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# Bio-management of fungal leaf spot of tomato (*Solanum lycopersicum* L.) using indigenous *Trichoderma* isolates

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**ABSTRACT**

One of the main obstacles to feeding the world is the fungal leaf spot disease, which has a negative impact on plants' photosynthetic areas and significantly lowers crop quality and output. To manage fungi that pose a serious hazard to both humans and the environment, various chemical fungicides are utilized. The goal of the current study was to determine whether local isolates of three *Trichoderma* spp. (*Trichoderma viride*, *Trichoderma harzianum*, and *Trichoderma asperellum*) had any antagonistic effects in vitro against the pathogenic fungi *Alternaria alternata*, *Fusarium solani*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus sydowii*, and *Alternaria* sp. *Trichoderma viride* showed the greatest growth inhibition against all of the tested pathogenic fungi, followed by *Trichoderma harzianum* and *Trichoderma asperellum*. It was shown that all three *Trichoderma* species strongly inhibited the mycelial growth of fungal pathogens. Compared to other isolated fungi, *Trichoderma* species inhibited *Alternaria alternata* mycelial growth more, whereas in *Fusarium oxysporum* least amount of mycelial growth inhibition was observed. These findings imply that Trichoderma species can function as an effective biocontrol agent against the fungi responsible for tomato leaf spot disease.

**Keywords:** Biocontrol, Pathogenic fungi, Mycelial growth, Antagonistic activity

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## INTRODUCTION

An important solanaceous crop that is widely farmed worldwide is the tomato (*Solanum lycopersicum* L.). It is a good source of numerous antioxidants, including vitamin A, vitamin K, lycopene, ascorbic acid, potassium, folate, and many more that are crucial for supporting human health (Capobianco-Uriarte *et al.*, 2021). The tomato is used in a variety of ways in the food, medicine, decorative, and horticultural industries (Bhowmik *et al.*, 2012). The quality and quantity of tomato fruit are significantly reduced as a result of a number of biotic stresses, including leaf spot fungal infections in the field (Ochilo *et al.*, 2019). The application of various chemical fungicides has been the most popular method of defending tomato plants against fungal diseases, but doing so has led to environmental contamination, negative effects on organisms that aren't the target, and the evolution of pathogen resistance (Kishor *et al.*, 2012). Therefore, it is essential to create a management approach that is effective, economical, and environmentally friendly in order to stop crop losses caused by pathogen damage.

The biocontrol fungi including *Trichoderma* are the promising biocontrol fungi. The fungus

*Trichoderma* strengthens both the direct and indirect plant defence systems, which increases fungal resistance (Poveda, 2021). It is an opportunistic, avirulent plant parasite fungus that serves as an antagonist and parasite fungus against a number of pathogenic fungi, defending plants against a variety of phytopathogenic plant diseases (Naher *et al.,* 2014). *Trichoderma* inhibits the growth of phytopathogens, in particular fungi, either directly through hyperparasitism, competition for nutrients and space, and antibiosis (Zhang *et al.,* 2017) or indirectly through promoting plant growth, enhancing plant resistance to stress, active nutrient uptake, and bioremediation of contaminated rhizosphere, as well as providing plants with a variety of secondary metabolites, cell wall degrading enzymes, and pathogenesis-related proteins (Kumar, 2013). The goal of the current study was to evaluate the antagonistic potential of several local *Trichoderma* isolates under in vitro conditions against chosen fungal pathogens that cause tomato leaf spot disease.

## MATERIALS AND METHODS

### Isolation and identification of pathogenic fungi

In order to better understand the fungi that cause the tomato leaf spot disease in Kashmir Valley, India, samples of infected tomato plant leaves were collected from several locations in Kashmir Valley and placed in sterilised zip-lock plastic bags. After being delivered to the lab, the leaf samples were properly cleaned with running water to get rid of any impurities before being chopped up. The diseased leaf fragments were first surface sterilised for two minutes with a 1% (v/v) NaOCl solution, followed by three to four rinses with sterile distilled water. Following sterilisation, the leaf pieces were aseptically placed on potato dextrose agar (PDA) media that also contained chloramphenicol (50 g/mL). Each plate had a single piece that was incubated for up to six days at 25±2°C in dark (Hassan *et al.*, 2022). Following Koch's postulates, the isolated pathogenic fungi were identified based on their cultural, morphological, and reproductive properties (Watanabe, 2002; Gilman, 2008).

### Antagonistic activity of different *Trichoderma* species against phytopathogenic fungi

Pure cultures of *Trichoderma* species such as *Trichoderma viride* (Pers), *Trichoderma harzianum* (Rifai), and *Trichoderma asperellum* (Samuels, Lieckf. & Nirenberg) were obtained from the Plant Pathology and Mycology Laboratory, Department of Botany, University of Kashmir. To evaluate the antimycotic effect of the isolated species of *Trichoderma* against fungi isolated from leaf spot disease of tomato plants the dual culture technique was used (Prince *et al.*, 2011). *Alternaria alternata, Fusarium oxysporum, Aspergillus sydowii, Aspergillus flavus, Alternaria* sp., *Fusarium solani*, and test antagonistic species were inoculated simultaneously on Petri plates with PDA and incubated at 25±2°C for seven days (Figure 1A-C). For each treatment retaining control with no treatment, there were three duplicates. On both treated and control plates, the pathogenic fungi's mycelial growth was determined. The formula provided by Vincent was used to compute the growth inhibition percentages (1947).

$$\text{Growth inhibition} = \frac{C-T}{C} \times 100$$

Where “C” is the mycelial growth of the pathogen in control and

“T” is the mycelial growth of the pathogen in the treatment group.

### Statistical analysis

The data was subjected to the analysis of variance (ANOVA) using SPSS Statistics Version 23. Mean values were compared using Tukey’s posthoc test at 5% level.

## RESULTS

According to the findings, all *Trichoderma* isolates significantly slowed the mycelial growth of pathogenic fungi such *Alternaria alternata, Fusarium oxysporum*, *Aspergillus sydowii*, *Aspergillus flavus*, *Alternaria* sp., and *Fusarium solani* (Table 1, Figure 1 A-C). The effectiveness of the *Trichoderma* isolates against the studied pathogenic fungi varied significantly. The study also reported that *Trichoderma viride* isolate (T1) greatly reduced *Alternaria alternata* mycelial growth by 78.04%, followed by *Trichoderma harzianum* isolate (T2) and *Trichoderma asperellum* isolate (T3) respectively (Table 1; Figure 1A-C). Similarly, T1 isolate reduced *Fusarium oxysporum* mycelial growth by 58.98%, T2 isolate reduced mycelial growth by 46.03%, and T3 by 38.13% respectively. T1, T2, and T3 isolates reduced *Alternaria* sp. mycelial growth by 64.61%, 59.20% and 57.28% respectively. Additionally, T1, T2, and T3 each isolate inhibited *Aspergillus flavus* mycelial growth by 61.64%, 58.00%, and 55.71%, respectively. *A. sydowii* mycelial growth was reduced effectively by T1, T2, and T3 isolates to the extent of 63.82%, 61.70% and 56.37% respectively. The *Trichoderma* isolate T1effectively inhibited *Fusarium solani* development at a rate of 68.16%, followed by T2 and T3 (Figure 1A-C). The mycelial growth inhibition was maximum by *Trichoderma viride* (T1), followed by *T. harzianum* (T2) and *T. asperellum* (T3), however all isolates caused the greatest growth inhibition of all the isolated pathogenic fungi.

**Table 1.** Effect of different *Trichoderma* species on the mycelial growth (mm) of pathogenic fungi causing leaf spot disease of tomato

| Treatment | Mycelial growth inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | ***Alternaria alternata*** | ***Fusarium oxysporum*** | ***Alternaria* sp.** | ***Aspergillus flavus*** | ***Aspergillus sydowii.*** | ***Fusarium solani*** |
| ***Trichoderma viride* (T1)** | 15.00 ± 5.0a (78.04%) | 19.00 ± 0.17a (58.98%) | 15.12 ± 1.5a (64.61%) | 28.00 ± 2.6a (61.64%) | 19.21 ± 1.5a (63.82%) | 23.66±2.5a (68.16%) |
| ***Trichoderma harzianum* (T2)** | 20.33 ± 1.5b (70.24%) | 25.00 ± 0.5b (46.03%) | 17.43 ± 2.0a (59.20%) | 30.66 ± 2.5a (58.00%) | 20.34 ± 1.0a (61.70%) | 24.00 ± 3.6a (67.71%) |
| ***Trichoderma asperellum* (T3)** | 21.33 ± 1.5b (68.78%) | 28.66 ± 0.25c (38.13%) | 18.25 ± 3.2ab (57.28%) | 32.33 ± 2.0ab (55.71%) | 23.17 ± 2.3b (56.37%) | 27.66 ± 5.8b (62.78%) |
| **Control** | 68.33 ± 3.5c | 46.33 ± 0.7d | 42.73 ± 4.0c | 73.00 ± 4.0c | 53.11 ± 5.5c | 74.33 ± 4.0c |

Data presented is the mean±SD (n=3). Mean values followed by the same lowercase alphabets in the column did not differ statistically by Tukey HSD test. Values in parenthesis are the mycelial growth inhibition (%).

## DISCUSSION

Under *in vitro* circumstances, the mycelial growth of isolated fungal pathogens was greatly reduced by all *Trichoderma* species, indicating that *Trichoderma* spp. may be effective for biocontrol of the pathogenic fungi that cause tomato leaf spot disease (Figure 1A-C). One of the most common fungal genera, *Trichoderma*, is well-known for its antagonistic action against pathogenic fungi, making it helpful in managing economically significant crop diseases, such as tomato leaf spot (Olowe *et al*., 2022). *Trichoderma* species and other bioagents may be used as a biological control agent against a number of plant diseases, according to several studies. These agents are becoming more and more popular in agriculture because they have dramatically reduced crop loss (Sharon *et al*., 2001; Jegathambigai *et al*., 2009; Abdel Wahab *et al*., 2020; Jan *et al*., 2022a; Nisa *et al*., 2022). These findings make it evident that local isolates of *Trichoderma* were successful in combating every pathogenic fungus tested that is connected to tomato plant leaf spot disease. Our findings are in line with earlier research by Bashar and Rai (1994), who discovered that *Trichoderma* isolates such *Trichoderma harzianum*, *T. hamatum*, and *T. viride* effectively inhibited *Fusarium oxysporum* development in chickpea plants. Studies have demonstrated that *Trichoderma* isolates boost plant tolerance to several foliar diseases by increasing micronutrient availability, hence promoting plant development and defence against pathogens (Harman *et al*., 2004; Gravel *et al*., 2007; Pandya *et al*., 2011). *Trichoderma* spp. also prevent pathogen invasion through competition, antibiosis, and mycoparasitism (Anwar *et al*., 2008; Jan *et al*., 2022b). *Trichoderma* spp. enhance tomato crop development and productivity, according to Haque *et al*. (2012).

**Figure 1A.** Antagonistic effect of *Trichoderma harzianum* on the pathogenic fungi.


**Figure 1B.** Antagonistic effect of *Trichoderma asperellum* on the pathogenic fungi.

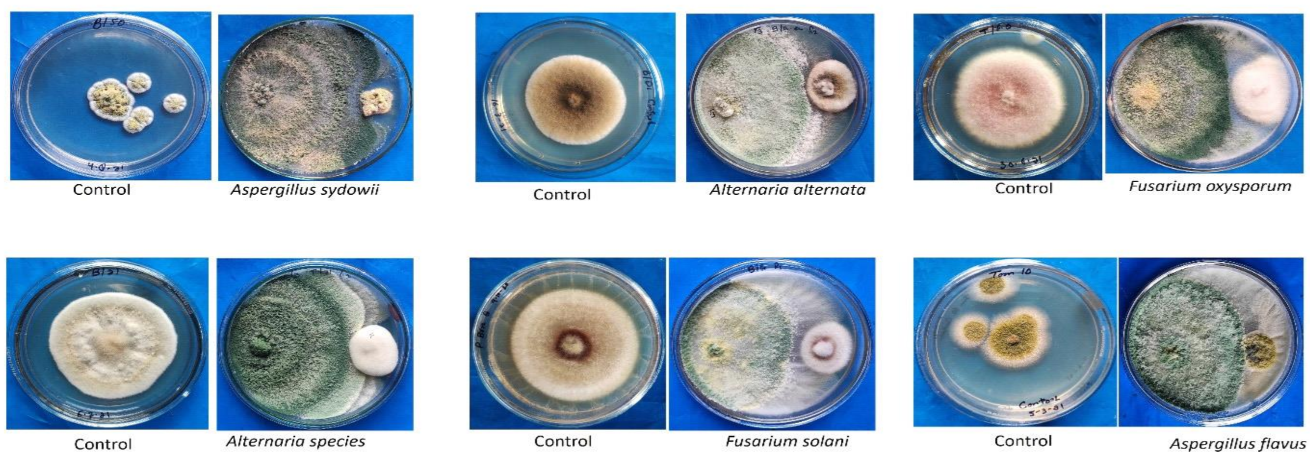


**Figure 1C.** Antagonistic effect of *Trichoderma viride* on the pathogenic fungi.

Similar findings were made by Sundaramoorthy and Balabaskar (2013), who discovered that *Trichoderma harzianum* dramatically reduced *Fusarium oxysporum's* ability to grow its mycelium on tomato plants. The effectiveness of *Trichoderma* species may be attributable to protective elements such as lytic enzymes, chitinases, 1, 3-glucanases, and other substances that guarantee the complete and efficient destruction of fungal mycelial or conidial walls (Fesel and Zuccaro, 2016). Additionally, Koka *et al*. (2017) discovered that various *Trichoderma* isolates prevented the pathogenic fungi that cause brinjal fruit rot in the Kashmir Valley from growing their mycelium. Similar to this, La Spada *et al.* (2020) reported *Trichoderma* species defended tomato plants from *Phytophthora nicotianae* infection by promoting plant defence mechanisms and expression of crinkler, necrosis-inducing phytophthora protein 1, and cellulose-binding elicitor lectin pathogenic effectors. These findings imply that *Trichoderma* spp. may function as an efficient biological control agent against pathogenic fungi that cause tomato leaf spot disease and other crop diseases, and that it can be used in sustainable, environmentally friendly disease management programmes to lessen environmental and health risks in the context of the current climate change scenario.

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