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EVALUATION OF BIOLOGICAL CONTROL AGENTS AND NEEM CAKE FOR THE MANAGEMENT OF *SCLEROTIUM ROLFSII* INDUCED COLLAR ROT IN BRINJAL

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ABSTRACT
Brinjal (*Solanum melongena* L.) collar rot, caused by *Sclerotium rolfsii*, is an important soilborne disease that adversely affects crop establishment and productivity. The present study aimed to develop an effective and environmentally sustainable management strategy by integrating native isolates of *Trichoderma asperellum* and *Bacillus subtilis* with neem cake. A field survey was conducted to assess the occurrence and distribution of collar rot in selected brinjal-growing locations in Tamil Nadu, followed by pathogenicity assessment of the collected *S. rolfsii* isolates. The antagonistic potential of native *T. asperellum* and *B. subtilis* isolates was evaluated through dual-culture assays, volatile-mediated inhibition and culture-filtrate studies. Neem cake and other oil cakes were also assessed for their inhibitory effects on pathogen growth. The most effective fungal and bacterial antagonists were subsequently evaluated individually and in combination with neem cake through seed treatment and soil application under pot-culture conditions. The integrated treatment improved seed germination, seedling vigour and plant growth while reducing collar rot incidence. Furthermore, the combined application enhanced the activities of defence-related enzymes, including peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase along with total phenolic content in brinjal plants. These findings highlight the potential of integrating native microbial antagonists with neem cake as an eco-friendly approach for managing brinjal collar rot. Further validation under field conditions is necessary to establish the effectiveness and practical applicability of this integrated disease management strategy.

KEYWORDS: *Solanum melongena, Sclerotium rolfsii;* collar rot, *Trichoderma asperellum*, *Bacillus subtilis,* neem cake, integrated disease management, defence enzymes.

1.INTRODUCTION

Eggplant (*Solanum melongena* L.), commonly known as brinjal or aubergine, is an economically important vegetable belonging to the family Solanaceae. Originating from the Indian subcontinent, it is widely cultivated across tropical and subtropical regions and is considered one of the major vegetable crops in Asia and the Mediterranean region. Often referred to as the “poor man’s vegetable” and the “king of vegetables” in India, eggplant is valued for its adaptability to diverse agroclimatic conditions and year-round cultivation potential (Zeven and Zhukovsky, 1975; Singh et al., 2014; Tiwari et al., 2023). It is a frost-sensitive, warm-season crop generally cultivated as an annual, with a growth cycle of approximately 100–120 days. The plant typically grows to 0.4–1.5 m in height, exhibiting a branched growth habit, broad leaves, and sometimes spiny stems. Its fruits are botanically classified as berries and vary considerably in shape, including spherical, oblong, ovoid, and oval forms (Kumar et al., 2011; Arya Kaniyassery et al., 2023).China and India are the leading eggplant-producing countries, together accounting for a substantial proportion of global production (FAO, 2022). In India, brinjal is cultivated across several states, particularly West Bengal, Odisha, Gujarat, Bihar, and Madhya Pradesh. The crop is also well suited to the agro climatic conditions of Tamil Nadu. Indian brinjal varieties are characterized by diverse fruit shapes and colours, with purple-skinned fruits being particularly common, and the produce is exported to countries including Sri Lanka, the Maldives, Singapore, Sudan, and Mali (Tiwari et al., 2023). In addition to its economic importance, eggplant contributes to human nutrition by providing dietary fibre, carbohydrates, protein, vitamins, and minerals, including potassium, magnesium, manganese, and copper. Its nutritional composition and broad adaptability make it an important component of vegetable-based diets and agricultural production systems.

Despite its economic and nutritional significance, eggplant cultivation is constrained by several biotic stresses, particularly diseases caused by fungal, bacterial, and phytoplasma pathogens. Important diseases include damping-off caused by *Pythium* spp., Phomopsis blight caused by *Phomopsis vexans*, Cercospora leaf spot caused

by *Cercospora melongenae*, Alternaria leaf spot caused by *Alternaria melongenae*, little leaf associated with phytoplasma infection, and collar rot caused by *Sclerotium rolfsii* (Srividya et al., 2018). Among these, collar rot is a destructive soil-borne disease that can affect eggplant from the seedling stage through maturity, resulting in plant mortality and substantial yield losses (Siddique et al., 2016).

Sclerotium rolfsii is a soil-borne basidiomycetous fungus with a broad host range and a predominantly necrotrophic mode of infection. It causes collar rot, also known as southern blight, as well as several other diseases, including damping-off, stem rot, foot rot, crown rot, rhizome rot, and blight (Nalim et al., 1995; Tang et al., 2015). The pathogen is particularly prevalent in tropical and subtropical regions and favours warm soil conditions, with disease development reported at temperatures above 15°C (Koike et al., 2007). Its persistence in soil is largely attributed to the production of sclerotia, compact survival structures consisting of a resistant outer layer and internal hyphal tissues. These structures enable the pathogen to survive unfavourable environmental conditions and serve as primary sources of inoculum (Singh and Singh, 1994; Agrios, 2005). Collar rot caused by *S. rolfsii* was reported in India by Rao (1969) in the Vijayawada region of Andhra Pradesh, as subsequently cited by Jadon et al. (2009).

Infection typically occurs in the collar region near the soil surface, where the pathogen colonizes host tissues and induces extensive necrosis and rotting. The resulting disruption of stem and collar tissues impairs the movement of water and nutrients between the roots and aerial plant parts, ultimately causing wilting, collapse, and plant death (Begum et al., 1985; Babar, 1999). Disease symptoms may occur at both seedling and mature growth stages. Although seedling losses may be partially compensated for through gap filling, the death of mature plants results in irreversible reductions in plant population and yield. Collar rot incidence of 60–100% and yield losses of 16–30% have been reported in brinjal, while severe infections occurring between the seedling and flowering stages may result in complete crop loss under favourable conditions (Singh and Dhancholia, 1991; Jadon, 2009; Siddique et al., 2016; Hasan and Meah, 2019). Chemical fungicides are commonly used to manage soil borne diseases, including collar rot. However, their intensive and repeated application raises concerns regarding environmental contamination, pesticide residues in food, potential groundwater pollution, and the development of fungicide resistant pathogen populations. Restrictions or withdrawal of certain effective fungicides further highlight the limitations of chemical dependent disease management (Theertha et al., 2017; Aswathy et al., 2019). These challenges underscore the need for alternative approaches that provide effective disease suppression while reducing environmental risks and supporting sustainable crop production.

Biological control is a promising alternative for managing soil-borne plant pathogens because it can suppress pathogen activity while supporting plant health and beneficial soil processes (Reithner et al., 2011; Singh et al., 2011; Singh and Singh, 2012; Ray et al., 2017). Among microbial biocontrol agents, *Trichoderma* spp. suppress fungal pathogens through multiple mechanisms, including competition for nutrients and space, antibiosis, mycoparasitism, modulation of plant defence responses, and the production or regulation of phytohormones. These activities can contribute to disease suppression, plant growth promotion, and improved agricultural sustainability (Oyesola et al., 2024). Similarly, *Bacillus* spp. are widely investigated for their antimicrobial and plant growth promoting properties. Their biocontrol activities include the production of antibiotics, extracellular hydrolytic enzymes, siderophores, and volatile compounds, together with competition for resources and the induction of systemic resistance (Karacic et al., 2024).

In addition to microbial antagonists, organic amendments such as compost, green manure, farmyard manure, and oil cakes can contribute to the suppression of soil borne pathogens. Oil cakes, in particular, have demonstrated pathogen-suppressive potential, while beneficial soil microorganisms can further support integrated disease management (Veena et al., 2023). Therefore, integrating microbial biocontrol agents with suitable organic amendments represents a promising approach to reducing dependence on synthetic fungicides and improving the sustainability of eggplant production. Further evaluation of these approaches under appropriate experimental and field conditions is essential to establish their efficacy against *S. rolfsii* and develop reliable, environmentally sound management strategies for collar rot in brinjal.

2. MATERIALS AND METHODS

Survey of collar rot incidence in brinjal growing districts of Tamil Nadu

A roving survey was conducted during 2023–2024 in major brinjal growing areas of Ariyalur, Cuddalore, and Salem districts of Tamil Nadu to assess the incidence of collar rot. Three fields were selected at each location, and five plots measuring 1 m² each were randomly selected within each field. In each plot, 100 plants were randomly examined for disease symptoms. Disease incidence was calculated according to Mayee and Datar (1986) using the following formula:

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

Plants exhibiting typical collar rot symptoms caused by *S. rolfsii* were collected along with rhizosphere soil for pathogen isolation.

2.1. Isolation and maintenance of *S. rolfsii*

Collar rot infected brinjal tissues were collected from Ariyalur, Cuddalore, and Salem districts of Tamil Nadu. The samples were washed, and 5 mm tissue segments containing the advancing margins of infection were excised. The segments were surface-sterilized with 1% sodium hypochlorite for 1 min, rinsed three times with sterile distilled water, and blotted dry using sterile filter paper. Four segments were placed on potato dextrose agar (PDA)

medium supplemented with streptomycin and incubated at 28 ± 2°C for seven days (Ainsworth, 1961; Mohanty et al., 2016). Ten isolates, designated Sr1–Sr10, were obtained from the infected plant samples. Fungal growth emerging from sclerotia was transferred aseptically to PDA slants and incubated at 28 ± 2°C for approximately four days to obtain pure cultures. The cultures were maintained at 5°C and sub cultured periodically. The isolates were identified as *S. rolfsii* based on colony and morphological characteristics (Sharma et al., 2002; Watanabe, 2002; Latha and Rajeswari, 2019).

2.2. Cultural and morphological characterization

Sterilized PDA (15 mL) was poured into sterile Petri dishes and allowed to solidify. A 6 mm mycelial disc obtained from the actively growing margin of a four day old *S. rolfsii* culture was placed at the centre of each plate and incubated at 28 ± 2°C. Colony diameter was measured along two perpendicular axes after five days of incubation. Colony morphology, mycelial growth, and sclerotial development were recorded. Sclerotial characteristics, including initiation, number per plate, shape, and colour, were evaluated. Variations in colony morphology were recorded after seven days, while sclerotial characteristics were assessed after 30 days of incubation (Manu et al., 2018).

2.3. Scanning electron microscopy (SEM)

Scanning electron microscopy was performed to examine the hyphal morphology of the pathogen. Actively growing fungal material was fixed overnight at 28°C in 0.05 M phosphate buffer containing 4% glutaraldehyde. The samples were washed three times with phosphate buffer and dehydrated in ethanol for 15 min. The fixed samples were subsequently dried using CO_2 for 5 min, mounted on aluminium stubs, and carbon coated using a Polaron E-500 sputter coater. The specimens were examined under a scanning electron microscope at an accelerating voltage of 15 kV. The analysis was conducted at the Department of Physics, Annamalai University (Fig 4,5).

2.4. Mass multiplication of *S. rolfsii* isolates on sorghum grains

The ten *S. rolfsii* isolates were multiplied on sorghum grains following the method described by Bekriwala et al. (2016). Sorghum grains (200 g) were soaked in water overnight. Subsequently, 100 g of the soaked grains were transferred into 500 mL saline bottles, sealed, and sterilized at 121°C and 15 lb pressure for 20 min. After cooling, each bottle was inoculated with a 6 mm mycelial disc obtained from the advancing margin of a four day old culture. The inoculated bottles were incubated at 28 ± 2°C for ten days to facilitate fungal growth(Fig 6).

2.5. Assessment of virulence of *S.rolfsii* isolates

A pot experiment was conducted during 2023–2024 at the Department of Plant Pathology, Faculty of Agriculture, Annamalai University, to evaluate the virulence of the ten *S. rolfsii* isolates. A potting mixture comprising clay loam soil, sand, and farm yard manure (FYM) in a 1:1:1 ratio was prepared. Each isolate was multiplied on sorghum grains and incorporated separately into sterilized soil at 5% (w/w). The inoculated soil mixture was transferred to 30 cm-diameter earthen pots ten days before transplanting. Brinjal seeds were surface sterilized with 0.1% $HgCl_2$ for 30 min, rinsed twice with sterile water, and sown in nursery trays. After two weeks, two to three seedlings were transplanted into each pot, while pots containing uninoculated soil served as controls. Soil moisture was maintained at the moisture holding capacity by adding sterilized water throughout the experiment. Disease development was assessed 30 days after inoculation based on the appearance of typical collar rot symptoms and wilting. Disease incidence was calculated as the percentage of diseased plants among the total plants assessed. The pathogen was reisolated from symptomatic tissues and compared with the corresponding original isolate to confirm its identity. Based on the virulence assessment, isolate Sr3 was selected for subsequent experiments (Murmu et al., 2021).

2.6. Isolation of fungal antagonists from brinjal rhizosphere

Rhizosphere soil samples were collected from brinjal plants at six locations and serially diluted up to 10^{-4} using sterile distilled water. One millilitre of the final dilution was plated on Trichoderma Selective Medium (TSM) (Elad and Chet, 1983) and incubated at 28 ± 2°C for seven days. The emerging Trichoderma colonies were purified on PDA by the single hyphal tip method and maintained on agar slants at 4°C. Preliminary identification was based on colony morphology, including mycelial growth, colony and conidial colour, and shape (Leahy and Colwell, 1990). Further identification was carried out using taxonomic keys (Samuels, 1996) and microscopic examination of KOH mounts and lactophenol cotton blue stained slide cultures. The six isolates were designated TaM, TaG, TaE, TaP, TaS and TaPp.

2.7. Isolation and purification of bacterial antagonists

Bacterial isolates were obtained from six brinjal growing locations in Tamil Nadu. After removing loosely adhering soil from freshly collected roots, 1 g of root material was suspended in 10 mL of sterile distilled water to prepare the initial 10^{-1} dilution. Serial dilutions were prepared up to 10^{-7}. Aliquots (1 mL) from the 10^{-6} and 10^{-7}dilutions were plated on nutrient agar using the pour plate method and incubated at 28 ± 2°C for 48–72 hr. Colonies exhibiting characteristics of *Bacillus* spp. were selected individually and purified by repeated streak plating on nutrient agar (Rangaswami, 1973). Pure cultures were maintained on agar slants at 4°C. Six bacterial isolates provisionally identified as *B. subtilis* were selected for biochemical characterization.

2.8. Biochemical characterization of bacterial isolates

The six bacterial isolates were subjected to biochemical tests for characterization according to Bergey's Manual of Determinative Bacteriology (Breed et al., 1989).

2.8.1. Gram staining

A loopful of bacterial culture was transferred onto a clean glass slide, spread into a thin smear, air dried, and heat fixed. The smear was stained with crystal violet for 1 min, treated with Lugol's iodine for 1 min, decolorized with ethyl alcohol, and counterstained with safranin for 30 s. After rinsing with distilled water, Gram-positive cells appeared purple, whereas Gram negative cells appeared red (Cyrabree and Hindshill, 1975).

2.8.2. Siderophore production

Siderophore production was assessed using chrome azurol S (CAS) agar according to Schwyn and Neilands (1987). Freshly grown bacterial cultures were inoculated onto CAS agar plates and incubated at 28 ± 2°C for 48–72 h. The development of a yellow orange halo around the colonies was considered a positive reaction for siderophore production.

2.8.3. Catalase activity

Catalase activity was assessed according to Cheesbrough (1991). Bacterial culture was exposed to hydrogen peroxide, and the formation of gas bubbles was recorded as a positive reaction for catalase activity.

2.8.4. Chitinase production

Chitinase production was assessed according to Basha and Ulaganathan (2002). The bacterial isolates were inoculated onto Luria Bertani (LB) agar supplemented with 0.5% colloidal chitin and incubated at 30°C for three days. The formation of clear halo zones surrounding the colonies indicated chitinase activity.

2.8.5. Starch hydrolysis

The bacterial isolates were streaked onto peptone sucrose agar supplemented with 0.2% starch and incubated for five days. Starch hydrolysis was assessed by flooding the plates with Lugol's iodine solution. Clear zones surrounding the bacterial growth indicated a positive reaction (Iverson and Millis, 1974)

2.8.6. Citrate utilization

Each bacterial isolate was streaked separately onto Simmons' citrate agar slants and incubated at 28 ± 2°C for 24 h. A colour change from green to blue indicated a positive reaction for citrate utilization (Simmons, 1926).The six bacterial isolates were designated BsM, BsG, BsG, BsP, BsS, and BsPp for subsequent experiments.

2.9. *In Vitro* evaluation of biocontrol agents

2.9.1. Efficacy of antagonists against *Sclerotium rolfsii* (Sr3) using the dual-culture technique

The antagonistic potential of *Trichoderma asperellum* (TaE) and *Bacillus subtilis* (BsE) against the virulent *S. rolfsii* isolate Sr3 was evaluated using the dual-culture technique described by Dennis and Webster (1971). Each treatment was maintained in triplicate, and plates containing the pathogen alone served as controls. The plates were incubated at 28°C, and pathogen growth was recorded by measuring the colony diameter. The percentage inhibition of mycelial growth was calculated according to Vincent (1927):

Percent inhibition (I) = C-T/C × 100

where (C) represents the radial growth of the pathogen in the control and (T) represents its radial growth in the treatment.

2.9.2. Inverted Plate Assay

The effect of volatile compounds produced by the fungal and bacterial antagonists on *S. rolfsii* was evaluated using the inverted plate assay (Dennis and Webster, 1971). The antagonists and pathogen were inoculated separately onto Potato Dextrose Agar (PDA) and Nutrient Agar (NA), respectively. The inoculated plates were positioned face to face and sealed with adhesive tape or parafilm to prevent the escape of volatile compounds. Plates containing the pathogen alone served as controls. The plates were incubated at 28 ± 1°C, and pathogen mycelial growth and sclerotial production were recorded. The percentage inhibition of mycelial growth was calculated using the formula described by Vincent (1927).

Percent inhibition (I) = C-T/C × 100

where (C) represents the radial growth of the pathogen in the control and (T) represents its radial growth in the treatment.

2.10. Preparation of Culture Filtrates of Fungal and Bacterial Antagonists

2.10.1. Preparation of *Trichoderma* Culture Filtrate

Six *Trichoderma* isolates were grown in 100 mL of sterile Potato Dextrose Broth (PDB) in Erlenmeyer flasks for 10 days at 28 ± 2°C. Following incubation, the cultures were filtered under vacuum through a bacteriological filter to remove mycelia and spores. The resulting culture filtrates were collected for further experiments.

2.10.2. Preparation of *Bacillus* Culture Filtrate

Six bacterial isolates were individually inoculated into 100 mL of Nutrient Broth in Erlenmeyer flasks and incubated on a rotary shaker at 100 rpm for 48 h at 28 ± 2°C. The cultures were subsequently filtered under vacuum through a bacteriological filter, and the resulting filtrates were collected for further experiments.

2.10.3. Effect of Culture Filtrates on the Mycelial Growth of *S. rolfsii* (Sr3)

The culture filtrates of the fungal and bacterial antagonists were separately incorporated into sterile PDA at concentrations of 10, 20, 30, and 40% (v/v). The amended medium was dispensed into sterile Petri plates at 15 mL per plate and allowed to solidify. Each plate was inoculated centrally with a 6 mm mycelial disc obtained from a four day old culture of *S. rolfsii* grown on PDA. Three replications were maintained for each treatment, while PDA without culture filtrate served as the control. Mycelial growth was measured in millimetres when the pathogen completely covered the control plates.

2.11. Effect of Water Extracts of Organic Amendments on the Mycelial Growth of *S. rolfsii* (Sr3)

The procedure described by Dubey and Patel (2000) was followed to evaluate the antifungal activity of five locally available oil cakes: Coconut (*Cocos nucifera*), Cotton (*Gossypium herbaceum*), Groundnut (*Arachis hypogaea*), Neem (*Azadirachta indica*), and Sesame (*Sesamum indicum*).Each oil cake was powdered and soaked overnight in sterile distilled water at a ratio of 1 g per mL. The material was subsequently ground and filtered through cheesecloth to obtain a standard 100% extract. The extracts were incorporated separately into sterile PDA at concentrations of 10, 20, 30, and 40% (v/v). The amended medium was dispensed into sterile Petri plates at 15 mL per plate and allowed to solidify. A 6 mm disc of *S. rolfsii* was placed at the centre of each plate, and the plates were incubated at 28 ± 2°C. PDA without organic amendment extract served as the control. Pathogen mycelial growth was measured after incubation.

3.12. Formulation of Biocontrol Agents

3.12.1. Solid Formulation of *T. asperellum* (TaE)

The selected *T. asperellum* isolate TaE was inoculated into molasses yeast medium and incubated for 15 days. The fungal mycelial mat was then harvested and oven-dried. The dried biomass was mixed with talc powder at a ratio of 1:2 (biomass). Carboxymethyl cellulose (CMC) was added at 5 g kg^{-1} of carrier material as a binding agent. The formulation was shade dried, packed in polythene bags, sealed, and stored at 28 ± 2°C. The viable population of *T. asperellum* was assessed at 15-day intervals for up to 120 days using a dilution-plate method with *Trichoderma*-specific medium (Ramakrishnan et al., 1994).

3.12.2. Solid Formulation of *B. subtilis* (BsE)

The selected *B. subtilis* isolate BsE was grown in Nutrient Broth at 28 ± 2°C for 48 h. Under sterile conditions, 400 mL of bacterial suspension was mixed with 1 kg of sterile talc powder and 10 g of CMC (Vidhyasekaran and Muthamilan, 1995). The pH of the carrier material was adjusted to 7.0 using 15 g of calcium carbonate. The formulation was shade dried to reduce the moisture content to approximately 35% and then packed in sealed polythene bags. The viable bacterial population was assessed at 15 day intervals for up to 120 days using Nutrient Agar and the dilution plate method. Colony forming units (CFU) were enumerated using a colony counter, following the method of Schmidt and Caldwell (1967).

2.13. Plant Growth Promotion Assay

The plant growth promoting potential of the antagonists was evaluated using the standard roll towel method, following ISTA (1993). One hundred brinjal seeds treated with the respective antagonists were placed on pre soaked germination paper. A second strip of pre soaked paper was placed over the seeds, and the sheets were rolled, secured, and incubated in a growth chamber for 10 days. Three replications were maintained for each treatment. Germination percentage, root length, and shoot length were recorded.

2.13.1. Germination Percentage

Germination percentage was calculated using the following formula:

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100$$

2.13.2. Seedling Height

Ten normal seedlings were randomly selected from each replication, and their seedling heights

2.13.3. Seedling Vigour Index

The seedling vigour index (VI) was calculated according to Abdul-Baki and Anderson (1973):

Vigour index = (Mean root length+ Mean shoot length) × Germination (%)

The treatment schedule was as follows:

Treatment	Seed treatment
T1	*T. asperellum* (TaE), 6 g kg^{-1} seed
T2	*B. subtilis* (BsE), 12 g kg^{-1} seed
T3	Oil cake, 2 g kg^{-1} seed
T4	T1 + T2
T5	T1 + T3
T6	T2 + T3
T7	T1 + T2 + T3
T8	Seed treatment with carbendazim 50% WP, 2 g kg^{-1} seed
T9	Untreated control

2.14. Pot Culture Studies

2.14.1. Effect of Seed Treatment with Different Doses of Antagonists on Collar Rot Incidence in Brinjal

Pot-culture experiments were conducted using sterilized potting soil consisting of red soil, sand, and farmyard manure (FYM) at a ratio of 1:1:1, with 5 kg of potting mixture per pot. The soil was amended with sorghum grain-based inoculum of *S. rolfsii* at 8 grains per pot, 10 days before sowing. Surface-sterilized seeds of brinjal (*Solanum melongena*) cv. PKM 1 were treated separately with talc-based formulations of the antagonists at the specified doses.

Treatment	Seed treatment
T1	*T. asperellum*, 4 g kg^{-1} seed
T2	*T. asperellum*, 5 g kg^{-1} seed
T3	*T. asperellum*, 6 g kg^{-1} seed
T4	*B. subtilis*, 10 g kg^{-1} seed
T5	*B. subtilis*, 11 g kg^{-1} seed
T6	*B. subtilis*, 12 g kg^{-1} seed
T7	Untreated control

2.14.2. Effect of seed treatment and soil application of antagonists and oil cake on collar rot incidence in brinjal

Based on the in vitro results, a pot culture experiment was conducted in a completely randomized design (CRD) with three replications using brinjal cv. PKM 1. Two plants were maintained per pot. The potting soil was amended with *S. rolfsii* inoculum before transplanting. Seed treatments and soil applications of biocontrol agents, neem oil cake, and the chemical fungicide were applied according to the treatment schedule. Recommended agronomic practices were followed. Disease incidence was recorded at 40 and 60 days after transplanting (DAT) and at harvest, and percentage disease incidence was calculated using the previously described formula.

Effect of treatments on brinjal growth and yield under pot culture conditions

The effects of the treatments on plant growth and yield were assessed by recording plant height, number of branches per plant, and fruit yield per plant (kg). The most effective treatments identified in the pot-culture experiment were selected for further evaluation.

The treatment schedule was as follows:

Treatment	Treatment details
T1	*T. asperellum* (TaE) seed treatment, 6 g kg^{-1} seed
T2	*B. subtilis* (BsE) seed treatment, 12 g kg^{-1} seed
T3	Neem oil cake seed treatment, 2 g kg^{-1} seed
T4	*T. asperellum* soil application, 5 g pot^{-1}
T5	*B. subtilis* soil application, 5 g pot^{-1}
T6	Neem oil cake soil application, 20 g pot^{-1}
T7	T1 + T4
T8	T2 + T5
T9	T3 + T6
T10	T7 + T8 + T9
T11	Seed treatment with carbendazim 50% WP, 2 g kg^{-1} seed, plus soil drenching at 0.1%
T12	Untreated control

3.15. Assay of defence-related enzymes and total phenols

3.15.1.Sample collection

Plant samples were collected from selected treatments in the pot culture experiment to assess the induction of defence-related enzymes following pathogen inoculation under glasshouse conditions. Leaf tissues were collected from plants treated with *T. asperellum*, *B. subtilis*, or neem cake, as well as from pathogen-inoculated plants maintained under the same conditions. Samples were collected at intervals from 0 to 7 days after inoculation.

3.15.2. Enzyme Extraction

Root tissue (1 g) was homogenized in 2 mL of 0.1 M sodium citrate buffer (pH 5.0) at 4°C. The homogenate was centrifuged at 1,000 rpm for 20 min, and the supernatant was collected as the crude enzyme extract. For the estimation of peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL), enzyme extracts prepared in 0.1 M sodium phosphate buffer (pH 7.0) were used. The extracts were stored at −70°C until biochemical analysis.

3.15.3. Assay of Peroxidase Activity

Peroxidase activity was determined according to Hammerschmidt et al. (1982). The reaction mixture contained 1.5 mL of 0.05 M pyrogallol, 0.5 mL of enzyme extract, and 0.5 mL of 1% hydrogen peroxide. The mixture was

incubated at 28 ± 3°C, and changes in absorbance were recorded at 420 nm at 30s intervals for 3 min. A boiled enzyme preparation served as the blank. Enzyme activity was expressed as reported in the original method, with the unit requiring verification.

3.15.4. Assay of Polyphenol Oxidase Activity

Polyphenol oxidase activity was determined following Mayer et al. (1965). The reaction mixture consisted of 0.2 mL of enzyme extract and 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5). The reaction was initiated by adding 0.2 mL of 0.01 M catechol. Changes in absorbance were recorded at 495 nm at 30s intervals for 3 min. Enzyme activity was expressed as reported in the original method, with the unit requiring verification.

3.15.5. Assay of Phenylalanine Ammonia-Lyase Activity

Phenylalanine ammonia-lyase activity was determined according to Dickerson et al. (1984). One gram of sample was homogenized in 1 mL of 25 mM borate–HCl buffer (pH 8.8) containing 5 mM mercaptoethanol. The homogenate was centrifuged at 15,000 rpm for 15 min, and the supernatant was used as the enzyme source.The assay mixture contained 0.2 mL of enzyme extract, 1.3 mL of water, and 0.5 mL of borate buffer (pH 8.8). The reaction was initiated by adding 1 mL of L-phenylalanine solution and incubating the mixture at 32°C for 1 h. The reaction was terminated by adding 0.5 mL of 2 N HCl. For the blank, L-phenylalanine was added after the addition of HCl. PAL activity was estimated by measuring the formation of trans-cinnamic acid at 290 nm and was expressed as µmol of cinnamic acid min^{-1} g^{-1} fresh tissue, as described in the original method.

3.15.6. Estimation of Total Phenolic Content

Total phenolic content was estimated following Zieslin and Ben-Zaken (1993). Brinjal leaf tissue (1 g) was homogenized in a mortar and pestle with 10 mL of 80% methanol and agitated at 70°C for 15 min. The supernatant was evaporated to dryness, and the residue was dissolved in 5 mL of distilled water.An aliquot of 0.2 mL was diluted to 3 mL with distilled water, followed by the addition of 0.25 mL of 1 N Folin–Ciocalteu reagent. After 3 min, 1 mL of 20% (w/v) sodium carbonate was added and mixed thoroughly. The tubes were placed in boiling water for 1 min and then cooled. Absorbance was measured at 650 nm against a reagent blank. Catechol was used as the standard, and total phenolic content was expressed as µg catechol equivalents g^{-1} leaf tissue.

3. RESULTS AND DISCUSSION

3.1. Survey for the Incidence of Brinjal Collar Rot

A survey conducted during 2023–2024 in the major brinjal growing areas of Ariyalur, Cuddalore, and Salem districts of Tamil Nadu revealed that collar rot incidence ranged from 9.78% to 31.59%. The highest incidence was recorded in Elayaperumalnallur village, Ariyalur district (31.59%), followed by Meensurutti (28.05%) in the same district. The lowest incidence was observed in Kattukottai village, Salem district (9.78%).

Similar geographical variation in disease incidence was reported by Kirankumar et al. (2024), who surveyed five districts of northern Karnataka during the 2020–2021 rabi season. Disease incidence was highest in Narendra village, Dharwad district (35.07%), whereas no disease was observed in some locations, including Hanchinal and Pettur. The authors associated disease spread with edaphic factors and agronomic practices, noting that flooded irrigation increased disease incidence while mulching reduced it. They also reported greater crop vulnerability during flowering and fruiting stages. Abinaya et al. (2020) recorded collar rot incidence ranging from 8.8% to 42.4% across locations in Madurai, Tiruchirappalli, and Theni districts, with the highest incidence in Vilangudi (42.4%) and the lowest in Kottampatty (8.8%). Similar findings were reported by Koshariya et al. (2020), Mahesha et al. (2022), Kamthe et al. (2023), and Meena et al. (2024b), supporting the present observations of variation in collar rot incidence among locations. **(**Table 1,Fig 1)

3.2. Cultural and Morphological Characteristics of *Sclerotium rolfsii* on PDA

3.2.1. Mycelial Growth

All ten *S. rolfsii* isolates produced profuse, cottony mycelial growth with a radial, fan-like appearance. The mycelium was initially silky white and later became dull white. Isolates Sr3 and Sr1 recorded the maximum colony diameter of 90 mm at 5 days after inoculation (DAI). Similar cultural characteristics were reported by Ganesan et al. (2024), who described initially silky white mycelium that subsequently became dull white with radial spreading. Microscopic observations in the present study revealed hyaline, thin walled, sparsely septate hyphae in young cultures. The pathogen was further characterized by extensive hyphal branching and the formation of small mycelial knots that developed into mustard seed like sclerotia. The mature sclerotia were shiny, hard, and deep brown to brownish black, with spherical to irregular shapes. These observations agree with the findings of Paul et al. (2023) and Shree et al. (2024).

3.2.2. Sclerotial Number

Sclerotial production varied among the ten *S. rolfsii* isolates grown on PDA. Isolate Sr3 produced the highest number of sclerotia, whereas Sr8 produced the lowest number (Table 3). These observations were consistent with the findings of Paul et al. (2023) and Shree et al. (2024), who also reported variation in sclerotial characteristics among isolates.

3.2.3. Sclerotial Colour

Variation in sclerotial colour was observed among the ten *S. rolfsii* isolates. Isolates Sr1, Sr6, and Sr9 produced pale brown sclerotia; Sr4 and Sr8 produced brown sclerotia; and Sr2, Sr3, Sr5, Sr7, and Sr10 produced dark-brown sclerotia.Paul et al. (2023) reported that spherical sclerotia began to develop after 10 days of incubation

and gradually changed from white or pale brown to cinnamon or Dresden brown. Similarly, Paparu et al. (2020) observed variation in sclerotial colour, with 69.6% of isolates producing light brown sclerotia and 30.4% producing dark brown sclerotia. These reports support the observed variation in sclerotial pigmentation among the isolates examined in the present study.

3.2.4. Sclerotial Shape

Sclerotial shape varied among the *S. rolfsii* isolates. The original observations recorded shaped sclerotia for Sr1, Sr5, Sr9, and Sr10; spherical sclerotia for Sr3, Sr4, and Sr8; and round sclerotia for Sr2, Sr3, and Sr9. Paul et al. (2023) described sclerotia as round, spherical, or irregularly elliptical, with smooth and shiny surfaces. Similarly, Hawaladar et al. (2022) reported that sclerotial bodies ranged from round to oval and varied in colour from light to dark brown. These findings indicate variation in sclerotial morphology among the isolates. **(**Table 2, Fig 2,3)

3.3. Pathogenicity of *S. rolfsii* on Brinjal under Pot-Culture Conditions

Pathogenicity tests conducted under greenhouse conditions revealed differences in virulence among the collected *S. rolfsii* isolates. Isolate Sr3, collected from Elayaperumalnallur village, recorded the highest collar rot incidence (62.13%) and was identified as the most virulent isolate. This was followed by Sr1, collected from Meensurutti, which recorded 59.03% disease incidence. Isolate Sr8, collected from Kattukottai, exhibited the lowest virulence, with 25.50% disease incidence. Geographical variation in the virulence of *S. rolfsii* has also been reported in other crops. Shanmugaraj et al. (2024) evaluated 50 isolates collected from tomato plants and classified them into highly, moderately, and less virulent groups based on the disease severity index. Boopathi et al. (2023) reported pathogenicity in all eight *S. rolfsii* isolates tested on tuberose, with SR1 recording the highest disease incidence (88%), followed by SR2 (57%). Mondal et al. (2022) identified isolate SRC2 as the most virulent among the isolates tested on lentil varieties, based on disease incidence and mortality. Similarly, Hawaladar et al. (2022) demonstrated that all ten isolates collected from five districts of Karnataka colonized the susceptible groundnut variety TMV-2 and induced stem rot symptoms. Comparable findings were reported by Parwanayoni et al. (2021) and Akash et al. (2022b), supporting the observed variation in pathogenicity among *S. rolfsii* isolates. (Table.3)

3.4.Cultural and Morphological Characteristics of *Trichoderma* Species

As presented in Table 8, isolates TaE and TaS attained a colony diameter of 90 mm within five days, whereas TaM, TaG, TaP and TaPp reached approximately 89 mm. Colony colour varied from dark green to pale white, with mycelial growth ranging from floccose to compact and scattered. The conidiophores were branched, exhibiting regular or irregular arrangements, while the conidia varied in colour from dark green to dull green and in shape from globose to ellipsoidal and obovoid.

These observations are consistent with Shanmugaraj et al. (2023), who characterized *T. asperellum* isolates by their branched, predominantly paired conidiophores, ampulliform phialides and light green, slightly ovoid to globose conidia. Raja et al. (2023) classified 35 *Trichoderma* isolates collected from groundnut growing areas of Rajasthan into four groups based on cultural characteristics, including compact dark green colonies, white green colonies with irregular conidiophores, dark olive coloured colonies and dusky yellowish green colonies. Similarly, Singh and Sharma (2020) reported considerable morphological variation among 15 *Trichoderma* isolates, with colony colours ranging from dark green and light green to whitish green, sometimes accompanied by concentric rings. Overall, the present findings demonstrate variation in the cultural and morphological characteristics of the tested isolates, consistent with the diversity reported in previous studies (Table 4, Fig 7)

3.5. *In Vitro* antagonistic activity of native *T. asperellum* isolates against *S. rolfsii* (sr3) using the dual culture technique

The dual-culture assay revealed considerable variation in antagonistic activity among the tested *T. asperellum* isolates. TaE exhibited the highest mycelial growth inhibition of *S. rolfsii* (83.48%), whereas TaG recorded the lowest inhibition (71.02%). These findings indicate differences in the antagonistic potential of the native isolates. Gahlot et al. (2023) reported that *T. harzianum* inhibited *S. rolfsii* by 70.78%, followed by *T. viride* at 57.67%. Akash et al. (2022) found that 30 of 104 *Trichoderma* isolates inhibited the pathogen by more than 80% under in vitro conditions. Similarly, Adhikari et al. (2022) evaluated nine *Trichoderma* isolates and recorded the highest inhibition (70.37%) with isolate Tricho5. They also observed lytic zones associated with the destruction of *S. rolfsii* mycelium. Comparable findings were reported by Montalvao et al. (2023). Aryal et al. (2022) described several mechanisms through which *Trichoderma* suppresses *S. rolfsii*, including the production of antifungal compounds and hydrolytic enzymes, as well as mycoparasitism involving host recognition, direct contact, hyphal coiling, penetration and degradation of pathogen mycelium and sclerotia. Compared with the inhibition values reported by Gahlot et al. (2023) and Adhikari et al. (2022), the higher inhibition recorded by TaE highlights its promising antagonistic potential against *S. rolfsii*. Its performance also falls within the range of highly antagonistic isolates identified by Akash et al. (2022). Thus, TaE emerged as the most effective isolate in the present dual-culture assay and warrants further evaluation under additional experimental conditions. **(**Table 5, Fig 8,9)

3.6. Efficacy of volatile compounds produced by *Trichoderma* species against *S. rolfsii* (Sr3) using the inverted plate assay

All six *Trichoderma* isolates produced volatile compounds that significantly inhibited the mycelial growth of *S. rolfsii*. TaE was the most effective isolate, restricting mycelial growth to 23.91 mm and achieving 73.43% inhibition. In contrast, the control recorded the maximum mycelial growth of 58.35 mm and the minimum inhibition value of 35.16%, as reported in the present study. Similar inhibitory effects of *Trichoderma* derived volatiles have been reported previously. Das and Mahapatra (2023) observed 44.25% inhibition of *S. rolfsii*

mycelial growth by *Trichoderma* isolate 5 using an inverted plate assay. Ayyandurai et al. (2023) reported that volatile compounds produced by *T. longibrachiatum* T(SP)-20 and *T. asperellum* T(AR)-10 inhibited the growth of *S. rolfsii* by 61.11% and 54.44%, respectively. Sridharan et al. (2020) likewise demonstrated the inhibitory activity of volatile organic compounds (VOCs) produced by *T. longibrachiatum* strain EF5.Rembinski et al. (2022) discussed the diversity of volatile compounds and secondary metabolites produced by *Trichoderma*, including epipolythiodioxopiperazines (ETPs), peptaibols, pyrones, butenolides, pyridines, azaphilones, steroids, anthraquinones, lactones, trichothecenes and harzianic acid. These compounds can interfere with the growth and development of other microorganisms, affecting processes such as sporulation, spore germination and sclerotial viability. Similar observations were reported by Kushwaha et al. (2018) and Sunil and Thara (2020). The 73.43% inhibition recorded for TaE exceeded the values reported by Das and Mahapatra (2023) and those reported for the two isolates evaluated by Ayyandurai et al. (2023). This comparison identifies TaE as a promising producer of inhibitory volatile compounds against *S. rolfsii* under the conditions tested. However, the specific compounds responsible for inhibition cannot be established from the inverted plate assay alone and require further chemical characterization. **(**Table 6,Fig 11)

3.7. Effect of culture filtrates of native *Trichoderma* Sp. on the mycelial growth of *S. rolfsii* (Sr3)

The culture filtrates of six *Trichoderma* isolates were evaluated against *S. rolfsii* (Sr3) using the poison food technique. Among the tested isolates, TaE exhibited the highest inhibitory activity at a 40% culture filtrate concentration, reducing mycelial growth to 1.06 mm and achieving 98.82% inhibition. Hossain et al. (2023) reported that incorporating culture filtrates of *T. harzianum* and *T. viride* into potato dextrose agar significantly reduced the colony diameter of *S. rolfsii*, with values ranging from 1 to 2.92 cm across the observation period. Similarly, Sunil and Thara (2022) recorded 84.44% and 71.11% inhibition of *S. rolfsii* by *T. harzianum* and *T. viride*, respectively. Rembinski et al. (2022) also discussed the potential roles of *Trichoderma*-derived volatile compounds and secondary metabolites in suppressing microbial growth and affecting spore germination and sclerotial viability. Further supporting evidence was reported by Vineela et al. (2020), Sekhar et al. (2020b) and Thomas et al. (2022). The 98.82% inhibition achieved by TaE at a 40% culture filtrate concentration indicates strong suppression of *S. rolfsii* under the tested in vitro conditions. This value is higher than the inhibition percentages reported by Sunil and Thara (2022), suggesting that TaE culture filtrate has considerable potential for further investigation. Nevertheless, the active compounds responsible for this effect remain to be identified, and their contribution should be confirmed through chemical analysis and further biological validation. **(**Table 7, Fig 10)

3.8. Biochemical Tests for the Identification of Native *Bacillus* Isolates

The biochemical tests and Gram staining results showed similar characteristics among all native *Bacillus* isolates. All isolates were positive for Gram staining, siderophore production, catalase activity and citrate utilization, whereas starch hydrolysis and chitinase production were negative in all isolates. These findings can be compared with the characterization studies reported by Sunkad et al. (2023), who evaluated *Bacillus subtilis* based on morphological, physiological and biochemical characteristics. Maina et al. (2021) also identified and characterized *Bacillus* spp. using Gram staining, morphological observations and biochemical tests, including catalase activity, nitrate reduction, methyl red, Voges–Proskauer, indole production, motility, hydrogen sulphide production, citrate utilization, triple sugar iron agar reactions and starch hydrolysis. In contrast, Zhu et al. (2020) reported positive results for nitrate reduction, glucose fermentation, starch hydrolysis, gelatin liquefaction and citrate utilization in two bacterial strains. Overall, the present results establish a consistent biochemical profile among the six tested isolates, particularly for siderophore production, catalase activity and citrate utilization. However, the negative results for starch hydrolysis and chitinase production distinguish their biochemical profiles from those reported for some other bacterial strains. These characteristics provide preliminary support for isolate characterization, although biochemical tests alone are insufficient for definitive species level identification. **(**Table 8)

3.9.Cultural and Morphological Characteristics of *Bacillus* Isolates

All six *Bacillus* isolates exhibited similar cultural and morphological characteristics. The bacterial cells were rod shaped, and the colonies were cream coloured, circular and characterised by undulate margins. Growth rates ranged from fast to slow, and no visible reaction was observed under UV light.

Similar observations were reported by Mirzabaev et al. (2023), who classified *B. subtilis* isolates into four groups based on colony morphology, with colony colours ranging from creamy white to dull white and variations in colony margins. Zhu et al. (2020) also reported that the antagonistic bacterial strains IBFCBF-4 and HZ-72 were rod-shaped and formed yellowish, circular colonies with undulate margins on Luria Bertani (LB) agar.

The similarities between the present observations and previous reports support the use of cultural and morphological characteristics for preliminary characterization of the native isolates. Nevertheless, molecular identification would be required to confirm their taxonomic identity. **(**Table9, Fig 12)

***3.10.In Vitro* antagonistic activity of native *B. subtilis* isolates against *S. rolfsii* (Sr3) using the dual culture technique**

The dual culture assay revealed differences in the antagonistic activity of the six bacterial isolates against *S. rolfsii* (Sr3). BsE exhibited the highest mycelial growth inhibition (84.5%), with a reported mycelial growth of 13.95 mm and the widest inhibition zone of 13.03 mm.

Li et al. (2023) reported that six bacterial strains exhibited strong antifungal activity against *S. rolfsii*, with inhibition zones exceeding 10 mm. Among these, strains F-1 and R-11 produced inhibition zones of 20.25 mm and 15.49 mm, respectively. Similarly, Zou et al. (2023) demonstrated that *B. subtilis* strain JY-7-2L suppressed hyphal growth, sclerotial production and sclerotial germination *in vitro*.

Farhaoui et al. (2022) evaluated 49 bacterial isolates against *S. rolfsii* associated with sugar beet and identified *B. amyloliquefaciens* MW644651 as the most effective isolate, with 70.35% inhibition, followed by *B. subtilis* MW644678, with 67.68% inhibition after six days of incubation. Sharf et al. (2021) described the role of antifungal compounds and competition for nutrients and space in the suppression of *S. rolfsii* by *Bacillus* spp. Similar findings were reported by Rakh et al. (2020) and Kumari et al. (2024).

Compared with the inhibition percentages reported by Farhaoui et al. (2022), the 84.5% inhibition achieved by BsE indicates strong antagonistic activity under the conditions tested. The observed inhibition zone further supports its potential to suppress the pathogen in vitro. However, direct comparisons among studies should be interpreted cautiously because assay conditions, incubation periods and bacterial strains may differ. Further evaluation is required to establish the effectiveness of BsE under greenhouse and field conditions. **(**Table 10,Fig 13)

3.11.Efficacy of volatile compounds produced by *B. subtilis* against *S. rolfsii* (Sr3) using the inverted plate assay

All six *B. subtilis* isolates produced volatile compounds that significantly inhibited the mycelial growth of *S. rolfsii*. BsE was the most effective isolate, restricting mycelial growth to 21.13 mm and achieving 76.52% inhibition. Sunkad et al. (2023) reported that volatile compounds produced by *Bacillus* spp. inhibited the growth of the pathogen associated with stem and pod rot of groundnut in a paired plate assay. Zou et al. (2023) demonstrated that the cell free culture filtrate of *B. subtilis* JY-7-2L completely inhibited the germination of *S.rolfsii* sclerotia, even after 120 hours of incubation. These findings indicate that *Bacillus* strains can produce inhibitory substances that affect different stages of pathogen development. Banakar et al. (2017) attributed the fungistatic activity of *Bacillus* spp. against *S. rolfsii* to the production of volatile compounds, antibiotics, siderophores and hydrogen cyanide. Chen et al. (2020) also reported that *B. velezensis* strain LHSB1 had potential for managing stem rot of groundnut through the production of antifungal metabolites, including bacillomycin L, surfactin A and fengycin A. Similar observations were reported by Massawe et al. (2018) and Hossain et al. (2023).

The 76.52% inhibition recorded for BsE demonstrates its strong inhibitory effect in the inverted-plate assay. Together with the dual-culture results, this finding suggests that BsE may suppress *S. rolfsii* through multiple antagonistic mechanisms. **(**Table 11,Fig 15)

3.12.Effect of Culture Filtrates of *B. subtilis* Isolates on the Mycelial Growth of *S. rolfsii* (Sr3)

Culture filtrates from six *B. subtilis* isolates were evaluated against *S. rolfsii* using the poison food technique. Among the isolates tested, BsE exhibited the highest inhibitory activity at a culture-filtrate concentration of 40%, reducing mycelial growth to 2.95 mm and achieving 96.72% inhibition. Nayak et al. (2023) reported antagonistic activity against *S. rolfsii* among 19 *Bacillus* isolates, with five isolates exhibiting comparatively high inhibition of the pathogen. Similarly, Ankitha et al. (2023) demonstrated that *Bacillus* strain NBAIR-BSWG1 inhibited *S. rolfsii* growth by 82.73–100% at concentrations ranging from 50 to 100 µL/mL using the poison-food technique.Kumari et al. (2021) described several mechanisms underlying the antagonistic activity of *Bacillus* spp., including the production of secondary metabolites such as iturin, surfactin and fengycin, as well as hydrolytic enzymes such as chitinases, proteases and glucanases. These compounds and enzymes can disrupt fungal structures and interfere with pathogen growth. Caulier et al. (2019) also discussed the potential roles of antimicrobial substances, including subtilin, bacilysin, mycobacillin and iturin, together with chitinolytic enzymes, hydrogen cyanide, siderophores and competition for nutrients, in the suppression of fungal pathogens. Similar findings were reported by Kumar et al. (2021) and Li et al. (2023).

The 96.72% inhibition achieved by BsE at a 40% culture-filtrate concentration indicates substantial suppression of *S. rolfsii* under the tested in vitro conditions. This result is comparable to the high inhibition reported by Ankitha et al. (2023). Collectively, the dual culture, volatile compound and culture-filtrate assays identify BsE as a promising candidate for further investigation. **(**Table 12,Fig 14)

3.13. Efficacy of Different Oil Cake Extracts against *S. rolfsii* (Sr3)

Among the five oil cake extracts evaluated at concentrations of 10%, 20%, 30% and 40%, neem cake extract at 40% was the most effective, reducing mycelial growth to 16.02 mm and achieving 82.20% inhibition compared with the control. In contrast, coconut oil cake extract was the least effective, recording 57.03 mm of mycelial growth and 36.63% inhibition.These findings are consistent with Babariya et al. (2023), who reported the highest in vitro inhibition of *S. rolfsii* with castor oil cake among five organic amendments. Ayyandurai et al. (2023) evaluated mahua, neem, pungam, coconut and castor oil cake extracts against the stem rot pathogen of groundnut and found that neem oil cake extract produced the greatest inhibition at 5% and 10% concentrations, with 73.33% and 78.88% inhibition, respectively. Bhagat et al. (2022) also reported that neem extracts significantly reduced the mycelial dry weight of *S. rolfsii*. However, their findings indicated that the combination of neem cake and cow dung produced a higher disease index than the biocontrol agent and plant extract treatments applied individually.The antifungal activity of neem cake may be associated with bioactive compounds such as azadirachtin, nimbin, salannin and nimbolinin, which have been reported to interfere with fungal growth and cellular integrity (Anitha et al., 2019; Manasa et al., 2022). Similar findings were reported by Dutta et al. (2022)

and Saini et al. (2023). Overall, neem cake extract at 40% showed the highest inhibitory activity against *S. rolfsii* among the oil cake extracts tested in the present study. Its 82.20% inhibition supports the potential of neem cake as an organic amendment for suppressing the pathogen. **(**Table.13,Fig.16)

3.14. Effect of seed treatment with biocontrol agents on seedling growth under the roll towel method

In the present study, the combined seed treatment designated T_7, consisting of *T. asperellum* TaE at 6 g/kg seed, *B. subtilis* BsE at 12 g/kg seed and neem cake, recorded the highest germination percentage, shoot length, root length and seedling vigour index of brinjal.

Pooja et al. (2024) evaluated different seed-dressing fungicides and biocontrol agents for improving green gram seed quality using the paper-towel method. Treatment with *T. harzianum* at 10 g/kg seed recorded the greatest shoot length (24.3 cm) and root length (13.42 cm). Similarly, Yamunarani et al. (2023) reported improvements in germination percentage, root length, shoot length and seedling vigour index in black gram seeds treated with *T. asperellum* ECK and *Streptomyces* sp.

Sunilkumar et al. (2021) found that the combined application of *Pseudomonas fluorescens* with coir pith and jelly improved soybean seed germination to 95.67% and increased the vigour index to 972.64. Konappa et al. (2020) evaluated rhizosphere microorganisms for their potential to induce resistance against bacterial wilt in tomato and reported that *P. fluorescens* Pf3 produced 93% germination and a vigour index of 1609, followed by *T. asperellum* T8, with 91% germination and a vigour index of 1593.

The growth promoting effects of seed-applied biocontrol agents may be associated with improved nutrient uptake, phytohormone production and root development. Trichoderma spp. can contribute to plant growth through improved nutrient acquisition and the production of growth-regulating compounds, including indole-3-acetic acid and gibberellins (Kthiri et al., 2020). Similarly, *Bacillus* spp. may promote growth through nutrient solubilization, nitrogen fixation and the production of auxins and gibberellins (Tsotetsi et al., 2022; Patani et al., 2024). Qessaoui et al. (2019) also reported that *Pseudomonas* spp. can promote germination and plant growth through phytohormone and siderophore production, phosphate solubilization, ammonia production and root colonization. The superior performance of T_7 in the present study suggests that combining *T. asperellum*, *B. subtilis* and neem cake may improve brinjal seedling establishment compared with individual treatments. This observation is consistent with previous reports demonstrating the benefits of microbial seed treatments for seed quality and early plant growth. However, the relative contribution of each component cannot be established without comparisons against the corresponding individual and combination treatments. **(**Table15)

3.15. Effect of seed treatment and soil application of a consortium of biocontrol agents and neem cake on collar rot incidence in brinjal under pot culture

In the present study, the combined treatment designated T10, involving seed treatment and soil application of *T. asperellum* and *B. subtilis*, together with neem cake, recorded the lowest collar rot incidence in brinjal plants challenged with *S. rolfsii*. The treatment included *T. asperellum* seed treatment at 6 g/kg and soil application at 2.5 kg/ha, *B. subtilis* seed treatment at 12 g/kg and soil application at 2.5 kg/ha, and neem cake seed treatment at 2 g/kg and soil application at 150 kg/ha.Meena et al. (2024) reported that combined seed treatment and soil application of *T. harzianum* and *P. fluorescens* at 10 g/kg seed and 10 kg/ha, respectively, resulted in 7.40% disease incidence and 69.37% disease control in groundnut. A combination of *T. viride* and *P. fluorescens* produced 8.0% disease incidence and 66.88% disease control. Similarly, Ayyandurai et al. (2023) reported that the combined application of *T. longibrachiatum* T(SP)-20 and *T. asperellum* T(AR)-10 as seed treatment at 4 g/kg, together with soil application at 50 g/pot and mahua oil cake at 100 g/pot before sowing and 30 days after sowing, resulted in the lowest stem rot incidence (11.34%) in groundnut under pot culture. The results of the present study support the potential of combining microbial antagonists with organic amendments for managing soil borne diseases. The lowest collar rot incidence recorded under T10 suggests that integrating *T. asperellum*, *B. subtilis* and neem cake may provide effective disease suppression under pot-culture conditions. **(**Table 16)

3.16.Effect of seed treatment and soil application of a consortium of biocontrol agents and neem cake on brinjal growth and yield under pot culture

The combined treatment designated T10, consisting of *T. asperellum* seed treatment at 6 g/kg and soil application at 5 g/pot, *B. subtilis* seed treatment at 12 g/kg and soil application at 5 g/pot, together with neem cake soil application at 20 g/pot, recorded the highest plant height, number of fruits per plant and yield per plant. The respective values were 136.61 cm, 14.97 fruits per plant and 2.29 kg per plant.

Farhaoui et al. (2022) reported significant increases in shoot length, root length, and fresh and dry plant weight following seed treatment with *Bacillus* isolates. Similarly, Murmu et al. (2021) observed improvements in plant height, number of branches and number of leaves in brinjal treated with biocontrol agents and organic amendments. Among the treatments, the combination of *T. viride* and castor oil produced the greatest improvement in growth parameters, followed by the combination of *T. viride* and neem oil. Similar findings were reported by Mbarki et al. (2016) and Zahim et al. (2018).

The growth-promoting effects of *Trichoderma* may be associated with enhanced nutrient uptake, phytohormone production and root development (Kthiri et al., 2020). Likewise, *Bacillus* spp. may improve plant growth through nutrient solubilization, nitrogen fixation and the production of growth regulating compounds such as auxins and gibberellins (Tsotetsi et al., 2022). Neem cake may additionally contribute to disease suppression through its bioactive constituents, including azadirachtin, nimbin, salannin and nimbolinin (Anitha et al., 2019; Manasa et al., 2022).

The higher plant height, fruit number and yield recorded under T10 indicate the potential benefits of integrating *T. asperellum*, *B. subtilis* and neem cake in brinjal production. These results are consistent with previous studies reporting improved plant growth following the combined application of biocontrol agents and organic amendments. However, the relative contributions of disease suppression and direct growth promotion cannot be separated from the reported results alone. **(**Table 17,Fig 17)

3.17. Induction of defense enzymes in brinjal plants treated with biocontrol agents and neem cake under greenhouse conditions

In the present study, the combined treatment designated T4, comprising *T. asperellum* seed treatment at 6 g/kg and soil application at 2 g/pot, *B. subtilis* seed treatment at 12 g/kg and soil application at 2 g/pot, together with neem cake seed treatment at 2 g/kg and soil application at 20 g/pot, resulted in higher activities of peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), as well as increased total phenol content, compared with the individual biocontrol-agent treatments and pathogen-inoculated plants.

Shanmugaraj et al. (2024) reported that treating tomato plants with *T. asperellum* against *S. rolfsii* upregulated several defence-related genes, including PR1, PR2, PR3, PR5, PR12, thioredoxin peroxidase, catalase, PPO, PAL, isochorismate synthase, laccase, prosystemin, multicystatin, WRKY31, MYC2, lipoxygenase A, lipoxygenase C, proteinase inhibitor I, proteinase inhibitor II and ethylene response 1. These genes were associated with defence-signalling pathways involving salicylic acid and jasmonic acid/ethylene. The response was most pronounced 48 hours after inoculation in plants primed with isolate A10 and subsequently challenged with *S. rolfsii*.

Similarly, Sharma et al. (2024) reported enhanced biochemical defence responses in seeds bioprimed with *T. harzianum*, *T. atroviride* and *P. fluorescens* in combination with neem seed kernel extract. The treatments increased the activities of PPO and PO, total phenolic content and PAL. Ayyandurai et al. (2021) also found that groundnut plants treated sequentially with *Trichoderma* spp. and mahua oil cake exhibited the highest PAL, PO and PPO activities and phenol content following challenge inoculation with *S. rolfsii*, whereas inoculated control plants recorded the lowest levels.

Singh et al. (2021) reported that root colonization by *Trichoderma* spp. contributed to enhanced resistance in brinjal. Combined treatment with *T. harzianum* and *T. asperellum* increased total phenolic content and shikimic acid levels at 72 hours after inoculation, accompanied by higher activities of PAL, PO and PPO. Similar findings were reported by Bisen et al. (2019), Kumari et al. (2020) and Maddu et al. (2022). Overall, the increased activities of defence-related enzymes and the higher total phenol content observed in the combined treatment suggest that *T. asperellum*, *B. subtilis* and neem cake may contribute to the activation of biochemical defence responses in brinjal challenged with *S. rolfsii*. These findings are consistent with previous studies demonstrating enhanced defence-related enzyme activity following the application of biocontrol agents and organic amendments (Fig 18,19, 20, 21).

4. CONCLUSION

The present study demonstrated the potential of integrating biological control agents and organic amendments for the eco-friendly management of collar rot of brinjal caused by *Sclerotium rolfsii*. Among the evaluated biocontrol agents, *Trichoderma asperellum* (TaE) exhibited promising antagonistic activity against the pathogen, while *Bacillus subtilis* (BsE) showed considerable potential as a bacterial antagonist. The incorporation of neem cake further provides an opportunity to strengthen an integrated disease management approach through the combined use of beneficial microorganisms and organic amendments. Overall, the findings highlight the importance of integrating *Trichoderma*, *Bacillus* and neem cake as potential components of sustainable collar rot management in brinjal. Further validation under field conditions would help establish the consistency and practical applicability of this approach.

Conflict of Interest

The authors declare that they have no conflict of interest regarding the publication of this research work.

REFERENCES

1. Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria. *Crop Sci* 13: 630-633
2. Abinaya A, Kalpana K, Ebeneza EG, Thiruvudainambi, Theradimani M, Arunkumar R (2020) Evaluation of Antagonistic Potential of Bacillus spp. and Trichoderma spp. against *Sclerotium rolfsii* Sacc. causing Collar Rot Disease in *Solanum melongena* L. *Int J Curr Microbiol App Sci* 9(12): 694-703
3. Adhikari P, Shrestha SM, Manandhar HK, Marahatta S (2022) Morphology and cross infectivity of *Sclerotium rolfsii* Sacc. isolated from different host plants in Nepal
4. Agrios GN (2005) Plant pathology. Acad Press, New Delhi.
5. Agrios GN (2005) Plant pathology. Acad Press, New Delhi.
6. Ainsworth GC (1961) Dictionary of fungi. Common Wealth Mycological Institute, Kew Burrey. England: pp 547
7. Akash AU, Ramya V, Uma Devi G, Pushpavalli, SNCVL, Triveni S (2022 a) Antagonist activities of native rhizosphere micro-flora against groundnut stem rot pathogen, *Sclerotium rolfsii* Sacc. *Egypt J Biol Pest Control* 32 (1): 133

8. Akash AU, Ramya V, Umadevi G, Triveni S, Pushpavalli SN (2022 b) Morphological and virulence variability of *Sclerotium rolfsii* Sacc. collected from groundnut growing areas of Nagarkurnool district of Telangana state. *Agric Res J* 59 (5): 974-980
9. Anita K, Thiruvudainambi S, Harish S, Kumar RA (2019) Bio-efficacy of organic amendments and plant oils against *Sclerotium rolfsii* Sacc.: The incitant of Southern blight of tomato. *J Pharmacogn Phytochem* 8(3): 2202-2205
10. Ankitha KS, Radha TK, Ruqiya S, Kukreti A, Aarthi N, Nanditha S, Manjunatha C (2023) Exploring the impact of cyclic lipopeptides from *Bacillus subtilis* NBAIR-BSWG1 through *in vitro* and *in planta* studies against *Sclerotium rolfsii*. *J Biol Control* 37(3)
11. Arya Kaniyassery, Sachin Ashok Thorat, Kodsara Ramachandra Kiran, Thokur Sreepathy Murali, Annamalai Muthusamy (2023) Fungal diseases of eggplant (*Solanum melongena* L.) and components of the disease triangle: A review. *J Crop Improv* 37(4): 543-594
12. Arya Kaniyassery, Sachin Ashok Thorat, Kodsara Ramachandra Kiran, Thokur Sreepathy Murali, Annamalai Muthusamy (2023) Fungal diseases ofeggplant (Solanum melongena L.) and components of the disease triangle: A review. J Crop Improv 37(4): 543-594
13. Aryal L, Baidya S, Subedi S (2022) Efficacy of *Trichoderma* isolates against *Sclerotium rolfsii* causing collar rot of lentil. *J Agric Nat Resour* 5(1): 191-197
14. Aswathy B Nai, Veena S, Sheela MN, Karthikeyan S, Sreelatha GL, Vishnu VR (2019) Microbial diversity in rhizosphere soils of tropical tuber crops: utilization for pathogen suppression and growth promotion. *J Root Crops* 45(1): 53-63
15. Ayed F, Jabnoun-Khiareddine H, Abdallah RAB, Daami-Remadi M (2020) Effect of different carbon and nitrogen sources on *Sclerotium rolfsii* Sacc. mycelial growth and sclerotial development. *Int J Phytopathol* 9(1): 17-27
16. Ayyandurai M, Akila R, Manonmani K, Harish S, Mini ML, Vellaikumar S (2023) Deciphering the mechanism of *Trichoderma* spp. consortia possessing volatile organic compounds and antifungal metabolites in the suppression of *Sclerotium rolfsii* in groundnut. *Physiological and Molecular Plant Pathology* 125: 102005
17. Ayyandurai M, Akila R, Manonmani K, Mareeswari P, Mini ML, Kalpana K, Brindhadevi S (2021) Upregulation of defense enzymes in groundnut (*Arachis hypogaea* L.) by combined application of *Trichoderma* spp. and Mahua oil cake against *Sclerotium rolfsii*. *The Pharma Innovation* 10: 1916
18. Ayyandurai M, Akila R, Mini ML, Manonmani K (2022) Gas chromatography–mass spectrometry (GC-MS) analysis of antimicrobial compounds produced by mahua oil cake against the stem rot pathogen - *Sclerotium rolfsii*. *J Appl Nat Sci* 14(2): 600-606
19. Babar HM (1999) Studies on collar rot of sunflower (Doctoral dissertation, Ph.D. Thesis, Dept. Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh)
20. Babar HM (1999) Studies on collar rot of sunflower (Doctoral dissertation, Ph.D. Thesis, Dept. Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh
21. Babariya et al. (2023) *(Note: Cited in text as Babariya et al., 2023, but corresponding full entry is generalized in references)*
22. Banakar SN, Sanath Kumar VB, Thejesha AG (2017) *In vitro* evaluation of bio-agents and fungicides against foot rot pathogen (*Sclerotium rolfsii* Sacc.) of tomato. *Int J Current Microbiol Appl Sci* 6(3): 1591-1598
23. Basha SU, Ulaganathan K (2002) Antagonism of *Bacillus* species (strain BC121) towards *Curvularia lunata*. *Curr Sci* 1457-1463
24. Begum SN, Chowdhury B, Ahamed HU (1985) Screening of brinjal varieties for resistance to *Sclerotium rolfsii*. *Abst. 1st Natl. Conf. Plant Pathol*
25. Begum SN, Chowdhury B, Ahamed HU (1985) Screening of brinjal varieties for resistance to Sclerotium rolfsii. Abst. 1st Natl. Conf. Plant Pathol
26. Bekriwala TH, Nath K, Chaudhary DA (2016) Effect of age on susceptibility of groundnut plants to *Sclerotium rolfsii* Sacc. caused stem rot disease. *J Plant Pathol Microbiol* 7: 386
27. Bhagat I, Yadav LK (2022) Integrated management of leaf spot of brinjal caused by *Alternaria alternata*. *J Mycopathol Res* 61(4): 537-544
28. Bisen K, Ray S, Singh SP (2019) Consortium of compatible *Trichoderma* isolates mediated elicitation of immune response in *Solanum melongena* after challenge with *Sclerotium rolfsii*. *Archives of Phytopathology and Plant Protection* 52: 733-756
29. Boopathi P (2023) Morphological and physiological characteristics of *Sclerotium rolfsii* causing stem rot or rhizome rot disease in tuberose (*Polianthes tuberosa*). *J crop weed* 19(1): 276-284
30. Breed RS, Murray EG, Smith NR (1989) Bergey's Manual of Determinative Bacteriology, 9th ed. William and Wilkins Co., Baltimore, Maryland. p. 1094
31. Caulier S, Nannan C, Gillis A, Licciardi F, Bragard C, Mahillon J (2019) Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Front Microbiol* 10: 302
32. Cheesebrough M (1991) Medical Laboratory Manual for Tropical Countries. University Press Cambridge
33. Chen L, Wu YD, Chong XY, Xin QH, Wang DX, Bian K (2020) Seed-borne endophytic *Bacillus velezensis* LHSB1 mediate the biocontrol of peanut stem rot caused by *Sclerotium rolfsii*. *J Appl Microbiol* 128(3): 803-813
34. Cyrabree T, Hindshill G (1975) Fundamental Experiments in Microbiology

35. Das A, Mahapatra SS (2023) Screening of native *Trichoderma* isolates against soilborne pathogens of green gram. *The Pharma Innovation J* 12(5): 2832-2835
36. Dennis L, Webster J (1971) Antagonistic properties of species group of Trichoderma I. Production of non-volatile antibiotics. *Trans Br Mycol Soc* 57: 25-39
37. Dickerson DP, Pascholari SF, Hagerman AE, Butler LG, Nicholson RL (1984) Phenylalanine ammonia lyase and hydroxyl cinnamate CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiol Mol Plant Pathol* 25: 111-123
38. Dubey SC, Patel B (2000) *In vitro* evaluation of some oil cakes and plant extracts against *Thanatephorus cucumeris*, *Gliocladium virens* and *Trichoderma viride*. *J Mycol Plant Pathol* 30: 411-413
39. Elad Y and Chet I (1983). Improved selective media for isolation of Trichoderma or Fusarium spp. Phytoparasitica 11: 55–58.
40. Farhaoui A, Adadi A, Tahiri A, El Alami N, Khayi S, Mentag R, Lahlali R (2022) Biocontrol potential of plant growth-promoting rhizobacteria (PGPR) against *Sclerotium rolfsii* diseases on sugar beet (*Beta vulgaris* L.). *Physiol Mol Plant Pathol* 119: 101829
41. Food and Agriculture Organization (2022) The state of food security and nutrition in the world 2021: Transforming food systems for food security, improved nutrition, and affordable healthy diets for all. FAO.
42. Food and Agriculture Organization (2022). The state of food security and nutrition in the world 2021: Transforming food systems for food security, improved nutrition, and affordable healthy diets for all.https\://www\.fao.org/publications
43. Gahlot VS, Kumhar DR, Meena AK, Dilip S, Reddy K, Kumar D (2023) Role of bioagents and plant extracts in resistance induction against *Sclerotium rolfsii* under *in vitro* condition.
44. Ganesan S, Sonavane P, Devadarshini D, Gadnayak A, Sahu S, Petikam S, Acharya GC (2024) Characterization of *Athelia rolfsii* associated with southern blight of pointed gourd (*Trichosanthes dioica*) in India. *Can J Plant Pathol Mar* 3: 1-8
45. Hammerschmidt R, Nuckles EM, Kuc J (1982) Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol Mol Plant Pathol* 20: 73-82
46. Hasan MM, Meah MB (2019) Anatomy and biochemical study of collar rot resistance in eggplant. *Int J Pathog Res* 2(2): 1-13
47. Hasan MM, Meah MB (2019) Anatomy and biochemical study of collar rot resistance in eggplant. Int J Pathog Res 2(2): 1-13
48. Hawaladar S, Nandan M, Vinaykumar HD, Hadimani RH, Hiremath S, Venkataravanappa V, Reddy CL (2022) Morphological and molecular characterization of *Sclerotium rolfsii* associated with stem rot disease of groundnut (*Arachis hypogaea* L.). *Indian Phytopathology* 1-12
49. Hossain MM, Azad MAK, Khalequzzaman KM (2023) Antifungal activity of botanicals and biological agents against *Sclerotium rolfsii* causing foot and root rot disease of lentil. *Bhartiya Krishi Anusandhan Patrika* 38(1): 71-75
50. Iverson JL, Millis NF (1974) *(Note: Cited in text for Starch hydrolysis, full entry follows standard author listings)*
51. Jadon KS (2009) Eco-friendly management of brinjal collar rot caused by Sclerotium rolfsii Sacc. Indian Phytopathol 62(3): 345-347
52. Kirankumar SJ, Yadahalli KB, Hegde GM, Purushotham P (2024) An investigation on the occurrence of brinjal wilt caused by *Sclerotium rolfsii* in Northern Karnataka, India. *Int J Environ Climate Change* 14(1): 54-66
53. Koike ST, Gladders P, Paulus AO (2007) Vegetable diseases: A color handbook. Gulf Professional Publishing, Texas
54. Koike ST, Gladders P, Paulus AO (2007) Vegetable diseases: A color handbook. Gulf Professional Publishing, Texas
55. Konappa N, Krishnamurthy S, Arakere UC, Chowdappa S, Ramachandrappa NS (2020) Efficacy of indigenous plant growth-promoting rhizobacteria and *Trichoderma* strains in eliciting resistance against bacterial wilt in tomato. *Egypt J Biol Pest Control* 30: 1-13
56. Koshariya AK, Neelu N (2020) Survey and collection of lentil collar rot in Chhattisgarh. *J Pharmacogn Phytochem* 9: 3191-3196
57. Kthiri Z, Jabeur MB, Machraoui M, Gargouri S, Hiba K, Hamada W (2020) Coating seeds with *Trichoderma* strains promotes plant growth and enhance the systemic resistance against Fusarium crown rot in durum wheat. *Egypt J Biol Pest Control* 30:1-0
58. Kumar K, Sharma JP, Chopra S (2011) Studies on variability, heritability and genetic advance for morphological and yield traits in brinjal (*Solanum melongena* L.). *Mysore J Agricult Sci* 45(1): 63-66
59. Kumar K, Sharma JP, Chopra S (2011) Studies on variability, heritability and genetic advance for morphological and yield traits in brinjal (Solanum melongena L.). Mysore J Agricult Sci 45(1): 63-66
60. Kumar R, Raja S, Arulselvi A, Immanuel R, Christopher D, Jaiganesh V, Udhayakumar R (2021) Cultural, morphological, and pathogenicity characters of *Sclerotium rolfsii* causing stem rot in groundnut. *Plant Archives* 21
61. Kumari A, Ghatak A, Srinivasaraghavan A (2020) Biochemical responses of soilborne necrotroph *Sclerotium rolfsii* during the pathogenesis on chickpea. *IJCS* 8(1): 2596-2601

62. Kumari R, Kumar V, Arukha AP, Rabbee MF, Ameen F, Koul B (2024) Screening of the biocontrol efficacy of potent *Trichoderma* strains against *Fusarium oxysporum* f. sp. *ciceri* and *Sclerotium rolfsii* causing wilt and collar rot in chickpea. *Microorganisms* 12(7): 1280
63. Latha P, Rajeswari E (2019) Evaluation of biocontrol agents, fungicides and organic amendments against Sclerotium wilt (*Sclerotium rolfsii* Sacc) of jasmine (*Jasminum sambac* (L.) Aiton). *J Pharmacognosy Phytochem. SP2*: 897-902
64. Leahy JG and Colwell RR (1990). Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54(3): 305–315.
65. Li L, Wang J, Liu D, Li L, Zhen J, Lei G, Yang W (2023) The antagonistic potential of peanut endophytic bacteria against *Sclerotium rolfsii* causing stem rot. *Braz J Microbiol* 54(1): 361-370
66. Mahesha HS, Keerthi MC, Manjunatha N, Vinaykumar HD, Singh T, Bhargavi HA, Saini RP, Ahmed S, Yadav VK (2022) Occurrence of collar rot on berseem (*Trifolium alexandrinum* L.) caused by *Sclerotium rolfsii* and its molecular characterization in India. *Arch Phytopathol Plant Prot* 55(1): 89-97
67. Maina J, Mathara JM, Kikuvi GM, Wafula E (2021) Isolation and identification of bacteriocin-producing *Bacillus* spp. from *Rastrineobola argentea* (Omena) with activity against bovine mastitis bacterial pathogens.
68. Manasa P, Senapati AK, Dwibedi SK, Kumar S (2022) Bio-efficacy of fungicides, botanicals, bioagents, and organic amendments against *S. rolfsii* causing stem rot disease of groundnut. *BFAIJ* 14(4a): 715-722
69. Manu TG, Nagaraja A, Manjunatha SV (2018) Morphological and cultural variability among the *Sclerotium rolfsii* isolates. *Journal of Pharmacognosy and Phytochemistry* 7: 904-907
70. Mayee CD, Datar VV (1986) Phytopathometry
71. Mayer AM, Harel E, Shaul RB (1965) Assay of catechol oxidase: a critical comparison of methods. *J Phytochem* 5: 783-789
72. Mbarki S, Cerda A, Brestic M, Mahendra R, Abdelly C, Pascual JA (2016) Vineyard compost supplemented with *Trichoderma harzianum* 178 improve saline soil quality. *Land Degrad* 1028-1037
73. Meena PK, Sharma RS, Dhayal S, Ghasolia RP, Nain Y (2024 b) Roving survey of collar rot disease of chickpea caused by *Sclerotium rolfsii* Sacc. in Rajasthan, India. *J adv biol biotechnol* 27(9): 208-218
74. Meena PN, Meena AK, Tiwari RK, Lal MK, Kumar R (2024 a) Biological control of stem rot of groundnut induced by *Sclerotium rolfsii* Sacc. *Pathogens* 13(8): 632
75. Mirzabaev BM, Sergazina AY, Mukhtarov AK (2023) Isolation and identification of promising strains of *Bacillus subtilis*.
76. Mohanty A, Behera P, Sundarkar D (2016) Antifungal activity of plant extracts against *Sclerotium rolfsii* (collar rot pathogen) in tuberose. *Int J Current Res* 8: 25946-25948
77. Mondal A, Debnath D, Das T, Das S, Samanta M, Mahapatra S (2022) Pathogenicity study of *Sclerotium rolfsii* isolates on popular lentil varieties in net house condition. *LR* 45: 1452-1458
78. Montalvao SC, Marques E, Martins I, Da Silva JP, De Mello SC (2023) Suppression of the phytopathogens *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* by *Trichoderma* spp. *Biologia* 78(10): 2941-2952
79. Murmu JJ, Tiwari S, Singh GJS (2021) Eco-friendly Management of *Sclerotium rolfsii* causing Collar Rot of Brinjal (*Solanum melongena* L.). *Int J Curr. Microbiol App Sci* 10: 373-381
80. Nalim FA, Starr JL, Woodard KE, Segner S, Keller NP (1995) Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. *Phytopathology* 85(12): 1507–1512
81. Nalim FA, Starr JL, Woodard KE, Segner S, Keller NP (1995) Mycelial compatibility groups in Texas peanut field populations of Sclerotium rolfsii. Phytopathology 85(12): 1507–1512
82. Nayak A, Harshitha M, Dubey S, Munangandu HM, Chakraborty A, Karunasagar I, Maiti B (2023) Evaluation of probiotic efficacy of *Bacillus subtilis* RODK28110C3 against pathogenic *Aeromonas hydrophila* and *Edwardsiella tarda* using *in vitro* studies and *in vivo* gnotobiotic zebrafish gut model system. *Probiotics Antimicrob Proteins* 1-15
83. Oyesola OL, Tonjock RK, Bello AO, Taiwo OS, Obembe OO (2024) *Trichoderma*: A review of its mechanisms of action in plant disease control.
84. Paparu P, Acur A, Kato F, Acam C, Nakibuule J, Nkuboye A, Mukankusi C (2020) Morphological and pathogenic characterization of *Sclerotium rolfsii*, the causal agent of southern blight disease on common bean in Uganda. *Plant Dis* 104(8): 2130-2137
85. Parwanayoni NMS, Suprapta DN, Darsini N, Sudirga SK (2021) Isolation and molecular identification of fungi causing stem rot disease in Bali's local legumes. *Biogen J Ilmiah Biologi* 9: 73-80
86. Patani A, Patel M, Islam S, Yadav VK, Prajapati D, Yadav AN, Sahoo DK, Patel A (2024) Recent advances in Bacillus-mediated plant growth enhancement: a paradigm shift in redefining crop resilience. *World J Microbiol Biotechnol* 40(2): 77
87. Paul SK, Gupta DR, Mahapatra CK, Rani K, Islam T (2023) Morpho-molecular, cultural, and pathological characterization of *Athelia rolfsii* causing southern blight disease on common bean. *Heliyon* 9: 5
88. Pooja D, Deshpande V, Patil NB, Rao M (2024) Bio-efficacy of seed dressing fungicides and bio-agents on seed-borne fungal infections and seed quality of greengram. *JFS* 37(01): 64-67
89. Qessaoui R, Bouharroud R, Furze JN, El Aalaoui M, Akroud H, Amarraque A, Chebli B (2019) Applications of new rhizobacteria *Pseudomonas* isolates in agroecology via fundamental processes complementing plant growth. *Scientific Reports* 9: 12832

90. Raja M, Sharma RK, Jambhulkar PP, Thava Prakasa Pandian R, Sharma P (2023) Comparative evaluation of native *Trichoderma* species from groundnut rhizosphere against stem rot caused by *Sclerotium rolfsii* Sacc. *Indian Phytopathology* 76(2): 459-471
91. Rakh RR, Raut LS, Dalvi SM (2024) *Bacillus* spp. for *in-vitro* microbiological control of *Sclerotium rolfsii* Sacc., a stem rot pathogen of groundnut.
92. Ramakrishnan G, Jeyarajan R, Dinakaran D (1994) Talc-based formulation of *Trichoderma viride* for biocontrol of *Macrophomina phaseolina*. *J Biol Control* 8: 41-44
93. Rangaswami G, Sowmini Rajagopalan (1973) Bacterial Plant Pathology TNAU, Coimbatore: 129
94. Rao MP (1969) Foot and root-rot of brinjal caused by *Sclerotium rolfsii* in Vijaywada. *Indian Phytopath* 22: 414-415
95. Rao MP (1969). Foot and root-rot of brinjal caused by Sclerotium rolfsii in Vijaywada. Indian Phytopath 22: 414-415
96. Rembinski J, Moreira IS, De Souza JT, Souza CA, Dorigan AF, Alves E, Julliati FC (2022) Using *Trichoderma* to manage sclerotia-producing phytopathogenic fungi. *IntechOpen*
97. Saini D K, Bunker R N, Maurya S, Saini A, Yadav R, Meena B S (2023) *In vitro* evaluation of fungicides, organic amendments and botanical against powdery mildew of chilli caused by *Leveillula Taurica*.
98. Samuels GJ (1996). Trichoderma: A review of biology and systematics of the genus. Mycol. Res. 100: 923–935.
99. Schmidt EL, Caldwell AC (1967) A practical manual of soil microbiology laboratory methods FAO of the UN
100. Schwyn B, Neilands J (1987) Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 160: 47-56
101. Shanmugaraj C, Kamil D, Gogoi R, Das A, Kumar GS, Hussain Z, Iquebal MA (2024) Characterizing genetic diversity of *Sclerotium rolfsii* isolates by biomapping of mycelial compatibility groupings and multilocus sequence analysis. *World J Microbiol Biotechnol* 40(8): 251
102. Shanmugaraj C, Kamil D, Kundu A, Singh PK, Das A, Hussain Z, Gogoi R, Shashank PR, Gangaraj R, Chaithra M (2023) Exploring the potential biocontrol isolates of *Trichoderma asperellum* for management of collar rot disease in tomato. *Horticulturae* 9(10): 1116
103. Shanmugaraj C, Kamil D, Parimalan R, Singh PK, Shashank PR, Iquebal MA, Nishmitha K (2024) Deciphering the defense response in tomato against *Sclerotium rolfsii* by *Trichoderma asperellum* strain A10 through gene expression analysis. *3 Biotech* 14: 210
104. Sharf W, Javaid A, Shoaib A, Khan IH (2021) Induction of resistance in chili against *Sclerotium rolfsii* by plant-growth-promoting rhizobacteria and *Anagallis arvensis*. *Egyptian J Biol Pest Control* 31: 1-11
105. Sharma BK, Singh UP, Singh KP (2002) Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia* 94: 1051-1058
106. Sharma D, Gautam HR, Chandel S, Thakur K, Sharma PL (2024) Seed biopriming with antagonistic microbes and their defense activity against damping-off disease in solanaceous vegetable crops. *Indian Phytopathol* 15:1-7
107. Shree D, Manjula CP, Anandakumar J, Punith G, Anusree K, Harish J, Lakshmeesha R (2024) Morphological and cultural characterization of *Sclerotium rolfsii* Sacc. on chickpea and its management using combined fungicide molecules. *J Sci Res Rep* 30: 268-276
108. Siddique MNA, Ahmmed ANF, Mazumder Md GH, Khaiyam MO, Islam Md R (2016) Evaluation of some fungicides and bio-agents against *Sclerotium rolfsii* and foot and root rot disease of eggplant (*Solanum melongena* L.). *The Agriculturists* 14(1): 92-97
109. Siddique MNA, Ahmmed ANF, Mazumder Md GH, Khaiyam MO, Islam Md R (2016) Evaluation of some fungicides and bio-agents against Sclerotium rolfsii and foot and root rot disease of eggplant (Solanum melongena L.). The Agriculturists 14(1): 92-97
110. Singh AM, Singh D (1994) Biocontrol of *Sclerotium rolfsii* Sacc. causing collar rot of brinjal. *J Biol Control* 8(2): 105-110
111. Singh AM, Singh D (1994) Biocontrol of Sclerotium rolfsii Sacc. causing collar rot of brinjal. J Biol Control 8(2): 105-110
112. Singh B, Singh S, Yadav S (2014) Some important plant pathogenic diseases of brinjal (*Solanum melongena* L.) and their management. *Plant Pathol J (Faisalabad)* 13(3): 208-213
113. Singh B, Singh S, Yadav S (2014) Some important plant pathogenic diseases of brinjal (Solanum melongena L.) and their management. Plant Pathol J (Faisalabad) 13(3): 208-213
114. Singh D, Dhancholia S (1991) A noteworthy disease of brinjal caused by *Sclerotium rolfsii* Sacc. *Himachal J Agric Res* 17: 119-120
115. Singh P, Sharma M (2020) Cultural and Morphological Characterization of Antagonistic *Trichoderma* Isolates. *Int J Curr Microbiol Appl Sci* 9(3): 1041-1048
116. Singh SP, Keswani C, Singh SP, Sansinenea E, Hoat TX (2021) *Trichoderma* spp. mediated induction of systemic defense response in brinjal against *Sclerotinia sclerotiorum*. *Curr Res Microbiol Sci* 2: 100051
117. Singh SP, Singh HB (2012) Effect of consortium of *Trichoderma harzianum* isolates on growth attributes and *Sclerotinia sclerotiorum* rot of brinjal. *Vegetable Sci* 39(2): 144–148

118. Sridharan AP, Thankappan S, Karthikeyan G, Uthandi S (2020) Comprehensive profiling of the VOCs of *Trichoderma longibrachiatum* EF5 while interacting with *Sclerotium rolfsii* and *Macrophomina phaseolina*. *Microbiol Res* 236: 126436
119. Srividya PV, Lal Ahamed M, Ramana JV, Khayum Ahmmed S (2018) Characterization of *Sclerotium rolfsii* Sacc. causing collar rot in chickpea isolates using cultural and morphological traits. *Int J Curr Microbiol App Sci* 7(06): 3912-3922
120. Srividya PV, Lal Ahamed M, Ramana JV, Khayum Ahmmed S (2018) Characterization of Sclerotium rolfsii Sacc. causing collar rot in chickpea isolates using cultural and morphological traits. Int J Curr Microbiol App Sci 7(06): 3912-3922
121. Sunil N, Thara SS (2022) Evaluation of biocontrol agents and chemical fungicides against *Sclerotium rolfsii* causing stem rot and foliar blight of cowpea. *J Krishi Vigyan* 10: 141-145
122. Sunilkumar DS, Rao MSL, Manjunatha SE (2021) *In vitro* evaluation of seed dressing fungicides, bioagent, and priming agents against pod blight complex of soybean (*Glycine max* (L.) Merrill) by rolled towel method. *J Pharmacogn Phytochem* 10(1): 1552-1555
123. Sunkad G, Patil MS, Joshi R (2023 b) Exploration of the potential of *Bacillus* spp. as an antagonist and PGPR against stem and pod rot of groundnut. *Legume Res* 46: 1501-1509
124. Sunkad G, Patil MS, Yenjerappa ST, Kulkarni SA, Rao S, Naik NM (2023a) *Trichoderma asperellum*: A treasure house of bioactive compounds with antagonistic activity against *Rhizoctonia bataticola*, a causal agent of dry root rot in chickpea. *J Animal Plant Sci* 33(5): 1109-1114
125. Theertha V, Kumar Veena SS, Karthikeyan S, Sreekumar J (2017) Compatibility of *Trichoderma asperellum* with fungicides, insecticides, inorganic fertilizers and biopesticides. *J Root Crops* 43(2): 68-75
126. Thomas J, Devi RS, Ahammed SK, Jayalakshmi V, Reddy VL (2022) Efficacy of botanical extracts and organic amendments against *Sclerotium rolfsii* Sacc. incitant of collar rot of chickpea and its compatibility with potential *Trichoderma* isolate.
127. Tiwari JK, Karkute SG, Tiwari SK, Singh M (2023) Brinjal breeding and genomics. *Vegetable Science*: 166-176
128. Tiwari JK, Karkute SG, Tiwari SK, Singh M (2023) Brinjal breeding and genomics. Vegetable Science: 166-176
129. Tsotetsi T, Nephali L, Malebe M, Tugizimana F (2022) *Bacillus* for plant growth promotion and stress resilience: what have we learned? *Plants* 11(19): 2482
130. Veena SS, Krishna N, Karthikeyan S (2023) Synergistic effect of oil cakes and *Trichoderma asperellum* in the suppression of *Sclerotium rolfsii*. *J Root Crops* 49(1): 39-45
131. Vidhyasekaran P, Muthamilan M (1995) Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt.
132. Vincent JM (1927) Distribution of fungal hyphae in the presence of certain inhibitors. *Nature* 159: 850
133. Vineela DRS, Beura SK, Dhal A, Swain SK (2020) Efficacy of bio-agents, botanicals, organic amendments against groundnut pathogens *Sclerotium rolfsii* and *Aspergillus niger in vitro*. *J Pharmacogn Phytochem* 9(3): 1206-1210
134. Watanabe T (2002) Sclerotium sp. morphologies of cultured fungi and key species: Pictorial Atlas of Soil and Seed fungi. 2nd Edn., CRC Press, New York
135. Yamunarani K, Sangeetha S, Chitra K, Dhanalakshmi K, Dhanushkodi V, Kalyanasundaram A (2023) Ecofriendly Management of Dry Root Rot in Black Gram Caused by *Macrophomina phaseolina*. *Int J Environ Climate Change* 13(12): 1360-5
136. Zahim S, Bekkar AA, Belabid L (2018) Efficacy of *Bacillus subtilis* and *Tricoderma harzianum* combination on chickpea Fusarium wilt caused by *F. oxysporum* f. sp. *ciceris*. *J Plant Prot Res* 53: 177-183
137. Zeven AC, Zhukovsky PM (1975) Dictionary of cultivated plants and their centres of diversity. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands: 62–63
138. Zeven AC, Zhukovsky PM (1975) Dictionary of cultivated plants and their centres of diversity. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands: 62–63
139. Zhu J, Tan T, Shen A, Yang X, Yu Y, Gao C, Li Z, Cheng Y, Chen J, Guo L, Sun X (2020) Biocontrol potential of *Bacillus subtilis* IBFCBF-4 against Fusarium wilt of watermelon. *J Plant Pathol* 102: 433-441
140. Zieslin N, Ben-Zaken R (1993) Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. *Plant Physiol Biochem* 31: 333-339
141. Zou L, Wang Q, Wu R, Zhang Y, Wu Q, Li M, Huang J (2023) Biocontrol and plant growth promotion potential of endophytic *Bacillus subtilis* JY-7-2L on *Aconitum carmichaelii* Debx. *Front Microbiol* 13: 1059549

Table 1. Survey on the incidence of collar rot of brinjal incited by *S. rolfsii* in major brinjal growing areas of Tamil Nadu

Si. No	Isolate identification code	Name of the district	Name of the village	Soil type	Type of irrigation	Variety	Stage of crop	Percent disease incidence
01	Sr_1	Ariyalur	Meensurutii	Sandy loam	Furrow	Local	Fruiting	28.05^{b}(31.97)
02	Sr_2		Gundaveli	Sandy loam	Furrow	C02	Flowering	18.89^{f}(25.76)
03	Sr_3		Elayaperumalnallur	Red soil	Furrow	Pkm 1	Fruiting	31.59^{a}(34.19)
04	Sr4	Cuddalore	Kurinjipadi	Sandy loam	Furrow	Local	Vegetative	14.78^{h}(22.60)
05	Sr_5		Periyapattu	Sandy soil	Furrow	Local	Fruiting	26.27^{c}(30.83)
06	Sr_6		Sivapuri	Clay loam	Furrow	Au1	Flowering	23.76^{d}(29.17)
07	Sr_7		Jayakontapattinam	Sandy soil	Furrow	Au1	Vegetative	17.69^{g}(24.87)
08	Sr_8	Salem	Kattukottai	Black soil	Furrow	Co2	Vegetative	9.78^{j}(18.22)
09	Sr_9		Ammampalayam	Black soil	Furrow	C02	Fruiting	12.99^{i}(21.12)
10	Sr_{10}		Pakkampadi	Black soil	Furrow	Pkm1	Flowering	20.63^{e}(27.01)
							SE(m)	0.23
							CD(p=0.05)	0.70

*Mean of three replications.
Data in Parentheses indicate angular transformed values.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table 2. Cultural characteristics of different isolates of *S. rolfsii* on PDA

Si.No	Isolate	Colony morphology	Mycelia growth (mm)*	No of sclerotia/plate (20 DAI) *	Colour of sclerotia	Shape of sclerotia	Sclerotial arrangement
1	Sr_1	White cottony growth of mycelium	90.00^{a}	298^{b}	Pale brown	oval	Scattered
2	Sr_2	Fluffy white growth of mycelium	87.84^{f}	162^{f}	Dark Brown	Round	Periphery
3	Sr_3	White cottony growth of mycelium	90.00^{a}	350^{a}	Dark brown	Spherical	Scattered
4	Sr_4	Dull white profuse mycelium	87.03^{g}	127^{h}	Brown	Spherical	Centre
5	Sr_5	Fluffy white growth of mycelium	89.59^{b}	263^{c}	Dark brown	Oval	Periphery
6	Sr_6	White cottony growth of mycelium	88.86^{c}	229^{d}	Pale brown	Round	Periphery
7	Sr_7	White cottony growth of mycelium	87.98^{e}	145^{g}	Dark brown	Round	Centre
8	Sr_8	Dull white profuse mycelium	86.03^{i}	107^{j}	Brown	Spherical	Scattered
9	Sr_9	Fluffy white growth of mycelium	86.45^{h}	112^{i}	Pale brown	Oval	Centre
10	Sr_{10}	Fluffy white growth of mycelium	88.27^{d}	186^{e}	Dark brown	Oval	Periphery

		SE(m)	0.13	2.94			
		CD(p=0.05)	0.39	9.06			

DAI-Days After Incubation*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table 3. Pathogenicity of *S.rolfsii* isolates on brinjal

Si.No.	Isolate number	Collar rot incidence (%)			Mean
		30DAT*	60DAT*	90DAT*	
1.	Sr_1	38.73^{b} (38.48)	64.06^{b} (53.16)	74.30^{ab} (59.53)	59.03
2.	Sr_2	23.11^{f} (28.73)	50.10^{e} (45.05)	62.06^{ef} (51.97)	45.07
3.	Sr_3	41.43^{a} (40.06)	68.22^{a} (55.68)	76.74^{a} (61.16)	62.13
4.	Sr_4	21.15^{g} (21.15)	43.00^{g} (40.97)	54.10^{g} (47.35)	39.41
5.	Sr_5	36.70^{c} (27.28)	59.41^{c} (50.42)	71.50^{bc} (57.73)	55.87
6.	Sr_6	33.42^{d} (35.31)	53.70^{d} (47.12)	68.88^{cd} (56.09)	52.66
7.	Sr_7	18.83^{h} (25.71)	46.21^{fg} (42.82)	58.79^{f} (50.79)	41.94
8.	Sr_8	12.21^{j} (20.45)	26.15^{i} (30.75)	38.15^{i} (38.15)	25.50
9.	Sr_9	15.60^{i} (23.26)	33.17^{h} (35.16)	42.37^{h} (40.61)	30.57
10.	Sr_{10}	29.83^{e} (33.10)	49.11^{ef} (44.49)	65.86^{de} (65.86)	48.28
	SE(m)	0.29	0.40	0.32	-
	CD(p=0.05)	0.89	1.23	0.99	-

DAT-Days After Transplanting *Mean of three replications.

Data in Parentheses indicate angular transformed values. Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table 4.Cultural and morphological characteristics of native isolates of *Trichoderma* spp.

Si.No	Locality	Isolate	Mycelial colony characters (5DAI)			Conidial characters (7DAI)		
			Growth rate (mm)	Colony color	Mycelial pattern	Conidiophore	Conidial shape	Conidial color
1	Meensurutii	(TaM)	89	Whitish green to dull green	Compact and cottony	Moderately branched, Regular	Globose	Dull green
2	Gundaveli	(TaG)	89	Light Green to bright green	Floccose	Highly branched, Regular	Ellipsoidal	Green
3	Elayaperumalnallur	(TaE)	90	Dark green	Floccose	Highly branched	Globose	Dark green
4	Periyapattu	(TaP)	89	Whitish green to dull green	Compact and cottony	Highly branched, Irregular	Ellipsoidal	Dull green
5	Sivapuri	(TaS)	90	Yellow to green	Arachnoid	Branched Regular	Globose to ellipsoidal	Green
6	Pakkampadi	(TaPp)	89	Whitish green to dull green	Floccose	Rarely branched, Regular	Globose	Light green

DAI-Days After Incubations
(TaM) - *T.asperellum* Meensurutii, (TaG) - *T.asperellum* Gundaveli, (TaE) - *T.asperellum* Elayaperumalnallur,(TaP) -*T.asperellum* Periyapattu, (TaS) - *T.asperellum* Sivapuri, (TaPp) - *T.asperellum* Pakkampadi

Table 5. *In vitro* antagonisms of native isolates of *T. asperellum* against *S. rolfsii* (Sr_3) by Dual culture technique.

Si. No	Isolates	Mycelia growth (mm)* (5DAI) *S.rolfsii*	Percent inhibition over control
1	TaM	16.94^{b}	81.17
2	TaG	26.08^{f}	71.02
3	TaE	14.86^{a}	83.48
4	TaP	18.53^{c}	79.41
5	TaS	20.59^{d}	77.12
6	TaPp	23.71^{e}	73.65

7	Control	90.00[g]	-
SE(m)		0.99	-
CD (P=0.05)		3.04	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table 6. Efficacy of Volatile compounds produced by *Trichoderma* spp. against *S. rolfsii* (Sr_3) (Inverted plate assay)

Si. No	Isolates	Mycelia growth (mm)* (5DAI) *S. rolfsii*	Percent inhibition over control
1	TaM	29.74[b]	63.62
2	TaG	58.35[f]	35.16
3	TaE	23.91[a]	73.43
4	TaP	32.48[c]	63.91
5	TaS	41.67[e]	53.70
6	TaPp	38.86[d]	55.71
7	Control	90.00[g]	-
SE(m)		0.92	-
CP(p=0.05)		2.85	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table 7. Effect of culture filtrate of native *Trichoderma* species on the mycelial growth of *S. rolfsii* (Sr_3)

i.No	Concentration of culture filtrate (%)							
	10		20		30		40	
	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control	Mycelial growth (mm)	Per cent inhibition over control
aM	24.95[b]	72.27	15.82[b]	82.42	8.76[b]	90.26	3.85[b]	95.72
aG	40.09[f]	55.45	31.03[f]	67.74	23.54[f]	76.06	10.21[f]	88.65
aE	18.23[a]	79.74	10.62[a]	88.20	4.38[a]	95.13	1.06[a]	98.82
aP	29.21[c]	67.27	21.63[c]	69.61	14.56[c]	78.18	05.91[c]	93.43
aS	34.10[d]	58.52	27.35[d]	68.87	19.63[d]	76.77	8.23[d]	90.85
aPp	37.33[e]	67.54	29.10[e]	75.96	21.90[e]	83.82	9.12[e]	89.86
ontrol	90.00[g]	-	90.00[g]	-	90.00[g]	-	90.00[g]	-
E(m)	0.51	-	0.96	-	1.23	-	1.74	-
D(p=0.05)	1.56	-	2.96	-	3.79	-	4.54	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05)

Table 8. Biochemical test for the identification of native *Bacillus* isolates

Si. No.	locality	Isolates	Gram staining	Siderophore production	Starch hydrolysis	Catalase production	Chitinase production	Citrate utilization
1	Meensurutii	BsM	+ve	+ve	-ve	+ve	-ve	+ve
2	Gundaveli	BsG	+ve	+ve	-ve	+ve	-ve	+ve
3	Elayaperumalnallur	BsE	+ve	+ve	-ve	+ve	-ve	+ve
4	Periyapattu	BsP	+ve	+ve	-ve	+ve	-ve	+ve
5	Sivapuri	BsS	+ve	+ve	-ve	+ve	-ve	+ve
6	Pakkampadi	BsPp	+ve	+ve	-ve	+ve	-ve	+ve

BsM- *B.subtilis* Meensurutii, BsG- *B.subtilis* Gundaveli, BsE- *B.subtilis* Ellayaperumanallur, BsP- *B.subtilis* Periyapattu, BsS-*B.subtilis*
Sivapuri, BsPp- *B.subtilis* Pakkampadi

Table 9. Cultural characteristics of native Bacillus isolates

Si No.	Isolates	Cell shape	Colony type	Colony colour	Growth type	Reaction to UV light fluorescence emission
1	BsM	Rod shape	Circular undulated	Cream colour	Fast	No reaction
2	BsG	Rod shape	Circular undulated	Cream colour	Fast	No reaction
3	BsE	Rod shape	Circular undulated	Cream colour	Slow	No reaction
4	BsP	Rod shape	Circular undulated	Cream colour	Fast	No reaction
5	BsS	Rod shape	Circular undulated	Cream colour	Fast	No reaction
6	BsPp	Rod shape	Circular undulated	Cream colour	Fast	No reaction

BsM- *B.subtilis* Meensurutii, BsG- *B.subtilis* Gundaveli, BsE- *B.subtilis* Ellayaperumanallur, BsP- *B.subtilis* Periyapattu, BsS-*B.subtilis* Sivapuri, BsPp- *B.subtilis* Pakkampad

Table 10. In vitro antagonisms of native isolates of *B. subtilis* against *S. rolfsii* (Sr_3) by Dual culture technique

Si.No	Isolates	Mycelial growth(mm)*	Inhibition zone(mm)*	Per cent inhibition over control
1	BsM	17.00^{b}	12.92^{b}	81.11
2	BsG	25.19^{f}	9.02^{f}	72.01
3	BsE	13.95^{a}	13.03^{a}	84.5
4	BsP	18.53^{c}	12.05^{c}	79.41
5	BsS	20.56^{d}	11.81^{d}	77.15
6	BsPp	22.85^{e}	10.41^{e}	74.61

7	Control	90.00[g]	_	_
	SE(m)	0.99	-	-
	CD(p=0.05)	3.04	-	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table11. Efficacy of volatile compounds produced by *B. subtilis* against *S.rolfsii* (Sr_3)

Si.No	Isolate	Mycelial growth(mm)*	Percentage of inhibition
1	BsM	26.33[b]	70.74
2	BsG	41.12[f]	54.31
3	BsE	21.13[a]	76.52
4	BsP	35.01[d]	61.1
5	BsS	31.35[c]	65.16
6	BsPp	37.85[e]	57.94
7	Control	90.00[g]	-
	SE(m)	0.94	-
	CD(p=0.05)	2.91	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table 12. Effect of culture filtrate of *B. subtilis* isolates on the mycelial growth of *S. rolfsii* (Sr_3)

Si.No	Concentration of culture filtrate (%)							
	10		20		30		40	
	Mycelial growth (mm)*	Per cent inhibition over control	Mycelial growth (mm)*	Per cent inhibition over control	Mycelial growth (mm)*	Per cent inhibition over control	Mycelial growth (mm)*	Per cent inhibition over control
BsM	27.45[b]	67.27	11.43[b]	87.30	08.23[b]	90.85	4.53[b]	94.96
BsG	46.77[f]	48.03	39.94[f]	55.62	31.23[f]	64.96	15.26[f]	83.04
BsE	17.31[a]	80.76	10.21[a]	88.65	5.21[a]	94.21	2.95[a]	96.72
BsP	31.47[d]	65.03	27.43[d]	69.52	23.74[d]	73.62	9.20[d]	89.77
BsS	20.81[c]	77.98	13.43[c]	85.07	7.21[c]	91.98	8.73[c]	90.30
BsPp	37.52[e]	58.31	33.24[e]	63.07	26.97[e]	70.03	11.62[e]	87.08
Control	90.00[g]	-	90.00[g]	-	90.00[g]	-	90.00[g]	-
SE(m)	0.43	-	0.92	-	1.13	-	1.40	-
CD(p=0.05)	1.33	-	2.82	-	3.47	-	4.31	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (p=0.05)

Table 13. Efficacy of different oil cake extracts on the growth *S. rolfsii* (Sr_3)

Si.No	Source	Concentration of culture filtrate (%)					
		10%		20%		30%	
		Mycelial growth* (mm)	Per cent inhibition over control	Mycelial growth* (mm)	Per cent inhibition over control	Mycelial growth* (mm)	P o
1	Coconut oil cake	86.12[e]	04.31	73.18[e]	18.68	65.12[e]	2
2	Cotton oil cake	78.74[d]	12.51	65.84[d]	26.84	58.15[d]	3
3	Groundnut oil cake	72.80[c]	19.11	61.92[c]	31.20	46.62[c]	4
4	Neem oil cake	46.90[a]	47.89	29.46[a]	67.26	23.53[a]	7
5	Sesame oil cake	55.78[b]	38.02	36.65[b]	59.27	29.38[b]	6
6	Control	90.00[g]	-	90.00[g]	-	90.00[g]	-
	SE(m)	0.82	-	0.77	-	0.67	-

	CD(p=0.05)	2.54	-	2.36	-	2.07	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table14. Effect of seed treatment with different dosages of antagonists on the incidence of collar rot of brinjal under pot culture

Si.No	Treatment	Disease incidence (%) *
T_1	*T.asperullum* (TaE) seed treatment@4 g /kg	27.86 d
T_2	*T.asperullum* (TaE) seed treatment@5 g /kg	25.14^{e}
T_3	*T.asperullum* (TaE)seed treatment@6 g /kg	24.18^{e}
T_4	*B.subtilis* (BsE)seed treatment@10 g /kg	33.73^{b}
T_5	*B.*subtilis (BsE)seed treatment@11 g /kg	31.08^{c}
T_6	*B.subtilis* (BsE)seed treatment@12 g /kg	30.87^{c}
T_7	Control	50.26^{a}
	SE(m)	0.52
	CD(p=0.05)	1.61

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Table 15. Efficacy of seed treatment with selective antagonists on the seed germination and plant growth promotion under *in vitro* conditions (Roll towel method)

Si.no	Treatment	Germination (%) *	Shoot length(cm)*	Root length(cm)*	Vigour index*
T_1	*T.asperellum* (TaE) ST@6g/kg of seed	86.35^{d}	11.87^{e}	7.35^{e}	1707.13^{d}
T_2	*B.subtilis* (BsE) ST@12g/kg of seed	83.07^{f}	9.45^{g}	5.42^{g}	1418.26^{e}
T_3	Neem cake ST@2g/kg	81.43^{g}	8.46^{h}	4.63^{h}	1171.77^{f}
T_4	T_1+T_2	89.84^{bc}	14.07^{c}	9.48^{c}	2097.76c

T_5	T_1+T_3	87.46[c]	10.58[f]	6.45[f]	1664.36[d]
T_6	T_2+T_3	84.20[e]	13.08[d]	8.56[d]	1799.42[cd]
T_7	$T_1+T_2+T_3$	93.65[a]	16.97[a]	12.15[a]	2633.43[a]
T_8	ST with carbendazim 50%WP@2g/kg of seeds	93.18 [ab]	15.14[b]	11.43[b]	2601.58[a]
T_9	Control	70.65[h]	6.12[i]	4.03[i]	929.04[g]
	SE(m)	0.73	0.14	0.11	
	CD(p=0.05)	2.25	0.44	0.34	

ST-Seed Treatment
*Mean of three replications.
Values in each column followed by the same letter are not significantly different, According to the DMRT method (P=0.05).

Table 16. Effect of seed treatment and soil application with a consortium of antagonists and oil cake on the incidence of collar rot of brinjal under pot culture

Tr no	Name of the treatment	Disease incidence*	Percent disease reduction over control	Disease incidence*	Percent disease reduction over control	Disease incidence*	Percent disease reduction over control
		30 DAT	30 DAT	60 DAT	60DAT	At harvest	At harvest
T_1	*T.asperullum* TaE seed treatment@6 g /kg	25.18[f]	51.31	22.92[f]	59.02	20.22[f]	65.89
T_2	*B.subtilis* BsE seed treatment@12 g /kg	27.79[e]	46.62	24.76[e]	55.73	22.18[e]	62.58
T_3	Neem cake seed treatment@ 2g/kg	36.83[b]	28.87	34.41[b]	38.47	31.91[b]	46.17
T_4	*T. asperullum* TaE soil application @5 g per pot	20.58[h]	60.20	17.62[h]	68.49	15.01[h]	74.67
T_5	*B.subtilis* BsE soil application@5 g per pot	23.19[g]	55.16	20.71[g]	62.97	17.57[g]	70.36
T_6	Neem cake soil application@ 20 g per pot	34.02[c]	34.22	31.17[c]	44.26	27.28[c]	53.98
T_7	T_1+T_4	14.53[j]	69.97	12.57[j]	77.52	8.14j	86.26
T_8	T_2+T_5	16.84[i]	67.44	14.81[i]	73.52	10.71i	81.93
T_9	T_3+T_6	29.92[d]	42.15	27.57[d]	50.70	24.83[d]	56.42
T10	$T_7+T_8+T_9$	09.49[l]	79.71	07.65[k]	85.35	6.29[k]	89.38
T11	ST with carbendazim 50%WP@2g/kg of seeds+Soil drenching @ 0.1%	12.03[k]	76.54	08.78[k]	84.30	7.03[jk]	88.14
T12	control	51.72[a]	-	55.93[a]	-	59.2[a]	-
	SE(m)	0.23	-	0.26	-	0.31	-
	CD(p=0.05)	0.70	-	0.81	-	0.96	-

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, According to the DMRT method (P=0.05).

Table 17. Effect of seed treatment and soil application with consortium of antagonists and oil cake on the biometrics of brinjal under pot culture

Tr.No	Treatments	Plant biometrics		
		Plant height(cm)	No branches/plant	Yield/plant (kg/plant)
T_1	*T.asperellum*(TaE) seed treatment@ 6 g /kg	112.28^{f}	10.63ef	1.19^{g}
T_2	*B.subtilis*(BsE) seed treatment@12g/kg	110.81^{fg}	10.07fg	1.08^{h}
T_3	Neem cake seed treatment@2g/kg	99.92hi	08.64ij	0.79^{j}
T_4	*T. asperellum*(TaE) soil application @ 5g per pot	120.69^{de}	11.83^{d}	1.43^{e}
T_5	*B.subtilis*(BsE) soil application@ 5g per pot	117.52^{ef}	11.09^{e}	1.31^{f}
T_6	Neem cake soil application@ 20g per pot	104.81^{gh}	09.06hi	0.86ij
T_7	T_1+T_4	127.92^{bc}	13.02^{c}	2.04^{c}
T_8	T_2+T_5	124.71^{cd}	12.39cd	1.87^{d}
T_9	T_3+T_6	102.62^{h}	09.65gh	0.91^{i}
T10	T_7+T_8+T_9	136.61^{a}	14.97^{a}	2.29^{a}
T11	ST with carbendazim 50%WP@ 2g/kg of seeds+Soil drenching @ 0.1%	132.55^{ab}	13.80^{b}	2.18^{b}
T12	Control	94.84^{i}	08.01^{j}	0.60^{k}
	SE(m)	0.31	0.16	0.21
	CD(p=0.05)	0.96	0.46	0.63

*Mean of three replications.
Values in each column followed by the same letter are not significantly different, according to the DMRT method (P=0.05).

Fig 1. Symptoms of brinjal collar rot incited by *sclerotium rolfsii*



Mustard like sclerotia formation **White mycelial mat on stem**

Fig 2. Axenic culture of *S. rolfsii*

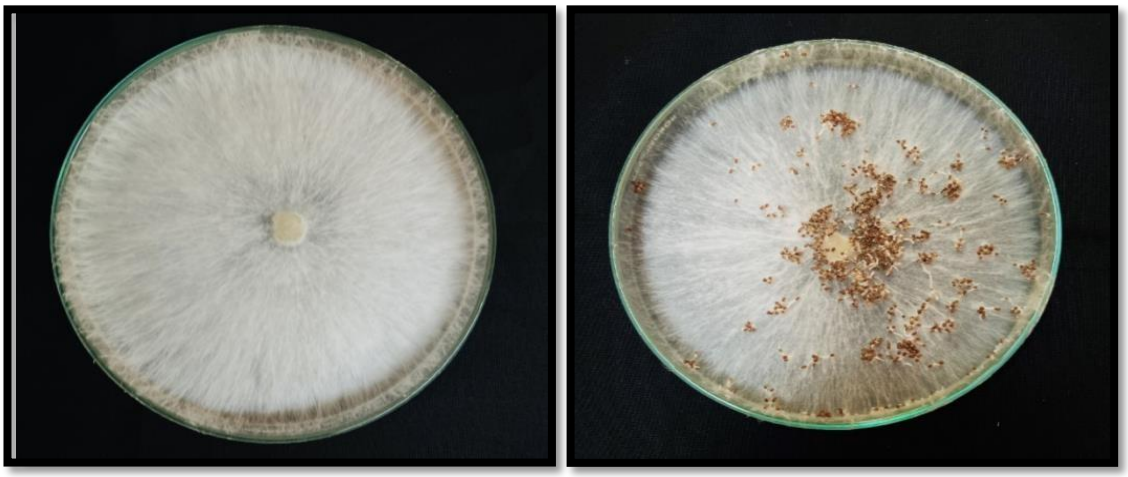


Fig 3. Different isolates of *S. rolfsii*

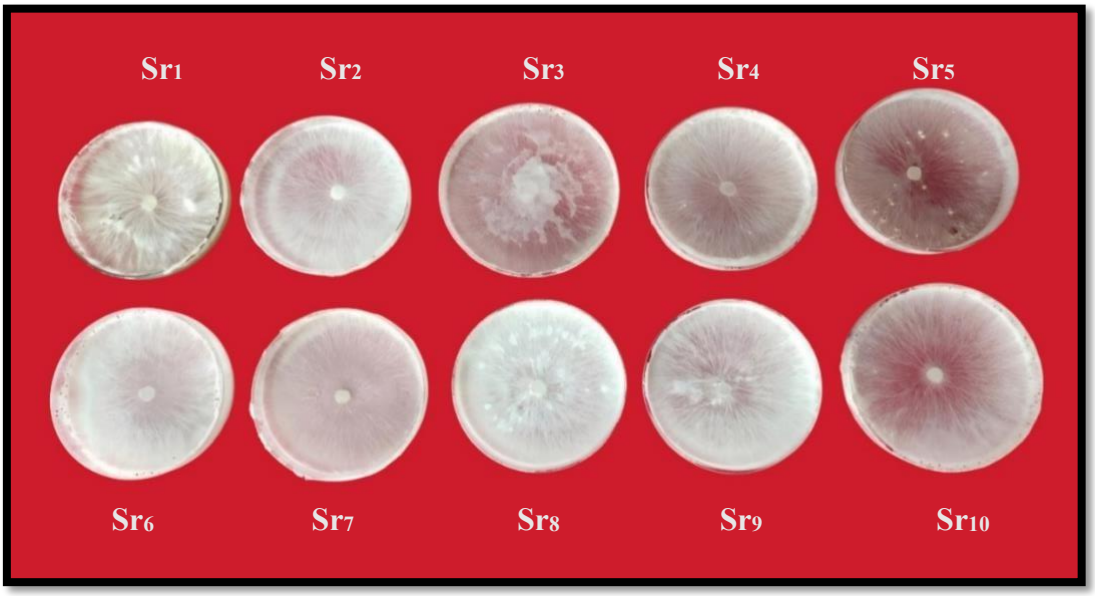


Fig 4. Microscopic observation of *S. rolfsii* (Sr3)

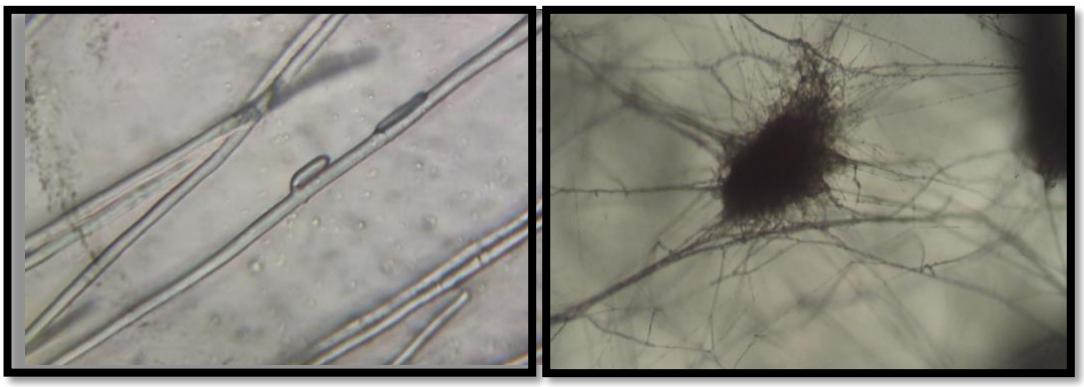




Fig 5. Scanning electron Microscope image of *S. rolfsii* (Sr_3) hyphae and Sclerotia

Fig 7. Native different isolates of *Trichoderma* spp. from brinjal rhizosphere.



Fig 6. Mass multiplication of *S.rolfsii* on sorghum grains



Fig 8 Scanning electron microscopic observations of Trichoderma asperellum (TaE) against *S.rolfsii*

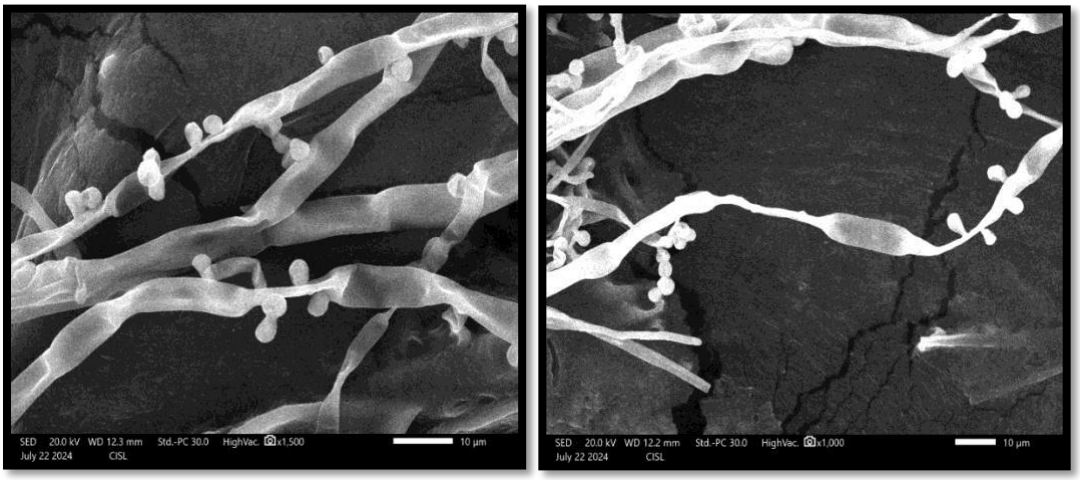


Fig 9 *In-vitro* efficacy of T. asperellum (TaE) against S. rolfsii (Sr$_3$) (Dual culture Technique)



Fig 10 Efficacy of cultural filtrate of *T. asperellum* (TaE) against *S. rolfsii* (Sr$_3$) (Poison food Technique)

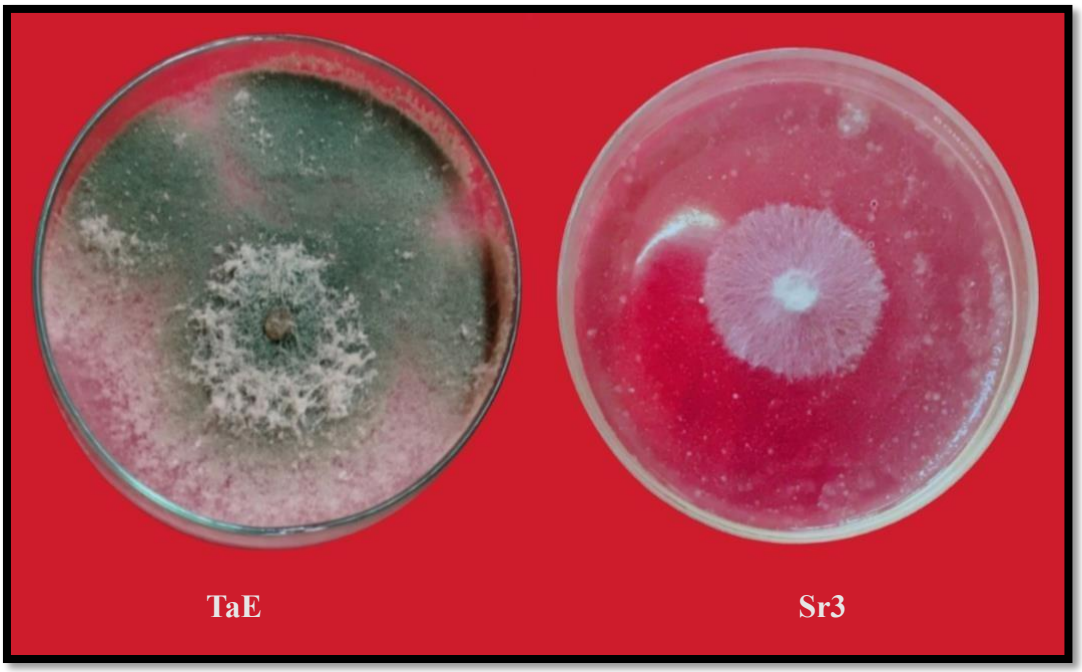


Fig 11 In-vitro efficacy of T. asperellum (TaE) against S. rolfsii (Sr_3) (inverted plate assay)

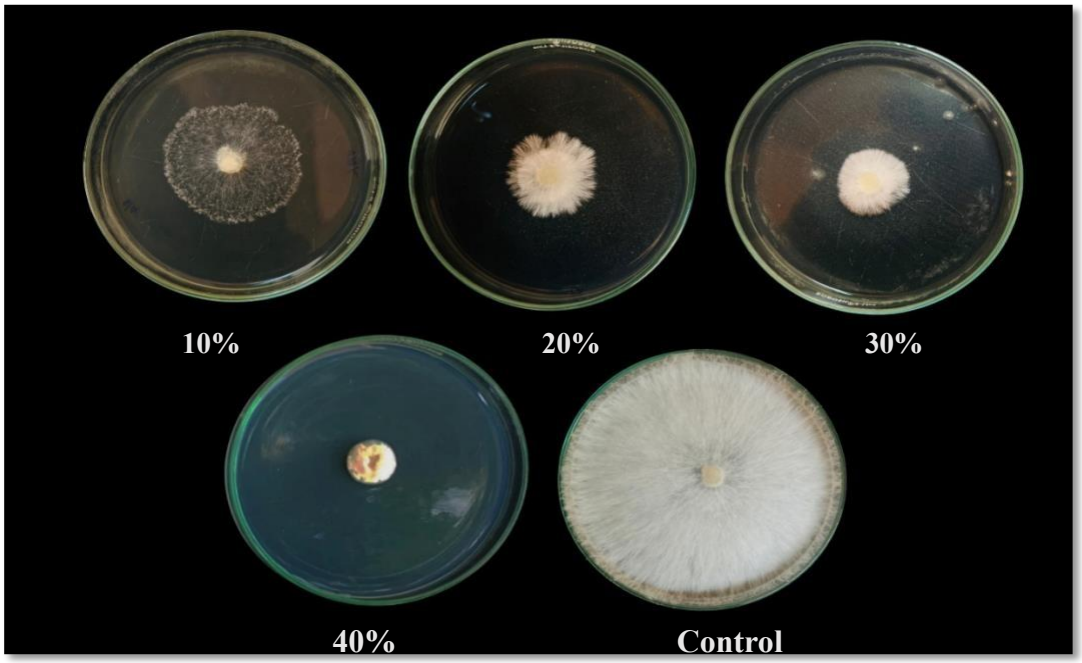


Fig 12 Native different isolates of *bacillus* spp. from brinjal rhizosphere

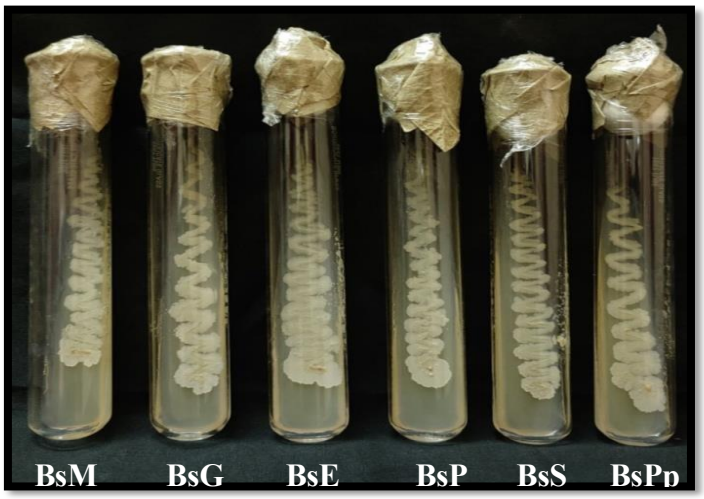




Fig 13 *In-vitro* efficacy of Bacillus subtilis (BsE) against S. rolfsii (Sr_3) (Dual culture Technique)

Fig 14. Efficacy of cultural filtrate of *Bacillus subtilis* (BsE) against *S. rolfsii* (Sr_3) (Poison food Technique)

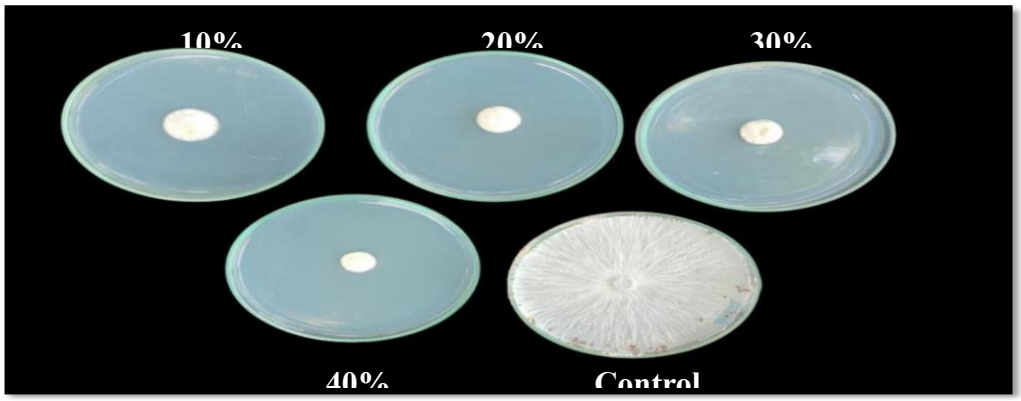


Fig15. *In-vitro* efficacy of Bacillus subtilis (BsE) against S. rolfsii (Sr_3) (inverted plate assay)

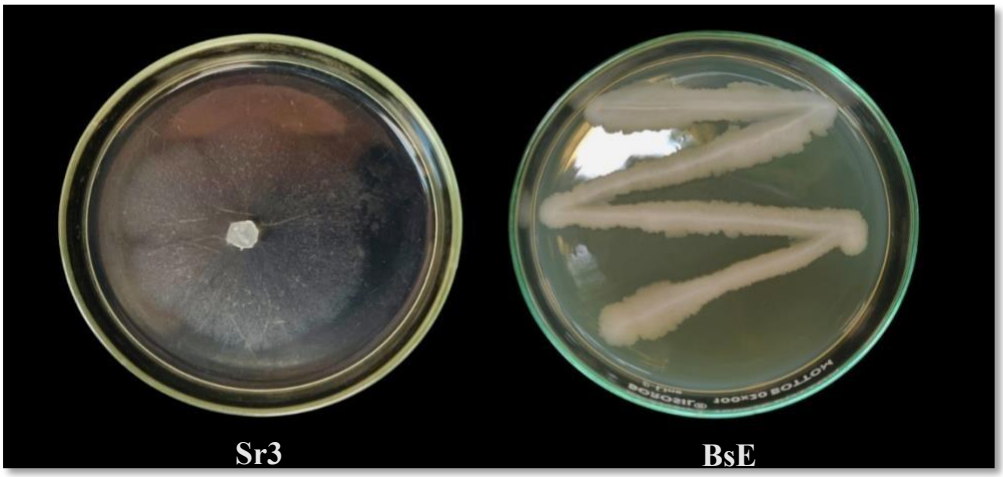


Fig16. *In-vitro* assay of Neem oil cake extract against S. rolfsii (Sr_3)

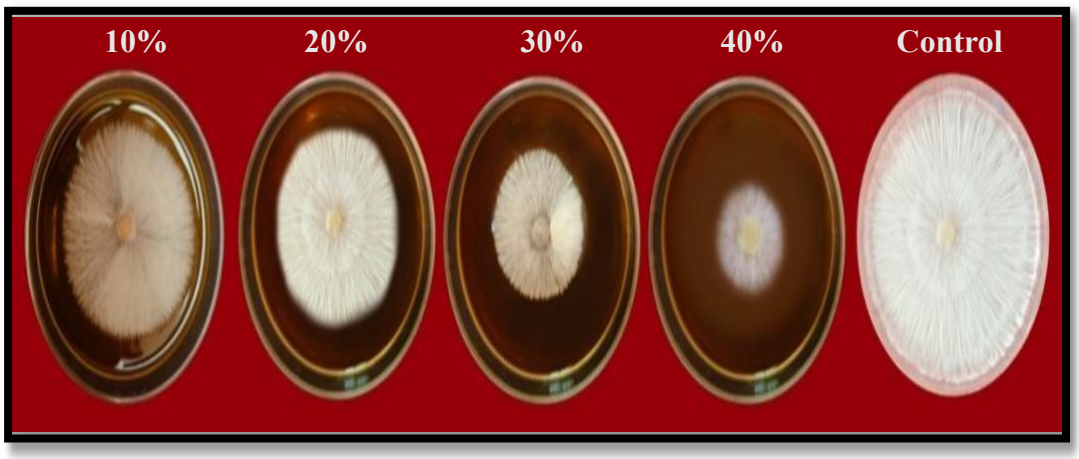
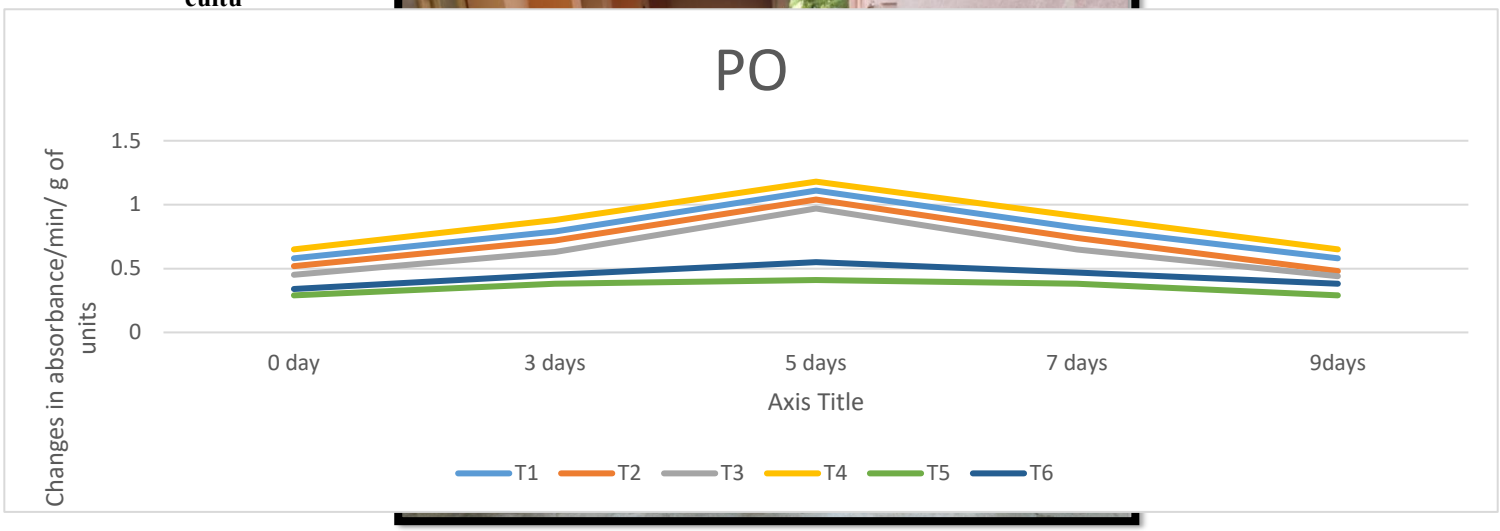


Figure 18. Induction of peroxidase(PO) activity in brinjal plants treated with bioagents and neem cake under pot culture experiment

Fig17.General cultu **view of Pot**

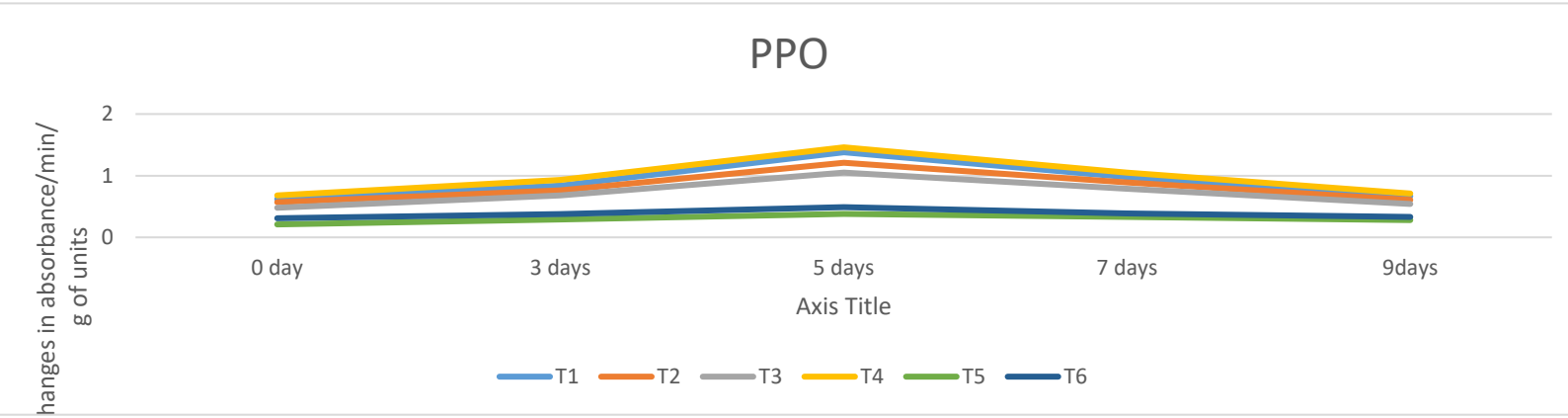


T_1-*T.*asperullum(TaE) seed treatment@6 g /kg+SA@5g/pot **T_2-*B.subtilis* (BsE) seed treatment@12 g /kg+SA@5g/pot**

T_3-Neem cake seed treatment@ 2g/kg+SA@20g/pot **T_4-T1+T2+T3**

T_5-Healthy control **T_6-Inoculated control**

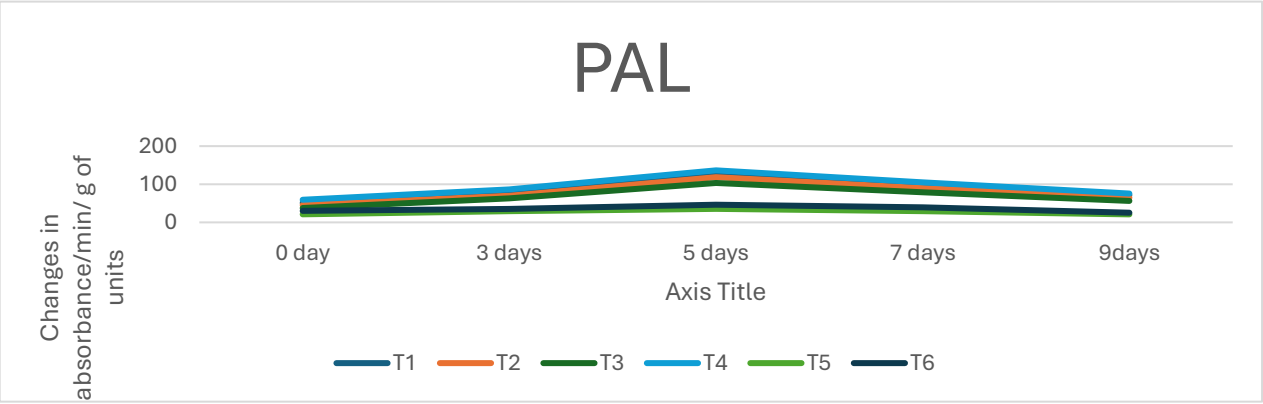
Figure 19. Induction of polyphenol oxidase (PPO) activity in brinjal plants treated with bio-agents and neem cake under pot culture experiment



T_1-*T.*asperullum(TaE) seed treatment@6 g /kg+SA@5g/pot **T_2-*B.subtilis* (BsE) seed reatment@12g/kg+SA@5g/pot**

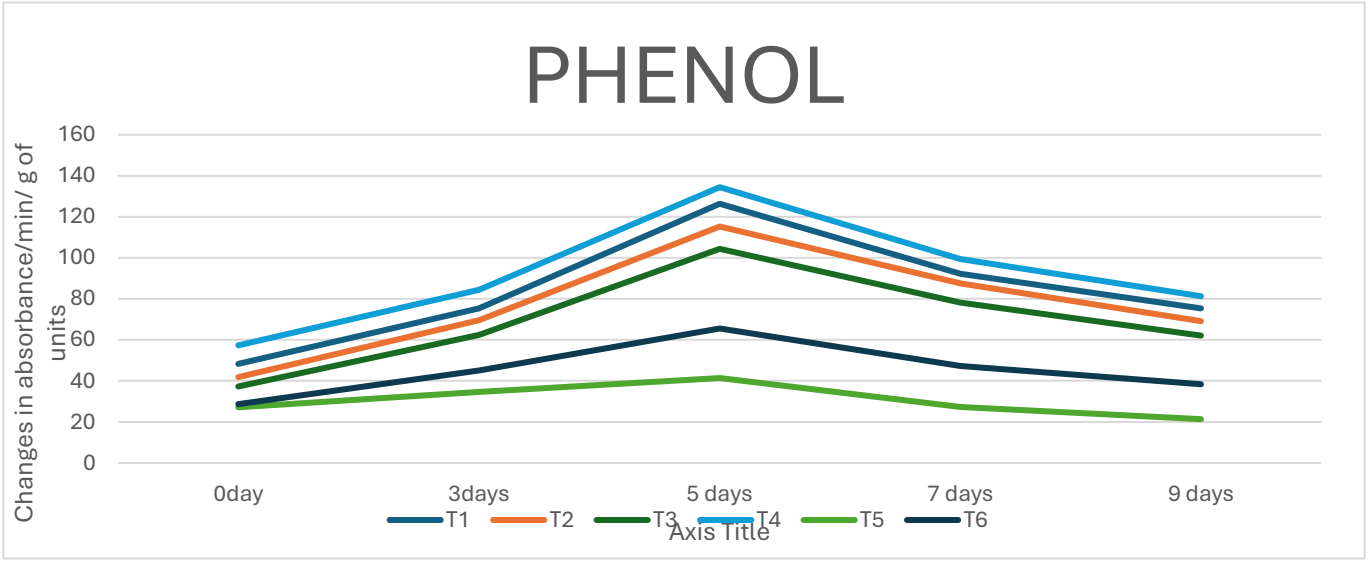
T_3-Neem cake seed treatment@ 2g/kg+SA@20g/pot **T_4-T1+T2+T3**

Figure 20. Induction of phenylalanine ammonia-lyase (PAL) activity in brinjal plants treated with bio-agents and neem cake under pot culture experiment condition



T_1-*T.*asperullum(TaE) seed treatment@6 g /kg+SA@5g/pot **T_2-*B.subtilis* (BsE) seed treatment@12g/kg+SA@5g/pot**

Figure 21. Induction of total phenol activity in brinjal plants treated with bio-agents and neem cake under pot culture experiment



T_1-*T.*asperullum(TaE) seed treatment@6 g /kg+SA@5g/pot **T_2-*B.subtilis* (BsE) seed treatment@12g/kg+SA@5g/pot**

T_3-Neem cake seed treatment@ 2g/kg+SA@20g/pot **T_4-T1+T2+T3**

T_5-Healthy control **T_6-Inoculated control**