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Research

In vitro Evaluation of *Trichoderma* Isolates and Botanical Extracts Against Early Blight of Tomato Caused by *Alternaria solani*

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Abstract

Early blight, caused by *Alternaria solani*, is an important fungal disease that serves as a serious limitation to tomato production, mainly in high-humidity and warm areas of Nepal. The present study was conducted to explore the in vitro efficacy of *Trichoderma* isolates and botanical extracts against *A. solani*. The pathogen was isolated from infected tomato plants and subjected to dual culture and poisoned food assays in a completely randomized design with five replications. Three species of *Trichoderma* (*T. asperellum*, *T. harzianum*, and *T. viride*) and four selected plant extracts (*Azadirachta indica*, *Ocimum basilicum*, *Cymbopogon citratus*, and *Psidium guajava* leaves) were tested for their effectiveness to inhibit *A. solani* mycelial growth over a period of 10 days. All the treatments significantly inhibited the mycelia growth in comparison to the control. *T. asperellum* showed the highest inhibitory activity (0.21), followed by *T. harzianum* and *T. viride*, thus showing their good antagonistic potential against *A. solani*. Similarly, among botanical extracts, *Azadirachta indica* leaf extract was most effective in inhibiting the mycelia growth (83.78), followed by *O. basilicum* and *C. citratus* extracts, with *P. guajava* leaf extract being comparatively less effective. The results indicate that *T. asperellum* and *A. indica* leaf extract had the highest mycelial growth inhibition capacity and proved to be effective in in vitro conditions. Therefore, these options must be evaluated further in field conditions against early blight of tomato, which can be a promising eco-friendly approach alternative to synthetic chemical fungicides.

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Statement of Sustainability: This study demonstrates that the use of *Trichoderma* isolates and botanical extracts as biological control agents supports sustainable agriculture by reducing chemical fungicide use, minimizing environmental impact, and promoting eco-friendly tomato production.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is a vegetable that is extensively grown in the family of Solanaceae and is highly valued for its nutritional value. In Nepal, the crop is colloquially called the "poor man's apple," with an average reported consumption at the national level of 11.97 kilograms per capita per year (Yogi et al., 2024; Wagle, 2023).

Although tomato plants are economically and nutritionally valuable, they are highly prone to diseases, one of which, early blight caused by *Alternaria solani*, is one of the most devastating fungal diseases in tomato production worldwide (Naz et al., 2025; Maurya et al., 2022; Singh, 2022; Dhaval et al., 2021). In favorable conditions, early blight causes dark necrotic spots with concentric rings on leaves, stems, and fruits, which causes defoliation, drying of twigs, early fruit loss, and yield of up to 50-86 percent (Chaudhary et al., 2021; Dhaval et al., 2021; Solankey et al., 2021). The pathogen also secretes mycotoxins that increase the severity of the disease and cause rapid spread of the disease when subjected to warm and humid climates (Casu et al., 2024; Kos et al., 2023; Alam et al., 2022). Causing a severe impact on the revenue, food security, and food availability of tomatoes, which is a significant source of multiple vitamins and minerals, early blight affects the yield and the quality of the fruit greatly (Jindo et al., 2021; Shoaib and Awan, 2021).

The rapid spread of the pathogen and rising resistance to chemical fungicides make the management of early blight difficult

(Sharma et al., 2025). Sustainable strategies involving the use of eco-friendly alternatives, including plant extracts and biological control agents, have become a focus (Jaiswal et al., 2022). Plant extracts exhibit antifungal properties against pathogens and provide alternatives to chemical pesticides, which are cheap, readily available, and eco-friendly (Jan et al., 2025; Ngegba et al., 2022). On the same note, *Trichoderma* species (*T. asperellum*, *T. harzianum*, and *T. viride*) are also known as being antagonistic to pathogenic fungi by several mechanisms: mycoparasitism, nutrients and space competition, enzyme release, and inhibitory metabolites (Adhikari, 2023; Dutta et al., 2023; Manzar et al., 2022). *Trichoderma*, as well as botanicals, can coexist with organic and sustainable farming methods, where reducing the application of synthetic pesticides is essential to ensure the proper health of soil and the environment (Kaushik et al., 2025; Ngegba et al., 2022; Bhandari et al., 2021).

Considering the severity of the early blight and the need for sustainable management practices, this research was conducted to determine the efficacy of various *Trichoderma* isolates and botanical extracts against *A. solani*. Particularly, the experiment was designed to assess the impact of these treatments on the radial growth and mycelial inhibition of the pathogens. The research aims to offer viable solutions to farmers to control early blight in a manner that is environmentally friendly and minimizes the utilization of chemical fungicides, as well as to improve the sustainability and productivity of tomato production in Nepal by determining the most effective biological control measures that can be used under local conditions.

2. Materials and Methods

2.1. Description of Experimental Site

This research was done in the Central Agricultural Laboratory, Hariharbhawan, Lalitpur, between April and June, 2025 AD. The site is located at 27° 40′ 43″ N latitude and 85° 18′ 58″ E longitude, at an elevation of 1,420 m above sea level, with a mild warm-temperate climate.

2.2. Disease Sample Collection

An early blight disease sample was collected from the Srijana variety of tomato, which was grown at the National Plant Pathology Research Centre, NARC, Khumaltar, Lalitpur. The samples were examined under laboratory conditions for the confirmation of the disease and kept at -20 °C after the sample was air-dried at ambient temperature.

2.3. Experimental Design

Eight treatments were used in the experiment, each of which was replicated five times using a Completely Randomized Design (CRD) (Table 1). The study was done with the Srijana variety of tomato. The *Trichoderma spp.* The isolates used in the study were collected from the National Plant Pathology Research Centre, and the isolates were sub-cultured and maintained under laboratory conditions before being used in the experiments.

Table 1. Details of treatment adopted in this study.

No. of Treatments	Name of Treatment	Concentration
T1	*Trichoderma asperellum*	5 mm colony diameter
T2	*Trichoderma harzianum*	5 mm colony diameter
T3	*Trichoderma viride*	5 mm colony diameter
T4	*Psidium guajava* leaf extract	15%
T5	*Cymbopogon citratus* leaf extract	15%
T6	*Azadirachta indica* leaf extract	15%
T7	*Ocimum basilicum* leaf extract	15%
T8	Control	—

2.4. Isolation and Identification of Pathogen

Pieces of diseased tissues (2-5 mm) with characteristic symptoms, and adjacent normal tissues were cut and sterilized on the surface with 1% sodium hypochlorite solution for one minute, followed by three sterile distilled water rinses, similar to that of Sigdel et al. (2022). Isolation was done by means of the moistened blotter paper technique, and pure cultures were acquired via single spore and hyphal tip methods (Agrios, 2005; Booth, 1971).

2.5. Maintenance of Culture

A. solani isolated from a single spore or hyphal tip method was grown on a 90 mm petriplate having PDA media and incubated at 25 ± 1 °C until the plate was fully grown with mycelial growth, following the method described by Pun et al. (2020). A fully grown culture plate was stored at 5 °C in a refrigerator and sub-cultured at 30-day intervals throughout the study (Agrios, 2005; Booth, 1971).

2.6. In vitro Evaluation Using Dual Culture Technique

In vitro response of *A. solani* to *Trichoderma* isolates (*T. viride*, *T. harzianum*, and *T. asperellum*) was assessed by the dual culture method (Figure 1). Potato dextrose agar (PDA) was made by dissolving 39 g of PDA powder in 1L of distilled water, mixing well to achieve uniformity, and sterilizing using an autoclave at 121 °C and 15 psi pressure for 15 min. The medium was allowed to cool, and 20 ml was added to 9 cm sterile Petri dishes. An active growth of 5mm mycelial discs of the pathogen and the *Trichoderma* isolates was placed on different sides of the Petri plates at an equal distance from the edge. Plates were incubated at temperatures of 25 °C at a 7-10 day time interval. Radial growth of the pathogen and *Trichoderma* was recorded at different intervals to determine inhibition (Singh et al., 2005; Dennis and Webster, 1971).

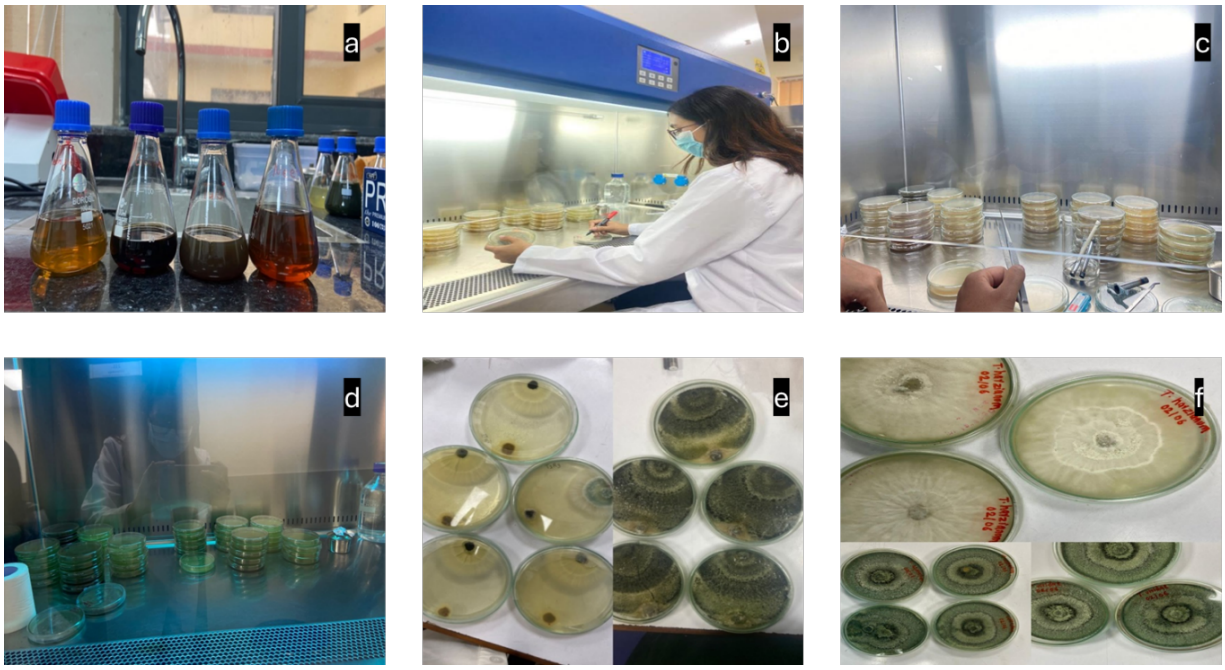

Figure 1. (a) Botanical extracts; (b) Inoculation of *Trichoderma*; (c) Inoculation of pathogen in prepared botanicals (treatment); (d) Sterilization under UV light in a laminar air flow chamber; (e) Interaction of mycelial growth against a pathogen; (f) Interaction of mycelial growth against a pathogen.

2.7. Preparation of Botanicals

A. indica, *O. basilicum*, *C. citratus*, and *P. guajava* were rinsed with tap water, then sterile distilled water, and dried in the air. The leaves were ground using a pestle and mortar at a 1:1 (w/v) ratio. One milliliter of distilled water was combined with one gram of the final extract, filtered through muslin cloth, and heated at 100 °C for 10 min to prevent contamination, following procedures for plant extract preparation in the control of plant diseases (Gurjar et al., 2012).

2.8. Data Recording

Colony diameter was measured at various periods of incubation using a scale under Petri plates. The mean growth of the pathogen (*A. solani*) in centimeters in a radial manner was measured. Mycelial growth inhibition percent in each of the treatments was determined by the formula (Eq. 1) presented by Vincent (1947).

$$I = \frac{(C - T)}{C} \times 100 \tag{1}$$

Where, I = Inhibition of radial mycelial growth of pathogen; C = Diameter of fungus colony (cm) in control plate; T = Diameter of fungus colony (cm) in treated plate.

2.9. Statistical Analysis

The recorded data were entered into MS Excel, and statistical analyses were made using R-Studio. ANOVA was performed at a level of significance of 5%, and a comparison of means was made using Duncan Multiple Range Test (DMRT) (Gomez & Gomez, 1984).

3. Results and Discussion

3.1. Radial Growth of *A. solani* as Affected by *Trichoderma* spp. and Botanicals

Table 2 shows the comparative efficacy of three *Trichoderma* species (*T. asperellum*, *T. harzianum*, and *T. viride*) and four botanical extracts (*P. guajava* leaf, *C. citratus*, *A. indica* leaf, and *O. basilicum* leaf) against *A. solani* based on radial growth inhibition within the period of 10 days of incubation. Radial growth in the untreated control grew progressively from the first day (0.69 cm) to the tenth day (4.09 cm), which is a normal development of the pathogen. All treatments had a strong negative effect on fungal growth as compared to the control.

Among the *Trichoderma* species, *T. asperellum* exhibited the strongest antagonistic effect throughout the experimental period, recording the lowest radial growth (0.21 cm) on day 10, followed by *T. harzianum* (0.30 cm) and *T. viride* (0.39 cm). Stracqudanio et al. (2020) have also reported similar dominance of *T. asperellum* over *A. solani*, where mycelial growth was greatly suppressed under in-vitro conditions. The high performance of *Trichoderma* species has been explained by their ability to colonize fast, high mycoparasitism, and the generation of hydrolytic enzymes and antifungal metabolites (Dhal et al., 2017).

Competition for space and nutrients, release of Enzymes that break down cell walls, such as β-1, 3-glucanases and chitinases and β-1, 3-glucanases and antibiosis through both volatile and non-volatile substances are also linked to the antagonistic activity of *Trichoderma* spp. (Hashem et al., 2023; Bais et al., 2019). These mechanisms collectively explain the consistent and strong inhibition observed in the present study.

Of the botanical treatments, *P. guajava* leaf extract (15%) had moderate inhibition in the early days, with the radial growth stabilizing at 1.80 cm after day 7. *C. citratus* extract (15%) gave a relatively good suppression, limiting fungal growth at 1.34 cm between days 6 and 10 of incubation. *A. indica* leaf (15%) and *O. basilicum* leaf (15%) extracts inhibited similarly, with the final radial growth at day 10 being 0.99 cm and 1.09 cm, respectively. *A. indica* has been reported to be active against *A. solani*, and in the study by Bhanage et al. (2019), the action of antifungals was considerable and was not only in vitro but also in vivo. Similar outcomes were observed by SC et al. (2020), who demonstrated that *A. indica* extract was more effective than other botanicals in the treatment of early blight.

The growth of pathogens was inhibited by all botanical extracts in contrast to the control, but their time-inhibitory activities were different because of the variations in the stability and concentrations of bioactive phytochemicals. This variability of antifungal properties of plant extracts has also been observed by Amsaraj et al. (2020) during the assays under the poisoned food technique.

In general, the findings validate that *Trichoderma* species and botanical extracts are effective in inhibiting the growth of *A. solani* in vitro, though *T. asperellum* is the most promising biocontrol agent.

Table 2. Effect of *Trichoderma* and botanicals on radial growth (cm) of *A. solani* at different period of incubation.

Treatment	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI
Trichoderma asperellum	0.22^{f}	0.54^{f}	0.88^{d}	0.72^{e}	0.80^{f}	0.60^{g}	0.54^{h}	0.56^{g}	0.30^{h}	0.21^{h}
Trichoderma harzianum	0.45^{cde}	0.75^{d}	1.05^{c}	0.92^{d}	0.94^{e}	0.85^{f}	0.76^{g}	0.59^{g}	0.42^{g}	0.30^{g}
Trichoderma viride	0.50^{b}	0.84^{b}	1.18^{b}	1.06^{c}	1.03^{d}	1.00^{e}	0.93^{f}	0.69^{f}	0.51^{f}	0.39^{f}
Psidium guajava (15%)	0.41^{e}	0.81^{c}	1.04^{c}	1.32^{b}	1.49^{b}	1.70^{b}	1.80^{b}	1.80^{b}	1.80^{b}	1.80^{b}
Cymbopogon citratus (15%)	0.46^{bcd}	0.66^{e}	0.86^{d}	1.06^{c}	1.25^{c}	1.34^{c}	1.34^{c}	1.34^{c}	1.34^{c}	1.34^{c}
Azadirachta indica (15%)	0.44^{de}	0.81^{c}	0.83^{d}	0.89^{d}	0.92^{e}	0.98^{e}	0.98^{e}	0.99^{e}	0.99^{e}	0.99^{e}
Ocimum basilicum (15%)	0.48^{bc}	0.76^{d}	0.84^{d}	0.91^{d}	1.03^{d}	1.09^{d}	1.09^{d}	1.09^{d}	1.09^{d}	1.09^{d}
Control	0.69^{a}	1.08^{a}	1.46^{a}	1.76^{a}	2.04^{a}	2.54^{a}	3.00^{a}	3.10^{a}	3.79^{a}	4.09^{a}
SEm(±)	0.001	0.0008	0.002	0.002	0.002	0.003	0.002	0.003	0.003	0.003
LSD	0.03	0.01	0.06	0.04	0.04	0.07	0.05	0.07	0.08	0.08
F value	***	***	***	***	***	***	***	***	***	***
CV,%	6.13	1.93	5.10	3.53	3.02	4.90	3.09	4.64	5.11	5.11
Grand mean	0.46	0.78	11.02	1.08	1.19	1.26	1.30	1.27	1.28	1.28

Note: DAI, Days after inoculation; LSD: least significant differences, SEm (±): Standard error of mean, C.V: Coefficient of variation, Treatment means separated by DMRT and columns represented with the same letter(s) are not significantly different from each other at 5% level of significance. '***' 0.001, '**' 0.01 '*'0.05 of p value

3.2. Inhibition Percentage of *A. solani* as Affected by *Trichoderma* spp. and Botanicals

Table 3 shows the percentage inhibition of *A. solani* by three *Trichoderma* species (*T. asperellum*, *T. harzianum*, and *T. viride*) and four botanical extracts (*P. guajava* leaf, *C. citratus*, *A. indica* leaf, and *O. basilicum* leaf) after 10 days of incubation. The untreated control had 0 percent inhibition during the experiment, which showed the active growth of the pathogen.

Among the *Trichoderma spp.*, *T. asperellum* had the highest inhibitory activity against *A. solani*. Colony inhibition by *T. asperellum*

was 67.14% on day 1 and 94.18% on day 10.*T. harzianum* and *T. viride* were also strongly antagonistic, with a 93.61 and 92.91 percent inhibition, respectively, after ten days, and were statistically similar to *T. asperellum*. The better performance of *Trichoderma* species can be explained by quick colonization, mycoparasitism, the release of hydrolytic enzymes, and antifungal metabolites (Ajiboye & Sobowale, 2022; Bais et al., 2019; Dhal et al., 2017).

Among the botanical treatments, the highest colony inhibition (83.78%) was recorded by the *A. indica* leaf extract (15%) followed by Basil leaf extract (15%) and *C. citratus* extract (15%) with 76.97% and 67.26% inhibition respectively at 10 days after incubation Lowest colony inhibition (56.02%) was recorded by *P. guajava* leaf extract (15%). Although the efficacy was less than that of *Trichoderma* species, all of the botanical extracts had an impact in inhibiting the colony of the pathogens as compared to the control.

The general average inhibition became more and more elevated, amounting to 33.45 percent on day 1 and 70.21 percent on day 10. These results are in accordance with earlier findings on the antifungal activity of plant extracts, especially *A. indica*, against *A. solani* (SC et al., 2020; Bhanage et al., 2019).

In general, the findings indicate that the *Trichoderma* species, as well as the botanical extracts, were effective in suppressing *A. solani* in the in vitro environment, where *T. asperellum* is the most effective antagonist. The botanical extracts, albeit with low effectiveness, are potential elements of combined biological disease management strategies.

Table 3. Effect of *Trichoderma* and botanicals on the % inhibition of *A. solani* at different periods of incubation.

Treatment	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI	6 DAI	7 DAI	8 DAI	9 DAI	10 DAI
Trichoderma asperellum	67.14^{a}	49.99a	39.28^{a}	58.87^{a}	60.44^{a}	76.40^{a}	81.99^{a}	81.77^{a}	91.91^{a}	94.67^{a}
Trichoderma harzianum	34.86^{bc}	30.44c	27.87^{b}	47.74^{b}	53.80^{b}	66.38^{b}	74.63^{b}	80.93^{a}	88.92^{b}	92.57^{b}
Trichoderma viride	27.09^{d}	22.24e	18.70^{c}	39.72^{c}	49.37^{c}	60.44^{c}	68.92^{c}	77.71^{b}	86.48^{c}	90.42^{c}
Psidium guajava (15%)	40.33^{b}	24.90d	28.27^{b}	24.67^{d}	26.66^{e}	33.15^{f}	40.07^{g}	41.99^{f}	52.55^{g}	56.02^{g}
Cymbopogon citratus (15%)	32.55^{cd}	38.96b	40.60^{a}	39.34^{c}	38.50^{d}	47.26^{e}	55.38^{f}	56.81^{e}	64.66^{f}	67.26^{f}
Azadirachta indica (15%)	35.70^{bc}	24.72^{d}	42.58^{a}	49.15^{b}	54.48^{b}	61.35^{c}	67.17^{d}	67.96^{c}	73.78^{d}	83.78^{d}
Ocimum basilicum (15%)	29.90^{cd}	29.76^{c}	42.43^{a}	48.06^{b}	49.28^{c}	56.76^{d}	63.44^{e}	64.61^{d}	71.01^{e}	76.97^{e}
Control	0.00^{e}	0.00^{f}	0.00^{d}	0.00^{e}	0.00^{f}	0.00^{g}	0.00^{h}	0.00^{g}	0.00^{h}	0.00^{h}
SEm(±)	0.23	0.08	0.20	0.12	0.10	0.12	0.07	0.12	0.09	0.07
LSD	5.48	2.01	4.61	2.81	2.49	2.89	1.72	2.79	2.26	1.62
F value	***	***	***	***	***	***	***	***	***	***
CV,%	12.72	5.65	11.96	5.68	4.66	4.47	2.37	3.67	2.66	1.80
Grand mean	33.45	27.63	29.97	38.44	41.56	50.22	56.45	58.97	66.16	70.21

Note: DAI, Days after inoculation; LSD: least significant differences, SEm (±): Standard error of mean, C.V: Coefficient of variation, Treatment means separated by DMRT and columns represented with the same letter(s) are not significantly different from each other at 5% level of significance. '***' 0.001, '**' 0.01 '*' 0.05 of p value

4. Conclusion

In vitro experiment demonstrated that among microbial agents, *T. asperellum* and among botanicals, *A. indica* leaf extract have a potent effect in inhibiting radial growth of *A. solani*, causing early blight of tomato. To validate the efficacy of these two biocontrol agents against *A. solani*, they must be tested in field conditions. Effectiveness in field conditions can be a promising, eco-friendly approach alternative to synthetic chemical fungicides in local conditions.

Authors' Contributions

Shanta Dawadi: Conceptualization, Methodology, Investigation, Software, Data curation, Writing original draft; Suraj Singh Karkee: Validation, Supervision, Review and editing; Naran Prasad Devkota: Resources, Writing original draft, Review and editing; Bindu Sharma: Writing original draft, Review and editing; Simran Poudel: Writing original draft, Review and editing

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Declarations

Conflicts of Interest: The author(s) declare no conflict of interest.

Institutional/Ethical Approval: This study did not involve human or animal subjects, and no institutional or ethical approval was required.

Data Availability/Sharing: The datasets used and analysed during the current study will be made available from the corresponding author upon a reasonable request.

Supplementary Information Availability: Not applicable.

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