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BIO-EFFICACY OF NUTRIENT-ENRICHED *Trichoderma harzianum* CONSORTIA AGAINST EARLY BLIGHT OF TOMATO *IN-VIVO* CONDITION

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ABSTRACT

Early blight (*Alternaria solani*) is a significant fungal disease that causes 50-86% yield losses in regions growing tomato and has resulted in increased environmental and residue concerns due to high levels of fungicide use for control. To assess the effectiveness of nutrient enriched consortia of *T. harzianum* which was produced by growing *T. harzianum* on calcium chloride, boric acid, potassium dihydrogen phosphate, ferric chloride, phosphoric acid and urea at 1% and 2% respectively, for seedling treatment and foliar spray for control of early blight under field conditions. A field experiment was conducted at SIF, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, using randomized block design for two seasons of *rabi* 2024-25 and 2025-26 and the disease severity index were recorded at 30, 45 and 60 days after transplanting (DAT). In both seasons the consortium with enriched calcium chloride (T2: 2g) always showed the lowest disease incidence and severity followed by the consortium with enriched potassium dihydrogen phosphate (T6: 2g) at all progress stages. At 60 DAT, T2 achieved the maximum disease reduction over control (83.91% in 2024-25 and 82.89% in 2025-26), followed by T6 (80.72% and 80.10%, respectively), while the untreated control recorded the highest disease severity (77.50% and 79.80%). The increased biocontrol activity of T2 and T6 can be explained by the synergism of the *Trichoderma* antagonism and the calcium and phosphate mediated strengthening of plant cell walls and defense metabolism. These results prove that the nutrient enriched consortia of *T. harzianum*, especially calcium chloride and potassium dihydrogen phosphate are effective, eco-friendly and alternative to chemical fungicides for sustainable management of early blight in tomato.

***Keywords*:** Early blight, Trichoderma harzianum, nutrient-enriched consortia, biocontrol, disease severity, tomato

Introduction

Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetable crops belonging to the family Solanaceae. It is originated from Mexico to Peru and grown throughout central, southern and southern North America (Nelson, 2008). It was Robert Gibbon Johanson, an ordinary farmer in the U.S.A who first ate tomato on a hot day of August 1820 to describe its edibleness. From then onwards, the tomato spread throughout the world. In India, it was introduced from Europe, in the seventeenth century (Bhujbal *et al.,* 2012). India is a major producer of tomatoes, which are the world's second-most significant vegetable after potatoes and the top crop for processing. The annual production of tomato in India during 2023-24 was 213.20 lakh tonnes with an area of about 864 thousand hectare and productivity of 27.8 MT/ha (FAOSTAT, 2023). It is cultivated in well drained sandy loam soil with pH range between 6.5 and 6.9. Tomatoes are vulnerable to frost injury and thrive in temperatures between 21-30°C during the day and 18-21°C at night. Too high or too low

temperatures can have a detrimental effect on fruit set, growth and quality (Shamshiri *et al.,* 2018).

Tomato is called the "poor man's apple" because it is inexpensive, widely available and rich in nutrients such as vitamins A, C, K, potassium, fiber and antioxidants like lycopene, which provide health benefits similar to apples but at a lower cost (Canene-Adams *et al.,* 2005; Willcox *et al.*, 2003). The mature tomato fruits are rich in minerals such as Mg, Ca, P, Fe, Na, K, Cu and S as well as vitamins A, B and C. It contains 1.9g protein, 0.1g fat, 0.6g minerals, 0.7g fiber and 3.7g carbohydrates per 100g of edible part. It is also a great source of many different micronutrients and posses anticancer properties and cholesterol control (Lenucci *et al.,* 2006; Agrawal and Rao, 2000; Beecher, 1998). Tomato fruit provides 3-4% total sugar, 4-7% total solids, 15-30 mg/100gm ascorbic acid, 7.5-10mg/100ml titrable acidity and 20-50mg/100g fruit weight of lycopene (Hand book of Horticulture, ICAR, 2007).

India ranks second in the area as well as in production of tomato. The major tomato growing countries are China (30.7%), India (11.5%), USA (8.1%), Turkey (7.0%) and Egypt (5.3%) (Bhujbal *et al.,* 2012).

In India, the leading producer states of tomato are Madhya Pradesh, Karnataka, Andhra Pradesh, Gujarat, Odisha, Tamil Nadu, West Bengal, Bihar. Chhattisgarh, Maharashtra, Uttar Pradesh, Haryana, Himanchal Pradesh and Telangana. Together, these states produce 90% of total tomato yield of India. Madhya Pradesh is the top among the tomato producing state, which contributing about 16.80% of the total production, followed by Karnataka (11.30%), Andhra Pradesh (10.50%), Gujarat (6.93%), Odisha (6.8%), Tamil Nadu (76.3%) and West Bengal (6.2%), respectively (Horticultural Statistics at a Glance, 2023).

Generally, tomatoes and their conversion products are the most important in conversion industries of the world. Approximately 30-35 million tons of fresh tomatoes are processed in factories, and tomato paste is their main product. A total of 37 countries in the world have the possibility of processing tomatoes, of which 12 countries do processing up to 90 percent (Niya *et al.,* 2015).

The tomato crop is affected by several diseases such as early blight, late blight, damping off, root rot, wilt, and several viruses which have a great impact on the growth and yield of this crop. The early blight of tomato (*Alternaria solani*) is one of the most serious diseases affecting tomato crops in the major tomato-producing areas of the world. Disease losses from various countries range from 20 to 80 per cent. Early blight of tomato has become a severe disease in the last few years. Nowadays, Early blight of tomato is world's most destructive disease, which causes 50 to 86 percent loss in fruit yield in different parts of the country. (Lal *et al*., 2017).

A lot of fungicides are recommended for the management of late blight of tomato, which poses a great environmental stress including residual effect of fungicides due to higher doses used by the farmers. With the ongoing impacts of climate change, global agriculture is encountering unprecedented challenges in disease management, particularly in efforts to reduce dependence on chemical herbicide pesticides, and fungicides (Meena *et al.,* 2021).

The management of disease can be done through biological control (Kokalis Burelle., 2002). The importance of the beneficial microbes in improving nutrient availability and promoting plant growth, antagonizing, and priming the plant's immune system is well established and abundantly used in biocontrol strategies (Zamioudis *et al.,* 2021) The root associated mutualistic microbes, besides impacting on plant nutrition and growth, can further boost plant defences, rendering the entire plant more resistant to pathogens (Romera *et al.,* 2019). To cope with biotic stresses incited by biological agents, like insects and pathogens, plants develop responses, and some of these responses systemically spread far from the infected tissue into the whole plant. These responses include the systemic acquired resistance (SAR) and the induced systemic resistance (ISR) (Shoresh *et al.,* 2010; Pieterse *et al.,* 2014). Systemic Acquire Resistant is induced by insects and pathogens, while induced systemic resistance is mediated by beneficial microbes present in the rhizosphere, like bacteria and fungi (Mukherjee *et al.,* 2023). Early blight diseases can be controlled by using natural performant antagonistic. *Trichoderma spp*. is worldwide known for their antifungal, for pathogenic fungi. This potency is expressed by several mechanisms involved in antagonistic interactions like competition, antibiosis and mycoparasitism. (Guirgiu *et al.,* 2018; Benlamoudi *et al.,* 2021)

Therefore, the present investigation was conducted to reduce the use of harmful chemical fungicide and use of biological microorganism for controlling of early blight disease.

Material and Method

The experiment was conducted during *rabi* season at SIF (Student's Instructional Farm) of Chandra Sekhar Azad University of Agriculture and

Technology, Kanpur situated at latitude of 26.49ºN and longitude 80.30º E and at around 126 mete MSL during 2024-25 and 2025-26 crop season. The field experiment was set up using a randomized block design (RBD) with three replications and thirteen treatments, each plot measuring roughly three meters by two meters. T-6, an early blight-prone cultivar, was employed in this study.

Prepared different *Trichoderma harzianum* based consortia for seed treatment and foliar spray

The *Trichoderma harzianum* culture will be collected from laboratory Department of Plant Pathology C.S.A.U.A& T Kanpur. *Trichoderma harzianum* will multiply on PDA poured Petri plate by hyphal tip culture method (Tutte, 1969). The PDB will be prepared and mixed with 1% and 2% chemicals of boric acid as source of boron, calcium chloride as source of calcium, potassium dihydrogen phosphate as source of potassium, ferric chloride anhydrous as source of iron, phosphoric acid as source of phosphorus, urea (liquid) as source of nitrogen. The prepared culture will be autoclaved at 121ºC temperature and 15 psi pressure for 15 min, after autoclaved the filled PDB conical flask kept for cooldown and 1 bits of 5 mm diameters size mycelia discs, taken from 7 days old culture of *Trichoderma harzianum* culture plates and inoculate in PDB. The inoculated substrate will be kept in BOD incubator at 25±1ºC allow to mycelium growth for 21 days. The culture will be prepared at two different concentrations 1% and 2% and filter the substance and use seedling treatment and foliar spray.

Table 1: Treatment details

Treatments	Treatment details
T1	Seedling treatment + foliar spray with calcium chloride @ 1gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T2	Seedling treatment + foliar spray with calcium chloride @ 2gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T3	Seedling treatment + foliar spray with boric acid @ 1gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T4	Seedling treatment + foliar spray with boric acid @ 2gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T5	Seedling treatment + foliar spray with potassium dihydrogen phosphate @ 1gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T6	Seedling treatment + foliar spray with potassium dihydrogen phosphate @ 2gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T7	Seedling treatment + foliar spray with ferric chloride anhydrous @ 1gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T8	Seedling treatment + foliar spray with ferric chloride anhydrous @ 2gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T9	Seedling treatment + foliar spray with phosphoric acid @ 1gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T10	Seedling treatment + foliar spray with phosphoric acid @ 2gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T11	Seedling treatment + foliar spray with urea (liquid) @ 1gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T12	Seedling treatment + foliar spray with urea (liquid) @ 2gm +100 ml of PDB in rich culture filtrate of *Trichoderma harzianum* based consortia
T13	Control

Field experiment was conducted during crop session 2024-25 and 2025-26. Seed treatment were given at time of seed sowing and two spray were given at 10 days interval.

Observation

Disease severity: The number of infected leaves were observed at 30, 45 and 60 days after transplanting. Severity of early blight was recorded on the basis of 0-5 rating scales as described by Horsefall and Barret, (1945). The rating scales were converted percent disease severity index for the analysis of disease severity using the following formula.

Table 2 : Disease rating scale by Horsefall and Barret, (1945).

Category	Grade/ Numerical rating	Description of the Symptoms
I	0	Leaves free from infection
II	1	Small irregular spots covering 1-10% leaf area
III	2	Small irregular brown spots with concentric rings covering 11-25% leaf area
IV	3	Lesions enlarge to form irregular brown with concentric rings covering 26-50% leaf area.
V	4	Lesions coalease to farm irregular and appears as a typical blight symptom covering 50-75% leaf area
VI	5	Lesions coalease to farm irregular and appears as a typical blight symptom covering >76% leaf area.

Disease severity and prevalence will be calculated by using the Wheeler' s formula (1996) as given bellow:

$$\text{Percent disease incidence} = \frac{\text{No of infected leaves}}{\text{Total no of leaves observed}} \times 100$$

$$\text{Disease Severity} = \frac{\text{Sum of Numerical ratings}}{\text{Total number of leaves observed} \times \text{Maximum disease rating}} \times 100$$

The data generated on various aspects of research were subjected to statistical analysis as per the methods described by Panse and Sukhatame (1978).

Result and Discussion

The data on disease incidence (DI) of early blight of tomato (*Alternaria solani*) reported for both the crop seasons 2024-25 and 2025-26 in Table 3. There were significant differences among the treatment of both cropping season. During 2024-25, the lowest disease incidence at 30 DAT was recorded under T2 (*T. harzianum* consortia enriched with $CaCl_2$ @ 2 g: 10.82%), followed by T6 (potassium dihydrogen phosphate @ 2 g: 11.33%) and T1 ($CaCl_2$ @ 1 g: 14.50%). It is noted that the highest disease incidence was found in the untreated control T13 (33.48%). The similar trends were observed in 2025-26, T2 again recorded the minimum DI (13.73%), followed by T6 (15.57%) and T8 ($FeCl_3$ @ 2 g: 20.02%), while T13 again recorded the maximum DI (34.45%).

The disease incidence was progressive increased advancement of crop age under all treatments from 30 DAT to 45 DAT. During 2024-25, T6 (18.21%) recorded the lowest DI, closely followed by T2 (18.78%), while T13 recorded the maximum (47.48%), disease incidence. In 2025-26, T6 (24.43%) and T2 (22.75%) were recorded lowest incidence, while T13 (control) recorded the highest disease incidence (46.72%).

Early blight (EB) is a progressive and epidemic disease, with the greatest disease severity occurring at 60 DAT for all treatments in both seasons. During 2024-25, T2 (27.90%) and T6 (28.09%) were recorded the minimum disease incidence, while T13 (control) recorded the maximum (71.66%). In 2025-26, T2 were recorded the minimum Disease incidence (27.44%) followed by T6 (28.92%), while T13 (control) recorded the highest (72.90%).

Table 3 : Effect of *T. harzianum* based nutrient-enriched consortia on disease incidence (%) of early blight of tomato

Treatments	Disease incidence % after transplanting					
	Season 2024-25			Season 2025-26		
	30 DAT	45 DAT	60 DAT	30 DAT	45 DAT	60 DAT
T1	14.5	20.2	30.2	16.2	26.67	31.23
T2	10.82	18.78	27.9	13.73	22.75	27.44
T3	21.12	26.66	39.4	23.08	30.1	37.56
T4	15.83	21.19	30.88	20.08	28.1	35.56
T5	18.37	25.96	35.02	22.28	30.23	32.9
T6	11.33	18.21	28.09	15.57	24.43	28.92
T7	23.84	29.69	43.11	25.63	34.44	41.37
T8	16.81	22.33	32.62	20.02	28.98	31.16
T9	22.06	27.81	44.58	24.85	36.18	42.26
T10	18.25	22.19	32.67	19.31	28.12	32.7
T11	27.69	31.75	46.66	27.14	38.02	47.58
T12	17.4	24.8	39.2	21.14	32.02	42.58
T13	33.48	47.48	71.66	34.45	46.72	72.9
CD at 5%	**0.84**	**1.21**	**1.81**	**0.84**	**1.11**	**1.4**
SE(m)	**0.29**	**0.41**	**0.62**	**0.29**	**0.38**	**0.48**
CV	**2.5**	**2.8**	**2.8**	**2.3**	**2.1**	**2.1**

DAT = Days After Transplanting; CD = Critical Difference; SE(m±) = Standard Error of Mean; CV = Coefficient of Variation.

Disease Severity (DS) and Disease Reduction over Control at 60 DAT

Disease severity at 60 DAT and the percentage of disease reduction over the control were presented in Table 4. During 2024-25, the minimum Disease severity was recorded in T2 (12.47%), followed by T6 (14.94%) and T1 (17.63%). The untreated control (T13) was recorded the maximum disease severity of 77.50% and T2 achieved the highest disease reduction over control (83.91%), followed by T6 (80.72%) and T1 (77.25%). In 2025-26, T2 were recorded the minimum disease severity (13.65%) with 82.89% disease reduction, followed by T6 (DS: 15.88%; reduction 80.10%) and T8 (DS: 18.82%; reduction: 76.42%), while T13 recorded the maximum DS (79.80%).

Table 4 : Effect of *T. harzianum*-based nutrient-enriched consortia on disease severity index (DSI, %) and disease reduction over control (%) of early blight of tomato at 60 DAT

Treatments	Season 2024-25		Season 2025-36	
	Disease severity @ 60 DAT	Disease reduction over control	Disease severity @ 60 DAT	Disease reduction over control
T1	17.63	77.25	19.8	75.19
T2	12.47	83.91	13.65	82.89
T3	23.81	69.28	25.63	67.88
T4	18.8	75.74	27.63	65.38
T5	22.89	70.46	22.74	71.50
T6	14.94	80.72	15.88	80.10
T7	26.5	65.81	26.82	66.39
T8	19.33	75.06	18.82	76.42
T9	28.23	63.57	28.91	63.77
T10	20.45	73.61	22.23	72.14
T11	35.96	53.60	36.92	53.73
T12	28.3	63.48	35.92	54.99
T13	77.5	-	79.8	-
CD at 5%	**0.85**		**1.84**	
SE(m)	**0.29**		**0.63**	
CV	**1.9**		**3.8**	

DS = Disease Severity; DAT = Days After Transplanting; CD = Critical Difference; SE(m±) = Standard Error of Mean; CV = Coefficient of Variation.

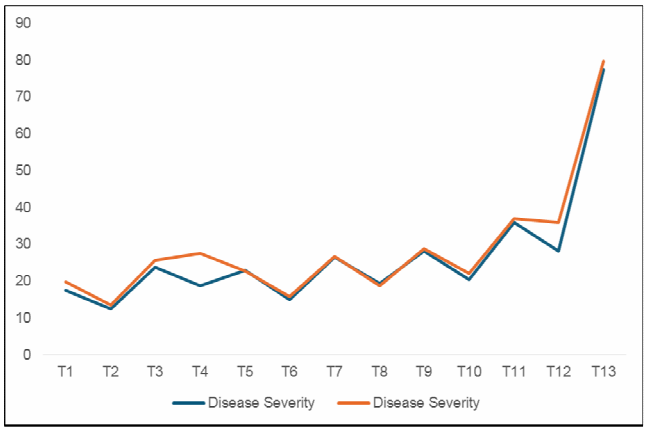

Fig. 1 : Disease severity of early blight of tomato in both growing season

Discussion

The present study shows that *Trichoderma harzianum* based nutrient enriched consortia can suppress early blight of tomato effectively under field conditions, with the best performance consistently observed in T2 and T6 across both seasons. In your data, T2 recorded the lowest disease incidence at 30, 45, and 60 DAT in 2024-25 and again in 2025-26, while T6 was usually the next best treatment, indicating stable season-to-season efficacy. This agrees with earlier reports that *Trichoderma spp.* can significantly suppress *Alternaria solani* and reduce early blight severity in tomato through biocontrol mechanisms such as competition, antibiosis, and mycoparasitism. (Ramakrishna *et al.,* 2017)

The lower disease incidence and severity in the best treatments may be due to the combined action of *Trichoderma* and nutrient enrichment, which can improve plant vigor and help the crop tolerate infection better. In your results, disease pressure increased with crop age in all treatments, but the treated plots still stayed well below the untreated control at every observation stage, showing that the treatment delayed disease progress rather than only reducing early infection. This pattern is consistent with literature showing that *Trichoderma* can improve plant growth and disease suppression, making it useful in integrated disease management programs. (Aljabali *et al.,* 2025; Imran *et al.,* 2023)

The superiority of CaCl enriched culture filtrate in T2 and the strong performance of KH PO - enriched filtrate in T6 suggests that mineral enrichment may have enhanced the biocontrol potential of the fungal consortium. Calcium is known to strengthen cell walls and membranes, while phosphate can support plant metabolism and defense responses, which may help explain the lower disease severity in these treatments. Your findings are in line with studies reporting that *Trichoderma*-based bioagents can markedly reduce early blight and perform well under field conditions compared with untreated control plots.

Conclusion

The study concludes that *Trichoderma harzianum* based nutrient enriched consortia are effective for managing early blight of tomato and can substantially

reduce disease incidence and severity compared with the control. Among the tested treatments, T2 was the most effective overall, followed closely by T6, making them the best options for eco-friendly management of *Alternaria solani* in tomato.

These results support the use of *Trichoderma* based formulations as a safer alternative to heavy chemical dependence in tomato disease management. Their consistent performance across two crop seasons suggests that they can be integrated into sustainable production systems for tomato cultivation.

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Competing interests

Authors have declared that no competing interest exist.

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