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# *In-vitro* efficacy of biocontrol agents against *Alternaria solani* (Early Blight of Tomato)

**Naik SC, Narute TK, Narute TT and Khaire PB**

**Abstract**
Cherry tomato (*Solanum lycopersicum* var. *cerasiformae* (Dunnel) A. Gray) is a cultivated species of tomato belonging to family Solanaceae. The crop cherry tomato is infected by many diseases including fungi, bacteria and viruses. Among the fungus diseases, Early blight of cherry tomato caused by *Alternaria solani* (EII and Martin) (Jones and Grout) during all stages of plant growth. In the experiment of *In vitro* efficacy of biocontrol agents against *Alternaria solani* all treatments showed significant effect to inhibit growth of the pathogen. The maximum inhibition was obtained by *Trichoderma harzianum* (85.13%) and *Trichoderma viride* (80.67%) growth inhibition over all other bioagents. The next best bioagent was *Pseudomonas fluorescens* (72.87%).

**Keywords:** Cherry tomato, *Alternaria solani*, *in-vitro*, *Trichoderma*, *Pseudomonas*.

## Introduction
The Cherry tomato [*Solanum lycopersicum* var. *cerasiformae* (Dunnel) A. Gray] is a cultivated species of tomato. Is one of the most popular high value exotics, it is favorite among the chefs who cook for high profile restaurants and hotels. Nowadays, it is increasingly popular among the common people for garnishing their dishes. In terms of food value, it is the second largest vegetable crop and ranks first in processed food products. India is one of the world's largest tomato producers, second only to China, with an estimated production of 1,8735,91 thousand metric tons in 2013-14, and about 11 per cent of the world's total tomato production is grown in India. The major rising states in India are Andhra Pradesh, Karnataka, Uttar Pradesh, Maharashtra, Haryana, Punjab, Bihar, and Himachal Pradesh. Fungi, bacteria, viruses, nematodes and abiotic factors are responsible for many tomato diseases (Balanchard, 1992; Gomaa, 2001; Abdel-Sayed, 2001; 2006, and 2008 by Abada *et al*.) [3, 6, 2, 1]. *Alternaria solani* (Ellis and Martin) Jones and Grout, causing early blight, is the most damaging of the fungal diseases (El-Abyad *et al*., 1993; Gomaa, 2001; Abdel-Sayed, 2006 and Abada *et al*., 2008) [9, 6, 2, 1], resulting in a major decrease in crop quantity and quality.
Tiny dark brown spots on the lowest and oldest leaves are an initial symptom of early blight. The tissue around the primary lesions will turn bright yellow and the entire leaves can become necrosis and chlorotic if the lesions are multiple. The spots are enlarged and they form concentric rings that give them the eye of a bull. Diseases grow in favorable weather conditions, accidents can become numerous and plants defoliate, destroying the quantity and quality of tomato fruits (Kouyoumjian, 2007) [8]. Every part of the plant (causing foliage blight, fruit lesions and stem collar rot) can be infected by Alternaria solani and can be affected at all stages of plant growth (Abada *et al*., 2008) [1]. So, it is really important to treat this disease. The ultimate control of this disease is the use of resistant varieties. Farmers in search of high yield, however are inclined to cultivate some varieties which may be less disease-resistant. In addition to affecting the health of users and customers, unplanned and wide-ranging use of fungicides also leads to significant environmental problems. Thus, the use of chemicals for disease control needs to be reduced. Therefore, the attempt against *Alternaria solani* was made to test bioagents to establish an eco-friendly management strategy to control the early tomato blight. In view of the *in vitro* evaluation of fungicides, bioagents and botanicals, the value of this disease was preserved in order to understand their bio-efficacy.

## Material and Methods
### 1. Evaluation of different biocontrol agents against *Alternaria solani*
The efficacy of five biocontrol agents *viz*., *Trichoderma viride* (Prasun *et al.,* (2013) [10] and *Pseudomonas fluorescens* were tested against causal organism by dual culture technique. Biocontrol agents were obtained from Department of Plant Pathology and Agricultural Microbiology, MPKV, Rahuri.

The fungal biocontrol agent was grown in potato dextrose agar media and bacterial biocontrol agent in nutrient agar media to get fresh active culture for the experiment. To study the antagonistic effect, the fungal culture *viz*., *Trichoderma* species were grown separately on PDA plates for seven days. The test fungus (5 mm diameter) and bioagent were cut with sterilized cork borer and transferred aseptically in Petri plates, which were already poured with PDA. The disc of the test fungus was placed exactly opposite to the disc of bioagent. The disc was placed in such manner that both the test fungus and biocontrol agent would get an equal opportunity for their growth and the plates were then incubated at temperature 28±1°C for next five days. In case of bacterial biocontrol agent *viz*., *Pseudomonas fluorescens* was used to test against *Alternaria solani* in *In vitro* on PDA medium. The streaks of 24 hrs. old bacterial isolates were made at both side of Petri plate equidistantly by placing the disc test pathogen (5 mm diameter) at the centre of the Petri plate. After that plates were incubated at temperature 28±1°C for next five days. The data was analysed statistically. The efficacy of biocontrol agents was expressed as percentage inhibition of mycelia growth over control and calculated as (Vincent, 1927) [19]:

$$I=\frac{C-T}{C}\times 100$$

Where,
I= Percent Inhibition,
C= Radial growth in control,
T= Redial growth in treatment.

### 1.1 Experimental Details for biocontrol agents:

Design : Completely Randomized Design, Replication: Three, Treatments : Six
Treatment details:
$T_1$-*Trichoderma viride*, $T_2$-*Trichoderma harzianum*, $T_3$-*Trichoderma asperellum*, $T_4$-*Trichoderma hamatum*, $T_5$-*Pseudomonas fluorescens*, $T_6$-*Alternaria solani* Observations recorded for colony diameter of *Alternaria solani* in (mm) and percent growth inhibition due to biocontrol agents.

## Result and Discussion

### 1. *In vitro* evaluation of antifungal bio control agents against *Alternaria solani*

The data pertaining the *In vitro* evaluation of biocontrol agents against pathogen *Alternaria solani* causing early blight of cherry tomato presented in (Table 1, Plate I, Fig.1).Among antifungal biocontrol agents tested, $T_2$, *T. harzianum* was found most effective with statistically significant least mycelial growth (13.33mm) and highest mycelial growth inhibition of (85.13%) against *Alternaria solani* followed by $T_1$, *T. viride* with (17.33mm) mycelial growth with highest mycelial growth inhibition (80.67%)and at par with each other.

**Table 1:** *In vitro* evaluation of bio control agents against *A. solani*

| Tr. No. | Treatments | Mean Colony Diameter (mm) of *Alternaria solani* | *Growth inhibition (%) |
|---|---|---|---|
| $T_1$ | *Trichoderma viride* | 17.33 | 80.67 |
| $T_2$ | *Trichoderma harzianum* | 13.33 | 85.13 |
| $T_3$ | *Trichoderma asperellum* | 27.00 | 69.89 |
| $T_4$ | *Trichoderma hamatum* | 24.33 | 66.18 |
| $T_5$ | *Pseudomonas fluorescens* | 24.33 | 72.87 |
| $T_6$ | Control | 89.67 | 00.00 |
| S.E.± | | 0.34 | |
| CD at 5% | | 1.02 | |

*The growth inhibition percent calculated on the basis of mean colony diameter (mm) recorded in control showing 89.67mm growth

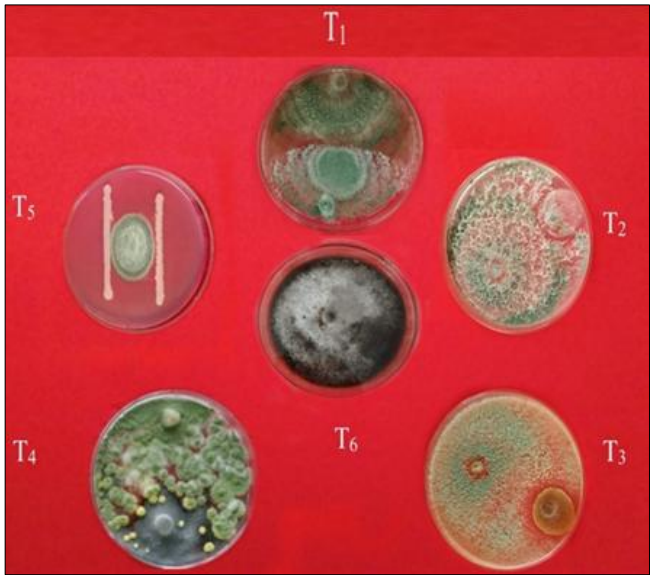


**Plate 1:** *In vitro* efficacy of antifungal biocontrol agents against *Alternaria solani*

The third best biocontrol agent $T_3$, *Pseudomonas fluorescens* with mycelial growth (24.33mm) and mycelial growth inhibition (72.87%) which are at par with $T_1$, *Trichoderma viride* as well as with $T_3$, *Trichoderma asperellum*. $T_4$, *Trichoderma hamatum* was found comparatively less effective and yielded more mycelium growth (24.33mm) and inhibit minimum mycelium growth inhibition (66.18%) as compared to control. Similar type of results were also obtained by Babu *et al*., (2000 a) [4] who evaluated the efficacy of six *Trichoderma* species on early blight of tomato. Among the six species of *Trichoderma* he tested, *T. harzianum* exerted the highest inhibition of the mycelial growth (50.22%) of the pathogen over control followed by *T. viride*.
Babu *et al.*, (2000b) [4] reported that all the six *P. fluorescens* used were significantly inhibited the growth of *A. solani* compared to control.
Also, Ganie *et al*., (2013) [7] evaluated some bioagents against *Alternaria solani* through dual culture technique and maximum mycelial growth inhibition of *Alternaria solani* was observed in the case of *Trichoderma harzianum* (71.85%), followed by *Trichoderma viride* (65.93%) and *Trichoderma virens* (58.65%).
Rani *et al.*, (2017) evaluated fungicides and plant extracts against *Alternaria solani*. Amongst the bio agents tested using dual culture technique, *Trichoderma harzianum* showed

maximum growth inhibition of the pathogen and appeared to be most effective. It is also proved by Vaibhav Pratap Singh *et al*., (2018) [17] evaluated seven fungicides along with seven biocontrol agents and seven plant extracts in *In vitro* condition against *Alternaria solani* and in this experiment *Trichoderma harzianum* was found to be effective and recorded highest inhibition (80.37%) of the mycelial growth of the pathogen followed by *T. viride* (77.41%), *T. koningii* (71.48%) and least mycelial inhibition shown by *T. hamatum* (27.41%).

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