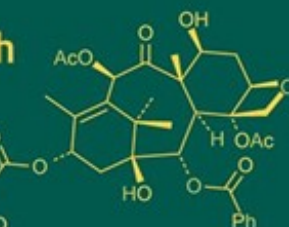


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In vitro evaluation of bioagents and their consortia against *Sclerotium rolfii* causing collar rot of chickpea

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ABSTRACT

Microbial consortia (combinations of bioagents) are increasingly preferred over single bioinoculants for crop protection as they overcome the inconsistent performance frequently encountered with individual biocontrol agents. The collar rot of chickpea caused by *Sclerotium rolfii* is a destructive soil borne disease that survives in the soil as sclerotia for several years and is difficult to manage. In the present studies four biocontrol agents *Trichoderma asperellum* UASR strain, *Bacillus subtilis* SE76, *Streptomyces nenu* DS-11 and *Pseudomonas fluorescens* UASR strain were evaluated alone and in all compatible combinations against *S. rolfii* under *In vitro* conditions. The antagonistic potential of the bioagents against the pathogen was distinctly higher in consortia than in single treatments. The four member consortium T_{15} (*T. asperellum* + *B. subtilis* + *Streptomyces nenu* + *P. fluorescens*) recorded the highest inhibition of *S. rolfii* mycelial growth (73.33 %) among all bioagent treatments and was statistically on par with the best three agents consortium T_{13} (*T. asperellum* + *Streptomyces nenu* + *P. fluorescens*) with inhibition of 72.22 per cent followed by T_{12} (*T. asperellum* + *B. subtilis* + *P. fluorescens*) with 70.11 per cent inhibition. Among two member consortia, T_7 (*T. asperellum* + *P. fluorescens*) recorded the highest inhibition (70.55 %). Among individual antagonists, *T. asperellum* proved most effective (61.00 %) followed by *Streptomyces nenu* (28.88 %) and *B. subtilis* (23.88 %), while *P. fluorescens* recorded the least inhibition (23.00 %). These results demonstrate that rationally designed, compatible microbial consortia provide substantially greater *In vitro* suppression of *S. rolfii* than individual bioagents.

Keywords: Bioagents, Chickpea, Collar rot, Dual culture, *Microbial consortia*, *Sclerotium rolfii*

Introduction

Growing awareness of residue free produce and environmental safety has made chemical free plant health management a major challenge for present day agriculture. Antagonistic microorganisms and other biological technologies are therefore receiving greater attention in crop protection (Sulaiman *et al.*, 2024) [1]. The biocontrol agents colonize the rhizosphere and leave behind no hazardous residues (Tewari *et al.*, 2001) [2]. Most biocontrol strategies, however, have been built around a single antagonist directed against a single pathogen which partly accounts for their variable performance under field conditions (Rajasekhar *et al.*, 2016) [3]. A single bioagent can rarely be expected to perform uniformly across soil types and against all phytopathogens (Nandakumar *et al.*, 2001) [4]. Consequently, microbial consortia, defined as two or more microbial groups that interact synergistically and act together as a community, are being favoured over single bioinoculants for crop protection, since single strains of diverse microorganisms are generally less effective than consortia (Sudharani *et al.*, 2014) [5].

Among the soil borne diseases of chickpea, collar rot caused by *Sclerotium rolfii* (teleomorph- *Athelia rolfii*) ranks among the most damaging alongside *Fusarium oxysporum* f. sp. *ciceris* and *Rhizoctonia bataticola* and is responsible for yield losses that range 60 - 70 per cent when conditions favour the pathogen (Patra *et al.*, 2023) [6]. The pathogen is remarkably polyphagous attacking more than 500 plant species and persists in soil and crop debris as sclerotia that remain viable for several years making it exceptionally difficult to eradicate once established in a field (Chowdary *et al.*, 2024) [7]. Infection at the collar region is accompanied by profuse white silky mycelium and mustard like sclerotia resulting in pre and post emergence seedling mortality and appreciable plant stand loss. The prolonged survival of sclerotia, the saprophytic ability of the fungus and the limited reach of foliar fungicides at the collar region together make chemical management of this disease inadequate. In recent years, therefore, increasing emphasis has been placed on the combined use of biocontrol agents possessing different mechanisms of action, so as to improve the level of disease suppression and overcome the inconsistency of single bioagents (Thakkar *et al.*, 2015) [8]. This approach has recently found field level support with integrated application of *Trichoderma* alongside soil amendments substantially lowered collar rot incidence in chickpea under natural field conditions (Paveen *et al.*, 2025) [9], reinforcing the case for consortium and integrated strategies over reliance on any single biocontrol component. In view of the potential benefits of microbial consortia, the present investigation was undertaken to evaluate

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selected fungal and bacterial bioagents, alone and in compatible combinations against *S. rolfii* under *In vitro* conditions.

Material and methods

The laboratory study was conducted in the Department of Plant Pathology, College of Agriculture, Raichur University of Agricultural Sciences, Raichur during 2025-26. The biocontrol agents viz., *Trichoderma asperellum* UASR strain, *Bacillus subtilis* SE76, *Streptomyces nenu* DS-11 and *Pseudomonas fluorescens* UASR strain used for the studies were collected from Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, Raichur. The *Sclerotium rolfii* was isolated from chickpea plants showing typical collar rot symptoms and maintained on potato dextrose agar (PDA) at 27 ± 2 °C. The bio-efficacy of the fungal and bacterial biocontrol agents was evaluated under *In vitro* conditions against *S. rolfii* using the dual culture technique (Dennis *et al.*, 1971) [10]. The 20 ml of sterilized and cooled PDA were poured into sterile Petri plates and allowed to solidify. For evaluation of the fungal biocontrol agent, a 5 mm mycelial disc of *S. rolfii* was placed at one end of the plate and the antagonist at the opposite end. For bacterial and actinomycete antagonists, a 5 mm pathogen disc was placed on one side and the antagonist was streaked or spread on the opposite side.

For testing combinations of compatible bioagents, a 5 mm pathogen disc was placed at the centre of the PDA plate and the compatible bioagents were placed or streaked on either side of the pathogen at equal distances. Three replications were maintained for each treatment along with a control containing only the test fungus. Plates were incubated at 27 ± 1 °C and the radial growth of the pathogen in dual culture and control plates was measured when the pathogen in the control plates had fully covered the medium. Percent inhibition of mycelial growth was calculated using the formula given by Vincent (1927) [11].

$$I = \frac{C-T}{C} \times 100$$

Where,

I = percent inhibition of mycelial growth,

T = radial growth of pathogen in treatment (cm),

C = radial growth of pathogen in control (cm).

Results

All the biocontrol agents either alone or in combination significantly inhibited the mycelial growth of *Sclerotium rolfii* under *In vitro* conditions (Table 1, Fig. 1 and Plate 1). The antagonistic activity of the fungal and bacterial bioagents was higher in consortia than when the agents were applied individually. Among the individual antagonists, *Trichoderma asperellum* (T₁) recorded the maximum inhibition of *S. rolfii* (61.00 %) and was statistically superior to all other single treatments. It was followed by *Streptomyces nenu* (T₃) with 28.88 per cent inhibition and *Bacillus subtilis* (T₂) with 23.88 per cent inhibition. The least inhibition among the individual treatments was recorded in *Pseudomonas fluorescens* (T₄) with 23.00 per cent which was statistically on par with T₂. Compared with the bacterial antagonists, the fungal antagonist showed a markedly higher level of suppression of the pathogen. Among the two agent combinations, *T. asperellum* + *P. fluorescens* (T₇) recorded the highest inhibition (70.55 %) which was statistically on par with *T. asperellum* + *Streptomyces nenu* (T₆) with 68.66 per cent and *T. asperellum* + *B. subtilis* (T₅) with 67.44 per cent. Among the three agent combinations, treatment *T. asperellum* + *Streptomyces nenu* + *P. fluorescens* (T₁₃) recorded the highest inhibition (72.22 %) and was statistically on par with *T. asperellum* + *B. subtilis* + *P. fluorescens* (T₁₂) with 70.11 per cent and *T. asperellum* + *B. subtilis* + *Streptomyces nenu* (T₁₁) with 66.88 per cent. *B. subtilis* + *Streptomyces nenu* + *P. fluorescens* (T₁₄) recorded the least inhibition (58.77 %) among the three agent combinations and T₁₃ was sta-

tistically superior to both T₁₁ and T₁₄. In case of the four agent combination, treatment *T. asperellum* + *B. subtilis* + *Streptomyces nenu* + *P. fluorescens* (T₁₅) recorded highest inhibition among all the bioagent treatments with 73.33 per cent which was statistically on par with the best performing three agent consortia (T₁₂ and T₁₃) and significantly superior to all the remaining treatments. Thus, the consortia of biocontrol agents consistently outperformed the individual bioagents under *In vitro* conditions and the level of suppression increased progressively from single agents to two, three and four agent combinations.

Discussion

The superior performance of *T. asperellum*, both alone and as the common component of every high performing consortium may be attributed to the production of antibiotic substances such as viridin, as reported by Brain (1951) [12] and to the penetration of the antagonist hyphae into the hyphae of the pathogen at the point of contact, as confirmed by Mukherjee *et al.* (2000). The present findings are comparable with those of Das *et al.* (2000) [13], who evaluated *Trichoderma harzianum*, *T. viride* and *T. koningii* against *S. rolfii* *In vitro* and found *T. harzianum* to be the most effective in inhibiting mycelial growth (61.50 %).

Similarly, Mundhe *et al.* (2009) [14] tested bioagents against *S. rolfii*, the causal organism of foot rot of finger millet and recorded the highest inhibition with *T. harzianum* (P) (73.77 %) followed by *T. harzianum* (JCR) (73.00 %), *Pseudomonas fluorescens* (72.66 %), *Bacillus subtilis* (71.55 %) and *T. viride* (63.33 %). Kulkarni (2007) [15] tested the antagonistic effect of *T. harzianum*, *T. viride*, *T. virens*, *T. koningii*, *P. fluorescens* and *B. subtilis* against *S. rolfii* and found *T. harzianum* to be the most effective in inhibiting mycelial growth (59.81 %). Comparable results were obtained by Muttappa (2010) [16], who evaluated microbial antagonists against *S. rolfii*, the causal agent of foot rot of finger millet by the dual culture plate method. Among the fungal antagonists, *T. harzianum* TH10 exhibited the best inhibition of the pathogen (72.44 %) while among the bacterial antagonists *B. subtilis* Bs1 showed the maximum inhibition (56.29 %) followed by *P. fluorescens* Pfl1 (46.78 %).

The author further reported that the fungal and bacterial bioagents used were capable of producing volatile compounds such as hydrogen cyanide, 6-pentyl- α -pyrone and benzaldehyde which are responsible for inhibition of the pathogen. The consistently higher suppression achieved by the fungal antagonist in the present study is in agreement with these reports. The advantage of combining antagonists observed here has also been reported for other soil borne pathogens. Most relevant to the present work, Jadav *et al.* (2022) [17] evaluated *T. harzianum* IOF strain (MH027645.1), *P. fluorescens* IOF strain (NAIMCC-B-01981), *B. subtilis* IOF strain (MT383652.1) and the endophyte *N. parvum* against *S. rolfii* under *In vitro* conditions and found the four agent combination (*T. harzianum* + *P. fluorescens* + *B. subtilis* + *N. parvum*) superior to all other treatments in arresting mycelial growth of the pathogen (80.37 %), followed among the three agent combinations by *T. harzianum* + *B. subtilis* + *N. parvum* (78.52 %) with the least inhibition in *T. harzianum* + *P. fluorescens* + *B. subtilis* (74.57 %). The same rank order of four agent over three agent over two agent and single treatments was obtained in the present study. The progressive increase in inhibition from single agents to two, three and four agent consortia observed here suggests additive or synergistic interactions among compatible antagonists. The mechanisms likely to be involved include the production of antibiotics, hydrogen cyanide and siderophores, secretion of lytic enzymes and competition for space and nutrients (Weller, 1988; Intana *et al.*, 2008) [19, 18]. It is notable that every consortium containing *T. asperellum* recorded higher inhibition than any consortium composed only of bacterial and actinomycete antagonists, indicating that mycoparasitism by the fungal antagonist contributes substantially to the performance of the consortia against *S. rolfii*.

Conclusion

Fungal and bacterial bioagents, alone and in combination were found effective against *Sclerotium rolfsii* under *In vitro* conditions. The microbial consortium comprising *Trichoderma asperellum* + *Bacillus subtilis* + *Streptomyces nenu* + *Pseudomonas fluorescens* (T₁₅) was the most promising bioagent treatment inhibiting mycelial growth of the pathogen by 73.33 per cent. *Microbial consortia* can therefore be

explored for the successful management of soil borne plant pathogens such as *S. rolfsii* in chickpea.

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Table 1. Bio-efficacy of bioagents and their consortia against *Sclerotium rolfsii*

Tr. No.	Treatment details	Inhibition over control (%)
T ₁	<i>Trichoderma asperellum</i> UASR	61.00 (51.35)
T ₂	<i>Bacillus subtilis</i> SE76	23.88 (29.25)
T ₃	<i>Streptomyces nenu</i> DS-11	28.88 (32.51)
T ₄	<i>Pseudomonas fluorescens</i> UASR	23.00 (28.66)
T ₅	<i>T. asperellum</i> + <i>B. subtilis</i>	67.44 (55.21)
T ₆	<i>T. asperellum</i> + <i>Streptomyces</i> sp.	68.66 (55.96)
T ₇	<i>T. asperellum</i> + <i>P. fluorescens</i>	70.55 (57.13)
T ₈	<i>B. subtilis</i> + <i>Streptomyces</i> sp.	50.00 (45.00)
T ₉	<i>B. subtilis</i> + <i>P. fluorescens</i>	49.55 (44.74)
T ₁₀	<i>Streptomyces</i> sp. + <i>P. fluorescens</i>	57.55 (49.34)
T ₁₁	<i>T. asperellum</i> + <i>B. subtilis</i> + <i>Streptomyces</i> sp.	66.88 (54.87)
T ₁₂	<i>T. asperellum</i> + <i>B. subtilis</i> + <i>P. fluorescens</i>	70.11 (56.86)
T ₁₃	<i>T. asperellum</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>	72.22 (58.19)
T ₁₄	<i>B. subtilis</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>	58.77 (50.05)
T ₁₅	<i>T. asperellum</i> + <i>B. subtilis</i> + <i>Streptomyces</i> sp. + <i>P. fluorescens</i>	73.33 (58.91)
	S. Em. ±	1.26
	C.D. at 1 %	3.69

Figures in the parenthesis are arc sine values

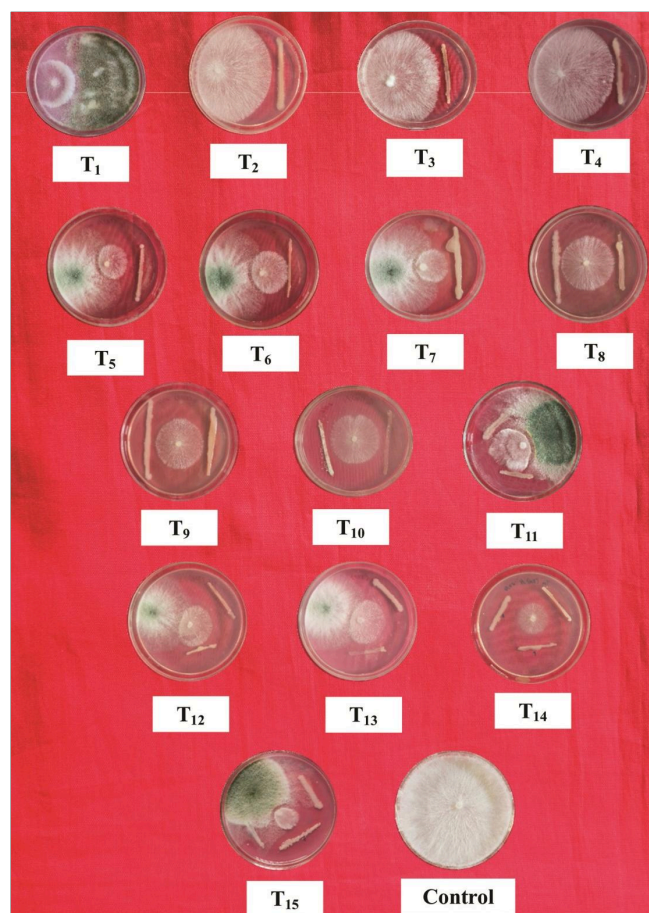


Plate 1. *In vitro* bio-efficacy of bioagents and their consortia against *Sclerotium rolfsii*

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