Archives of Current Research International

Volume 24, Issue 10, Page 35-52, 2024; Article no.ACRI.124451
ISSN: 2454-7077

Biological Suppression of *Sclerotium rolfsii* in Groundnut Cultivation: A Path Towards Sustainable Disease Management

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Authors' contributions

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

Article Information

DOI: https://doi.org/10.9734/acri/2024/v24i10906

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124451

Original Research Article

Received: 22/07/2024
Accepted: 24/09/2024
Published: 01/10/2024

ABSTRACT

Stem rot of groundnut caused by *Sclerotium rolfsii* Sacc, is a major soil borne disease which impact on groundnut cultivation both in India and globally. The primary objective of this study was to assess the antagonistic potential of biocontrol agents against the pathogen, both individually and in combination, under *In vitro* and glasshouse conditions. The results of the present investigation indicated that the application of microbial consortia was more effective against *Sclerotium rolfsii* than individual bioagents. Specifically, seed treatment with microbial consortia MC1, MC2, MC3, and MC4 resulted in lower disease incidence with 13.80%, 16.01%, 20.0%, and 22.60%

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Cite as: *Lavanya.K, Vidyasagar.B, Ameer Basha.S, and S. Triveni. 2024. "Biological Suppression of Sclerotium Rolfsii in Groundnut Cultivation: A Path Towards Sustainable Disease Management". Archives of Current Research International 24 (10):35-52. https://doi.org/10.9734/acri/2024/v24i10906.*

respectively, compared to the pathogen check, which had a 74.12% PDI. Additionally, these treatments also enhanced plant growth through improved plant growth-promoting traits under glasshouse conditions.

Keywords: Groundnut; stem rot; compatibility; consortia and Sclerotium rolfsii.

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.), is an annual leguminous plant and it is called as ―king of oil seeds. Groundnut has been widely distributed and cultivated in more than eighty countries in tropical and sub-tropical regions of the world [1]. India is the second largest producer of groundnut after China. India holds a notable place in the world with 25.5Mt of oilseeds production on 32.26 mha of land, [2]. Groundnut constitutes 2.61% of the total cropped area and 28.18 % of the total oil seeds cropped area in Telangana. Mahaboobnagar, Warangal and Nalgonda districts of Telangana together accounts for 86.66 % of groundnut area in the state [3]. Groundnut production is limited by many abiotic and biotic stresses including fungal, viral and nematodal diseases. Among the fungal diseases, stem rot (*Sclelrotium rolfsii* Sacc), have been recognized as major disease in the groundnut crop [4] causing yield losses up to 50% [5]. Stem rot disease is also known as, southern stem rot, southern blight, white mold, and sclerotium rot. *Sclerotium rolfsii* is a ubiquitous, polyphagous soil- borne pathogen responsible for destructive plant diseases of different crops.

The wide host range of *S. rolfsii* due to its prolific growth and ability to produce persistent ―sclerotia contribute to the large economic losses associated with this disease [6]. Since, these pathogen survives in the soil as resistant structures *i.e.* Sclerotia, that are found associated with plant debris or near the soil surface remaining viable for a long period i.e. 2 months to 3 years in the absence of a susceptible host. Management of soil borne diseases by chemical means is difficult and not economical and has already proved to be harmful to the environment. Increased public concern about pesticide utilization and the health hazards necessitates the exploitation of alternative methods of disease control like bioagents. These bioagents are less detrimental, eco-friendly and safer than synthetic pesticides [7]. Biological control strategy is one of the most promising alternative to protect plants from soil borne phytopathogens [8-10]. It not only reduces the negative consequences of phytopathogens but also promotes positive responses in host plants [11]. Use of single biocontrol agent against soil borne disease is effective, but when two or more compatible biocontrol agents (consortia) combinedly used against disease is more effective and economical. Studies revealed that plants treated with anagonistic microbial consortia showed a significant disease reduction compared to individual isolates. Biocontrol attributes are also more in consortia than using single isolates [12]. Therefore, the present study aimed to exploit the biocontrol agents with antagonistic ability for managing stem rot of groundnut individually as well as in combination i.e. consortia.

2. MATERIALS AND METHODS

2.1 Fungal and Bacterial Isolates

The test pathogen, *Sclerotium rolfsii*, a total of 31 biocontrol agents (13 fungi and 18 bacteria) were procured and the experiment was conducted at the Department of Plant Pathology, College of Agriculture, Rajendranagar.

2.2 *In vitro* Evaluation of the Efficacy of Biocontrol Agents Against *Sclerotium rolfsii* Causing Groundnut Stem Rot

The efficacy of 13 fungal isolates *viz*, Ts_1 – *Trichoderma* sp.1, Ta_1-*Trichoderma asperellum,* Tv_1- *T.viridae* 1, Tv_2 - *T.viridae* 2, Ts_2 - *Trichoderma* sp.2, Ts_3 - *Trichoderma* sp.3, T_{ar} - *T.arenarium,* Ta_2 - *T. asperellum* 5*,* Th_1- *T.harzianum* 2, Tv_3- *T.viridae,* Tv_4- *T. viridae,* Th_2 -*Trichoderma harzianum,* Th_3-*T.harzianum* 4D *and* 18 bacterial isolates *viz*, Bs_1- *B.subtilis* FSB16, Bs_2- *B.subtilis* ESB 9, Pf_1 - *P.florescenes* (s), Bs_3 - *B.subtilis* (A), Bs_4 - *B.subtilis* (AA), Bs_5 - *B.subtilis* (1), Bs_6 - *B.subtilis* FSB 2, Bs_7 - *B.subtilis* I, Pp- *P.putida*, Pf_2 - *P.florescenes,* As_1- Actinomycetes strain3, As_2- ActinomycetesN24, As_3- Actinomycetes strain2, Bs_8 - *B.subtilis* S4KB5, Bs_9 - *B.subtilis* 3, B_1 - *Bacillus* S8KB2, Bs_{10} - *B.subtilis* 26, Bs_2 - *Bacillus* S9KB4 were evaluated against *Sclerotium rolfsii* under *in vitro* conditions using dual culture technique [13].

2.3 Screening of Bacterial Isolates Against *S. rolfsii* under *In vitro* Conditions

A loopful of 24-hour-old pure bacterial cultures were streaked 1 cm from the edge of PDA plates, while a 5 mm mycelial disc from a 5-day-old pathogen culture was placed on the opposite side. The plates were then incubated at 25 ± 2ºC. A control plate containing only the pathogen was also maintained. Once the pathogen reached full growth on the control plate, its mycelial growth was measured in each Petri dish and recorded in millimeters.

2.4 Screening of Fungal Isolates Against *S. rolfsii* under *In vitro* Conditions

Five mm mycelial discs from 5- day-old cultures of both the pathogen and the fungal biocontrol agents were positioned on opposite sides of a Petri dish, 1 cm from the edge, and incubated at 25 ± 2ºC. The plate with only pathogen was served as control. After the pathogen attained full growth on the control plate, its mycelial growth in each Petri dish was measured in mm.

The inhibition percentage of the pathogen's mycelial growth by the fungal and bacterial biocontrol agents were calculated using the formula provided by Vincent [14].

$$I = \frac{C - T}{C} X\, 100$$

Where,

I = Per cent inhibition of mycelial growth over control
C = Radial growth of the Pathogen in control (mm)
T = Radial growth of the Pathogen in treatment (mm)

2.5 Screening for Compatibility among the Potential Isolates

Nine bacterial and nine fungal potential biocontrol isolates were identified as potential biocontrol agents due to their higher inhibition percentages compared to other bioagents. These isolates were tested for compatibility using the plate assay method described by Pierson and Weller [15].

The cross-streak method was used to assess bacterial isolate compatibility by streaking cultures on nutrient agar plates and observing inhibition zones after two days of incubation at 30 ± 2°C. Isolates with no growth inhibition were considered compatible. For bacteria-fungi interactions, a modified dual culture technique was used, with bacterial isolates streaked around the plate's edge and a fungal disc at the center; fungal overgrowth indicated compatibility.

For fungi-fungi interactions, two mycelial discs were placed on opposite corners of a plate, and overgrowth of one isolate signified compatibility. Control plates were used for fungi only.

2.6 Evaluation of Fungal and Bacterial Bioagents for Plant Growth-Promoting Traits and Biochemical Parameters Enhancing Antagonistic Activity

2.6.1 Production of IAA

IAA production was estimated using the method described by Gordon and Weber [16]. IAA production was measured using bacterial and fungal isolates. For bacteria, cultures in nutrient broth with 5 mM tryptophan were incubated for 4-6 days, centrifuged, and the supernatant was treated with orthophosphoric acid and Salkowski reagent. After 25 minutes, IAA was measured spectrophotometrically at 530 nm. For fungi, isolates were grown in Potato Dextrose Broth with 0.2g tryptophan for 7 days, followed by filtration. The filtrate was mixed with Salkowski reagent, incubated for 20 minutes, and IAA was measured similarly at 530 nm, following the method of Bric *et al*, [17].

2.6.2 Phosphate solubilization

Biocontrol agents were tested for phosphate solubilization by spot inoculating pure isolates onto Pikovskaya's agar plates under sterile conditions in a laminar air flow chamber. The plates were incubated at 30°C for 6 to 8 days. The presence of a clear zone around the colonies indicated positive phosphate solubilization [18].

2.6.3 Production of ammonia

The ammonia production test was conducted using peptone water broth (5 g peptone and 10 g sodium chloride in 1 liter of water). The peptone water broth was prepared in 10 ml test tubes and

sterilized in an autoclave. Biocontrol agent cultures were inoculated into each tube and incubated for 2-3 days. After incubation, Nessler's reagent was added to the tubes, and any color change was observed. A change from slight yellow to brownish indicated positive ammonia production [19].

2.6.4 HCN production

HCN production by the biocontrol agents were estimated using a modified method from Castric and Castric [20]. Modified nutrient agar plates were prepared by adding 4.4 g of glycine per liter. Bacterial isolates were streaked onto these plates, and for fungal isolates, a mycelial disc was placed at the center. A disc of Whatman's no.1 filter paper, the same diameter as the Petri plate, was soaked in an alkaline picric acid solution (0.2% picric acid in 1% sodium carbonate) and placed on the upper surface of the inoculated Petri plates under sterile conditions. Control plates did not receive any inoculum. The plates were incubated upside down at 30°C for 6 to 7 days. A color change from yellow to light brown, moderate, or strong reddish-brown indicates positive HCN production.

2.6.5 Siderophore production

To assess qualitative siderophore production, fungal and bacterial biocontrol agents were tested using Chrome Azurol S dye (CAS) agar medium following the method of Schwyn and Neilands [21]. CAS agar plates were prepared and spot inoculated with various biocontrol isolates. These inoculated plates were incubated at 28 ± 2°C for 3 to 6 days. A positive response for siderophore production was indicated by the appearance of a yellow to orange halo zone surrounding the colonies.

2.6.6 Pectolytic activity

Pectolytic activity of the fungal and bacterial isolates were evaluated using pectinase screening agar medium (PSAM) as described by Oumer and Abate [22]. Test isolates were spot inoculated onto PSAM agar plates and incubated at 30 ± 2°C for two days. Following incubation, the plates were flooded with Gram's iodine solution (prepared by dissolving 2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3 to 5 minutes. A clear zone around the colonies indicates positive pectinase production activity.

2.7 Developing the Consortia and Testing Its Efficacy Against Stem Rot in Pot Culture

Equal volumes of each selected isolate was combined to create a dual microbial consortium, while one-third volumes of each isolate was combined to form a triple microbial consortium [23]. Pot culture experiments were conducted under glasshouse conditions to evaluate the effectiveness of individual bioagents and microbial consortia for controlling stem rot disease in groundnut. For these experiment, the susceptible groundnut variety Kadiri-6 (K-6) was used. Pot covers were filled with a sterilized mixture of soil, sand, and vermicompost in a 2:1:1 ratio, with each pot containing 3 kg of this mixture. The seeds were surface-sterilized using 0.1 percent sodium hypochlorite before being sown, with five seeds per pot. Eventually, three seedlings were maintained in each pot. The experiment followed a completely randomized block design, including fourteen treatments with three replicates each. Additional replications were also maintained to study plant growth promotion activity.

The number of seeds germinated is recorded on the tenth day. Observations on germination percentage, shoot length, root length, fresh weight and dry weight were recorded subsequently vigour index I and vigour index II were calculated [24] and disease incidence at 50 DAS.

3. RESULTS AND DISCUSSION

3.1 *In vitro* Evaluation of the Efficacy of Biocontrol Agents Against *Sclerotium rolfsii* Causing Groundnut Stem Rot

3.1.1 Screening of bacterial isolates against *S. rolfsii* under *in vitro* conditions

Among the 18 bacterial biocontrol isolates tested, all the isolates recorded significant percent of inhibition, the *B. subtilis* FSB2 isolate recorded the highest percentage of growth inhibition at 60.37% compared to the control, followed by *Bacillus subtilis* S9KB4 (56.60%), *Bacillus subtilis* S4KB5 (52.57%), *Bacillus subtilis* 1 (51.11%), *B. subtilis* FSB16 (50.74%), *B. subtilis* A (50%), *B.subtilis* S8KB2 (47.33%), *Pseudomonas florescens*(S) (43.33%), Actinomycetes N24 (43.30%), *B.subtilis* ESB 9 (42.22%),

Pseudomonas putida (39.97%), *B.subtilis* AA (39.63%), *B.subtilis* I (39.62%), *P. florescens* (39.20%). The lowest percentage of growth inhibition were observed in the Actinomycetes strain AS3 (37.37%) over the control against *S.rolfsii* (Table .1, Fig. 1, Plate1). Our results are in confirmation with the, findings of, Akash *et al.* [25] tested 33 bacterial isolates. Among these 33 bacterial isolates, *Bacillus* isolates S3KB6 (62.82%), S9KB4(61.70%), and S1NA7(61.11%) recorded maximum inhibitions against *S.rolfsii* over the control, concluding that *Bacillus* spp. isolated from the soil inhibited the growth of *S. rolfsii* in groundnut. Rajkumar *et al.* [26] screened thirty *Bacillus subtilis* isolates *in vitro* against *S. rolfsii*. Among these, *Bacillus* strain BS16 inhibited the maximum mycelial growth (64.04%), followed by BS30 (47%), while the minimum inhibition was observed in BS17 (11.98%) compared to the check isolate with 47% inhibition. The genus *Bacillus was* found more potential than other biocontrol microorganisms due to its unique metabolic attributes, including the production of a diverse array of antimicrobial metabolites and its capability to form endospores. *Bacillus* performs different mechanisms such as antibiosis, parasitism, competition for space and nutrients with pathogens, or by directly inducing systemic resistance in host plants [27].

List 1. Treatments details

Treatment	Particulars
T1	ST with *Trichoderma asperellum* 5 + SA of *S. rolfsii* at 30 DAS
T2	ST with *Trichoderma harzianum* 2 +SA of *S. rolfsii* at 30 DAS
T3	ST with *B. subtilis* FSB16 + SA of *S. rolfsii* at 30 DAS
T4	ST with *B. subtilis* FSB2 + SA of *S. rolfsii* at 30 DAS
T5	ST with MC1(*Trichoderma asperellum* 5 +*B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS
T6	ST with MC2 (*Trichoderma harzianum* 2 +*B. subtilis* isolate FSB16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS
T7	ST with MC3 (*Trichoderma asperellum* 5 + *Trichoderma harzianum* 2)+SA of *S. rolfsii* at 30 DAS
T8	ST with MC4 (*B. subtilis* FSB 16 + *B. subtilis* FSB2) + SA of *S. rolfsii* at 30 DAS
T9	ST with *Trichoderma*sp. 1 + SA of *S. rolfsii* at 30 DAS
T10	ST with *Trichoderma asperellum* Ta1 + SA of *S. rolfsii* at 30 DAS
T11	ST with MC5 (*Trichoderma* sp. 1+ *Trichoderma asperellum* Ta1) + SA of *S. rolfsii* at 30 DAS
T12	ST carbendazim 50 WP @2g/Kg of seeds and SA of *S.rolfsii* at 30 DAS
T13	SA of *S. rolfsii* at 30 DAS
T14	Uninoculated control

Table 1. Efficacy of bacterial biocontrol isolates against *S. rolfsii* under *In vitro* conditions

Treatments	Particulars	Radial growth of thepathogen (mm)	Percent inhibition over control
T_1	Bs_1 - *B.subtilis* FSB16	44.33 ± 0.678	50.74^{c} (45.40)
T_2	Bs_2 - *B.subtilis* ESB 9	52.00 ± 1.082	42.22^{ef} (40.50)
T_3	Pf_1 - *P.florescenes* (s)	51.00 ± 0.779	43.33^{e} (41.15)
T_4	Bs_3 - *B.subtilis* (A)	45.00 ± 0.687	50.00^{cd} (44.98)
T_5	Bs_4 - *B.subtilis* (AA)	54.33 ± 0.830	39.63^{fg} (38.99)
T_6	Bs_5 - *B.subtilis* (1)	44.00 ± 0.508	51.11^{c} (45.61)
T_7	Bs_6 - *B.subtilis* FSB 2	35.66 ± 0.544	60.37^{a} (50.96)
T_8	Bs_7 - *B.subtilis* I	54.33 ± 1.130	39.62^{fg} (38.99)
T_9	Pp - *P.putida*	39.96 ± 0.611	39.97^{fg} (39.19)
T_{10}	Pf_2 - *P.florescenes*	39.20 ± 0.450	39.20^{fg} (38.74)
T_{11}	As_1 - Actinomycetes strain3	37.36 ± 0.776	37.37^{g} (37.66)
T_{12}	As_2 - ActinomycetesN24	43.30 ± 0.901	43.30^{e} (41.13)
T_{13}	As_3 - Actinomycetes strain2	42.20 ± 0.645	42.20^{ef} (40.49)
T_{14}	Bs_8 - *B.subtilis* S4KB5	52.56 ± 1.094	52.57^{c} (46.45)

Treatments	Particulars	Radial growth of thepathogen (mm)	Percent inhibition over control
T_{15}	Bs_9 - *B.subtilis* 3	50.70 ± 0.774	50.70 ^{c} (45.38)
T_{16}	B_1 - *Bacillus* S8KB2	47.33 ± 0.723	47.33^{d} (43.45)
T_{17}	Bs_{10} - *B.subtilis* 26	41.46 ± 0.632	41.47^{ef} (40.06)
T_{18}	B_2 - *Bacillus* S9KB4	56.60 ± 0.652	56.60 ^{b} (48.77)
	CD	2.229	2.807
	SE (m)	0.774	0.975
	CV	2.902	3.671

*Values expressed are mean of three replications; *Figures in parenthesis are arc sine transformed values*

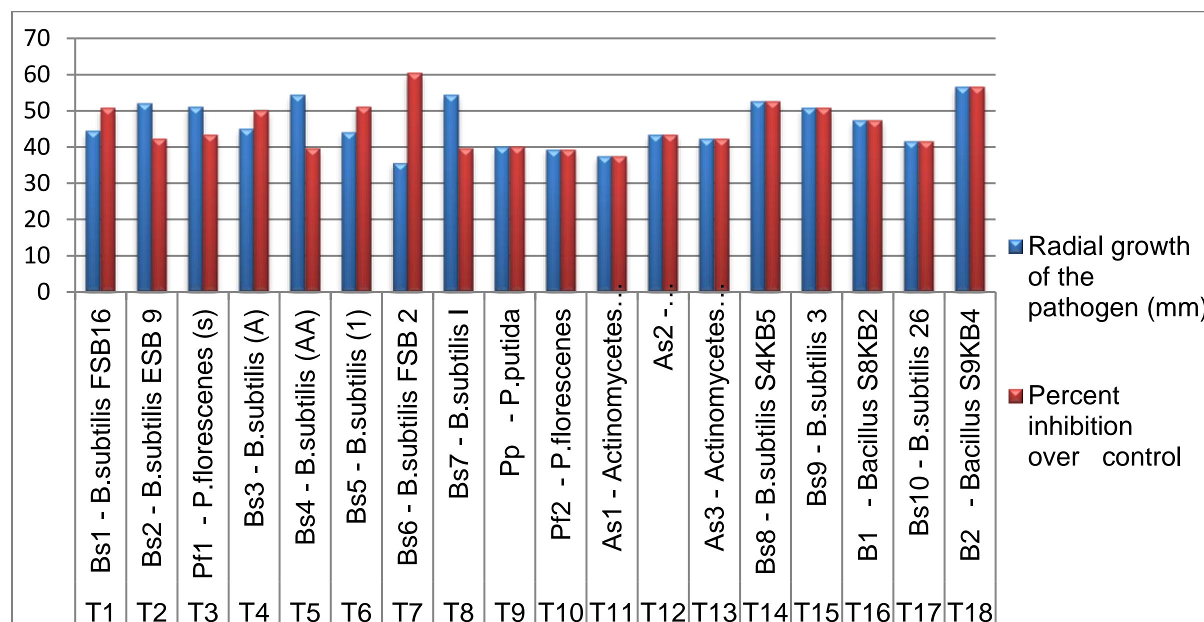

Fig. 1. Antogonistic activity of bacterial biocontrol agents against *Sclerotium rolfsii*

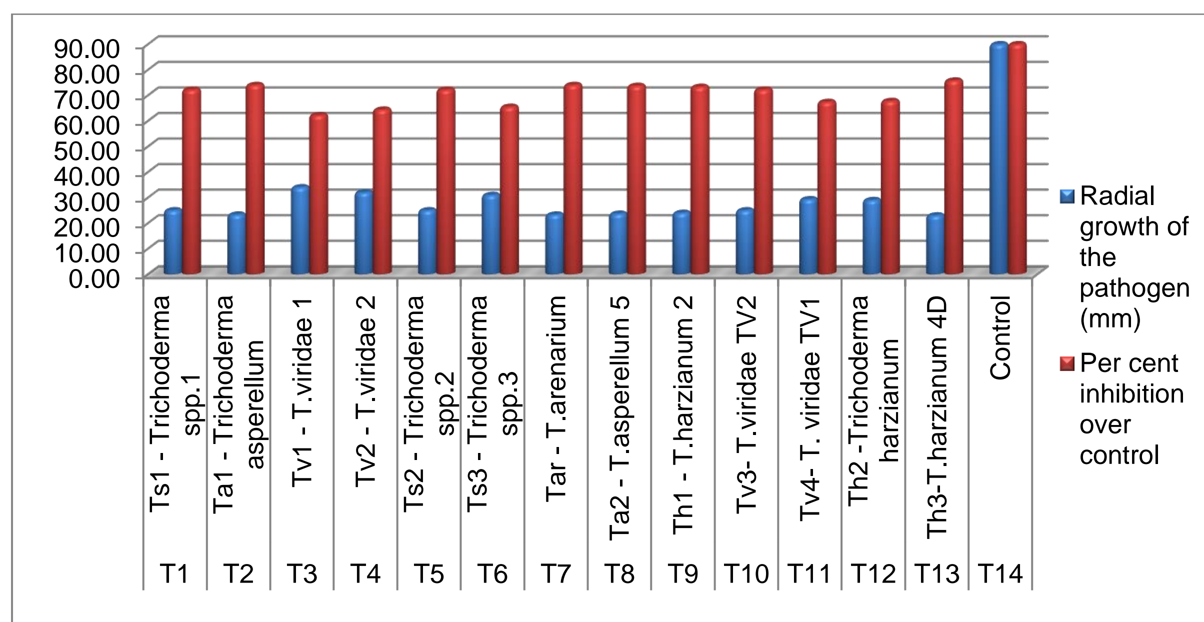

Fig. 2. Antogonistic activity of fungal biocontrol agents against *Sclerotium rolfsii*

Plate 1. Antogonistic activity of bacterial bioagents on radial growth of *Sclerotium rolfsii*

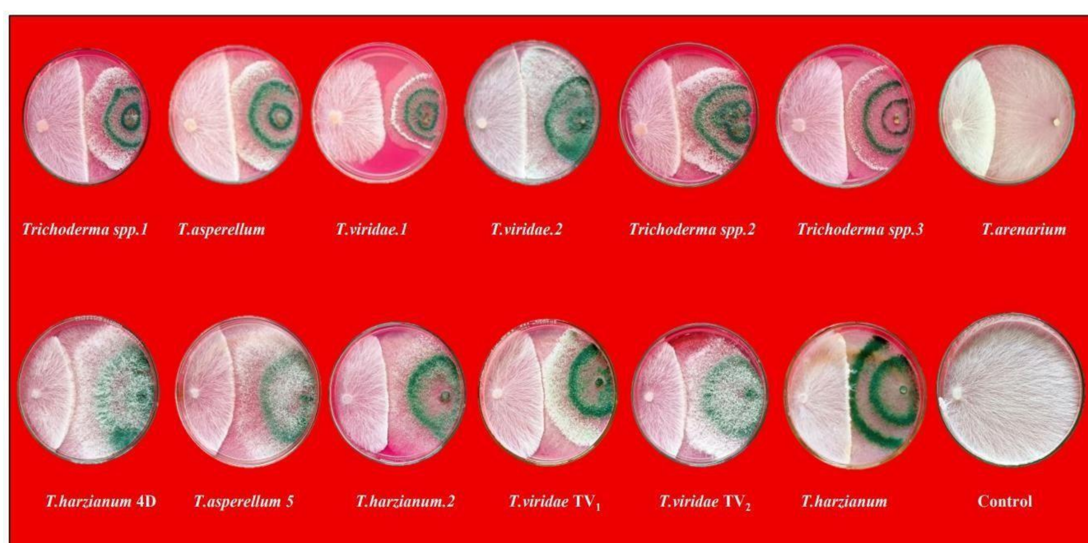

Plate 2. Antogonistic activity of fungal bioagents on radial growth of *Sclerotium rolfsii*

3.1.2 Screening of fungal isolates against *S. rolfsii* under *in vitro* conditions

Among the13 fungal isolates, *T. asperellum* 5 recorded the highest inhibition at 75.77%, followed by *Trichoderma* sp. 2 (74.03%), *T. harzianum* 4D (73.70%) compared to the control. The other isolates demonstrated the percent of inhibition as *T.harzianum* 2 with 73.33 %, *T.viridae* TV2 (72.22%), *Trichoderma* sp. 1 and *Trichoderma* sp.2 with (72.20%), *T.harzianum* (67.77%), *T.viridae* TV1 (67.40%), *Trichoderma* spp.3 (65.50%), *T.viridae* 2 (64.40%). The lowest inhibition was recorded by *Trichoderma viridae* 1 at 62.22% (Table 2, Plate 2, Fig. 2).

Our results are similar with the studies of, Bhuiyan *et al.* [28] reported that *T. harzianum* isolate Th-18 showed the highest (83.09%) reduction of the radial growth against *S. rolfsii*. This might be due to the production of secondary metabolites and antibiotics production, which diffused into the PDA which showed detrimental effect towards growth of *S. rolfsii* as well as due to higher antagonistic ability of potential *Trichoderma* mutants. Vrieze *et al.* [29] concluded the reason behind antagonistic property employed by *Trichoderma* spp. and other bioagents as competition as an indirect mechanism, where in pathogens is excluded by depletion of food or by physical occupation of

sites. Similarly, Rani *et al*. [30] conducted an *in vitro* evaluation of native fungal isolates, revealing that all tested isolates inhibited the growth of *S. rolfsii*. The highest inhibition rate (70.58%) were observed with the native bioagent *T. harzianum* (MBNRT-1). The highest pathogen growth inhibition were achieved with *T. harzianum* (Th-BKN) at 83.12%, followed by *T. viride* (Tv- BKN) at 73.16% using dual culture technique [31].

3.1.3 Screening for compatibility among the potential isolates

From the results of dual culture assay, 18 biocontrol agents (9 bacterial isolates, 9 fungal isolates) were identified as the most potential against the *Sclerotium rolfsii* and these were checked for their compatibility among them to prepare consortia. Among the 9 bacterial bioagents, five isolates *B. subtilis* FSB2, *Bacillus subtilis* FSB16, *Bacillus* S9KB4, *B. subtilis* A and *Bacillus subtilis* S4KB5 showed compatibility with each other and with all other bacterial isolates, indicated by the absence of inhibition zones at the points of interception with uniform bacterial growth.

Compatibility among the 9 fungal biocontrol agents were checked using the dual culture technique. Among all possible combinations, *Trichoderma asperellum* 5, *Trichoderma harzianum* 2, *Trichoderma* sp. 1 and *Trichoderma asperellum* Ta1 demonstrated compatibility, confirmed by the overlapping growth of one fungus over the other. The other isolates, *Trichoderma arenarium*, *Trichoderma* sp. 2, *Trichoderma harzianum*, *Trichoderma viridae* TV2 and *Trichoderma harzianum* 4D exhibited incompatible interactions, evident by the presence of inhibition zones at the points of intersection.

Among all possible combinations of fungal and bacterial biocontrol agents, *Bacillus* S9KB4, *Bacillus subtilis* S4KB5, *B. subtilis* FSB2 and *Bacillus subtilis* FSB16 showed compatibility with the *Trichoderma* isolates, *Trichoderma* sp. 1, *Trichoderma asperellum* Ta1, *Trichoderma asperellum* 5 and *Trichoderma harzianum* 2—confirmed by the growth of the fungal isolates over the bacterial streaks. Remaining were incompatible, as evidenced by the lack of overlapping fungal growth on the bacterial streaks.

The compatibility between two *Trichoderma* strains is primarily attributed to their ability to complement each other's metabolic activities, competitive strategies, and modes of action against pathogens. *Trichoderma* species were known for the production of enzymes, secondary metabolites, and their ability to induce systemic resistance in plants. When two strains are compatible, they often enhance each other's abilities through synergistic interactions. Contreras- Cornejo *et al*. [32] demonstrated that co-inoculation with two compatible *Trichoderma* strains resulted in improved plant growth and pathogen suppression compared to single strains, attributing this effect to their complementary modes of action. Similarly, Sivakumar *et al*. [33] identified the most effective isolates, *T. viride* (Tv3) and *P. fluorescens* (Pf5) and tested their compatibility for managing stem rot. The results showed that *T. viride* (Tv3) grew over *P. fluorescens* (Pf5) without any inhibition zone, indicating compatibility. Two bacterial strains are considered compatible when they can coexist and even benefit each other through various mechanisms such as metabolic cooperation, niche differentiation, or mutual protection, without antagonizing each other [34]. A recent study by Sarma *et al*. [35] demonstrated that co-inoculation of *Trichoderma* and *Bacillus* species enhanced both biocontrol efficiency and plant growth promotion, due to their complementary effects on pathogen suppression, nutrient solubilization, and hormone production. Druzhinina *et al*. [36] explored the ecological and genetic factors contributing to incompatibility between *Trichoderma* strains, highlighting how strain-specific antagonism and competition for resources shape their interactions.

3.2 Screening of Fungal and Bacterial Bioagents for Plant Growth-Promoting Activities and Antagonism Promoting Biochemical Parameters

IAA production was observed by the color change of 48-hour-old culture broth. Twelve fungal biocontrol agents tested positive for IAA production. Among the 18 bacterial isolates tested, all showed positive results for IAA production. Phytohormone IAA involves in cell enlargement, cell division, and root growth and development, resulting in a larger root surface area allowing the plant to acquire more nutrients from the soil. Ahemad and Kibret [37] reported that BCAs have the ability to produce plant growth promoting substances like Indole Acetic Acid (IAA) and antifungal substances, which favours better growth of crop plants. They

facilitate the plant growth directly or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of BCAs.

Phosphate solubilizing activity was seen in eleven fungal biocontrol isolates and 12 bacterial isolates. PSBs increase phosphorus availability by secreting phosphatases and organic acids which convert phosphate to plant available forms [38].

HCN is a secondary metabolite produced by certain strains of *Bacillus* and other biocontrol agents like *Pseudomonas*. It acts as a potent inhibitor of cellular respiration by interfering with the cytochrome c oxidase enzyme in the respiratory chain. By disrupting the electron transport chain, HCN inhibits energy production in fungal cells, leading to the suppression of growth and spread of pathogens like *Sclerotium*. HCN production was recorded in 8 fungal biocontrol isolates, eleven bacterial bioagents.

All the fungal biocontrol isolates showed positive results for ammonia production and all the bacterial isolates except one bacteria were shown ammonia production.

Siderophore production was observed in ten fungal biocontrol isolates and in all the bacterial isolates. Siderophores excreted by rhizosphere bacteria may promote plant growth by enhancing Fe nutrition and protecting plants against a variety of fungal and bacterial infections. Bacteria that produce siderophores can play a significant role in the biocontrol of several phytopathogens [39].

Trichoderma organisms release substances around root structures, enhancing the solubility of specific nutrients, thus facilitating their uptake by plants. One of these compounds, siderophores, plays a significant role in iron assimilation [40].

Ten fungal isolates and 10 bacterial bioagents tested positive for pectolytic activity. Eleven fungal isolates and 10 bacterial bioagents tested positive for cellulolytic activity (Plate 3, Table 3, Table 4). Cellulolytic and pectolytic enzymes produced by *Bacillus* and *Trichoderma* play a critical role in the suppression of groundnut stem rot caused by *Sclerotium rolfsii*. These enzymes degrade the pathogen's cell wall components, which are primarily composed of cellulose, hemicellulose, and pectin. A study by Meena *et al.* [41] demonstrated the effectiveness of cellulolytic and pectolytic enzyme-producing strains of *Bacillus subtilis* and *Trichoderma harzianum* in managing groundnut stem rot through the degradation of *Sclerotium rolfsii* cell walls. A study by Patel *et al.* [42] evaluated the combined effect of HCN production, phosphate solubilization, and ammonia production by *Bacillus* and *Trichoderma* species, which significantly reduced the incidence of groundnut stem rot by suppressing *Sclerotium rolfsii* while enhancing plant growth.

Table 2. Efficacy of fungal biocontrol agents against *S. rolfsii* under *in vitro* conditions

Treatments	Particulars	Radial growth of the pathogen (mm)	Per cent inhibition over control
T_1	Ts_1 - *Trichoderma* sp.1	25.00 ±0.382	72.20 (58.17)
T_2	Ta_1 - *Trichoderma asperellum*	23.33 ±0.484	74.03 (59.35)
T_3	Tv_1 - *T.viridae* 1	34.00 ±0.519	62.22 (52.05)
T_4	Tv_2 - *T.viridae* 2	32.00 ±0.489	64.40 (53.35)
T_5	Ts_2 - *Trichoderma* sp.2	25.00 ±0.382	72.20 (58.15)
T_6	Ts_3 - *Trichoderma* sp.3	31.00 ±0.358	65.50 (54.01)
T_7	Tar - *T.arenarium*	23.33 ±0.357	74.03 (59.34)
T_8	Ta_2 - *T.asperellum* 5	23.66 ±0.494	73.70 (59.13)
T_9	Th_1 - *T.harzianum* 2	24.00 ±0.367	73.33 (58.89)
T_{10}	Tv_3- *T.viridae* TV_2	25.00 ±0.289	72.22 (58.19)
T_{11}	Tv_4- *T. viridae* TV_1	29.33 ±0.609	67.40 (55.16)
T_{12}	Th_2 -*Trichodermaharzianum*	29.00 ±0.604	67.77 (55.38)
T_{13}	Th_3-*T.harzianum* 4D	23.00 ±0.351	75.77 (60.49)
T_{14}	Control	90.00	0
	CD	1.31	3.35
	SE (m)	0.44	1.14
	CV	2.90	2.825

*Values expressed are mean of three replications; *Figures in parenthesis are arc sine transformed values.*

Plate 3. Biochemical characterization of biocontrol agents

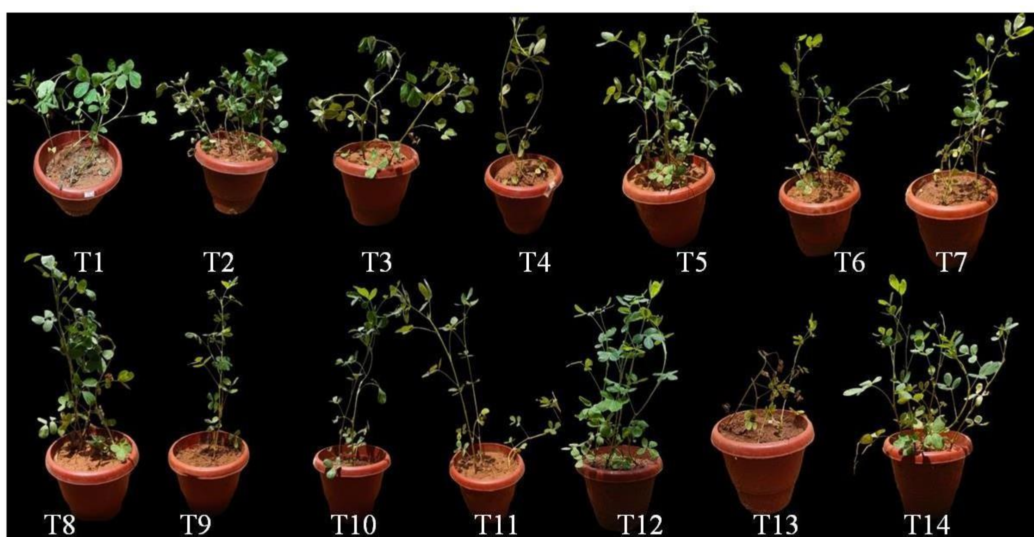

Plate 4. Evaluation of microbial consortia against groundnut stem rot under glasshouse conditions

3.3 Developing the Consortia and Testing Its Efficacy Against Stem Rot in Pot Culture

Six potential biocontrol isolates were selected for developing consortia, based on their antogonististic activity, plant growth promoting characteristics and their compatibility with each other. Selected isolates were combined to form microbial consortia.

A total of fourteen treatments were assessed for their effectiveness in managing stem rot disease of groundnut in pot culture under glasshouse conditions. Plant growth parameters were measured 10 DAS, and percent disease incidence at 50 DAS were recorded.

In the pot culture method, All the treatments showed significant difference in germination percentage compared to the pathogen check. The highest germination of 86.66% was recorded for treatment T12 par with treatments T5 with 84.44% and T6 with 82.5%. The highest shoot length of 19.12 cm was recorded for treatment T12, which was comparable to T5 with 18.12 cm, and T6 with 17.86 cm. The highest root length of 12.1 cm was observed in T12 which was comparable to T5 with 11.25 cm, and T6 with 10.92 cm. Among the fourteen treatments,

the highest fresh weight of 3.780 g was observed in T12 which was at par with T5 with 3.66 g, and T6 with 3.54 g. For dry weight, the highest value of 0.442 g was observed in T12 which was at par with T6 with 0.429 gm, and T5 with 0.418 gm. And the highest vigour index I of 2705.525 was observed in T12 which was comparable to T5 with 2480.002, and T6 with 2374.35. Also, the highest vigour index II of 38.303 were found in T12 which were comparable to T6 at 35.39, and T5 at 35.295 (Table 5). Minimum disease incidence were recorded in T12 with 12.61 percent which was at par with T5 with 14.23 percent followed by T6 with 16.34 percent (Table 4, Fig. 3, Plate 6).

Our results are similar with, Kumar *et al*. [43] also reported the effectiveness of several species of *Trichoderma* and *pseudomonas* in suppressing the incidence of *S.rolfsii* and encouraging plant growth parameters . Khan *et al*. [44] explored a consortium of *Trichoderma* spp. and *Bacillus* spp. for groundnut stem rot management. The results showed higher germination percentage, plant height, vigor index, fresh weight, and dry weight. The consortium achieved a disease reduction of 70%. Smith and Adams [45] investigated the use of a consortium of *Trichoderma harzianum*, *Pseudomonas fluorescens*, and *Bacillus subtilis* for managing groundnut stem rot. Germination was recorded as 95%. The plant height reached 39 cm, the vigor index was 1325, fresh weight per plant averaged 5.0 g, and dry weight was 1.4 g. Rathore *et al*. [46] used a microbial consortium of *Bacillus subtilis* and *Trichoderma viride* for controlling stem rot in groundnut. The combined application of these bioagents was found to reduce pathogen load and promote healthier plant growth through improved nutrient uptake and enhanced resistance to stress. A study by Ganesan *et al*. [47] reported the effectiveness of a microbial consortium involving *Trichoderma harzianum* and *Pseudomonas fluorescens* in controlling stem rot disease in groundnut. The consortium not only reduced disease severity but also improved plant growth, nodulation, and yield by enhancing nutrient uptake and producing plant growth-promoting hormones. Singh *et al*. [48] involved a consortium of *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, led to a higher germination percentage plant height, vigor index, fresh weight, and dry weight and higher disease reduction was noted at 75%.

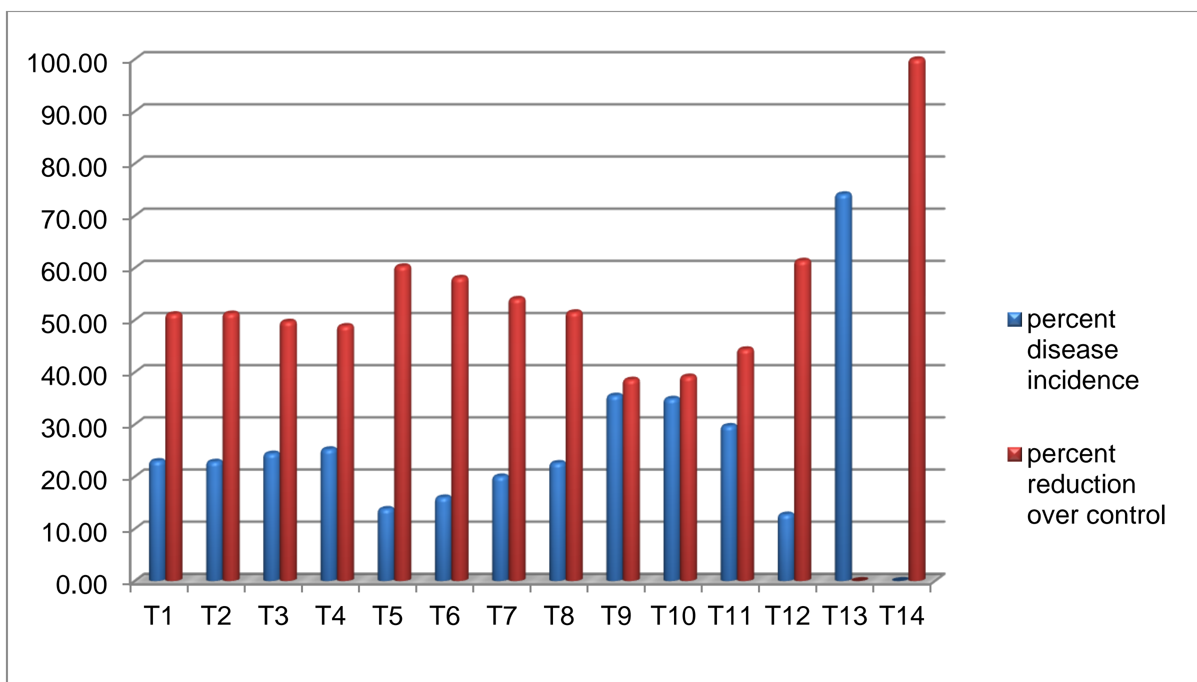

Fig. 3. Percent disease incidence of stem rot under glass house conditions

Table 3. Evaluation of fungal bioagents for plant growthpromoting biochemical traits

S.No	Isolate	Phosphate Solubilization	Ammonia production	HCN production	Siderophore production	IAA production	Pectolytic activity	Cellulolytic activity
1	Ts_1 - *Trichoderma* sp.1	+	+	+	+	+	+	-
2	Ta_1 - *Trichoderma asperellum*	+	++	-	-	++	+	+
3	Tv_1 - *T.viridae* 1	-	+	+	+	+	-	+
4	Tv_2 - *T.viridae* 2	-	+	-	-	+	-	+
5	Ts_2 - *Trichoderma* sp.2	+	+	+	+	+	+	+
6	Ts_3 - *Trichoderma* sp.3	+	+	+	+	++	+	+
7	Tar - *T.arenarium*	+	++	-	+		+	+
8	Ta_2 - *T.asperellum* 5	+	+++	+	+	+	-	+
9	Th_1 - *T.harzianum* 2	+	+++	+	+	++	+	+
10	Tv_3- *T.viridae* TV_2	+	+	+	+	+++	+	+
11	Tv_4- *T. viridae* TV_1	+	++	-	+	+	+	+
12	Th_2 -*Trichodermaharzianum*	+	+	-	+	+++	+	-
13	Th_3-*T.harzianum* 4D	-	++	+	-	+	+	+

-: negative; +: slightly positive; ++: moderately positive; +++: highly positive

Table 4. Evaluation of bacterial bioagents for plant growth promoting biochemical traits

S.No	Isolate	Phosphate Solubilization	Ammonia production	HCN production	Siderophore production	IAA production	Pectolytic activity	Cellulolytic activity
1	Bs_1 - *B.subtilis* FSB16	+	+	+	+	++	+	+
2	Bs_2 - *B.subtilis* ESB 9	-	-	-	-	+	+	-
3	Pf_1 - *P.florescenes* (s)	+	+	-	+	++	-	+
4	Bs_3 - *B.subtilis* (A)	-	+	+	+	+	+	+
5	Bs_4 - *B.subtilis* (AA)	+	+	+	+	++	+	+
6	Bs_5 - *B.subtilis* (1)	+	++	+	-	+	+	+
7	Bs_6 - *B.subtilis* FSB 2	+	++	+	+	+++	-	-
8	Bs_7 - *B.subtilis* I	+	+++	+	+	+	+	-
9	Pp - *P.putida*	+	++	+	+	+++	+	+
10	Pf_2 - *P.florescenes*	-	+	+	+	+++	-	+
11	As_1 - Actinomycetes strain3	+	+	+	+	+	-	+
12	As_2 - ActinomycetesN24	+	+	-	+	+	-	-
13	As_3 - Actinomycetes strain2	-	++	-	+	+	-	-
14	Bs_8 - *B.subtilis* S4KB5	-	++	-	+	+	-	-
15	Bs_9 - *B.subtilis* 3	-	++	-	+	+	+	-
16	B_1 - *Bacillus* S8KB2	+	++	-	+	+++	+	-
17	Bs_{10} - *B.subtilis* 26	+	++	+	+	+++	+	+
18	B_2 - *Bacillus* S9KB4	+	++	+	+	++	+	+

-: negative; +: slightly positive; ++: moderately positive; +++: highly positive

Table 5. Evaluation of microbial consortia for plant growth promoting activity under glasshouse conditions

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	Vigor index I	Fresh weight (g)	Dry weight(g)	Vigor index II
T_1	73.33 (58.89)	14.97 ± 0.22^{d}	8.91± 0.137^{de}	1751.12 ± 26.74^{f}	2.45 ± 0.039^{f}	0.36 ± 0.007^{cd}	26.54 ± 0.407^{d}
T_2	66.66 (54.71)	14.51 ± 0.30^{de}	9.12± 0.187^{d}	1575.17 ± 32.78^{g}	2.34 ± 0.048^{fg}	0.35 ± 0.009^{cd}	23.93 ± 0.500^{ef}
T_3	70.00 (56.77)	13.82 ± 0.21^{efg}	8.62± 0.132^{e}	1570.80 ± 23.99^{g}	2.92 ± 0.046^{de}	0.32 ± 0.003^{cd}	22.89 ± 0.351^{f}
T_4	72.43 (58.31)	13.63 ± 0.20^{fg}	7.96± 0.122^{f}	1563.76 ± 23.88^{g}	2.33 ± 0.036^{fgh}	0.32 ± 0.006^{cd}	23.82 ± 0.367^{ef}
T_5	84.44 (66.77)	18.12 ± 0.27^{b}	11.25± 0.173^{b}	2480.00 ± 37.87^{b}	3.66 ± 0.055^{ab}	0.41 ± 0.006^{ab}	35.29 ± 0.538^{b}
T_6	82.50 (65.26)	17.86 ± 0.20^{b}	10.92± 0.127^{b}	2374.35 ± 27.40^{c}	3.54 ± 0.040^{b}	0.42 ± 0.006^{ab}	35.39 ± 0.410^{b}
T_7	76.66 (61.10)	16.63 ± 0.25^{c}	9.62± 0.147^{c}	2012.32 ± 30.73^{d}	3.17 ± 0.048^{c}	0.37 ± 0.006^{bc}	29.05 ± 0.443^{c}
T_8	74.53 (59.68)	15.92 ± 0.33^{c}	9.73± 0.203^{c}	1911.69 ± 39.79^{e}	2.99 ± 0.062^{d}	0.35 ± 0.006^{cd}	26.16 ± 0.544^{d}
T_9	65.55 (54.04)	13.13 ± 0.19^{gh}	7.42± 0.112^{g}	1347.05 ± 20.57^{h}	2.27 ± 0.037^{gh}	0.32 ± 0.006^{cd}	21.04 ± 0.321^{g}
T_{10}	63.33 (52.71)	14.32 ± 0.16^{def}	7.98± 0.092^{f}	1412.25 ± 16.30^{h}	2.25 ± 0.026^{gh}	0.32 ± 0.003^{cd}	20.51 ± 0.237^{g}
T_{11}	71.50 (57.72)	15.03 ± 0.31^{d}	8.43± 0.175^{ef}	1677.39 ± 34.90^{f}	2.92 ± 0.062^{de}	0.34 ± 0.007^{cd}	24.52 ± 0.512^{e}
T_{12}	86.66 (68.63)	19.12 ± 0.39^{a}	12.10 ± 0.25^{a}	2705.52 ± 56.31^{a}	3.78 ± 0.078^{a}	0.44 ± 0.012^{a}	38.30 ± 0.797^{a}
T_{13}	56.66 (48.80)	12.61 ± 0.19^{h}	7.06± 0.107^{g}	1114.50 ± 17.02^{i}	2.17 ± 0.033^{h}	0.30 ± 0.006^{d}	17.05 ± 0.262^{h}
T_{14}	75.55 (60.36)	16.52 ± 0.34^{c}	9.95± 0.208^{c}	1999.80 ± 41.63^{de}	2.83 ± 0.057^{e}	0.34 ± 0.009^{cd}	26.14 ± 0.544^{d}
CD(0.05)	3.66		0.47	94.53	0.14	0.020	1.35
SE(m)	1.25	0.26	0.16	32.46	0.05	0.007	0.46
CV	2.99	3.00	3.03	3.08	3.03	3.269	3.05

*Values expressed are mean of three replications; *Figures in parenthesis are arc sine transformed values. where DAS – days after sowing; ST – seed treatment; SA- Soil application.*

(T_1) ST with Trichoderma asperellum5 + SA of S. rolfsii at 30 DAS, (T_2) ST with Trichoderma harzianum 2+SA of S. rolfsii at 30 DAS, (T_3) ST with B. subtilis FSB 16 + SA of S. rolfsii at 30 DAS, (T_4) ST with B. subtilis FSB 2 + SA of S. rolfsii at 30 DAS, (T_5) ST with MC1(Trichoderma asperellum 5 +B. subtilis FSB 16 + B. subtilis FSB2) + SA of S. rolfsii at 30 DAS, (T_6) ST with MC2 (Trichoderma harzianum 2 +B. subtilis FSB 16 + B. subtilis FSB2) + SA of S. rolfsii at 30 DAS (T_7) ST with MC3 (Trichoderma asperellum5 + Trichoderma harzianum 2)+SA of S. rolfsii at 30 DAS, (T_8) ST with MC4 (B. subtilis FSB 16 + B. subtilis FSB2) + SA of S. rolfsii at 30 DAS, (T_9) ST with Trichoderma sp. 1 + SA of S. rolfsii at 30 DAS, (T_{10}) ST with Trichoderma asperellum Ta1 + SA of S. rolfsii at 30 DAS, (T_{11}) ST with MC5 (Trichoderma sp. 1+ Trichoderma asperellum Ta1) + SA of S. rolfsii at 30 DAS, (T_{12}) ST carbendazim 50 WP @2g/Kg of seeds and SA of S. rolfsii at 30 DAS, (T_{13}) SA of S. rolfsii at 30 DAS, (T_{14}) Uninoculated control.

Table 6. Evaluation of microbial consortia against groundnut stem rot under glasshouse conditions

Treatments	Particulars	Per cent disease incidence at 50 DAS**		Percent reduction over control
T_1	ST with *Trichoderma asperellum* 5 + SA of pathogen at 30DAS	22.97	(29.11)	51.14
T_2	ST with *Trichoderma harzianum* 2+ SA of pathogen at 30DAS	22.85	(28.09)	51.27
T_3	ST with *B. subtilis* FSB 16 + SA of pathogen at 30DAS	24.42	(30.10)	49.70
T_4	ST with *B. subtilis* FSB 2 + SA of pathogen at 30DAS	25.24	(29.82)	48.88
T_5	ST with MC1(*Trichoderma asperellum* 5 +*B. subtilis* FSB 16 + *B. subtilis* FSB 2) + SA of pathogen at 30DAS	13.80	(22.14)	60.32
T_6	ST with MC2 (*Trichoderma harzianum* 2 +*B. subtilis* isolate FSB 16 + *B. subtilis* isolateFSB2) + SA of pathogen at 30DAS	16.01	(23.83)	58.11
T_7	ST with MC3 (*Trichoderma asperellum* 5 + *Trichoderma harzianum* 2) + SA of pathogen at30DAS	20.0	(26.30)	54.08
T_8	ST with MC4 (*B. subtilis* FSB 16 + *B. subtilis* FSB 2) + SA of pathogen at30DAS	22.60	(27.92)	51.51
T_9	ST with *Trichoderma* sp. 1 + SA of pathogen at 30DAS	35.53	(37.22)	38.59
T_{10}	ST with *Trichoderma asperellum* Ta1 + SA of pathogen at 30DAS	34.93	(35.80)	39.19
T_{11}	ST with MC5 (*Trichoderma* sp. 1+ *Trichoderma asperellum* Ta1) + SA of pathogen at 30DAS	29.71	(32.83)	44.41
T_{12}	ST with carbendazim 50 WP @2g/Kg of seeds + SA of pathogen at 30DAS	12.73	(20.79)	61.39
T_{13}	SA of pathogen at 30DAS	74.12	(60.93)	0
T_{14}	Untreated control	0		100
	CD	1.471		
	SE (m)	0.503		
	CV	3.172		

*Values expressed are mean of three replications; *Figures in parenthesis are arc sine transformed values. where DAS – days after sowing; ST – seed treatment; SA- Soil application.*

4. CONCLUSION

The consortial management of groundnut stem rot, which involves the use of combinations of biocontrol agents such as *Trichoderma* spp, *Bacillus* spp, has proven to be significantly more effective compared to the application of individual bioagents. The integration of multiple biocontrol agents into a consortium provides a synergistic effect that enhances the overall disease management and plant health. Our study concludes that seed treatment with microbial consortia with *Trichoderma* spp. and *Bacillus* spp. significantly reduced the stem rot incidence, compared to the pathogen check. Hence, the consortial management of groundnut stem rot is a more effective and sustainable approach compared to the application of individual biocontrol agents.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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