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In vitro antagonistic activity of fungal and bacterial bioagents against *Alternaria solani* causing early blight of tomato

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Abstract
Early blight caused by *Alternaria solani* is an important foliar disease of tomato. 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, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Selected fungal and bacterial bioagents were evaluated against *A. solani* under *in vitro* conditions. The pathogen was isolated from naturally infected tomato leaves. It was 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. 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. The untreated control recorded 90.00 mm colony diameter. *Trichoderma viride* (VNMKV-1) recorded the minimum colony diameter of 35.98 mm. It also showed maximum mycelial growth inhibition of 60.02%. *Pseudomonas fluorescens* (VNMKV-1) ranked next with 36.56 mm and 59.38% inhibition. *T. viride* 2.0% AS recorded 37.50 mm and 58.33% inhibition. *Bacillus subtilis* treatments produced moderate inhibition. *P. fluorescens*-1 showed the lowest inhibition among the bioagents at 45.00%. The findings demonstrated clear strain-dependent variation. *Trichoderma viride* (VNMKV-1) and *P. fluorescens* (VNMKV-1) were identified as promising antagonists. They warrant further evaluation in biological management of tomato early blight.

Keywords: *Bacillus subtilis*, biological control, dual culture, *Pseudomonas fluorescens*, *Trichoderma viride*, tomato early blight)

Introduction
Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops grown worldwide. The fruit is valued nutritionally because it supplies vitamins, minerals, dietary fibre and bioactive compounds. Lycopene, an important carotenoid antioxidant, is particularly notable [1]. In India, tomato production was estimated at about 20.60 million tonnes during 2024-25. This reflects the major role of the crop in vegetable production and farm income [2]. Diseases that reduce healthy foliage, fruit set and marketable yield have direct economic importance.

Among the fungal diseases of tomato, early blight caused by *Alternaria solani* is one of the most widespread and destructive. The disease generally begins on older leaves as small brown necrotic spots. These spots enlarge and develop characteristic concentric rings. Under favourable warm and humid conditions, lesions may coalesce. This results in extensive blighting and premature defoliation. Stems and fruits may also become infected. Loss of functional leaf area reduces photosynthetic capacity. This may adversely affect fruit development and yield. Recent reviews of the *Alternaria* disease complex on tomato emphasise the importance of *A. solani*. They also highlight the difficulty of obtaining durable control under favourable disease environments [3]. Maurya *et al*. (2022) [4] summarised diverse management tactics for early blight. Their review underscored the continuing need for effective, sustainable control options.

Management of early blight has traditionally depended heavily on fungicides. Fungicidal protection remains useful during severe disease pressure. However, repeated applications increase production costs. They may also contribute to environmental concerns, pesticide residues and selection pressure for fungicide-insensitive pathogen populations. The increasing occurrence of fungicide resistance in *A. solani* and related *Alternaria* populations has strengthened this need. Management components that reduce exclusive dependence on chemicals are therefore essential. Naz *et al*. (2025) [5] recently emphasised integrated laboratory and field evaluation of biocontrol agents. Their work supports the development of sustainable management programmes. Biological control represents an important complementary approach within integrated disease management.

Microbial bioagents suppress plant pathogens through several direct and indirect mechanisms. Trichoderma species are well-known fungal antagonists. They may suppress pathogens through rapid colonisation, competition for nutrients and space, antibiosis, mycoparasitism and production of cell-wall-degrading enzymes (Harman *et al*., 2004) [6]. Manzar *et al*. (2022) [7] reviewed the versatile biocontrol potential of Trichoderma species. They detailed the diverse mechanisms underlying antagonistic activity. Bacterial antagonists such as *Pseudomonas fluorescens* and *Bacillus subtilis* can restrict fungal pathogens. They act through competition, siderophore-mediated nutrient sequestration and production of diffusible or volatile antifungal metabolites. Ali *et al*. (2022) [8] reviewed the antagonistic potential of bacterial species against fungal plant pathogens. They highlighted siderophore production,

volatile compounds and induced systemic resistance. Alattas *et al*. (2024) [9] further highlighted the sustainable crop protection potential of Pseudomonas species. Such mechanisms make these organisms suitable candidates for biological suppression of foliar fungal pathogens, including *A. solani*.

Several studies have demonstrated the *in vitro* antagonistic potential of Trichoderma, Pseudomonas and Bacillus against tomato early blight. Naik *et al*. (2020) [10] recorded 80.67% inhibition of *A. solani* with *T. viride*. Ragupathi *et al*. (2020) [11] reported 77% inhibition with the same antagonist. Lalhruaitluangi *et al*. (2022) [12] observed marked variation among native Trichoderma and *P. fluorescens* isolates. The best isolates produced 73.34 and 77.73% inhibition, respectively. More recent work has also demonstrated effective suppression of *A. solani* by Trichoderma isolates, phylloplane antagonists and *P. fluorescens* strains (Wongamthing and Kurian, 2023; Saini *et al*., 2025; Abebe *et al*., 2026) [13,14,15]. Kumar *et al*. (2022) [16] documented the prevalence and morphological variability of *A. solani* isolates from Uttar Pradesh. Das (2025) [17] recently evaluated eco-friendly management strategies. That study used *T. viride* and *P. fluorescens* against tomato early blight with encouraging results.

However, the magnitude of antagonism reported for the same microbial species varies considerably among isolates and studies. This variation indicates that biocontrol efficacy is strain dependent. Promising cultures should be screened against the target pathogen under the same experimental conditions before further evaluation. Metz and Hausladen (2022) [18] similarly showed substantial differences among Trichoderma strains against *A. solani*. They emphasised that performance at one testing stage may not be directly transferable to another. Comparative screening of locally maintained and externally obtained bioagents was therefore considered necessary in the present investigation. The study was undertaken to evaluate the in *vitro* efficacy of selected fungal and bacterial bioagents against *A. solani*. It also aimed to identify the most promising antagonists for subsequent biological management studies.

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.

Isolation and maintenance of *Alternaria solani*

Tomato leaves showing typical early blight symptoms were collected from the Horticulture Research Station (Vegetable), VNMKV, Parbhani. The symptoms were characterised by brown necrotic lesions with concentric rings. Small tissue pieces of approximately 2-3 mm were cut from the advancing margins of lesions. Each piece contained both diseased and apparently healthy tissue. The pieces were surface sterilised with 1% sodium hypochlorite solution for 30 s. They were then washed three times with sterile distilled water. The sterilised pieces were aseptically transferred to Petri plates containing about 20 ml Potato Dextrose Agar (PDA). The medium was amended with streptomycin at 50 mg L^{-1} to minimise 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. It was then transferred to PDA slants and stored at 4°C. Periodic subculturing was performed to maintain viability.

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. It was 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* (Simmons, 2007) [19]. The isolate was identified as *A. solani*.

Bioagents used in the study

Seven bioagent treatments were evaluated against *A. solani*. The VNMKV cultures consisted of *Pseudomonas fluorescens* (VNMKV-1), *P. fluorescens*-1, *Trichoderma viride* (VNMKV-1) and *Bacillus subtilis* (VNMKV-1). These were maintained at the Department of Plant Pathology, VNMKV, Parbhani. The remaining bioagents and formulations were obtained from the Indian Institute of Vegetable Research (IIVR), Varanasi. These included *T. viride* 2.0% AS, *B. subtilis* and *P. fluorescens* 1.75% WP. Fungal antagonists were maintained on PDA. Bacterial antagonists were maintained on Nutrient Agar (NA). The treatments were as follows. T_1: *P. fluorescens*-1. T_2: *T. viride* 2.0% AS. T_3: *B. subtilis*. T_4: *P. fluorescens* (VNMKV-1). T_5: *T. viride* (VNMKV-1). T_6: *P. fluorescens* 1.75% WP. T_7: *B. subtilis* (VNMKV-1). T_8: untreated control.

In vitro evaluation of fungal bioagents

The fungal antagonists were evaluated by the dual culture technique following Dennis and Webster (1971) [20]. Approximately 20 ml of sterilised PDA was poured into sterile 90-mm Petri plates. The medium was allowed to solidify. A 5-mm mycelial disc was cut from the actively growing margin of an 8-day-old culture of *A. solani*. It was 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.

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. 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. Each treatment was replicated three times.

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 (1947) [21] as: I = [(C − T) / C] × 100. Here, I represented percentage inhibition of mycelial growth. C represented colony diameter of *A. solani* in the untreated control. T represented colony diameter of *A. solani* in the presence of the respective bioagent.

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) [22]. 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. Angular-transformed values are shown in parentheses.

Results

All seven bioagents restricted the colony growth of *A. solani* compared with the untreated control. Significant differences were observed among treatments. The untreated control (T_8) recorded the maximum colony diameter of 90.00 mm. It showed no growth inhibition. In the bioagent treatments, colony diameter ranged from 35.98 to 49.50 mm. This corresponded to 45.00–60.02% inhibition of mycelial growth (Table 1).

Among the tested bioagents, *T. viride* (VNMKV-1) (T_5) recorded the minimum colony diameter of 35.98 mm. It also showed the maximum mycelial growth inhibition of 60.02%. *Pseudomonas fluorescens* (VNMKV-1) (T_4) ranked next with 36.56 mm colony diameter and 59.38% inhibition. *Trichoderma viride* 2.0% AS (T_2) recorded 37.50 mm colony diameter and 58.33% inhibition. The differences among T_5, T_4 and T_2 were within the respective critical differences. Therefore, these leading treatments were statistically comparable.

Table 1: *In vitro* efficacy of bioagents against *Alternaria solani* causing early blight of tomato

Tr. No.	Treatment	Mean colony diameter (mm)	Growth inhibition (%)
T_1	*Pseudomonas fluorescens*-1	49.50	45.00 (42.11)*
T_2	*Trichoderma viride* 2.0% AS	37.50	58.33 (49.79)
T_3	*Bacillus subtilis*	43.15	52.06 (46.16)
T_4	*Pseudomonas fluorescens* (VNMKV-1)	36.56	59.38 (50.39)
T_5	*Trichoderma viride* (VNMKV-1)	35.98	60.02 (50.77)
T_6	*Pseudomonas fluorescens* 1.75% WP	40.33	55.19 (47.97)
T_7	*Bacillus subtilis* (VNMKV-1)	41.30	54.11 (47.34)
T_8	Untreated control	90.00	0.00
	SE(m) ±	1.88	1.21
	CD at 1%	5.69	3.65

*****Figures in parentheses are angular-transformed values.

P. fluorescens 1.75% WP (T_6) recorded 40.33 mm colony diameter and 55.19% inhibition. *B. subtilis* (VNMKV-1) (T_7) and *B. subtilis* (T_3) recorded 41.30 and 43.15 mm colony diameter. They showed 54.11 and 52.06% inhibition, respectively. *P. fluorescens*-1 (T_1) showed the lowest antagonistic activity among the bioagents. It recorded 49.50 mm colony diameter and 45.00% inhibition. The overall numerical order of mycelial growth inhibition was $T_5 > T_4 > T_2 > T_6 > T_7 > T_3 > T_1$.

Discussion

Trichoderma viride (VNMKV-1) produced the highest numerical inhibition of *A. solani* in the present study. It recorded 60.02% inhibition and restricted the pathogen colony to 35.98 mm. The strong antagonistic response may be associated with the well-established ability of Trichoderma to rapidly colonise the available substrate. It may also compete for nutrients and space, produce antifungal metabolites and directly interact with pathogen hyphae through mycoparasitic mechanisms. These mechanisms were not measured directly in the present experiment. They provide a plausible biological basis for the observed suppression (Harman *et al*., 2004) [6]. The present result is in agreement with Naik *et al*. (2020) [10]. They reported 80.67% inhibition of *A. solani* by *T. viride*. Ragupathi *et al*. (2020) [11] recorded 77% inhibition with the same antagonist. Sudarshan *et al*. (2020) [23] also observed strong inhibition with Trichoderma isolates. Bhalerao *et al*. (2021) [24] recorded 75.2% inhibition with *T. harzianum*. Saini *et al*. (2025) [14] similarly reported high antagonistic activity of Trichoderma against the tomato early blight pathogen. Alhadethy (2026) [25] recently demonstrated that *T. harzianum* can stimulate defensive enzymes. This stimulation enhances tomato plant resistance against *A. solani* infection.

Pseudomonas fluorescens (VNMKV-1) was the second-best treatment numerically. It recorded 59.38% inhibition and only 36.56 mm pathogen growth. Its performance was statistically comparable with *T. viride* (VNMKV-1). This indicates that the bacterial isolate possessed substantial antagonistic potential under the same laboratory conditions. The inhibitory response may be related to competition for nutrients and iron. It may also involve production of siderophores and release of diffusible or volatile antifungal metabolites. The present finding closely agrees with Abebe *et al*. (2026) [15]. They reported 57.65% inhibition with a standard *P. fluorescens* strain. They also recorded 56.04 and 55.04% inhibition with two selected isolates against *A. solani*. Lalhruaitluangi *et al*. (2022) [12] recorded a wider range of responses among Pseudomonas isolates. They obtained up to 77.73% inhibition. This further supports the importance of isolate-specific antagonistic capacity.

The two *Bacillus subtilis* treatments produced moderate but clear suppression of *A. solani*. *B. subtilis* (VNMKV-1) recorded 54.11% inhibition. The *B. subtilis* treatment obtained from IIVR recorded 52.06% inhibition. Such inhibition may be associated with the production of antifungal secondary metabolites and volatile compounds by Bacillus species. Awan *et al*. (2023) [26] demonstrated strong concentration-dependent inhibition of *A. solani* by metabolites produced by *B. subtilis* BS-01. This provides experimental support for metabolite-mediated antagonism. Wongamthing and Kurian (2023) [13] also recorded strong in *vitro* activity of a *B. subtilis* phylloplane isolate against *A. solani*. Zhang *et al*. (2022) [27] elucidated the transcriptome-level action mechanism of *B. subtilis* lipopeptide fengycins. Their work demonstrated how these compounds suppress *A. solani* in potato. Irmawatie *et al*. (2025) [28] also reported effective suppression of *A. solani* on potato. They found that *B. subtilis* at optimal concentrations significantly reduced disease intensity. Although the inhibition levels in the present study were lower, the results confirmed that both Bacillus treatments were capable of restricting pathogen growth.

A notable feature of the present experiment was the variation among strains or formulations belonging to the same microbial group. *P. fluorescens* treatments ranged from 45.00 to 59.38% inhibition. The VNMKV-1 strain of *T. viride* was numerically more effective than the *T. viride* 2.0% AS treatment. However, the two were statistically comparable. This variation may reflect differences in growth rate, metabolite production, competitive ability, formulation characteristics and interaction with the specific *A. solani* isolate used in the assay. Metz and Hausladen (2022) [18] also reported marked strain-to-strain variation among Trichoderma isolates tested against *A. solani*. Therefore, differences between the present inhibitions percentages and values reported in earlier studies should not be interpreted as contradictory. They underline the importance of screening individual bioagent strains under defined conditions.

Overall, the present results showed that *T. viride* (VNMKV-1), *P. fluorescens* (VNMKV-1) and *T. viride* 2.0% AS formed the leading group under *in vitro* conditions. The numerical superiority of *T. viride* (VNMKV-1) was modest. Its differences from *P. fluorescens* (VNMKV-1) and *T. viride* 2.0% AS were within the critical difference. Nevertheless, the consistent reduction in colony growth by all tested antagonists compared with the untreated control demonstrated the biological potential of *Trichoderma*, *Pseudomonas* and *Bacillus*. These organisms are suitable for suppressing *A. solani*. The better-performing strains merit selection for subsequent evaluation under plant and field conditions.

Conclusion

All seven bioagents significantly restricted the *in vitro* growth of *Alternaria solani* compared with the untreated control. *Trichoderma viride* (VNMKV-1) recorded the lowest colony diameter (35.98 mm) and highest numerical mycelial growth inhibition (60.02%). *Pseudomonas fluorescens* (VNMKV-1) followed with 59.38% inhibition. *Trichoderma viride* 2.0% AS recorded 58.33% inhibition. The leading treatments were statistically comparable. This indicates that more than one bioagent had strong antagonistic potential. The variation observed among strains and formulations confirmed the importance of isolate-level screening. On the basis of the present laboratory evaluation, *T. viride* (VNMKV-1) and *P. fluorescens* (VNMKV-1) were identified as promising candidates. They deserve further evaluation in biological management of tomato early blight.

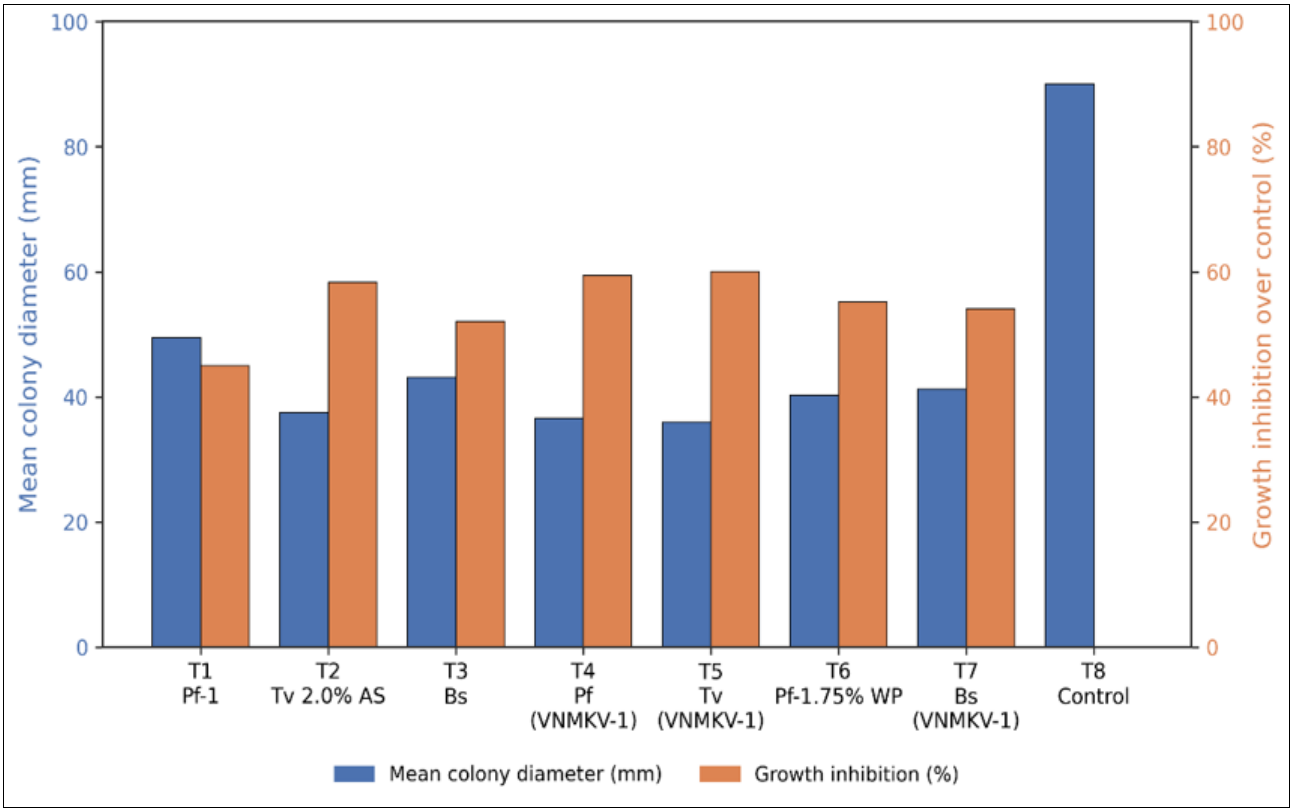

Fig 1: *In vitro* efficacy of bioagents against *Alternaria solani*, showing mean colony diameter and percentage growth inhibition of the pathogen

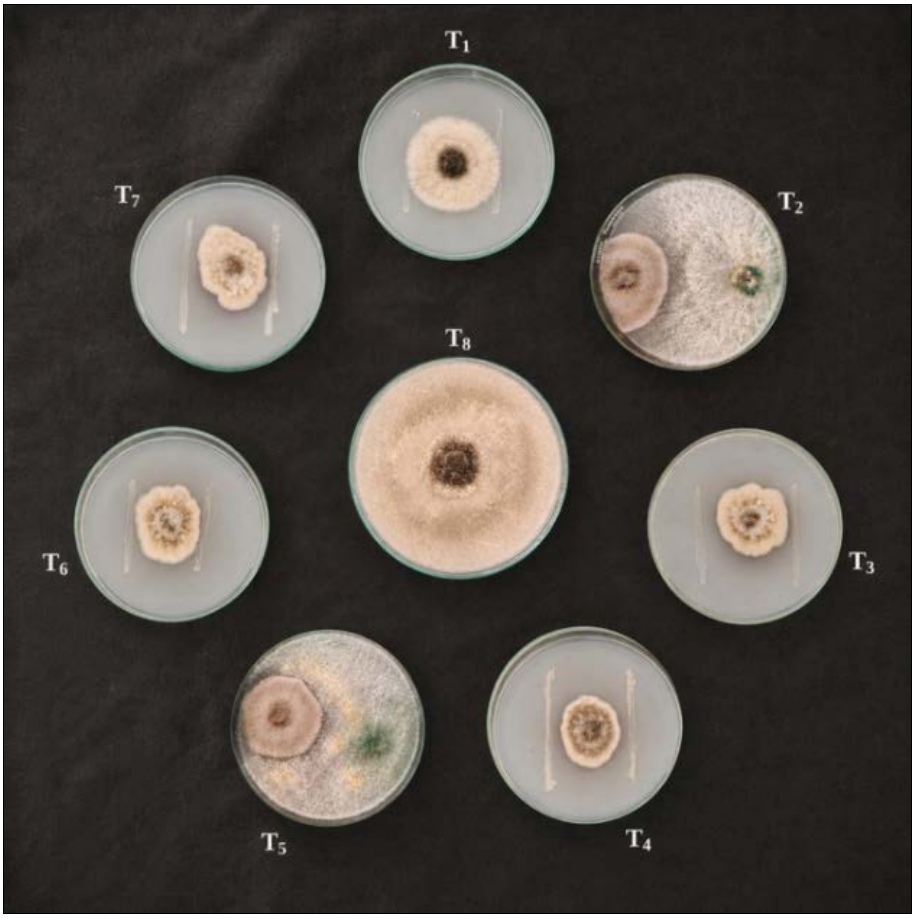

Plate 1: *In vitro* evaluation of bioagents against *Alternaria solani* using dual culture technique.

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