evaluated (Table-3), *Trichoderma viride*, exhibited the highest antagonistic activity against *S. rolfsii* with 77.8 per cent inhibition of mycelial growth. *Pseudomonas fluorescens* also suppressed pathogen growth effectively, recording 61.1 per cent inhibition.

The strong antagonistic effect of *Trichoderma viride*, may be attributed to its rapid growth, competition for nutrients and space, production of antifungal metabolites and

secretion of cell wall degrading enzymes such as chitinases and glucanases. In addition, *Trichoderma* is known to parasitize fungal pathogens directly through mycoparasitism. The inhibitory effect of *P. fluorescens* may be associated with the production of antibiotics, siderophores and other metabolites that restrict pathogen growth. These findings are consistent with reports of Harman *et al.* (2004)<sup>[5]</sup>, Singh *et al.* (2017)<sup>[17]</sup> and Sarita *et al.* (2018)<sup>[16]</sup>.

### Sclerotial Characteristics

Sclerotial production differed significantly among the isolates evaluated (Table-4). The isolate SR-3 produced dark brown sclerotia distributed throughout the culture plate and recorded the highest sclerotial weight (35.76 mg per 100 sclerotia). In contrast, SR-2 produced comparatively fewer and lighter sclerotia with the lowest weight (17.23 mg per 100 sclerotia).

Sclerotia serve as the principal survival structures of *S. rolfsii* and play a crucial role in disease perpetuation under field conditions. Isolates producing larger and heavier sclerotia may possess enhanced survival ability under adverse environmental conditions. The variability observed in the present study is in agreement with the findings of Sutha Raja Kumar *et al.* (2021)<sup>[18]</sup>, who reported considerable differences in sclerotial morphology and production among isolates of *S. rolfsii*.

### In vitro Evaluation of Fungicides

The fungicides tested exhibited varying levels of effectiveness against *S. rolfsii* under laboratory conditions (Table-3). Complete inhibition of mycelial growth was recorded with Nativo (Tebuconazole 50% + Trifloxystrobin 25%), indicating its superior efficacy against the pathogen. Custodia recorded 83.0 per cent inhibition, followed by UPL Saaf with 66.0 per cent inhibition. The lowest level of inhibition was observed with Thiophanate methyl (38.0%).

The excellent performance of Nativo may be attributed to the combined action of its active ingredients. Tebuconazole interferes with ergosterol biosynthesis, thereby affecting fungal cell membrane integrity, whereas trifloxystrobin inhibits mitochondrial respiration and energy production. The dual mode of action likely contributed to the complete suppression of fungal growth observed in the present study. Similar results have been reported by Khan and Javaid (2015)<sup>[8]</sup> and Wang *et al.* (2015)<sup>[20]</sup>, who demonstrated the effectiveness of fungicides in suppressing the growth of *S. rolfsii*.

### Pot Culture Studies

Integrated disease management treatments significantly influenced the growth and development of groundnut plants under pot culture conditions (Table-7). Considerable variation was observed among the treatments with respect to shoot length, root length, fresh weight, dry weight and disease incidence. Among the treatments evaluated, T<sub>2</sub> recorded the maximum shoot length (24.33 cm), root length (15.67 cm), fresh weight (6.84 g) and dry weight (0.855 g), and was significantly superior to the other treatments. The lowest disease incidence among the disease management treatments was recorded in T<sub>1</sub> (12.6%), followed by T<sub>2</sub> (16.5%). The pathogen-inoculated control (T<sub>5</sub>) recorded the highest disease incidence (88.5%) along with poor growth performance, whereas the uninoculated healthy control (T<sub>6</sub>)

remained free from disease. The enhanced plant growth observed under integrated treatments may be due to effective suppression of pathogen activity coupled with the growth-promoting effects of beneficial microorganisms. Bio-control agents not only reduce disease incidence but also improve nutrient availability, root development and overall plant vigour. When combined with effective disease

management practices, they provide both immediate disease suppression and long-term protection.

Similar beneficial effects of integrated disease management strategies against soil-borne pathogens have been reported by Hoitink *et al.* (2006) <sup>[6]</sup>, Singh *et al.* (2017) <sup>[17]</sup> and Chowdary *et al.* (2024) <sup>[4]</sup>.

**Table 1:** Survey Details

| S.No | Isolate Name | Isolated Plant Part | Geographical coordinates | Location     | Disease Incidence |
|------|--------------|---------------------|--------------------------|--------------|-------------------|
| 1.   | SR-1         | Collar Region       | 11.15°N 78.30°E          | Erumpatty    | 23.58             |
| 2.   | SR-2         | Collar Region       | 11.297°N 78.167°E        | Pudhuchatram | 18.52             |
| 3.   | SR-3         | Stem                | 11.31°N 78.14°E          | Karavalli    | 65.84             |
| 4.   | SR-4         | Branch              | 11.06°N 78.14°E          | Mohanur      | 33.75             |

\*Values are the means of three replicates; means in a column followed by the same letters are not significantly different according to Dunnett's multiple range test at P= 0.05.

**Table 2:** Morphological Characterization

| S.No          | Name of Isolates | Growth pattern of <i>S. rolfsii</i> mycelium               | Colony colour | Growth rate | Mycelial Fresh weight (g)* | Mycelial Dry weight (g)* |
|---------------|------------------|------------------------------------------------------------|---------------|-------------|----------------------------|--------------------------|
| 1.            | SR-1             | Thin Mycelial Growth With Aggregated Centre                | Dull White    | Moderate    | 4.53 <sup>c</sup>          | 0.42 <sup>c</sup>        |
| 2.            | SR-2             | Very Thin Mycelial Growth                                  | Dull White    | Moderate    | 3.54 <sup>d</sup>          | 0.33 <sup>d</sup>        |
| 3.            | SR-3             | Cottony Mycelial Growth                                    | Pure White    | Very Fast   | 5.75 <sup>a</sup>          | 0.78 <sup>a</sup>        |
| 4.            | SR-4             | Cottony Mycelial Growth With Aggregation Around The Centre | Pure White    | Very Fast   | 5.23 <sup>b</sup>          | 0.53 <sup>b</sup>        |
| CD (P = 0.05) |                  |                                                            |               |             | 0.14                       | 0.03                     |

\*Values are the means of three replicates; means in a column followed by the same letters are not significantly different according to Dunnett's multiple range test at P= 0.05.

**Table 3:** *In vitro* Evaluation of Bio-control Agents

| Treatment                      | Radial growth of pathogen (mm) | % Inhibition |
|--------------------------------|--------------------------------|--------------|
| Control                        | 90                             | 0            |
| <i>Trichoderma viride</i>      | 20                             | 77.8         |
| <i>Pseudomonas fluorescens</i> | 35                             | 61.1         |

**Table 4:** Sclerotial Characteristics

| S. No.        | Name of Isolates | Sclerotia shape                        | Colour of sclerotia | Sclerotial formation (No. of days) | Weight of 100 sclerotia (mg)* |
|---------------|------------------|----------------------------------------|---------------------|------------------------------------|-------------------------------|
| 1.            | SR-1             | Globous                                | Light Brown         | 8c                                 | 20.32c                        |
| 2.            | SR-2             | Globous and Aggregated on Upper Plate  | Light Brown         | 7d                                 | 17.23d                        |
| 3.            | SR-3             | Round Small Scattered Throughout Plate | Dark Brown          | 12a                                | 35.76a                        |
| 4.            | SR-4             | Round Small                            | Dark Brown          | 10b                                | 26.55b                        |
| CD (P = 0.05) |                  |                                        |                     | 0.80                               | 1.20                          |

**Table 5:** *In vitro* Evaluation of Fungicides

| S. No.         | Treatment schedule                               | Concentration | Percent inhibition over control |
|----------------|--------------------------------------------------|---------------|---------------------------------|
| T <sub>1</sub> | Bulton (Thiophante methy 70%)                    | 0.1%          | 38.00%                          |
| T <sub>2</sub> | UPL Saaf (Carbendazim 12% + Mancozeb 63%)        | 0.1%          | 66.00%                          |
| T <sub>3</sub> | Nativo(Tebuconazole 50% Trifloxystrobin 25%)     | 0.1%          | 100.00%                         |
| T <sub>4</sub> | Custodia (Azoxystrobin 11% + Tebuconazole 18.3%) | 0.1%          | 83.00%                          |

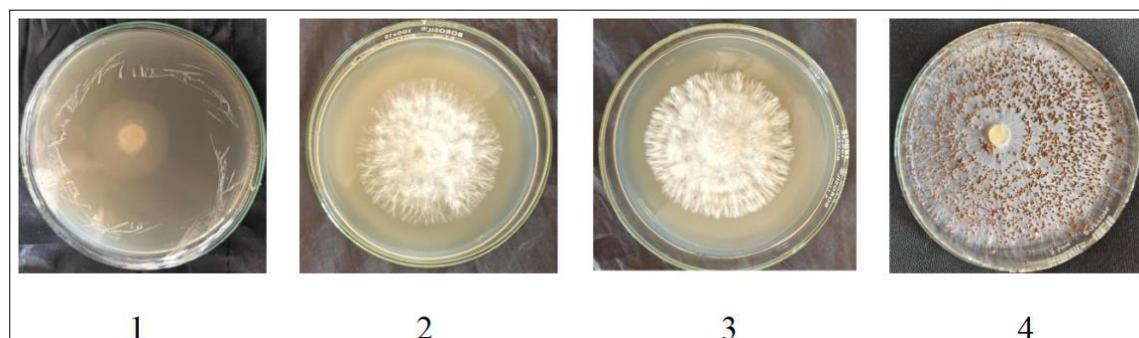
**Table 6:** Combination of Different Bio Control Agents and Chemicals for Pot Culture Studies

| Treatments     | Combinations                                                                                                                                                |
|----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------|
| T <sub>1</sub> | Seed Treatment With fungicide Nativo (Tebuconazole 50% Trifloxystrobin 25%) and soil application after 15,35,45 and 65 DAS                                  |
| T <sub>2</sub> | Seed treatment+Soil Application of Pathogen Specific Bio Control Agent ( <i>Trichoderma spp.</i> ) 4g/kg of seeds + soil application at 15,35,45 and 65 DAS |
| T <sub>3</sub> | Seed treatment + Soil Application of <i>Trichoderma</i> (4g/kg) + <i>Bacillus</i> (10g/kg) at 15,35,45 and 65 DAS                                           |
| T <sub>4</sub> | Soil Application of <i>Trichoderma</i> (2g) + <i>Rhizobium</i> (25g) + VAM (75g) + <i>Pseudomonas</i> (10g) at 15,35,45 and 65 DAS                          |
| T <sub>5</sub> | Control + Pathogen Inoculated                                                                                                                               |
| T <sub>6</sub> | Control                                                                                                                                                     |

**Table 7: Pot Culture Studies**

| S. No.        | Treatments     | Shoot Length (cm) | Root Length (cm) | Fresh weight (g) | Dry Weight (g) | Disease Incidence (%) |
|---------------|----------------|-------------------|------------------|------------------|----------------|-----------------------|
| 1.            | T <sub>1</sub> | 21.00b            | 12.67b           | 5.22b            | 0.65b          | 12.6c                 |
| 2.            | T <sub>2</sub> | 24.33a            | 15.67a           | 6.84a            | 0.855a         | 16.5bc                |
| 3.            | T <sub>3</sub> | 18.33c            | 12.67b           | 4.13c            | 0.516c         | 22.3b                 |
| 4.            | T <sub>4</sub> | 18.00c            | 12.33b           | 3.64c            | 0.42cd         | 26.6b                 |
| 5.            | T <sub>5</sub> | 11.33e            | 7.33d            | 1.47d            | 0.287d         | 88.5a                 |
| 6.            | T <sub>6</sub> | 16.00d            | 1.67c            | 2.92e            | 0.365d         | 0d                    |
| CD (P = 0.05) |                | 2.00              | 1.5              | 0.80             | 0.10           | 5.20                  |

\*Values are the means of three replicates; means in a column followed by the same letters are not significantly different according to Duncun's multiple range test at P= 0.05.

**Plate 1:** Different Isolates of *Sclerotium rolfsii* Obtained from Regions of Namakkal District**Plate 2:** Different Growth Stages of *Sclerotium rolfsii***Plate 3:** Comparison of Control and Treatments in Pot Culture Studies

## Conclusion

The present study demonstrated the existence of considerable cultural and morphological variability among *Sclerotium rolfsii* isolates associated with collar rot of groundnut in Namakkal district. Among the isolates evaluated, SR-3 exhibited greater aggressiveness as evidenced by higher disease incidence, enhanced mycelial growth and increased sclerotial production. *In vitro* studies revealed that Nativio (Tebuconazole 50% + Trifloxystrobin 25%) was highly effective in suppressing the pathogen, resulting in complete inhibition of mycelial growth. Among the biological control agents tested, *Trichoderma viride* showed superior antagonistic activity against *S. rolfsii* and

demonstrated significant potential as a component of integrated disease management.

The pot culture experiment further confirmed that integrated disease management treatments improved plant growth and reduced the impact of collar rot. Among the treatments evaluated, T<sub>2</sub> recorded the highest shoot length, root length, fresh weight and dry weight, indicating superior growth promotion. However, T<sub>1</sub> recorded the lowest disease incidence, suggesting better suppression of collar rot under pot culture conditions. The combined use of chemical and biological approaches offers a practical, environmentally safer and economically feasible strategy for sustainable management of the disease. The findings of the present

investigation may contribute to the development of integrated disease management programmes for groundnut cultivation and provide a basis for future field-level validation under diverse agro-climatic conditions.

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