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

ABSTRACT

Groundnut, a crucial oilseed crop in India, is widely cultivated across various regions of the country. Stem rot, caused by *Sclerotium rolfsii* Sacc, is a major disease affecting groundnut cultivation both in India and globally. The primary objective of this study were to assess the antagonistic potential of biocontrol agents against the pathogen, both individually and in combination, under in vitro and glasshouse conditions. The results indicated that the application of microbial consortia were 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% respectively, compared to the pathogen check, which had a 74.12% PDI. Additionally, these treatments 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.), also known as peanut, goober pea, pindad, jack nut, manila nut, pygmy nut, pignut and monkey nut (Rathnakumar *et al*, 2013) is an annual leguminous plant. It is called as –king of oil seeds. In recent times, groundnut has been widely distributed and cultivated in more than eighty countries in tropical and sub-tropical regions of the world (Madhusudhana, 2013). 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, (Reddy and Maiti, 2023). 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 together accounts for 86.66 % of groundnut area in the state (Jyothirmai *et al*, 2019). Groundnut production is limited by many abiotic and biotic stresses including bacterial, fungal and viral diseases. Among the fungal diseases, stem rot (*Sclelrotium rolfsii* Sacc), have been recognized as major disease in the groundnut crop (Jadon *et al*, 2017) causing yield losses up to 50% (Joshi *et al,*2020). 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. (Cilliers *et al*, 2003). 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 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 (Hashim *et al*, 2003). Biological control strategy is one of the promising alternative to protect plants from soil borne phytopathogens (Reithner *et al*. 2011; Singh *et al*. 2011; Singh and Singh 2012). It not only reduces the negative consequences of phytopathogens but also pro motes positive responses in host plants (Ray *et al*, 2017). 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 (Thakkar and Saraf, 2015). Therefore, the present study aimed to exploit the biocontrol agents with antagonistic ability for managing stem rot of groundnut both individually and 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 experiments were 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, Trichoderma* spp. 1, *T. asperellum, T. viride* 1, *T. viride* 2, *Trichoderma* spp. 2, *Trichoderma* spp. 3, *Trichoderma arenarium*, *T. asperellum* 5, *T. harzianum* 4D, *T. viridae* TV_1, *T. harzianum* 2, *T. viridae* TV_2 and *T. harzianum and* 18 bacterial isolates *viz, Bacillus subtilis* S4KB5, *B. subtilis* S8KB2, *B. subtilis* S9KB4, *Bacillus* spp. B3, *B. subtilis* 26, *Bacillus* spp. 1, *Bacillus* spp. I, *B. subtilis* A, *B. subtilis* AA, *Bacillus* spp. FSB16, *Bacillus* spp. FSB2, *Bacillus* spp. ESB9, *Pseudomonas fluorescens, P. putida*, Actinomycetes AS2, AS3 and N24, and *Pseudomonas fluorescens* (S) were evaluated against *Sclerotium rolfsii* under *in vitro* conditions using dual culture technique. (Dennis and Webster ,1971).

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 were placed on the opposite side. The plates were then incubated at 25 ± 2ºC. A control plate containing only the pathogen were 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 were served as control. After the pathogen attained full growth on the control plate, its mycelial growth in each Petri dish were 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 (1947).

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

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 1994).

The cross-streak method were 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 were 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 tests.

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 were estimated using the method described by Gordon and Weber (1951). IAA production were 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 were treated with orthophosphoric acid and Salkowski reagent. After 25 minutes, IAA were 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 were mixed with Salkowski reagent, incubated for 20 minutes, and IAA were measured similarly at 530 nm, following the method of Bric *et al*, (1991).

2.6.2. Phosphate solubilisation

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 (Pikovskaya, 1948).

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 (Gupta and Pandey, 2019).

2.6.4. HCN production

HCN production by the biocontrol agents were estimated using a modified method from Castric and Castric (1983). 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 were placed at the center. A disc of Whatman's no.1 filter paper, the same diameter as the Petri plate, were 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 Succinate (CAS) agar medium following the method of Schwyn and Neilands (1987). 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 were 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 (2018). 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 were combined to create a dual microbial consortium, while one-third volumes of each isolate were combined to form a triple microbial consortium (Syed *et al*, 2020). 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) were 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 (Abdul- Baki and Anderson, 1973) and diseaseincidence at 50 DAS.

List 1 :**Treatments details:**

Treatment	**Particulars**

T1	ST with *Trichoderma asperellum* + SA of *S. rolfsii* at 30 DAS
T2	ST with *Trichoderma harzianum* +SA of *S. rolfsii* at 30 DAS
T3	ST with *B. subtilis* isolate FSB – 16 + SA of *S. rolfsii* at 30 DAS
T4	ST with *B. subtilis* isolate FSB- 2 + SA of *S. rolfsii* at 30 DAS
T5	ST with MC1(*Trichoderma asperellum +B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS
T6	ST with MC2 (*Trichoderma harzianum +B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS
T7	ST with MC3 (*Trichoderma asperellum*+ *Trichoderma harzianum*)+SA of *S. rolfsii* at 30 DAS
T8	ST with MC4 (*B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS
T9	ST with *Trichoderma*spp. 1 + SA of *S. rolfsii* at 30 DAS
T10	ST with *Trichoderma asperellum* isolate Tricho 2 + SA of *S. rolfsii* at 30 DAS
T11	ST with MC5 (*Trichoderma* spp. 1+ *Trichoderma asperellum* isolate Tricho 2) + 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

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 showed significant percent of inhibition, the *B. subtilis* FSB2 isolate demonstrated 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. (2021), selected a total of 33 bacterial isolates (12 from KB, 15 from NA, and 6 from AIA) through primary screening. Observations were taken on the day when the radial growth of *S. rolfsii* in the control plate were complete. Among the 33 bacterial isolates tested, isolates S3KB6, S9KB4, and S1NA7 recorded maximum inhibitions of 62.82%, 61.70% and 61.11%, respectively, over the control, concluding that *Bacillus* spp. isolated from the soil inhibited the growth of *S. rolfsii* in groundnut. Rajkumar *et al*. (2018), screened thirty *Bacillus subtilis* isolates *in vitro* against *S. rolfsii*. The isolates showed different levels of inhibition of the mycelial growth of *S. rolfsii*. Among the isolates, 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* is often more effective than other potential 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* achieves disease suppression through various mechanisms such as antibiosis, parasitism, competition for space and nutrients with pathogens, or by directly inducing systemic resistance in host plants. Consequently, there is a strong demand for finding bio agents that not only combat disease-causing pathogens but also pose no environmental hazard (Fira *et al*, 2018).

Table.1- Evaluation of bacterial isolates against *S. rolfsii* under *in vitro* conditions

S.No	Particulars	Radial growth of the pathogen (mm)	Percent inhibition over control
1	*Sclerotium rolfsii* + *B.subtilis* FSB16	44.33 ± 0.678	50.74^{c} (45.40)
2	*Sclerotium rolfsii* +*B.subtilis* ESB 9	52.00 ± 1.082	42.22^{ef} (40.50)
3	*Sclerotium rolfsii* + *P.florescenes* (s)	51.00 ± 0.779	43.33^{e} (41.15)
4	*Sclerotium rolfsii* + *B.subtilis* (A)	45.00 ± 0.687	50.00^{cd} (44.98)
5	*Sclerotium rolfsii* + *B.subtilis* (AA)	54.33 ± 0.830	39.63^{fg} (38.99)
6	*Sclerotium rolfsii* + *B.subtilis* (1)	44.00 ± 0.508	51.11^{c} (45.61)
7	*Sclerotium rolfsii* + *B.subtilis* FSB 2	35.66 ± 0.544	60.37^{a} (50.96)
8	*Sclerotium rolfsii* + *B.subtilis* I	54.33 ± 1.130	39.62^{fg} (38.99)
9	*Sclerotium rolfsii* + *P.putida*	39.96 ± 0.611	39.97^{fg} (39.19)
10	*Sclerotium rolfsii* + *P.florescenes*	39.20 ± 0.450	39.20^{fg} (38.74)
11	*Sclerotium rolfsii* + Actinomycetes strain3	37.36 ± 0.776	37.37^{g} (37.66)
12	*Sclerotium rolfsii* + ActinomycetesN24	43.30 ± 0.901	43.30 ^{e} (41.13)
13	*Sclerotium rolfsii* + Actinomycetes strain2	42.20 ± 0.645	42.20^{ef} (40.49)
14	*Sclerotium rolfsii* + *B.subtilis* S4KB5	52.56 ± 1.094	52.57 ^{c} (46.45)
15	*Sclerotium rolfsii* + *B.subtilis* 3	50.70 ± 0.774	50.70 ^{c} (45.38)
16	*Sclerotium rolfsii* + *Bacillus* S8KB2	47.33 ± 0.723	47.33^{d} (43.45)
17	*Sclerotium rolfsii* + *B.subtilis* 26	41.46 ± 0.632	41.47^{ef} (40.06)
18	*Sclerotium rolfsii* + *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.

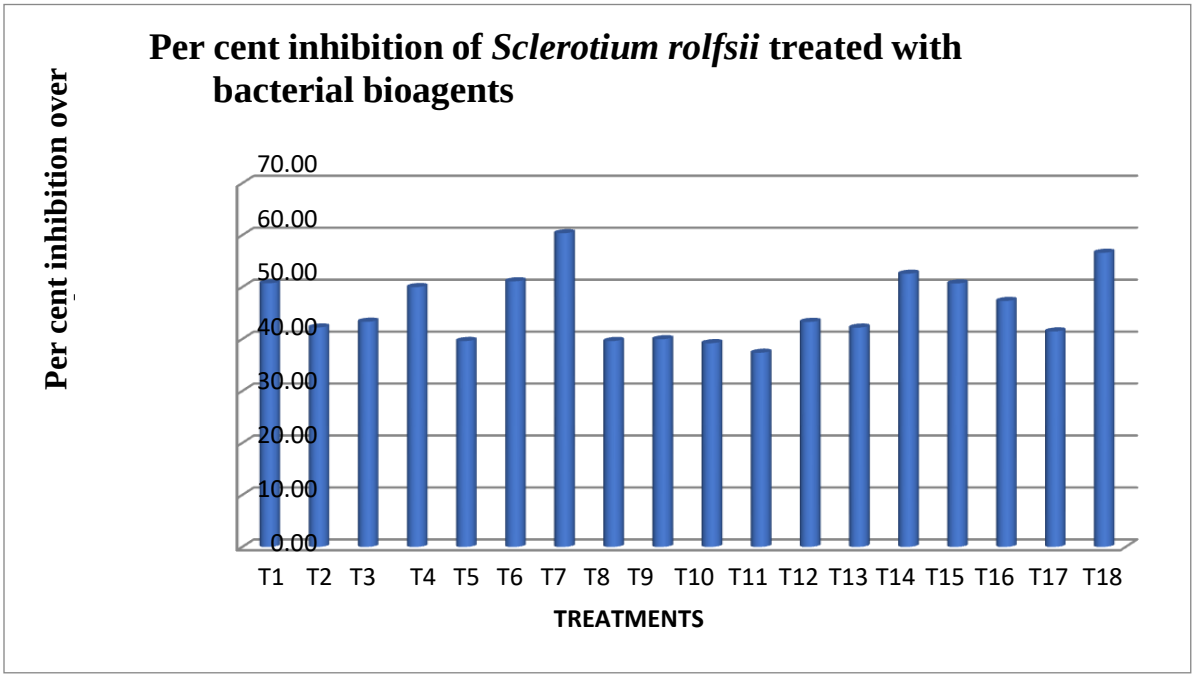

Fig 1: Per cent inhibition of *Sclerotium rolfsii* treated with bacterial bioagents under in vitro conditions

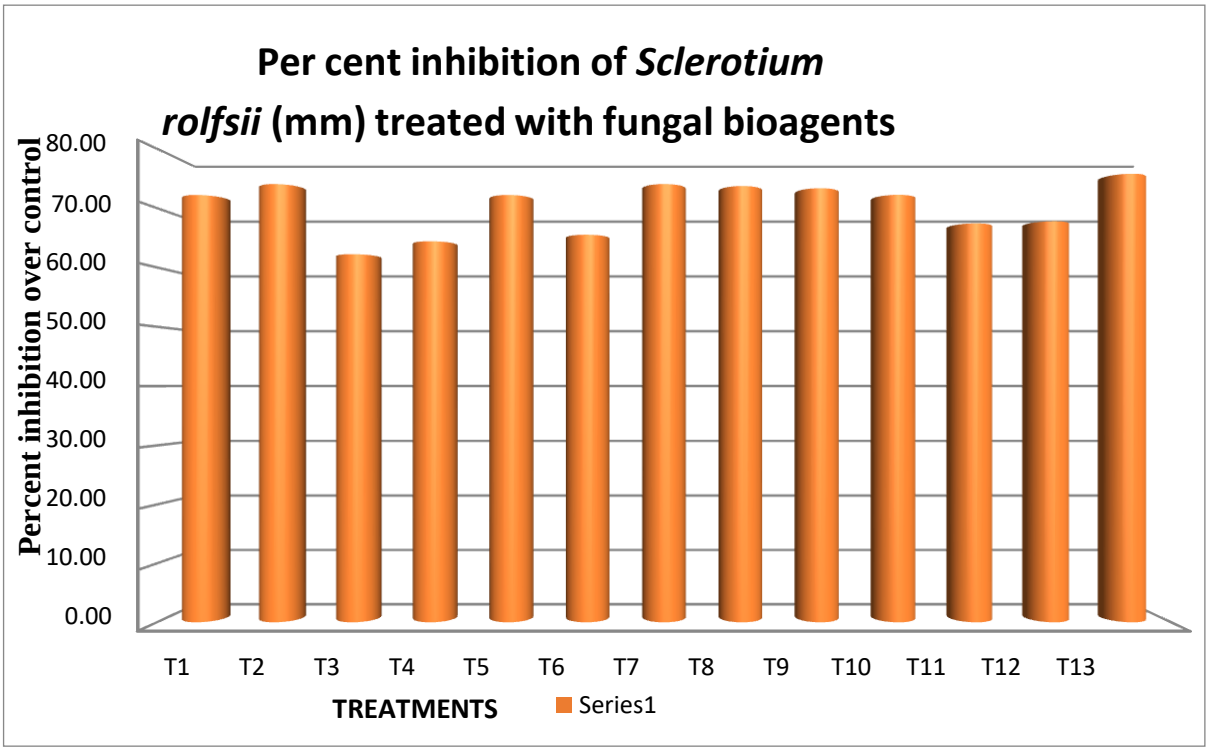

Fig 2: Per cent inhibition of *Sclerotium rolfsii* treated with fungal bioagents under in vitro conditions

Plate.1. Antogonistic activity of bacterial 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 exhibited the highest inhibition at 75.77%, followed by *Trichoderma* spp. 2 (74.03%), *T. harzianum* 4D (73.70%) compared to the control. The other isolates demonstrated the percent of inhibition as *T.harzianum* with 73.33 %, *T.viridae* TV_2 (72.22%), *Trichoderma* spp. 1 and *Trichoderma* spp.2 with (72.20%), *T.harzianum* (67.77%), *T.viridae* TV_1 (67.40%), *Trichoderma* spp.3 (65.50%), *T.viridae* 2 (64.40%). The lowest inhibition were recorded by *Trichoderma viridae* 1 at 62.22% (Table.2, Plate 2, Fig 2).

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

Table.2 - Evaluation of fungal isolates against *S. rolfsii* under *in vitro* conditions

S.No	Particulars	Radial growth of the pathogen (mm)	Per cent inhibition over control
1	*Sclerotium rolfsii*+ *T1-Trichoderma* spp.1	25.00 ± 0.382	72.20 ^{a} (58.17)
2	*Sclerotium rolfsii*+ T2-*T. aperellum*	23.33 ± 0.484	74.03 ^{a} (59.35)
3	*Sclerotium rolfsii* + T3-*T.viridae* 1	34.00 ± 0.519	62.22 ^{c} (52.05)
4	*Sclerotium rolfsii*+ T4-*T.viridae* 2	32.00 ± 0.489	64.40 ^{bc} (53.35)
5	*Sclerotium rolfsii* + T5- *Trichoderma* spp.2	25.00 ± 0.382	72.20 ^{a} (58.15)
6	*Sclerotium rolfsii* + T6 - *Trichoderma* spp.3	31.00 ± 0.358	65.50 ^{bc} (54.01)
7	*Sclerotium rolfsii* + T7- *T.arenarium*	23.33 ± 0.357	74.03 ^{a} (59.34)
8	*Sclerotium rolfsii* + Ta5 –*T.asperellum*	23.66 ± 0.494	73.70 ^{a} (59.13)
9	*Sclerotium rolfsii* + TH_2 – *T.harzianum* 2	24.00 ± 0.367	73.33 ^{a} (58.89)
10	*Sclerotium rolfsii*+ TV_2-*T.viridae*	25.00 ± 0.289	72.22 ^{a} (58.19)
11	*Sclerotium rolfsii* + TV_1-T. viridae	29.33 ± 0.609	67.40 ^{b} (55.16)
12	*Sclerotium rolfsii* + ERF2 –*Trichoderma harzianum*	29.00 ± 0.604	67.77 ^{b} (55.38)
13	*Sclerotium rolfsii* + C Cart –*T.harzianum* 4D	23.00 ± 0.351	75.77 ^{a} (60.49)
	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.

Our results are similar with the studies of, Bhuiyan *et al*. (2012) 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*. (2018) 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*. (2023), 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 (Meena *et al*,2024).

3.2. Screening for compatibility among the potential isolates

From the results of dual culture assay, 18 biocontrol agents (9 bacteria, 9 fungi) 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* spp. FSB16, *Bacillus subtilis* 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 pointsof interception with uniform bacterial growth.

Compatibility among the 9 fungal biocontrol isolates were checked using the dual culture technique. Among all possible combinations, *Trichoderma asperellum* 5, *Trichoderma harzianum* 2, *Trichoderma* spp. 1 and *Trichoderma asperellum* demonstrated compatibility, confirmed by the overlapping growth of one fungus over the other. The other isolates—*Trichoderma arenarium*, *Trichoderma* spp. 2, *Trichoderma harzianum*, *Trichoderma viridae* TV_2 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 isolates, *Bacillus subtilis* S9KB4, *Bacillus subtilis* S4KB5, *B. subtilis* FSB2 and *Bacillus* spp. FSB16 showed compatibility with the *Trichoderma* isolates—*Trichoderma* spp. 1, *Trichoderma asperellum*, *Trichoderma asperellum* 5 and *Trichoderma harzianum* 2—confirmed by the growth of the fungal isolates over the bacterial streaks. The other combinations 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 are known for their 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*. (2020), 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*. (2020), 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 (Marasco *et al*, 2018). A recent study by Sarma *et al*. (2022), 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*. (2018), explored the ecological and genetic factors contributing to incompatibility between *Trichoderma* strains, highlighting how strain-specific antagonism and competition for resources shape their interactions.

3.3. Screening of fungal and bacterial bioagents for plant growth- promoting activities and antagonism promoting biochemical parameters

IAA production were observed by the color change of 48-hour-old culture broth. Twelve fungal biocontrol isolates 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 (2014), 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 is seen in eleven fungal biocontrol isolates and 12 bacterial isolates. PSBs increase phosphorus availability by secreting phosphatases and organic acids whichconvert phosphate to plantavailable forms (Breedt *et al*, 2017).

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 were recorded in 8 fungal biocontrol isolates, elevenbacterial bioagents.

All the fungal biocontrol isolates showed positive results for ammonia production and 17 bacterial isolates were shown ammonia production.

Siderophore production were 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 severalphytopathogens (Javorekova *et al*, 2020).

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 (López-Bucio *et al*, 2015).

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). 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*. (2019), 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*. (2020), 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.

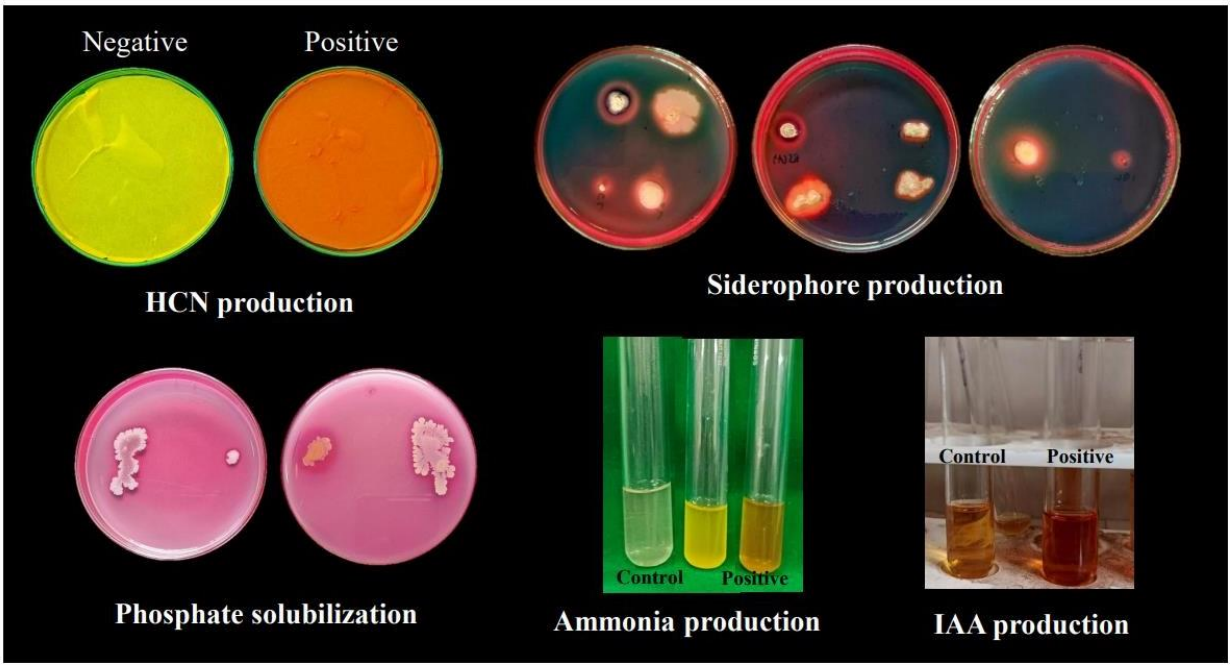

Plate.3. Biochemical characterization of biocontrol agents

3.4. 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. Selectedisolates 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 such as germination percentage, shoot length, root length, fresh weight, dry weight, vigour index I, and vigour index II 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.3). 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 4)

Our results are similar with, Kumar *et al*. (2017), evaluated a consortium of *Trichoderma harzianum* and *Pseudomonas fluorescens* for managing groundnut stem rot. The consortium resulted in a 92% germination percentage, an average plant height of 25 cm, a vigor index of 1050, fresh weight of 35 g per plant, and dry weight of 10 g per plant. Disease severity was reduced by 68% compared to untreated controls. Khan *et al*. (2017), explored a consortium of *Trichoderma* spp. and *Bacillus* spp. for groundnut stem rot management. The results showed a germination percentage of 88%, plant height of 27 cm, vigor index of 1250, fresh weight of 38 g, and dry weight of 11 g. The consortium achieved a disease reduction of 70%. Smith and Adams (2018), 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*. (2019), 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*. (2020), demonstrated 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*. (2021), This study involved a consortium of *Trichoderma harzianum*, *Bacillus subtilis*, and *Pseudomonas fluorescens*. The consortium led to a germination percentage of 90%, a plant height of 30 cm, a vigor index of 1350, fresh weight of 45 g, and dry weight of 14 g. disease reduction was noted at75%.

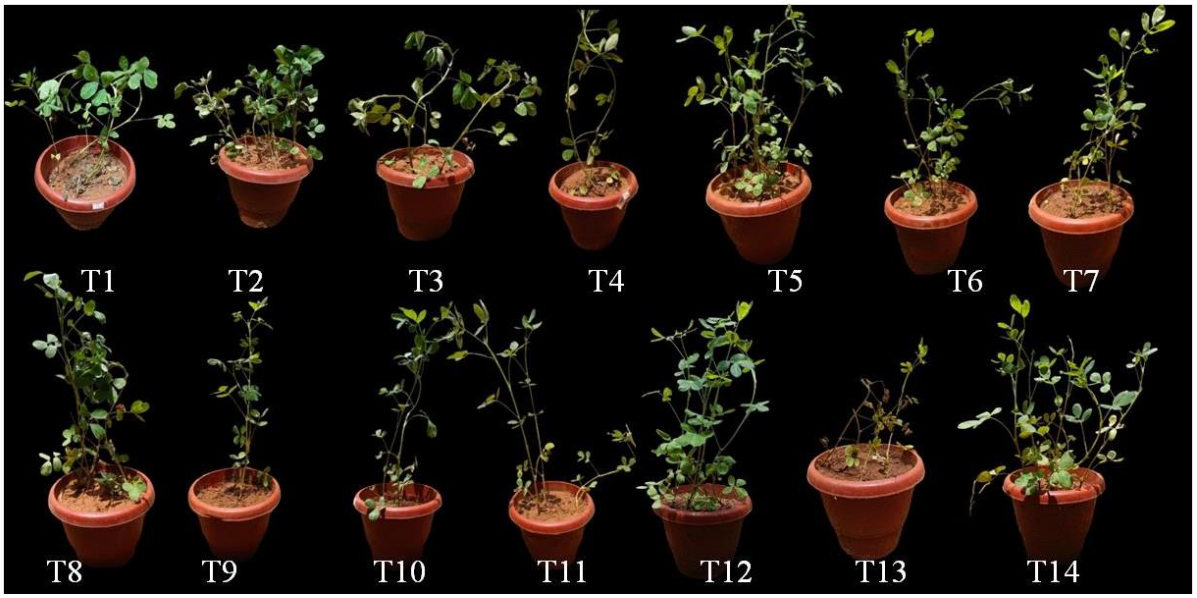

Plate.4. Evaluation of microbial consortia against groundnut stem rot under glasshouse conditions

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 MC1, MC2, MC3, and MC4 resulted in lower disease incidence with 13.80%, 16.01%, 20.0%, and 22.60% respectively, compared to the pathogen check, which had a 74.12% PDI. Hence, the consortial management of groundnut stem rot is a more effective and sustainable approach compared to the application of individual biocontrol agents.

Table.3- 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 asperellum* + SA of *S. rolfsii* at 30 DAS, (T_2) ST with *Trichoderma harzianum* +SA of *S. rolfsii* at 30 DAS, (T_3) ST with *B. subtilis* isolate FSB – 16 + SA of *S. rolfsii* at 30 DAS, (T_4) ST with *B. subtilis* isolate FSB- 2 + SA of *S. rolfsii* at 30 DAS, (T_5) ST with MC1(*Trichoderma asperellum* +*B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS, (T_6) ST with MC2 (*Trichoderma harzianum* +*B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS (T_7) ST with MC3 (*Trichoderma asperellum*+ *Trichoderma harzianum*)+SA of *S. rolfsii* at 30 DAS, (T_8) ST with MC4 (*B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of *S. rolfsii* at 30 DAS, (T_9) ST with *Trichoderma* spp. 1 + SA of *S. rolfsii* at 30 DAS, (T_{10}) ST with *Trichoderma asperellum* isolate Tricho 2 + SA of *S. rolfsii* at 30 DAS, (T_{11}) ST with MC5 (*Trichoderma* spp. 1+ *Trichoderma asperellum* isolate Tricho 2) + 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.

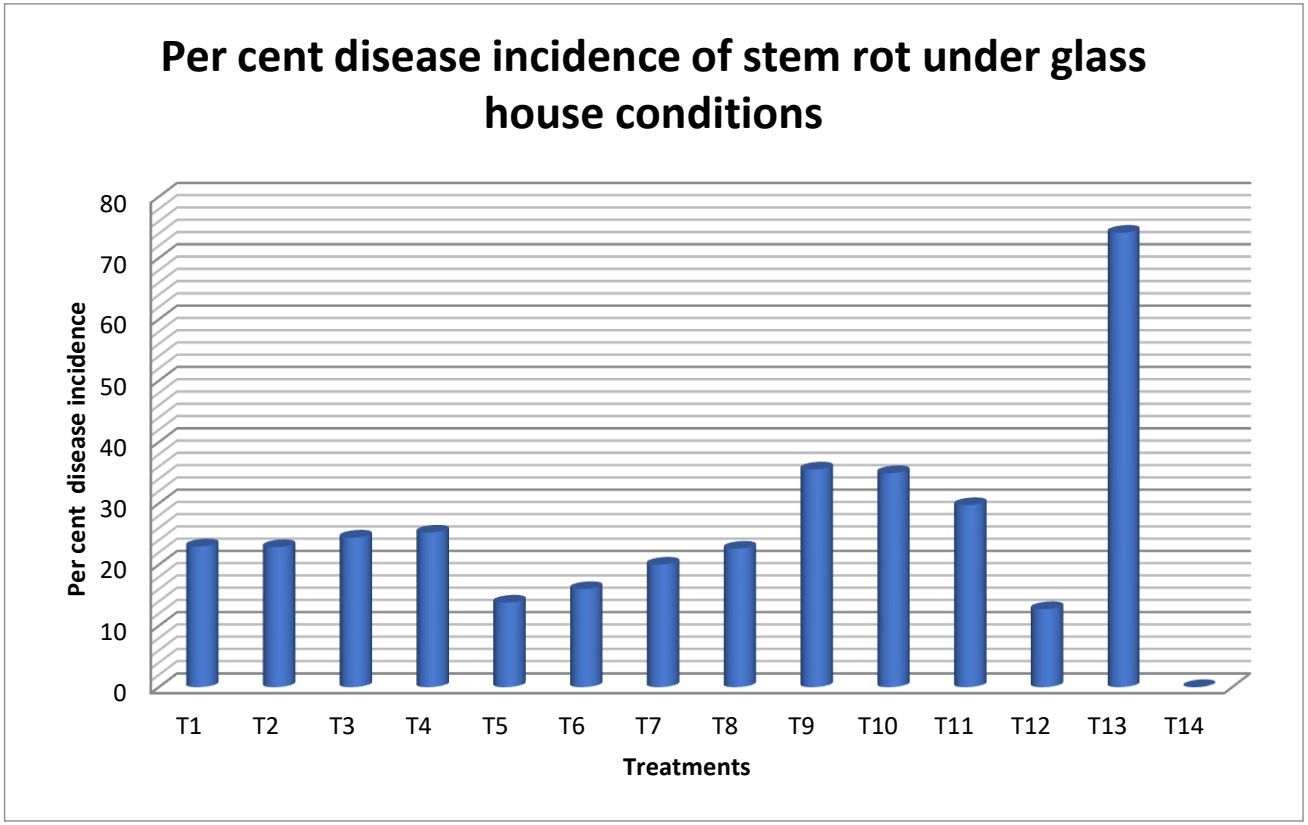

Fig.3.Percent disease incidence of stem rot under glass house conditions

Table.4 - 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* + SA of pathogen at 30DAS	22.97	(29.11)	51.14
T_2	ST with *Trichoderma harzianum* + SA of pathogen at 30DAS	22.85	(28.09)	51.27
T_3	ST with *B. subtilis* isolate FSB – 16 + SA of pathogen at 30DAS	24.42	(30.10)	49.70
T_4	ST with *B. subtilis* isolate FSB- 2 + SA of pathogen at 30DAS	25.24	(29.82)	48.88
T_5	ST with MC1(*Trichoderma asperellum* +*B. subtilis* isolate FSB 16 + *B. subtilis* isolateFSB2) + SA of pathogen at 30DAS	13.80	(22.14)	60.32
T_6	ST with MC2 (*Trichoderma harzianum* +*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*+ *Trichoderma harzianum*) + SA of pathogen at30DAS	20.0	(26.30)	54.08
T_8	ST with MC4 (*B. subtilis* isolate FSB 16 + *B. subtilis* isolate FSB2) + SA of pathogen at30DAS	22.60	(27.92)	51.51
T_9	ST with *Trichoderma* spp. 1 + SA of pathogen at 30DAS	35.53	(37.22)	38.59
T_{10}	ST with *Trichoderma asperellum* isolate Tricho 2 + SA of pathogen at 30DAS	34.93	(35.80)	39.19
T_{11}	ST with MC5 (*Trichoderma* spp. 1+ *Trichoderma asperellum* isolate Tricho 2) + SA ofpathogen 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.

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