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# IN-VITRO ANTAGONISTIC POTENTIAL OF SELECTED TRICHODERMA STRAINS AGAINST ANTHELIA ROLFSII OF TOMATO

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**Abstract:** Tomato (*Solanum lycopersicum*), a critical global crop, is highly vulnerable to soil-borne pathogens like *Athelia rolfsii*, which causes devastating diseases such as southern blight and damping-off. This study evaluated the antagonistic potential of 14 molecularly identified *Trichoderma* strains against *A. rolfsii* using in-vitro dual culture techniques. Experimental setups included simultaneous, prophylactic, and curative inoculation methods, with treatments assessed for their ability to suppress pathogen growth over nine days. The results revealed significant differences in the efficacy of inoculation methods. Prophylactic inoculation demonstrated superior performance, reducing mycelial growth of *A. rolfsii* to 3.48 mm by Day 9, compared to 4.68 mm and 5.96 mm for simultaneous and curative methods. Among the *Trichoderma* strains, T4, T29, and T22 consistently exhibited the highest antagonistic activity, achieving up to 54.6% inhibition of *A. rolfsii* growth by Day 9. The study also confirmed the statistical significance of these findings, emphasizing the critical role of early application in enhancing pathogen suppression. The data underscore the potential of specific *Trichoderma* strains as effective biological control agents, offering environmentally sustainable alternatives to chemical fungicides. These findings provide a foundation for integrating *Trichoderma* into tomato disease management programs, contributing to sustainable agricultural practices. Further research into field-level applications and strain-specific biocontrol mechanisms is recommended.
**Keywords:** Trichoderma spp; *Anthelia rolfsii*; Biological control; Tomato diseases; Dual culture techniques

## 1 INTRODUCTION

Tomato (*Solanum lycopersicum*), a globally important crop, is a vital source of vitamins, minerals, and antioxidants, contributing significantly to human nutrition [1, 2] However, several studies, including that of [3] and [4] have shown that its cultivation faces severe threats from soil-borne pathogens, with *Athelia rolfsii* (formerly *Sclerotium rolfsii*) emerging as a significant challenge. This destructive pathogen, responsible for southern blight, collar rot, and damping-off, causes substantial yield losses globally, particularly in regions with warm and humid climates [5, 6]. Effective management of *Athelia rolfsii* remains critical for sustainable tomato production [7, 8].

Conventional control strategies, such as chemical fungicides and cultural practices, are commonly employed but are increasingly limited by their environmental impact, high costs, and the emergence of resistant pathogen strains [9]. Consequently, the focus has shifted towards eco-friendly approaches, such as biological control agents, which offer a sustainable alternative [10]. Among these, species of the genus *Trichoderma* have shown great promise due to their diverse antagonistic mechanisms, including mycoparasitism, competition for nutrients, and production of antifungal metabolites [11].

Studies have demonstrated the biocontrol potential of *Trichoderma* spp. against various soil-borne pathogens, yet there remains a need for comparative evaluations of specific strains under different conditions [12, 13]. In the study by [14], highlighted the efficacy of *Trichoderma* in suppressing *Athelia rolfsii*; in contrast, [15], emphasized the variability in strain-specific performance and the influence of agroecological factors. Furthermore, limited research has explored the in-vitro antagonistic activity of multiple *Trichoderma* strains against *Athelia rolfsii*, an area critical for identifying the most effective candidates for biocontrol applications.

This study aims to investigate the in-vitro antagonistic potential of 15 selected and identified *Trichoderma* strains against *Athelia rolfsii* in Tomato. The study seeks to contribute to developing environmentally sustainable strategies for managing *Athelia rolfsii* infections by identifying highly effective strains. The findings are expected to provide insights into strain-specific biocontrol mechanisms and support the integration of *Trichoderma* into commercial crop protection programs, aligning with the global demand for sustainable agricultural practices.

## 2 MATERIALS AND METHODS

### 2.1 Experimental Design

From an initial collection of 24 identified *Trichoderma* strains previously isolated and molecularly identified from Southwestern Nigeria, 14 representative strains were strategically selected based on their potential biocontrol characteristics.

The in-vitro experiment was structured using a Completely Randomized Design (CRD) to ensure unbiased allocation of treatments and robust statistical analysis. Each treatment was replicated thrice to enhance the findings' reliability and reproducibility [16]. In total, 135 Petri dishes were prepared and utilized throughout the study to effectively accommodate all treatment combinations and replicates (Table 1).

**Table 1** Identity of Identified and Evaluated Trichoderma Strains

| Isolate Code | Identity and NCBI-GenBank Code | Accession No. | Organism with Closet Homology | Location | % Identity |
|---|---|---|---|---|---|
| T1 | *Trichoderma longibrachiatum*AAF1 | OR617404 | *Trichoderma longibrachiatum* PC1 | China | 100 |
| T2 | *Trichoderma breve* AAF2 | OR617405 | *Trichoderma breve*TWS48Abf(b) | Taiwan | 99.83 |
| T6 | *Trichoderma longibrachiatum*AAF4 | OR617407 | *Trichoderma longibrachiatum* PC1 | China | 100 |
| T7 | *Trichoderma asperellum* AAF5 | OR617408 | *Trichoderma asperellum* MMCC 1532.2 | Malaysia | 100 |
| T8 | *Trichoderma lixii* AAF6 | OR617409 | *Trichoderma lixii* ercha4 | China | 99.82 |
| T12 | *Trichoderma reesei* AAF8 | OR617411 | *Trichoderma reesei* GT-31 | Brazil | 100 |
| T14 | *Trichoderma reesei* AAF10 | OR617413 | *Trichoderma reesei*VMB23 | India | 100 |
| T15 | *Trichoderma longibrachiatum* AAF11 | OR617414 | *Trichoderma longibrachiatum* PC1 | China | 100 |
| T20 | *Trichoderma ghanense* AAF16 | OR617419 | *Trichoderma ghanense* CEN555 | Brazil | 100 |
| T21 | *Trichoderma reesei* AAF17 | OR617420 | *Trichoderma reesei* S4-P-2-3 | China | 99.83 |
| T23 | *Trichoderma longibrachiatum* AAF18 | OR617421 | *Trichoderma longibrachiatum* ASNBRI_F9 | India | 99.48 |
| T25 | *Trichoderma asperellum* AAF20 | OR617423 | *Trichoderma asperellum* Tasp-SR22 | Pakistan | 100 |
| T27 | *Trichoderma asperellum* AAF22 | OR617425 | *Trichoderma asperellum*MF1 | Turkey | 99.62 |
| T30 | *Trichoderma longibrachiatum* AAF24 | OR617427 | *Trichoderma longibrachiatum* Tl_2_Delhi | India | 99.66 |

These selected strains were subjected to detailed evaluation using dual culture techniques, a widely recognized method for assessing antagonistic interactions between fungal species. The experiment incorporated variations in inoculation timing to capture the dynamic interaction between *Trichoderma* strains and *A. rolfsii*, enabling a comprehensive understanding of temporal effects on biocontrol efficacy. This approach ensured that the experimental design provided a rigorous framework for comparing the inhibitory potential of the different *Trichoderma* strains under controlled conditions (Figure 1-2).

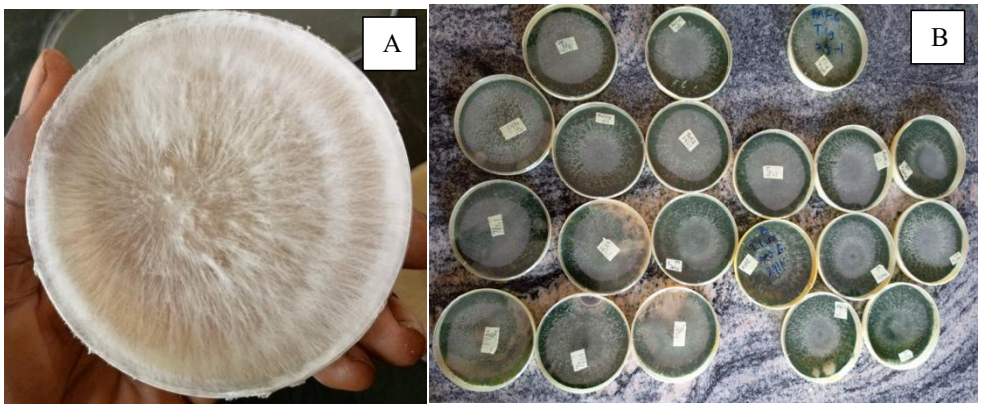


**Figure 1** A: 5 Day Old Pure Culture of *Anthelia rolfsii*, B: Pure Culture of Representative Trichoderma Strains

**Figure 2** C-D: A Cross Section of *in vitro* Laboratory Set-Up in a Completely Randomized Design Under Different Inoculation Approach

### 2.2 Dual Culture Techniques

The antagonistic activity of the 14 selected *Trichoderma* strains against *Anthelia rolfsii* was assessed using dual culture techniques on Potato Dextrose Agar (PDA) in Petri dishes, following the method described by [17], the experiment comprised three inoculation approaches and a control. For the simultaneous inoculation approach, a 5 mm agar disc of the *Trichoderma* isolate was placed 0.5 cm from one edge of the Petri dish. A 5 mm agar disc of *A. rolfsii* was inoculated at 0.5 cm from the opposite edge, with an 8 cm gap between the two discs. In the preventive approach, the *Trichoderma* disc was inoculated 0.5 cm from the edge of the Petri dish and incubated for 24 hours before introducing the *A. rolfsii* disc 0.5 cm from the opposite edge, maintaining the same 8 cm distance. In the curative approach, *A. rolfsii* was inoculated first, 0.5 cm from one edge of the dish, and incubated for 24 hours before introducing the *Trichoderma* disc 0.5 cm from the opposite edge, keeping the 8 cm distance. The control consisted of a 5 mm agar disc of *A. rolfsii* inoculated 0.5 cm from one edge of the Petri dish. All treatments were incubated for nine days at room temperature (Figure 3-4).

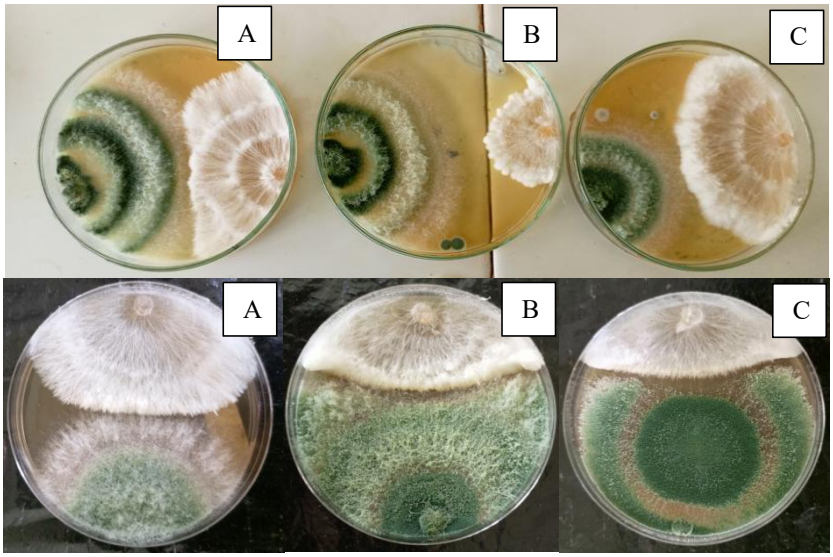


**Figure 3** A: Simultaneous Inoculation; B: Prophylactic Inoculation; C: Curative/ Delayed Inoculation Approach in Mycelia Inhibition Of Both *Trichoderma* and *Anthelia* Spp In Dual Culture Techniques

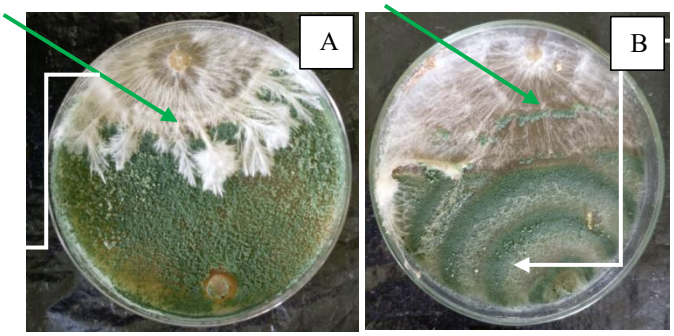


**Figure 4** Mycoparasitism Mechanisms Displayed by Both Isolates: (a) *Anthelia rolfsii*. (b) *Trichoderma* Species

### 2.3 Data Collection

The radial mycelia growth of both *A. rolfsii* and *Trichoderma* strains was monitored on days 3, 6, and 9 post-inoculation. To assess the effectiveness of treatments, the percentage inhibition of radial growth (PIRG) of *A. rolfsii* was determined for each treatment on the respective days. The PIRG
was calculated using the formula [18].

$$\text{PIRG}\ (\%) = \left[\frac{R_1 - R_2}{R_1}\right] X\ 100 \quad (1)$$

where $R_1$ represents the radial growth of *A. rolfsii* in the control plate, while $R_2$ denotes the radial growth of *A. rolfsii* in the presence of the antagonist, data obtained on mycelial growth and percentage inhibition were analyzed statistically to identify significant differences among the treatments.

## 3 RESULT AND DISCUSSIONS

**Table 2** Effects of Application Methods of *Trichoderma* Spp on Mycelia Growth of *A. rolfsii.*

| Application Methods | Day 3 | Day 6 | Day 9 |
|---|---|---|---|
| Simultaneous Inoculation Methods | 2.21556b | 4.07b | 4.68b |
| Prophylactic Inoculation Methods | 1.20667c | 3.08c | 3.48c |
| Curative/ Therapeutic Methods | 2.42667a | 5.17a | 5.96a |

### 3.1 Evaluation of Application Methods on Mycelial Growth of A. rolfsii

This *in-vitro* study evaluated the antagonistic potential of 14 selected *Trichoderma* strains against *A. rolfsii*, a significant pathogen of tomatoes. The results demonstrated different application methods for *Trichoderma spp.*, which markedly influenced the mycelial growth of *A. rolfsii* over a 9-day observation period. Prophylactic inoculation proved the most effective among the methods, significantly reducing mycelial growth to 3.48c by Day 9, compared to 4.68b and 5.96a observed for simultaneous and curative methods. Early application allowed *Trichoderma spp*. to establish dominance, effectively suppressing pathogen proliferation.

Simultaneous inoculation displayed moderate efficacy, providing a viable option when preventive measures are not feasible. Conversely, curative methods were least effective, with the highest mycelial growth observed across all time points, emphasizing the difficulty of reversing pathogen progression once it is established. The slower rate of mycelial growth of *A. rolfsii* in prophylactic treatments can be attributed to the early establishment of *Trichoderma spp.*, which preemptively colonizes the environment and inhibits *A. rolfsii* in conformity with the studies of [19] and [20]. *Trichoderma* effectively suppresses the pathogen through mechanisms such as competition for resources, production of antifungal metabolites, and mycoparasitism by gaining an initial advantage. In contrast, simultaneous and curative treatments are less effective because *Trichoderma* competes directly with an established pathogen (curative) or shares initial resources with it (simultaneous). This delay reduces *Trichoderma*'s ability to dominate the environment. Prophylactic applications provide a significant temporal advantage, enabling proactive control and curbing *A. rolfsii*'s growth more effectively. Tukey's test confirmed statistically significant differences among the application methods, highlighting distinct performance tiers.

As shown in Table 2, the results emphasize the critical role of preventive strategies, particularly prophylactic inoculation, in managing tomato wilt caused by *A. rolfsii*. This approach leverages competitive exclusion and other antagonistic mechanisms to inhibit pathogen establishment. The limited efficacy of curative methods reinforces the importance of early intervention.

### 3.2 Antagonistic Potential of Trichoderma Strains

Table 3 depicts the antagonistic potential of 14 *Trichoderma* strains against *A. rolfsii*, a pathogenic fungus that affects tomatoes. Table 2 presents the impact of these bio-agents on *A. rolfsii* mycelial growth over 9 days, emphasizing their effectiveness compared to the control, where no bio-agent was applied (Table 3).

**Table 3** Effects of Bio-Agent on Mycelia Growth of *A. rolfsii*

| Bio-agent | Day 3 | Day 6 | Day 9 |
|---|---|---|---|
| Control | 2.367a | 6.86a | 8.50a |
| T4 | 2.367a | 2.84f | 3.86ef |
| T29 | 2.244ab | 3.74c | 4.60cd |
| T22 | 2.133abc | 4.97b | 5.42b |
| T25 | 2.078a-d | 3.30c-f | 3.61efg |
| T12 | 2.067bcd | 3.22c-f | 3.48efg |
| T6 | 2.056bcd | 3.54cde | 3.74efg |
| T15 | 2.056bcd | 4.41b | 5.23bc |
| T8 | 1.944cde | 3.56cde | 4.13de |

| | | | |
|---|---|---|---|
| T7 | 1.811def | 3.68cd | 3.77efg |
| T21 | 1.744ef | 3.03ef | 3.24fg |
| T20 | 1.689efg | 3.10def | 3.23fg |
| T2 | 1.556fg | 3.44cde | 3.57efg |
| T30 | 1.411g | 3.11def | 3.57efg |
| T1 | 1.72ef | 2.84f | 3.18g |

On day 3, the control exhibited the highest mycelial growth (2.367), similar to most of the *Trichoderma* strains, with T4 showing the lowest growth (2.367), suggesting an early-stage equilibrium between the pathogen and the bio-agent. By day 6, notable differences emerged, with strains such as T4, T29, and T22 significantly reducing mycelial growth (2.84, 3.74, and 4.97, respectively), while the control still showed a higher value (6.86). The most inhibitory strains were T4, T29, and T22, which suppressed mycelial growth substantially.

On day 9, the bio-agent effects were more pronounced. T4 (3.86) showed the lowest mycelial growth, followed by T29 (4.60), demonstrating their consistent antagonism against *A. rolfsii*. Strains like T25 and T12 also showed significant inhibition, although not as much as T4 and T29. The control continued to have the highest mycelial growth (8.50), confirming the effectiveness of the *Trichoderma* strains in controlling the pathogen.

The results indicated the antagonistic effects of *Trichoderma* strains, particularly T4, T29, and T22, against *A. rolfsii*. These strains hold promise for further exploration in biological control strategies for managing tomato diseases caused by the pathogen. The statistical significance of the differences in mycelial growth, as indicated by Tukey's test at a 5% significance level, reinforces the effectiveness of these bio-agents in reducing pathogen growth.

### 3.3 Combined Effects of Application Methods and Bio-Agents

Table 4 shows the antagonistic potential of 14 *Trichoderma* strains against *A. rolfsii*, focusing on the effects of application methods and bio-agents on mycelial growth at days 6 and 9. Tukey's test at a 5% significance level revealed significant variations in growth depending on the bio-agent and the application method (Table 4).

**Table 4** Comparison of the Response of Application Methods and Bio-Agent on Mycelia Growth of *Anthelia rolfsii* at Days 6 and 9, Respectively

| Methods * bio-agent | Day 6 | Day 9 |
|---|---|---|
| SIM Control | 6.87a | 8.5a |
| SIM T4 | 2.57o-t | 3.23k-p |
| SIM T12 | 2.40p-t | 3.17l-q |
| SIM T2 | 3.37h-q | 3.43j-o |
| SIM T20 | 3.70g-o | 3.73i-o |
| SIM T21 | 2.96l-s | 3.20l-p |
| SIM T22 | 5.00c-f | 5.03d-i |
| SIM T25 | 3.37h-q | 3.47j-o |
| SIM T29 | 3.90e-m | 4.30f-l |
| SIM T30 | 3.00k-r | 3.16l-q |
| SIM T1 | 2.90l-s | 2.557o-r |
| SIM T6 | 3.30i-q | 3.80i-o |
| SIM T7 | 3.77g-n | 4.00h-n |
| SIM T8 | 4.23d-j | 5.43d-g |
| SIMT15 | 4.20d-k | 6.00bcd |

On day 6, the control treatment (SIM Control) exhibited the highest mycelial growth (6.87), consistent with untreated *A. rolfsii*. In contrast, the bio-agent treatments significantly reduced growth, with T4 (2.57) and T12 (2.40) showing the

lowest values. These strains demonstrated strong antagonistic effects, possibly due to their ability to inhibit or outcompete the pathogen early. Other strains, such as T21 (2.96) and T1 (2.90), also showed lower growth than the control, confirming the antagonistic potential of the *Trichoderma* strains.

By day 9, the pattern persisted, with the control still showing the highest mycelial growth (8.50). The bio-agent treatments maintained their inhibitory effects, with strains like T4 (3.23) and T12 (3.17) continuing to reduce *A. rolfsii* growth. Other strains, such as T22 (5.03) and T8 (5.43), exhibited significant suppression, though less pronounced. These results suggest that T4 and T12 can inhibit *A. rolfsii*, vital for effective biological control strategies.

The comparison of application methods further emphasized that specific *Trichoderma* strains, particularly T4 and T12, consistently exhibited more substantial antagonistic effects. Overall, the study highlights the potential of using *Trichoderma* strains to control tomato pathogens, with promising strains for further research and application in agricultural pest management.

These findings showed the importance of bio-agent selection and application methods in managing fungal pathogens, offering valuable insights into how these factors influence *A. rolfsii* growth.

Table 5 shows the results for the antagonistic potential of the 14 *Trichoderma* strains against *A. rolfsii*, focusing on comparing the effects of different application methods and bio-agents on mycelial growth at days 6 and 9. The results, analyzed using Tukey's test at a 5% probability level, reveal significant differences in the mycelial growth of *A. rolfsii* when treated with various *Trichoderma* strains compared to the control (Table 5).

**Table 5** Comparison Response of Application Methods and Bio-Agent on Mycelia Growth of *A. rolfsii* at Days 6 and 9

| Methods * bio-agent | Day 6 | Day 9 |
|---|---|---|
| PRO Control | 6.87a | 8.5a |
| PRO T1 | 2.40p-t | 2.53o-r |
| PRO T12 | 2.03rst | 2.03pqr |
| PRO T15 | 3.27j-r | 3.57j-o |
| PRO T2 | 2.50p-t | 2.83m-q |
| PRO T20 | 1.77st | 1.83pqr |
| PRO T21 | 1.87st | 2.03pqr |
| PRO T22 | 3.53g-p | 4.07h-n |
| PRO T25 | 2.83l-s | 2.90m-q |
| PRO T29 | 2.27rst | 2.50o-r |
| PRO T30 | 2.37q-t | 3.23k-p |
| PRO T4 | 1.60t | 1.4o-r |
| PRO T6 | 2.83l-s | 2.83m-q |
| PRO T7 | 2.73p-t | 2.73n-r |
| PRO T8 | 2.73n-t | 2.90m-q |

On day 6, the control treatment (PRO Control) exhibited the highest mycelial growth (6.87), consistent with untreated *A. rolfsii*. The bio-agent treatments significantly reduced pathogen growth, with strains T4 (1.60) and T20 (1.77) showing the lowest growth. These strains demonstrated strong antagonistic effects, effectively suppressing *A. rolfsii* growth. Strains such as T1 (2.40) and T12 (2.03) also showed notable reductions, further underscoring the antagonistic potential of these *Trichoderma* strains.

By day 9, the control still exhibited the highest mycelial growth (8.50), but the bio-agent treatments continued to exert inhibitory effects. T4 (1.40) remained the most effective, followed by strains T12 (2.03), T1 (2.53), and T30 (3.23), which also demonstrated significant suppression of *A. rolfsii*. Other strains, such as T22 (4.07) and T25 (2.90), exhibited some inhibition, but to a lesser degree than the leading strains.

The comparison of application methods and bio-agent combinations emphasized the effectiveness of certain *Trichoderma* strains, particularly T4, in significantly reducing mycelial growth. These findings suggest that bio-agents, particularly T4, can sustain the inhibition of *A. rolfsii*, highlighting their potential as candidates for biological control strategies against tomato pathogens.

As shown in Table 4, the results show the critical role of the choice of *Trichoderma* strain and the application method in controlling *A. rolfsii*. Strains such as T4 and T12 show considerable antagonistic potential, making them promising candidates for further research and practical applications in integrated pest management strategies.

Table 6 shows the in-vitro antagonistic potential of 14 *Trichoderma* strains against *A. rolfsii* in tomatoes, focusing on mycelial growth inhibition at days 6 and 9. Tukey's 5% probability level test revealed significant variations in pathogen suppression across strains and application methods (Table 6).

**Table 6** Comparison Response of Application Methods and Bio-Agent on Mycelia Growth of *A. rolfsii* at Days 6 and 9

| Methods * bio-agent | Day 6 | Day 9 |
|---|---|---|
| CUR Control | 6.87a | 8.5a |
| CUR T1 | 4.70c-g | 5.80cde |
| CUR T12 | 5.23bcd | 5.23d-h |
| CUR T15 | 5.77abc | 6.13bcd |
| CUR T2 | 4.47d-j | 4.43f-l |
| CUR T20 | 3.83f-m | 4.13g-m |
| CUR T21 | 4.27d-j | 4.50e-l |
| CUR T22 | 6.37ab | 7.17ab |
| CUR T25 | 3.70g-o | 4.47e-l |
| CUR T29 | 5.07cde | 7.0bc |
| CUR T30 | 3.97e-l | 4.30f-l |
| CUR T4 | 4.03d-l | 5.57def |
| CUR T6 | 4.50d-i | 4.60e-j |
| CUR T7 | 4.53d-h | 4.56e-k |
| CUR T8 | 3.70g-o | 5.43d-g |

At day 6, the control treatment (CUR CONTROL) recorded the highest mycelial growth (6.87), indicating uninhibited pathogen proliferation. In contrast, strains such as T20 (3.83), T25 (3.70), and T8 (3.70) exhibited strong antagonistic potential, significantly reducing mycelial growth. Strains like T1 (4.70) and T15 (5.77) showed moderate inhibition, while T22 (6.37) was less effective.

By day 9, the control remained the highest (8.5), confirming the absence of inhibition. Strains T20 (4.13), T25 (4.47), and T30 (4.30) sustained their inhibitory effects, with T20 remarkably consistent over time. Intermediate reductions were observed with T4 (5.57) and T1 (5.80), while T22 (7.17) and T29 (7.0) demonstrated lower efficacy.

These results emphasize the variability in antagonistic potential among *Trichoderma* strains. Effective strains like T20, T25, and T8 show promise for biological control strategies, while less effective strains (e.g., T22 and T29) may require further optimization. Strain selection and application methods are critical in developing sustainable management approaches for *A. rolfsii*. Future research should investigate the mechanisms driving these effects and assess the field applicability of the most effective strains.

The in-vitro experiment under different inoculation methods revealed varying mechanisms of action among the *Trichoderma* isolates. The ability of *Trichoderma* spp. to proliferate, covering the media surface within 3–4 days and overtaking the pathogenic fungus, irrespective of the inoculation method, is a mechanism described as mycoparasitism. This mechanism was observed in *Trichoderma longibrachiatum* isolates (T1, T6, T15, T18, T29, and T30). Several *Trichoderma* isolates, under various inoculation methods, exhibited mycelial inhibition of *A. rolfsii*, with percentage inhibition ranging from 64% to 52%. This mechanism, described as antagonism, was observed in *Trichoderma asperellum* isolates (AAF5, AAF22, AAF20, AAF19). The prophylactic approach in which *Trichoderma* strains was inoculated ahead of the pathogenic fungus recorded a higher inhibition percentage, this could be due to the ability of *Trichoderma* species to grow very fast in filling the agar plate. It could also be as a result of the possible secretion of secondary metabolites by *Trichoderma* spp. which antagonized the mycelia of A. *rolfsii* and inhibited its growth. This finding was reported [21, 22] that bio-control agents may grow faster or use its food source more efficiently than the pathogen, thereby out crowding the pathogen and taking over the growing surface, and that inhibition could probably also be due to the secretion of extracellular cell-degrading enzymes such as Chitinase B-1, 3-Glucanase, Cellulose and Lectin which could assist in mycoparasitism.

Under the therapeutic approach, *A. rolfsii* demonstrated the ability to establish faster than *Trichoderma*, which was used to assess the latter's capacity to control the pathogenic fungus. It was noted that *A. rolfsii* took advantage of its early establishment to parasitize specific slow-growing *Trichoderma* isolates (*T. reesei* and *T. ghanense*). However, other *Trichoderma* isolates counteracted this initial pathogenic growth, creating inhibition zones.

The frequent labouratory observation of *Trichoderma* hyphae wrapping around the pathogen's hyphae strongly indicates mycoparasitism as a critical mechanism in suppressing *A. rolfsii*. (Figure 4)

These structural modifications provide visual evidence of the antagonistic interactions and the ability of *Trichoderma* to disrupt the pathogen's growth and survival. However, [23] reported that interaction by *Trichoderma* strains begins before the two organisms (the antagonist and the pathogen) come into contact through production of sensing enzymes

and secondary metabolites that release cell wall fragments from the hyphae of the target pathogen. The possible secretion of secondary metabolites by *Trichoderma* strains in the inhibition of mycelial growth of pathogenic fungi agreed with the report by [24]. The two researchers stated that the inhibition of pathogen may be attributed to the production of secondary metabolites by antagonist (Trichoderma) such as Glioviridin, Viridin and Gliotoxin. Similarly, [25] revealed that inhibitory effect from *Trichoderma* spp was probably due to hyperparasitism/ mycoparasitism, competition for space, nutrient source and antagonistic biochemical produced and released into the environment by the fungus, leading to actual parasitism and coiling of the *Trichoderma* fungus around the pathogen. Additionally, secondary metabolites' role in Trichoderma's antagonistic activity was evident. Volatile organic compounds (VOCs), such as 6-pentyl-α-pyrone, and non-volatile metabolites, including gliotoxin and viridin, inhibited *A. rolfsii*. Strains producing higher concentrations of these metabolites effectively suppressed the pathogen's growth, inhibited spore germination, and reduced fungal propagule viability. The antifungal properties of these metabolites highlight their contribution to the multifaceted mechanisms employed by *Trichoderma* in pathogen suppression.

The implications of these findings for tomato cultivation are significant. Utilizing *Trichoderma* strains as biological control agents offers a sustainable alternative to chemical fungicides, reducing pesticide residues and mitigating environmental contamination. Moreover, applying *Trichoderma* as a seed treatment or soil amendment can improve plant health by promoting root growth and inducing systemic resistance to pathogens. These attributes underscore the potential of *Trichoderma* in integrated disease management programs, contributing to sustainable agriculture, enhanced crop yields, and reduced reliance on chemical inputs in tomato cultivation.

## 4 CONCLUSION

This study demonstrates the promising in-vitro antagonistic potential of selected *Trichoderma* strains against *A. rolfsii*, a significant pathogen in tomato cultivation. Prophylactic inoculation proved most effective, significantly reducing *A. rolfsii* mycelial growth compared to simultaneous and curative applications, emphasizing the advantage of early intervention in leveraging the competitive and antagonistic mechanisms of *Trichoderma* spp. Strains T4, T29, and T22 consistently exhibited inhibitory solid effects over the 9-day observation period, highlighting their suitability as candidates for biocontrol strategies. The variation in efficacy among strains and application methods suggests the need for tailored approaches to optimize the biocontrol potential of *Trichoderma* spp. Integrating *Trichoderma* into sustainable tomato production practices offers a viable alternative to chemical fungicides. Future research should focus on field trials to validate these findings under natural conditions, assess compatibility with other crop management practices, and expand their application to other soil-borne pathogens. Such efforts will advance eco-friendly agricultural disease management.

## COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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## REFERENCES

[1] Dorais M, Ehret D L, Papadopoulos A P. Tomato (Solanum lycopersicum) health components: From the seed to the consumer. Phytochemistry Reviews, 2008, 7(2): 231–250. DOI: 10.1007/s11101-007-9085-x.

[2] Ali M Y, Sina A A I, Khandker S S, et al. Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. Foods (Basel, Switzerland), 2020, 10(1). DOI: 10.3390/foods10010045.

[3] Paul S K, Gupta D R, Mahapatra C K, et al. Morpho-molecular, cultural and pathological characterization of Athelia rolfsii causing southern blight disease on common bean. Heliyon, 2023, 9(5): e16136. DOI: 10.1016/j.heliyon.2023.e16136.

[4] Patra G K, Acharya G K, Panigrahi J, et al. The soil-borne fungal pathogen Athelia rolfsii: Past, present, and future concern in legumes. Folia Microbiologica, 2023, 68(5): 677–690. DOI: 10.1007/s12223-023-01086-4.

[5] Paparu P, Acur A, Kato F, et al. Morphological and pathogenic characterization of Sclerotium rolfsii, the causal agent of southern blight disease on common bean in Uganda. Plant Disease, 2020 104(8): 2130–2137. DOI: 10.1094/pdis-10-19-2144-re.

[6] Sun S, Sun F, Deng D, et al. First report of southern blight of mung bean caused by Sclerotium rolfsii in China. Crop Protection, 2020, 130: 105055. DOI: 10.1016/j.cropro.2019.105055.

[7] Dade A, Kihara J, Ueno M. Control of tomato southern blight caused by Athelia rolfsii (syn. Sclerotium rolfsii) using the soil isolate Streptomyces sasae strain GT4041. Journal of General Plant Pathology, 2023, 89(3): 159–169. DOI: 10.1007/s10327-023-01122-8.

[8] Dafny Yelin, M, Graph S, Rabinovitz O, et al. Optimal treatment against Athelia rolfsii rot in processing tomatoes using pesticide and rootstock against key stages of the disease. Crop Protection, 2023, 176: 106480. DOI: 10.1016/j.cropro.2023.106480.
[9] Ahmad I, Malak H A, Abulreesh H H. Environmental antimicrobial resistance and its drivers: A potential threat to public health. Joural of Global Antimicrobial Resistance, 2021, 27. DOI: 10.1016/j.jgar.2021.08.001.
[10] Haq I U, Rahim K, Yahya G, et al. Eco-smart biocontrol strategies utilizing potent microbes for sustainable management of phytopathogenic diseases. Biotechnology Reports, 2024, 44: e00859. DOI: 10.1016/j.btre.2024.e00859.
[11] Panchalingam H, Powell D, Adra C, et al. Assessing the various antagonistic mechanisms of Trichoderma strains against the brown root rot pathogen Pyrrhoderma noxium infecting heritage fig trees. Journal of Fungi, 2022, 8(10)A: 1105. DOI: 10.3390/jof8101105.
[12] Mukesh S, Sonika P, Mohammad S, et al. Trichoderma: A magical weapon against soil-borne pathogens. African Journal of Agricultural Research, 2015, 10(50): 4591–4598. DOI: 10.5897/ajar2015.10192.
[13] Yao X, Guo H, Zhang K, et al. Trichoderma and its role in biological control of plant fungal and nematode disease. Frontiers in Microbiology, 2023, 14. DOI: 10.3389/fmicb.2023.1160551.
[14] Jegathambi V, Wijeratnam R S W, Wijesunder R L C. Effect of Trichoderma sp. on Sclerotium rolfsii, the causative agent of collar rot on Zamioculcas zamiifolia and an on-farm method to mass produce Trichoderma species. Plant Pathology Journal, 2010, 9(2): 47–55. DOI: 10.3923/ppj.2010.47.55.
[15] Ghali M, Maha Ben Jaballah, Nejla Ben Arfa, et al. Analysis of factors that influence adoption of agroecological practices in viticulture. Review of Agricultural, Food and Environmental Studies, 2022, 103(3): 179–209. DOI: 10.1007/s41130-022-00171-5.
[16] Hirsch C, Schildknecht S. In vitro research reproducibility: Keeping up high standards. Frontiers in Pharmacology, 2019, 10. DOI: 10.3389/fphar.2019.01484
[17] Olowe O M, Nicola L, Asemoloye M D, et al. Characterization and antagonistic potentials of selected rhizosphere Trichoderma species against some Fusarium species. Frontiers in Microbiology, 2022, 13. DOI: 10.3389/fmicb.2022.985874.
[18] Yadav D, Adhikari A, Babita D, et al. In-vitro efficacy of Trichoderma isolates on Sclerotium rolfsii causing collar rot of chili. Asian Journal of Agriculture, 2022, 6(2). DOI: 10.13057/asianjagric/g060206.
[19] Talavera-Ortiz A, Chaverri P, Diaz-Godinez G, et al. Mycelial inhibition of Trichoderma spp. isolated from the cultivation of Pleurotus ostreatus with an extract of Pycnoporus sp. Acta Botanica Mexicana, 2020, 127. DOI: 10.21829/abm127.2020.1537.
[20] Safae G, Chair I, Zineb B, et al. Harnessing Trichoderma spp.: A promising approach to control apple scab disease. Pathogens, 2024, 13(9): 752. DOI: 10.3390/pathogens13090752.
[21] Rodriguez-Kabana R. Enzymatic interactions of Sclerotium rolfsii and Trichoderma viride in mixed soil culture. Phytopathology, 1969, 59: 910–921.
[22] Samuels G J. Centenary review, Trichoderma: A review of biology and systematic of genus. Mycological Research, 1996, 100(8): 923–935.
[23] Harman G E. Overview of mechanisms and uses of Trichoderma spp. Phytopathology, 2006, 96(2): 190–194.
[24] Muhammad S, Amusa N A. In-vitro inhibition of growth of some seedling blight-inducing pathogens by compost-inhabiting microbes. African Journal of Biotechnology, 2003, 2(6): 161–164.
[25] Islam M T, Faruq A N. Effects of selected soil amendments on seed germination, seedling growth and control of damping-off of chilli seedlings. Journal of Sher-e-Bangla Agricultural University, 2008, 2(2): 12–16.