



***In-vitro* evaluation of selected bioagents against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate**

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Abstract

Pomegranate (*Punica granatum* L.) is an economically important fruit crop affected by several fungal diseases, among which anthracnose caused by *Colletotrichum gloeosporioides* is an important constraint to production and fruit quality. The present investigation was conducted during 2025-26 at the Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, to evaluate selected fungal and bacterial bioagents against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate. The pathogen was isolated from naturally infected fruits, purified on Potato Dextrose Agar and identified on the basis of cultural and morphological characteristics. Seven bioagent treatments and an untreated control were evaluated in a Completely Randomized Design with three replications. Fungal antagonists were tested by dual culture using opposing mycelial discs, whereas bacterial antagonists were evaluated by a modified dual culture assay using bacterial streaks and a central pathogen disc. All tested bioagents significantly restricted pathogen growth compared with the untreated control, which recorded 32.30 mm colony diameter. Among the seven bioagents evaluated, *Trichoderma asperellum* exhibited the highest antagonistic activity and restricted the pathogen growth to 5.97 mm, with 81.57 per cent inhibition of mycelial growth. It was followed by *T. harzianum*, which recorded 75.15 per cent inhibition. In comparison, *Bacillus subtilis* exhibited the lowest antagonistic activity, recording 19.30 per cent inhibition of pathogen growth. The results demonstrated that *T. asperellum* possessed substantial inhibitory potential against *C. gloeosporioides* and may serve as a promising bioagent for the biological management of pomegranate anthracnose. Further studies under greenhouse and field conditions are required to validate its efficacy and develop an effective biocontrol strategy for disease management.

Keywords: Pomegranate, anthracnose, *Colletotrichum gloeosporioides*, bioagents, *Trichoderma asperellum*, *Pseudomonas fluorescens*, biological control, dual culture, antagonistic activity

Introduction

Pomegranate (*Punica granatum* L.) is an economically important fruit crop cultivated extensively in tropical and subtropical regions and is valued for its nutritional, medicinal and commercial importance. India is one of the major producers and exporters of pomegranate, with Maharashtra being the leading pomegranate-producing state. The crop has considerable importance in Maharashtra, particularly in the semi-arid regions, where it provides an important source of income to fruit growers. However, production and fruit quality are constrained by several insect pests and diseases, among which fungal diseases constitute a major problem (Jain, K. and Desai, N. 2018)^[8].

Anthrachnose is an important disease of pomegranate and is traditionally associated with *Colletotrichum gloeosporioides*. The disease can affect different plant parts, including leaves, shoots, flowers and fruits, resulting in necrotic lesions, defoliation, flower and fruit drop, and deterioration of fruit quality. Severe infection may consequently result in considerable yield and economic losses. Surveys conducted in the Marathwada region of Maharashtra have documented the occurrence and severity of pomegranate anthracnose in major pomegranate-growing districts, demonstrating its importance under local production conditions (Chavan and Dhutraj 2017)^[1].

The association of *Colletotrichum* spp. with pomegranate anthracnose has also been documented through cultural, pathogenicity and molecular studies (Prasannakumar *et al.*, 2018)^[15] reported *C. gloeosporioides* as the causal organism

of pomegranate anthracnose and characterized its cultural and growth characteristics under laboratory conditions. More recent molecular investigations have demonstrated that pomegranate fruit anthracnose in India may involve multiple species belonging to the *C. gloeosporioides* species complex, emphasizing the importance of accurate pathogen identification in disease-management programmes.

Management of pomegranate anthracnose generally relies heavily on fungicides. Although chemical fungicides can provide effective disease suppression, repeated and intensive use may result in concerns related to fungicide resistance, environmental contamination and residue management. Recent investigations have continued to evaluate fungicides for pomegranate anthracnose, indicating the importance of effective disease-management strategies. Therefore, incorporation of biological control agents into integrated disease-management programmes is of considerable interest as a complementary approach to reducing dependence on chemical fungicides (Vandana *et al.*, 2021)^[21].

Biological control using antagonistic microorganisms offers an environmentally compatible approach for suppressing plant pathogens. Among the microbial bioagents, species of *Trichoderma* are widely investigated because of their rapid growth, competitive ability and multiple mechanisms of antagonism against phytopathogenic fungi (Jayalakshmi *et al.*, 2015)^[12]. These mechanisms include competition for nutrients and space, mycoparasitism, antibiosis through production of inhibitory metabolites, secretion of cell-wall-

degrading enzymes and, in some host–pathogen systems, induction of plant defence responses. Recent literature indicates considerable potential of *Trichoderma* spp. against *Colletotrichum*-associated diseases, although efficacy can vary with strain, pathogen, crop, environmental conditions and method of application.

Studies specifically addressing biological management of pomegranate anthracnose have also reported promising antagonistic activity. (Vandana *et al.*, 2021) [21] evaluated different bioagents against *C. gloeosporioides* causing anthracnose of pomegranate and reported significant differences among *Trichoderma* isolates in their ability to inhibit pathogen growth. Such findings provide a basis for further screening and selection of efficient bioagents for management of the disease.

In view of the economic importance of pomegranate and the occurrence of anthracnose in major production regions, identification of effective microbial antagonists is essential for developing sustainable disease-management strategies. Therefore, the present investigation was undertaken to evaluate the *in vitro* efficacy of selected bioagents against *Colletotrichum gloeosporioides* causing anthracnose of pomegranate, with particular emphasis on their ability to inhibit mycelial growth of the pathogen.

Materials and Methods

The laboratory investigation was conducted during 2025-26 at the Department of Plant Pathology, Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV), Parbhani, Maharashtra, India.

1. Isolation and maintenance of *Colletotrichum gloeosporioides*

Pomegranate fruits exhibiting characteristic symptoms of anthracnose caused by *Colletotrichum gloeosporioides* were collected from infected pomegranate fields and carefully placed in sterile paper bags for transportation. The collected diseased fruit samples were brought to the Department of Plant Pathology laboratory, where they were processed for pathogen isolation. Small pieces of approximately 5 × 5 mm from the advancing margin of the lesion were cut so that each piece contained both diseased and apparently healthy tissue. The pieces were surface sterilized with 1% sodium hypochlorite solution for approximately 1–2 minutes and washed three times with sterile distilled water. The sterilized pieces were aseptically transferred to Petri plates containing about 20 ml Potato Dextrose Agar (PDA) amended with streptomycin at 50 mg L⁻¹ to minimize bacterial contamination. The plates were incubated at 25 ± 2°C and observed for fungal growth. Emerging colonies were purified by transferring advancing hyphal tips to fresh PDA plates. The purified culture was allowed to develop adequate mycelial growth and sporulation, transferred to PDA slants, and stored at 4°C with periodic subculturing to maintain viability.

2. Identification of the pathogen

The purified fungus was identified on the basis of cultural and morphological characteristics. Colony colour, growth pattern, margin, pigmentation and mycelial texture were recorded on PDA. For microscopic examination, a small portion of actively growing culture was mounted in lactophenol cotton blue and examined under a compound microscope. *C. gloeosporioides* colonies varied from flat to

fluffy, with regular to irregular margins, and pigmentation ranging from white, yellow to orange. The observed characters were compared with standard descriptions and identification keys for *Colletotrichum* (Weir, Johnston & Damm (2012) [23], and the isolate was identified as *C. gloeosporioides*.

3. Bioagents used in the study

The biological control agents used in the present investigation, including isolates of *Trichoderma* spp., and other beneficial microorganisms, were obtained from the Department of Plant Pathology, VNMKV, Parbhani. The cultures were maintained under suitable laboratory conditions and used for the experimental studies. Fungal antagonists were maintained on PDA, whereas bacterial antagonists were maintained on Nutrient Agar (NA). The treatments were T₁: *Trichoderma asperellum*; T₂: *Trichoderma harzianum*; T₃: *Aspergillus awamori*; T₄: *Verticillium lecanii*; T₅: *Pseudomonas fluorescens*; T₆: *Pseudomonas striata*; T₇: *Bacillus subtilis*; and T₈: untreated control.

4. *In vitro* evaluation of fungal bioagents

The fungal antagonists were evaluated by the dual culture technique following Dennis and Webster (1971) [3]. Approximately 20 ml of sterilized PDA was poured into sterile 90-mm Petri plates and allowed to solidify. A 5-mm mycelial disc was cut from the actively growing margin of an 8-day-old culture of *C. gloeosporioides* and placed near one side of the plate. A similar 5 mm disc of the respective fungal antagonist was placed on the opposite side at an equal distance from the centre. In the untreated control, only the pathogen disc was placed on PDA. The inoculated plates were incubated at 25 ± 2°C in a BOD incubator. Each treatment was replicated three times.

5. *In vitro* evaluation of bacterial bioagents

A modified dual culture technique was used for bacterial antagonists. Active 24-h-old bacterial cultures were streaked on opposite sides of PDA plates, and a 5-mm disc from the actively growing margin of the *C. gloeosporioides* culture was placed at the centre of each plate. The untreated control consisted of the pathogen disc without bacterial streaking. The plates were incubated at 27 ± 2°C in a BOD incubator, and each treatment was replicated three times.

6. Observations recorded

The growth of *C. gloeosporioides* was observed at 24-h intervals. Colony diameter of the pathogen was recorded when the untreated control reached the full diameter of the Petri plate. Percentage inhibition of mycelial growth was calculated according to Vincent (1947) [22] as: $I = [(C - T) / C] \times 100$, where I represented percentage inhibition of mycelial growth, C represented colony diameter of *C. gloeosporioides* in the untreated control, and T represented colony diameter of *C. gloeosporioides* in the presence of the respective bioagent.

7. Statistical analysis

The experiment was laid out in a Completely Randomized Design (CRD) with eight treatments and three replications. The data were subjected to analysis of variance following the procedures described by Panse and Sukhatme (1978) [14]. Percentage inhibition values were subjected to angular

transformation before statistical analysis. Standard error of [SE(m) ±] and critical difference (CD) at 1% probability were calculated for comparison of treatment means. Original percentage values are presented in the table, with arcsine-transformed values in parentheses.

Results

All seven bioagents restricted the colony growth of *C. gloeosporioides* compared with the untreated control, and significant differences were observed among treatments. The untreated control (T₈) recorded the maximum colony diameter of 32.30 mm and showed no growth inhibition. In the bioagent treatments, colony diameter ranged from 5.97 to 26.07 mm, corresponding to 19.30 to 81.57 % inhibition of mycelial growth (Table 1).

Table 1: *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides*.

Tr. No.	Treatments	Mean colony Diameter (mm)	Growth inhibition (%)
T ₁	<i>Trichoderma asperellum</i>	5.97	81.57 (64.63)
T ₂	<i>Trichoderma harzianum</i>	8.03	75.15 (60.11)
T ₃	<i>Aspergillus awamori</i>	19.87	38.52 (38.36)
T ₄	<i>Verticillium lecanii</i>	18.07	44.06 (41.59)
T ₅	<i>Pseudomonas fluorescens</i>	16.10	50.17 (45.10)
T ₆	<i>Pseudomonas striata</i>	24.10	25.39 (30.26)
T ₇	<i>Bacillus subtilis</i>	26.07	19.30 (26.06)
T ₈	Untreated control	32.30	0.00 (0.00)
S. E. (m) ±		0.44	0.60
C.D @ 1%		1.83	2.49

* Figures in parentheses are arcsine-transformed values.

Among the tested bioagents *Trichoderma asperellum* (T₁) recorded the minimum colony diameter of 5.97 mm and the maximum mycelial growth inhibition of 81.57 per cent. *Trichoderma harzianum* (T₂) ranked next with 8.03 mm colony diameter and 75.15 per cent inhibition followed by *Pseudomonas fluorescens* (T₅), *Verticillium lecanii* (T₄), *Aspergillus awamori* (T₃), and *Pseudomonas striata* (T₆), with 50.17%, 44.06%, 38.52% and 25.39% inhibition respectively. In contrast, *Bacillus subtilis* (T₇) was the least effective bioagent, recording the highest colony diameter of 26.07 mm and the lowest mycelial growth inhibition of 19.30 per cent among the treated plates. The overall numerical order of mycelial growth inhibition was T₁ > T₂ > T₅ > T₄ > T₃ > T₆ > T₇.

Discussion

Among the bioagents evaluated against *C. gloeosporioides*, *Trichoderma asperellum* (T₁) exhibited the highest efficacy, recording the minimum colony diameter of 5.97 mm and the maximum mycelial growth inhibition of 81.57 per cent. The efficacy of T₁ was statistically at par with *Trichoderma harzianum* (T₂), which recorded a colony diameter of 8.03 mm and 75.15 per cent inhibition. Among the remaining treatments, *Verticillium lecanii* (T₄) was the next most

effective bioagent, with 44.06 per cent inhibition and a colony diameter of 18.07 mm, followed by *Aspergillus awamori* (T₃), which recorded 38.52 per cent inhibition with a colony diameter of 17.87 mm. Among the bacterial bioagents, *Pseudomonas fluorescens* (T₅) exhibited comparatively higher efficacy, resulting in 50.17 per cent inhibition and a colony diameter of 16.10 mm, followed by *Pseudomonas striata* (T₆), which recorded 25.39 per cent inhibition with a colony diameter of 24.10 mm. In contrast, *Bacillus subtilis* (T₇) was the least effective bioagent, recording the highest colony diameter of 26.07 mm and the lowest mycelial growth inhibition of 19.30 per cent among the treated plates. The untreated control (T₈) recorded maximum radial mycelial growth of 32.30 mm, corresponding to 0.00 per cent inhibition.

Overall, the results demonstrated considerable variation in the antagonistic efficacy of the bioagents against *C. gloeosporioides*, with *Trichoderma spp.* exhibiting superior inhibitory activity compared with the other bioagents evaluated. The results obtained in the present investigation agree with the findings of Rani *et al.*, (2018), who reported that *Trichoderma harzianum* exhibited the highest antagonistic activity against *C. gloeosporioides* and proved to be the most effective bioagent when evaluated through the dual culture technique.

Similarly, Dev *et al.*, (2016) [4], who evaluated bio-agents against *C. gloeosporioides* causing pomegranate anthracnose and reported 87–100 per cent inhibition with *Trichoderma viride*, *T. asperellum* and *T. harzianum*, whereas bacterial antagonists showed comparatively lower inhibition. Vandana *et al.*, (2021) [21] likewise reported that *T. harzianum* isolates produced the highest mean inhibition among the bio-agents evaluated against pomegranate anthracnose. Prashanth *et al.*, (2008) [16] also found strong suppression of *C. gloeosporioides* by *Trichoderma viride* and *Pseudomonas fluorescens*. Thus, the superior antagonistic activity of *T. asperellum* and *T. harzianum* observed in the present study is consistent with the broader evidence supporting *Trichoderma spp.* as effective *in-vitro* antagonists of pomegranate anthracnose pathogen.

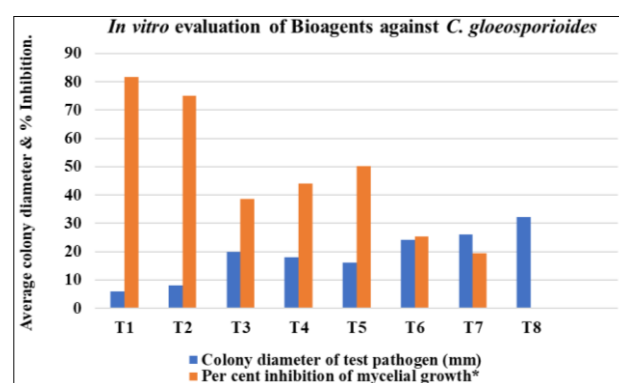


Fig 1: *In vitro* evaluation of Bioagents against *Colletotrichum gloeosporioides*, showing mean colony diameter and percentage growth inhibition of the pathogen

Conclusion

Seven bioagents comprising four fungal and three bacterial antagonists were evaluated against *C. gloeosporioides* using the dual culture technique. All the bioagents exhibited inhibitory activity against the pathogen, although their efficacy varied considerably. Among the treatments,

Trichoderma asperellum was the most effective, recording the minimum colony diameter of 5.97 mm and maximum mycelial growth inhibition of 87.57 per cent. It was statistically at par with *Trichoderma harzianum*, which recorded 8.03 mm colony diameter and 75.15 per cent inhibition. *Verticillium lecanii* and *Aspergillus awamori* recorded 44.06 and 38.52 per cent inhibition, respectively. Among the bacterial bioagents, *Pseudomonas fluorescens* showed comparatively greater inhibitory activity than *Pseudomonas striata* and *Bacillus subtilis*.

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