



Evaluation of antagonistic activity of selected bioagents against *Alternaria solani*

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Abstract

Potato (*Solanum tuberosum* L.) is an important tuber crop cultivated widely for food and nutritional purposes. Among the major biotic constraints affecting potato production, early blight is an important foliar disease caused by species of *Alternaria*, which adversely affects plant growth and productivity under favourable environmental conditions. Biological control offers a sustainable option for reducing dependence on repeated fungicide applications. The present investigation was conducted during 2025-26 at the Department of Plant Pathology, Vasantao Naik Marathwada Krishi Vidyapeeth, Parbhani, to evaluate selected fungal and bacterial bioagents against early blight of potato caused by *A. solani* under *in vitro* conditions. The pathogen was isolated from naturally infected potato leaves, 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 90.00 mm colony diameter. Among the tested bioagents, *Trichoderma asperellum* (PBN) was found to be the most effective, recording the minimum colony diameter of 6.43 mm and maximum mycelial growth inhibition of 91.96 per cent. It was statistically at par with *Trichoderma harzianum*, (PBN) which recorded a colony diameter of 7.50 mm and 90.62 per cent inhibition. *Aspergillus awamori* and *Verticillium lecanii* (PBN) recorded 84.77 and 77.28 per cent inhibition, respectively. Among the bacterial bioagents, *Pseudomonas fluorescens* (PBN) exhibited comparatively greater inhibitory activity, recording 63.53 per cent inhibition, followed by *Bacillus subtilis* (60.60%) and *Pseudomonas striata* (56.02%). as promising antagonists for further evaluation in biological management of potato early blight.

Keywords: *Trichoderma asperellum*, *Trichoderma harzianum*, *Pseudomonas fluorescens*, biological control, dual culture, Potato early blight, inhibition

Introduction

Potato (*Solanum tuberosum* L.), a member of the family *Solanaceae*, is one of the most important vegetable and food crops cultivated worldwide. Owing to its high productivity, nutritional value, and adaptability to diverse agro-climatic conditions, potato has become a staple food for millions of people across the globe. The crop originated in the Andean highlands of Peru and Bolivia in South America. It was introduced into Europe by Spanish explorers during the latter half of the sixteenth century and subsequently spread across other European countries and different parts of the world between the seventeenth and eighteenth centuries. In India, potato was introduced by the Portuguese during the seventeenth century. In India, potato cultivation was introduced during the early seventeenth century through the efforts of Portuguese traders and later expanded by British missionaries.^[7]

Potato is presently cultivated in more than 100 countries under temperate, subtropical, and tropical climatic conditions. It is predominantly regarded as a cool season crop, and temperature is one of the most critical environmental factors influencing its growth, development, and tuber formation. Tuber initiation and development are adversely affected when temperatures fall below 10°C or exceed 30°C, whereas optimum growth and maximum tuber yield are generally achieved at mean daily temperatures ranging from 18°C to 20°C (FAO, 2008).

Potato is widely recognized as the "King of Vegetables" and an excellent source of dietary energy. It is one of the most significant food crops in India, ranking after rice, wheat, and maize in terms of importance. As a staple vegetable, potato occupies a vital place in the Indian diet due to its versatility and widespread consumption. Owing to its high nutritional value, potato has considerable potential in combating malnutrition and undernutrition across the world. It is an excellent source of essential minerals such as potassium, phosphorus, and magnesium, as well as vitamins including thiamine (B1), niacin (B3), and pyridoxine (B6) (Camire *et al.*, 2009). In addition, potatoes provide appreciable amounts of protein, calcium, vitamin C, and a well-balanced amino acid composition, making them a nutritious food crop.

Several antagonistic microorganisms have demonstrated significant potential for the biological management of plant pathogens and are considered important components of sustainable disease management. Biocontrol agents enhance plant defence by inducing systemic resistance and stimulating the production of plant growth-promoting substances as well as defence-related enzymes, thereby improving the plant's ability to withstand pathogen attack (Srividya and Ashwini, 2014). In addition to suppressing diseases, these beneficial microorganisms promote overall plant growth and vigour by activating the plant's innate defence responses.

Among the various biocontrol agents, species of *Trichoderma* have been extensively studied for their broad-spectrum antagonistic activity against numerous phytopathogens and are recognized as environmentally friendly alternatives to chemical fungicides. Besides their antagonistic effects, *Trichoderma* spp. Enhance plant growth by improving nutrient availability and uptake, promoting root development, and maintaining soil health through mechanisms comparable to those of mycorrhizal fungi (Mukherjee *et al.*, 2022). Furthermore, *Trichoderma* exhibits mycoparasitic behaviour by directly attacking and colonizing pathogenic fungi, utilizing them as a nutrient source and thereby contributing effectively to disease suppression (Adnan *et al.*, 2019).

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 *Alternaria solani*

Potato leaves exhibiting characteristic symptoms of early blight caused by *Alternaria solani* were collected from infected potato fields and carefully placed in sterile paper bags for transportation. The collected diseased leaf samples were brought to the Department of Plant Pathology laboratory, where they were processed for pathogen isolation. Small tissue pieces of approximately 2-3 mm were cut from the advancing margins of lesions so that each piece contained both diseased and apparently healthy tissue. The pieces were surface sterilized with 1% sodium hypochlorite solution for 30 s 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. Hyphal branching and septation, conidial shape, pigmentation, septation pattern and beak formation were recorded. The observed characters were compared with standard descriptions and identification keys for *Alternaria* [21], and the isolate was identified as *A. solani*.

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.^[19] 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 *A. solani* 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 27 ± 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 *A. solani* 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 *A. solani* 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^[24] as: $I = [(C - T) / C] \times 100$, where I represented percentage inhibition of mycelial growth, C represented colony diameter of *A. solani* in the untreated control, and T represented colony diameter of *A. solani* 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.^[19] Percentage inhibition values were subjected to angular transformation before statistical analysis. Standard error of mean [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 angular-transformed values in parentheses.

Results

All seven bioagents restricted the colony growth of *A. solani* compared with the untreated control, and significant differences were observed among treatments. The untreated control (T8) recorded the maximum colony diameter of 80.00 mm and showed no growth inhibition. In the bioagent treatments, colony diameter ranged from 6.43 to 35.17 mm,

corresponding to 56.02 to 91.96% inhibition of mycelial growth (Table 1).

Table 1: *In vitro* evaluation of bioagents against *Alternaria solani*.

Tr. No.	Treatments	Mean colony Diameter (mm)	Growth inhibition (%)
T ₁	<i>Trichoderma asperellum</i>	6.43	91.96 (73.50)
T ₂	<i>Trichoderma harzianum</i>	7.50	90.61 (72.13)
T ₃	<i>Aspergillus awamori</i>	12.17	84.77 (67.02)
T ₄	<i>Verticillium lecanii</i>	18.17	77.28 (61.51)
T ₅	<i>Pseudomonas fluorescens</i>	29.17	63.53 (52.83)
T ₆	<i>Pseudomonas striata</i>	35.17	56.02 (48.43)
T ₇	<i>Bacillus subtilis</i>	31.50	60.60 (51.05)
T ₈	Untreated control	80.00	0.00 (0.00)
S. E. (m) ±		0.56	0.46
C.D @ 1%		1.70	1.40

* Figures in parentheses are angular-transformed values.

Among the tested bioagents *Trichoderma asperellum* (T₁) recorded the minimum colony diameter of 6.43 mm and the maximum mycelial growth inhibition of 91.96 per cent. *Trichoderma harzianum* (T₂) ranked next with 7.50 mm colony diameter and 90.62 per cent inhibition followed by *Aspergillus awamori* (T₃), *Verticillium lecanii* (T₄), *Pseudomonas fluorescens* (T₅) and *Bacillus subtilis* (T₇) with 84.77%, 77.28%, 63.53% and 60.60% inhibition respectively. In contrast, *Pseudomonas striata* (T₆) was the least effective bioagent, recording the highest colony diameter of 35.17 mm and the lowest mycelial growth inhibition of 56.02 per cent among the treated plates. The overall numerical order of mycelial growth inhibition was T₁ > T₂ > T₃ > T₄ > T₅ > T₇ > T₆.

Discussion

Among the bioagents evaluated against *Alternaria solani*, *Trichoderma asperellum* (T₁) exhibited the highest efficacy, recording the minimum colony diameter of 6.43 mm and the maximum mycelial growth inhibition of 91.96 per cent. The efficacy of T₁ was statistically at par with *Trichoderma harzianum* (T₂), which recorded a colony diameter of 7.50 mm and 90.62 per cent inhibition. The difference in their efficacy was within the critical difference (C.D.) value of 2.75 mm. Among the remaining treatments, *Aspergillus awamori* (T₃) was the next most effective bioagent, with 84.77 per cent inhibition and a colony diameter of 12.17 mm, followed by *Verticillium lecanii* (T₄), which recorded 77.28 per cent inhibition with a colony diameter of 18.17 mm. Among the bacterial bioagents, *Pseudomonas fluorescens* (T₅) exhibited comparatively higher efficacy, resulting in 63.53 per cent inhibition and a colony diameter of 29.17 mm, followed by *Bacillus subtilis* (T₇), which recorded 60.60 per cent inhibition with a colony diameter of 31.50 mm. In contrast, *Pseudomonas striata* (T₆) was the least effective bioagent, recording the highest colony diameter of 35.17 mm and the lowest mycelial growth inhibition of 56.02 per cent among the treated plates. The untreated control (T₈) recorded maximum radial mycelial growth of 80.00 mm, corresponding to 0.00 per cent inhibition.

Overall, the results demonstrated considerable variation in the antagonistic efficacy of the bioagents against *A. solani*, with *Trichoderma spp.* exhibiting superior inhibitory activity compared with the other bioagents evaluated. The low coefficient of variation indicated good experimental

precision and reliability. The results obtained in the present investigation are in agreement with the findings of Rani *et al.*, (2018), who reported that *Trichoderma harzianum* exhibited the highest antagonistic activity against *Alternaria solani* and proved to be the most effective bioagent when evaluated through the dual culture technique.

Similarly, Naik *et al.*, (2020) evaluated five bioagents, viz., *Trichoderma viride*, *T. harzianum*, *T. asperellum*, *T. hamatum* and *Pseudomonas fluorescens*, against *A. solani* under *in vitro* conditions. Among the bioagents tested, *T. harzianum* was found to be the most effective, restricting mycelial growth to 13.33 mm and recording 85.13 per cent inhibition. This was followed by *T. asperellum*, which restricted mycelial growth to 17.33 mm and exhibited 80.67 per cent inhibition; the two treatments were statistically at par with each other. The present findings are further corroborated by the reports of Mahantesh *et al.*, (2017), Kumar *et al.*, (2018) and Das *et al.*, (2023), who also documented significant antagonistic activity of different bioagents against *A. solani*.

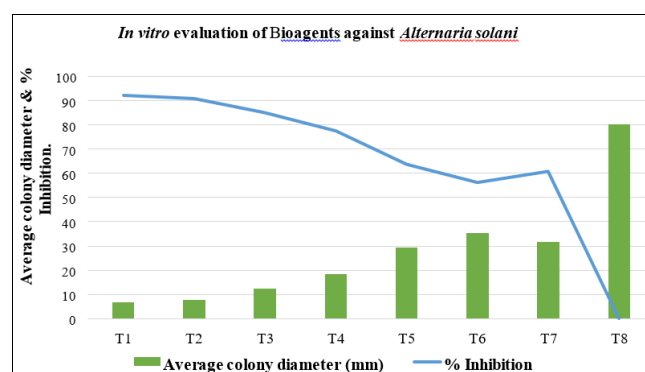


Fig 1: *In vitro* evaluation of Bioagents against *Alternaria solani*, showing mean colony diameter and percentage growth inhibition of the pathogen



Plate 1: *In vitro* evaluation of bioagents against *Alternaria solani* using dual culture technique.

Conclusion

Seven bioagents comprising four fungal and three bacterial antagonists were evaluated against *A. solani* 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 6.43 mm and maximum mycelial growth inhibition of 91.96 per cent. It was statistically at par with *Trichoderma harzianum*, which recorded 7.50 mm colony diameter and 90.62 per cent inhibition. *Aspergillus awamori* and *Verticillium lecanii* recorded 84.77 and 77.28 per cent inhibition, respectively. Among the bacterial bioagents, *Pseudomonas fluorescens* showed comparatively greater inhibitory activity than *Bacillus subtilis* and *Pseudomonas striata*.

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