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Kashti PD
Plant Pathology and Agricultural Microbiology Section, College of Agriculture, Pune, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Lohate SR
Junior Pathologist, AICRP on Floriculture, Zonal Agricultural Research Station, Ganeshkhind, Pune, Maharashtra, India

Dalvi SG
Scientist, Tissue Culture Agriculture Science and Technology, Vasantdada Sugar Institute, Manjari (Bk), Pune, Maharashtra, India

Karade VM
Professor of Plant Pathology College of Agriculture, Pune, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Chavanke SN
Scientific Officer Tissue Culture Agriculture Science and Technology, Vasantdada Sugar Institute, Manjari (Bk), Pune, Maharashtra, India

Kumbhar SC
Plant Pathology and Agricultural Microbiology Section, College of Agriculture, Pune, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

Corresponding Author:
Kashti PD
Plant Pathology and Agricultural Microbiology Section, College of Agriculture, Pune, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India

In vitro antagonistic activity of bioagents against *Sclerotium rolfsii* causing stem rot of Aster (*Callistephus chinensis* L.)

Kashti PD, Lohate SR, Dalvi SG, Karade VM, Chavanke SN and Kumbhar SC

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Abstract
Aster (*Callistephus chinensis*), a widely cultivated ornamental plant. The area, production and productivity of China aster have practically stagnated in recent decades. Among the various reasons the plants suffering from different diseases is one of the major reasons. Among the different diseases stem rot caused by *S. rolfsii* leads to extensive financial loss to growers. The pathogen was isolated from infected stems exhibiting the classic symptoms of stem rot disease. The Potato Dextrose Agar (PDA) medium was used to grow the fungal colony. A variety of morphological characteristics, including mycelium, colony, and sclerotia formation, were used to identify the pathogen. Conventional management using chemical fungicides poses significant environmental and health concerns, emphasizing the need for sustainable alternatives. This study explores eco-friendly disease management strategies through the use of antagonistic microorganisms against *S. rolfsii*. The integration of such biocontrol agents showing a promising and environmentally approach for managing stem rot in aster, reducing reliance on chemical fungicides, and promoting sustainable floriculture practices. The results indicated that *Trichoderma asperellum* (5.4cm) which was significantly superior over all other bioagents inhibited the growth of *S. rolfsii*, reducing mycelial expansion and sclerotia formation.
Future research should focus on field trials to validate these *in-vitro* findings and explore the potential synergistic effects of combining different bioagents. This study contributes to the development of integrated disease management strategies for stem rot in aster plants, promoting healthier and more resilient crops.

Keywords: Bioagents, Sustainable, Eco-Friendly, Integrated disease management

Introduction
The *Callistephus chinensis* Nees, commonly known as aster, is a member of the Asteraceae family, one of the most extensive family of angiosperms. This semi-hardy, prolific bloomer is a winter annual species cultivated for both cut flowers and loose blossoms.
Aster is an upright annual plant, with hairy branches and ovate or triangular ovate leaves that are attached to stem spirally (Cockshull, 1985) [1]. Aster is a diverse species with a variety of forms, types, and colours. It is a semi-hardy annual flowers crop and is part of the Asteraceae family, one of the largest family of flowering plants. It is an important annual crop in India and is cultivated worldwide (Navalinskien *et al*., 2005) [2].
In India, Aster cultivation is commonly in regions with favourable agro-climatic conditions, particularly in the states of Maharashtra (notably Pune and Nashik), Karnataka (Bangalore and Mysuru), Tamil Nadu, West Bengal, Punjab, Uttar Pradesh, and Himachal Pradesh (Jadhav *et al*., 2021) [3].
Aster is highly susceptible to several soil-borne pathogens, among which *Sclerotium rolfsii* causing stem rot is one of the most destructive diseases. The disease manifests as water-soaked lesions at the collar region, rapid plant wilting, and the development of cottony white mycelial mats along with the appearance of mustard seed-like sclerotia on the soil and infected plant tissue (Kumar *et al*., 2020) [4]. *S. rolfsii* infects the collar region of plants, producing characteristic white, cottony mycelium and brown sclerotia, ultimately leading to lodging and plant death. Traditional disease management practices predominantly involve the use of chemical fungicides. Although effective, these methods raise serious concerns regarding environmental contamination, residual toxicity, human health risks, and the

development of fungicide resistance (Pal *et al*., 2001) [5]; (Fravel, 2005) [6].

S. rolfsii is a ubiquitous and highly aggressive fungal pathogen with a broad host range, affecting over 500 plant species. It survives in the soil for long periods as dormant sclerotia, making its management challenging. Farmers and commercial growers often rely on synthetic fungicides for disease control; however, their indiscriminate use has led to negative environmental impacts, including soil degradation, chemical residues, harm to beneficial microorganisms, and the emergence of resistant pathogen strains.

In this scenario, fungicides offer the most reliable control for foliage diseases. However, increasing public concern and environmental risks are driving the search for alternative, eco-friendly disease management strategies. Numerous bioagents have been shown to possess strong antifungal properties and their potential as bio-fungicides has been highlighted as part of a broader strategy for environmental protection.

Materials and Methods

Present investigations entitled "*In vitro* antagonistic activity of bioagents against *Sclerotium rolfsii* causing stem rot of Aster (*Callistephus chinensis* L.)" was carried out during 2023- 2025 and experiments were carried out in the Department of Plant Pathology, College of Agriculture, Pune and field experiments were carried out at AICRP on floriculture, ZARS Ganeshkhind, Pune.

Collection of Disease Samples

Diseased samples of aster were collected from Aster field of AICRP on floriculture, ZARS Ganeshkhind Pune Maharashtra, India.

Preparation of culture media

The boiled and filtered extract of potatoes is mixed with dextrose and agar-agar, then dissolved by indirect heating (in boiling water). The media is sterilized in an autoclave at 15 lbs pressure for 15 minutes.

Isolation

The isolation was carried out by tissue isolation method on PDA medium and pure culture was obtained was maintained in in PDA slants.

Identification of pathogen

Pathogen was identified on the basis of morphological and cultural characteristics of the pathogen and identified as *S. rolfsii*

In vitro Evaluation of Bioagents

The bioagents was evaluated against *S. rolfsii* using dual culture technique.

Dual Culture Technique

Twenty ml of PDA medium was poured into sterile petri plate and allowed for solidification. Ten days old culture was taken and cut into 5mm disc by using sterile cork borer and placed near the periphery, on one side of PDA plate. For fungal bio-agents; the bio-agent and pathogen colonies were placed on exactly opposite sides of plate, 0.4 mm colony of ten days old culture of each was placed in PDA plate. The bacterial bio-agents were evaluated by placing pathogen colonies were placed on exactly opposite sides and bacterial colony then striking on media opposite sides of pathogen. All inoculated plates were incubated at 27 ± 1 ºC for seven days. Each treatment was replicated thrice. medium without use of plant extract served as control. The Petri Plates were then incubated at room temperature for seven days.

The radial growth of the pathogen was measured and per cent inhibition over control was worked out according to the equation given by Vincent (1947) [7]. Per cent inhibition of mycelial growth of the fungus was calculated by using the formula,

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

Where,

I = Per cent growth inhibition

C = Growth (mm) in control after ten days

T = Growth (mm) in treatment after ten days

Table 1: Bioagent treatments

Sr. No.	Treatment
T_1	*Trichoderma koningii*
T_2	*Trichoderma virens*
T_3	*Pseudomonas fluroscens*
T_4	*Trichoderma asperellum*
T_5	*Trichoderma harzianum*
T_6	*Trichoderma hamatum*
T_7	Control

Statistical analysis

The data collected for various observations was analyzed using CRD. The standard statistical methods as described by Panse and Sukhmate (1985) [8] was followed for statistical significance and an online OPSTAT application.

Results

During the present investigation on the "*In vitro* antagonistic activity of bioagents against *Sclerotium rolfsii* causing stem rot of Aster (*Callistephus chinensis* L.)", the results obtained were discussed as follows.

Morphological Studies of *S. rolfsii*

The morphological characters of *S. rolfsii* were studied on culture medium by growing the pathogen on Potato dextrose agar. The growth of mycelium was observed as cottony, fluffy, and compact, with a creamy white to dull white colour. Sclerotia formation begins after 10 days. The brown to chocolate coloured, round to spherical sclerotia ranging from 0.5 to 2.05 mm in size evenly distributed in Petri Plate were observed. The results are presented in the (Table 2, Plate 1).

Table 2: Morphological characters of *S. rolfsii*

Sr. No.	Characters	*S. rolfsii*
1	Mycelium	Cottony white mycelium
2	Mycelium shape	Fluffy and compact
3	Mycelium colour	Creamy white to dull white
4	Sclerotial colour	Brown to Chocolate
5	Sclerotial shape	Round to Spherical
6	Sclerotial size	0.5 to 2.05 mm
7	Days required for sclerotia formation	10
8	Sclerotial distribution	Overall

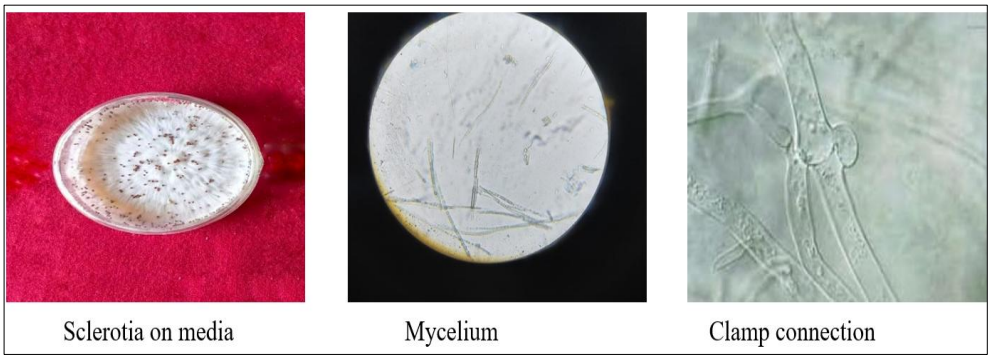

Plate 1: Morphology of *S. rolfsii*

Antimicrobial effect of bioagents against of *S. rolfsii in vitro*

Six bioagents were tested against S. *rolfsii*, including one bacterial agent, *Pseudomonas fluorescens*, and five fungal species *Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma hamatum*, *Trichoderma koningii*, and *Trichoderma virens*. PDA was used as the basal media in the dual culture procedure. All six bioagents showed antifungal action against *S. rolfsii*, greatly limiting its growth in comparison to the untreated control. All six bioagents showed antifungal action against *S. rolfsii*, greatly limiting its growth in comparison to the untreated control, according to the results, which are summarised in Table 3 and the corresponding figures.

Radial mycelial growth of bioagents

T. asperellum had the highest mean mycelial diameter (5.4 cm), It performed significantly superior over other bioagent. *T. hamatum* (4.5 cm), *T. virens* (4.0 cm), and *T. harzianum* (3.8 cm) came next. Notably, *T. koningi* had the lowest mean radial mycelial growth (2.8 cm). (Table 3, Plate 2, Fig 1).

Mycelial Growth Inhibition *S. rolfsii*

The *T. asperellum* was observed highest antifungal activity where significantly maximum mycelial growth inhibition of test pathogen (62.22%) was recorded. The next effective bioagents were *T. hamatum* (52.22%), *T. virens* (45.55%), and *T. harzianum* (43.33%). *T. koningii* (34.44%), on the other hand, was found to be less effective with the least amount of mycelial growth inhibition. (Table 3, Plate 2, Fig 2).

The results showed that every bioagents tested was determined to possess antifungal activity against *S. rolfsii* and to have a considerable inhibitory effect on its mycelial diameter.

Table 3: Antimicrobial effect of bioagents against *S. rolfsii in vitro*

Sr. No.	Treatment	Mean mycelial diameter* of the bio-agent (cm)	Mean mycelial diameter* of test pathogen (cm)	Per cent Growth inhibition
T_1	*Trichoderma koningii*	2.8	5.9	34.44
T_2	*Trichoderma virens*	4.0	4.9	45.55
T_3	*Pseudomonas fluroscens*	3.5	5.2	42.22
T_4	*Trichoderma asperellum*	5.4	3.4	62.22
T_5	*Trichoderma harzianum*	3.8	5.1	43.33
T_6	*Trichoderma hamatum*	4.5	4.3	52.22
T_7	Control	-	9.0	-
SE(m)±		0.07	0.04	
C.D(0.01)		0.22	0.13	

*Means of three replications

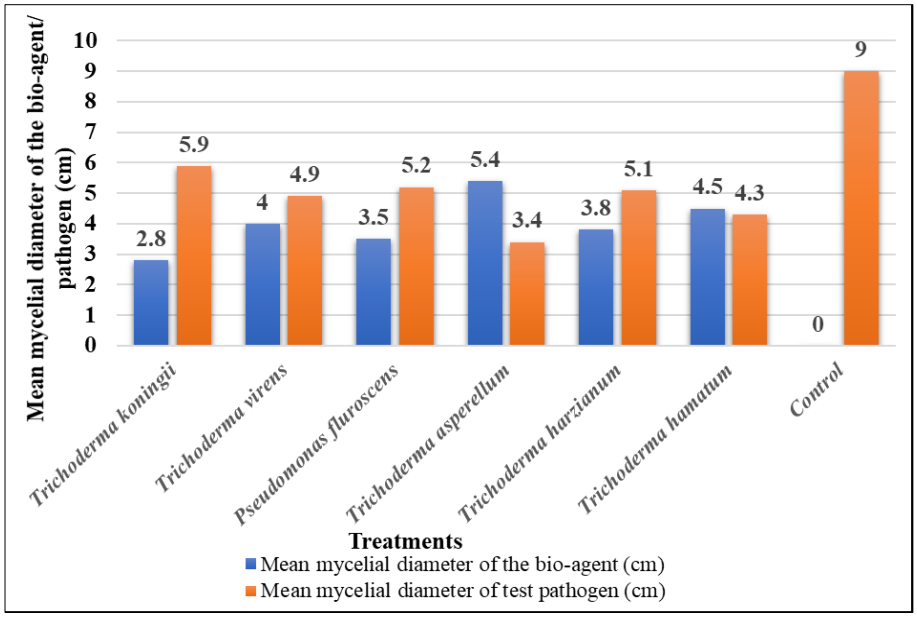

Fig 1: Antimicrobial effect of bioagent on mycelial diameter of *S rolfsii*

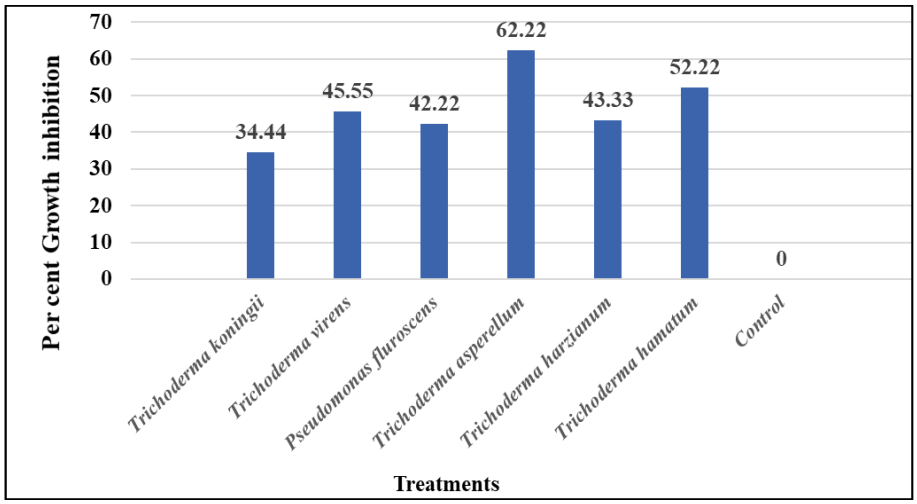

Fig 2: Per cent growth inhibition of *S. rolfsii* by bioagent

T_1: *Trichoderma koningii* T_2: *Trichoderma virens* T_3: *Pseudomonas fluorescens* T_4: *Trichoderma asperellum* T_5: *Trichoderma harzianum* T_6: *Trichoderma hamatum* T_7: Control

Plate 2: Antimicrobial effect of bioagents against of *S. rolfsii in vitro*

Discussion

The results of present investigation are in agreement with Kim (1974) [9] who reported the fungus produces floccos, snow white, cottony, fast-growing mycelium forming sclerotia. The mature sclerotia appears to mustard seed and the size was about 1-2 mm in diameter. Khalequzzaman and Hossain (2012) [10] found that radial mycelial growth ranged from 75.9 to 88.8 mm at 7 DAI and the colony colour was off white to creamy white. The shape of the colony was regular and compactness was fluffy to medium fluffy. The colour of sclerotia was found brownish and the shape was round. Prasad *et al.* (2012) [11] also reported that the pathogen produced cottony white mycelium with ropy strands, fluffy and wooly of *S. rolfsii* causing foot and root rot diseases in pulses. The sclerotia are small and round with dark reddish brown.

Pandi *et al.* (2017) [12] reported similar morphological characters of mycelium and sclerotial characters of *S. rolfsii* causing stem rot of groundnut and sunflower respectively. Similar finding by Manu *et al.* (2017) [13] who investigated and found that colony diameter ranged from 4.10 to 8.00 cm after 72 hrs of incubation, the sclerotial colour light to dark brown and shape spherical to round. Hiremath *et al.* (2022) [14] also reported same morphological characters of *S. rolfsii*.

The findings of the present investigation aligned with those of Kumar *et al.* (2021) [15], who evaluated multiple potent bio-control agents for their antagonistic effects on *S. rolfsii*, reporting significant mycelial growth inhibition by *T. asperellum* (72.22%), *Pseudomonas fluorescens* (76.3%), and *T. harzianum* (71.48%), with *Bacillus subtilis* showing the least inhibition (71.11%). Similarly, Meena *et al*. (2023) [16] observed the greatest inhibition of mycelial growth by the *T. asperellum* isolate, followed closely by *T. harzianum*. Bagul and Hasabnis (2024) [17] also reported that *T. harzianum* was the most effective bioagent in inhibiting the mycelial growth of *S. rolfsii*. These results are consistent with those of Deshmukh *et al*. (2024) [18], who reported that *S. rolfsii*, a polyphagous pathogen infecting over 500 plant species, can be effectively managed *in vitro* using bioagents. Their study demonstrated that *T. harzianum* achieved 82.40% inhibition of the pathogen, followed by *T. asperelloides* (80.92%), *T. atroviride* (80.55%), and *T. asperellum* (80.00%). Among bacterial bioagents, *B. subtilis* (44.25%) and *P. fluorescens* (41.11%) exhibited the highest percentages of mycelial growth inhibition.

Conclusion

The six different bioagents were tested against *S. rolfsii*, the highest mean mycelial diameter was recorded in *T. asperellum* (5.4 cm) which was significantly superior over all other bioagents. It was followed by *T. hamatum* (4.5 cm), *T. virens* (4.0 cm), *T. harzianum* (3.8 cm). Significantly the least mean mycelial diameter was recorded with T. koningi (2.8 cm). The mycelial diameter inhibition *T. asperellum* was found antifungal which recorded significantly maximum mycelial growth inhibition of 62.22%. This was followed by *T. hamatum* (52.22%), *T. virens* (45.55%), *T. harzianum* (43.33%). However, *T. koningii* (34.44%) was found less effective with minimum mycelial diameter inhibition.

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