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# Antagonistic Potential and Metabolomic Profiling of *Clonostachys rosea* against *Alternaria* spp. causing Early Blight in Tomato

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## Abstract

**Tomato cultivation faces significant challenges from foliar fungal diseases such as early blight caused by *Alternaria* spp., resulting in substantial reduction in yield. In this study, we examined the suppressive effects of the antagonistic fungus *Clonostachys rosea* on early blight of tomato under controlled experimental conditions. The results of dual culture experiments revealed the inhibitory effects of five *C. rosea* isolates on *Alternaria* spp., with the TNAU CR04 isolate exhibiting the highest inhibition (77.22%). Scanning electron microscopy provided good insight into antagonistic effects of *C. rosea* against *A. alternata*, revealing hyphal interactions and structural alterations. Further investigations focused on the suppression of *Alternaria alternata* mycelial growth (86.78%) by culture filtrates of *C. rosea*. The results revealed that TNAU CR04 at a 50% concentration strongly inhibited mycelial growth. Through GC-MS analysis, we identified key compounds involved in the interaction between *C. rosea* TNAU CR04 and *A. alternata*, shedding light on metabolic pathways and defense mechanisms. Overall, this study showed that *C. rosea* and its metabolites strongly act against *Alternaria* spp., revealing its mode of action and mechanisms underlying disease suppression in tomato plants.**

**Keywords:** Plant Pathogenic Fungi, Antifungal Activity, Metabolites, Pathways, Interaction, Defense

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## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a commonly cultivated vegetable from the Solanaceae family. It is a major crop grown extensively in China, India, the United States, Turkey and Italy. China ranks as the top producer, whereas United States and Mexico are key players in export markets. Its adaptability to both temperate and tropical regions makes it a crop of global importance. It is considered to be a crucial "protective food" because of its rich nutritional profile.[1] Various biotic obstacles including viruses, oomycetes, bacteria, fungi and root-knot nematodes limit the yield of tomatoes. Early blight induced by *Alternaria solani* and *Alternaria alternata* is one of the most harmful and prevalent diseases among the numerous biotic factors that degrade tomato quality and yield globally.[2] Due to its detrimental effects, this pathogen has received significant attention over the years, primarily because of the substantial yield loss of about 80%.[3] To manage early blight disease, farmers predominantly depend on fungicides. However, the excessive use and reliance on these chemicals have sparked concerns regarding food safety, resistance to pathogens, degradation of soil and environmental sustainability. Hence, innovative disease management strategies that promote sustainable agriculture without compromising the environment need to be developed.[4] Various mycoparasitic fungi can counteract *Alternaria* species in plants[5,6] and their utilization as biological control agents against foliar diseases induced by *Alternaria* is underexplored and needs further investigation.

*Clonostachys rosea* (*Gliocladium roseum*)[7] was initially identified as the anamorph of *Bionectria ochroleuca*, a teleomorph based on its morphology and supported by DNA sequence data. In temperate and tropical regions, the *Clonostachys* genus harbors predatory fungi that exhibit versatile lifestyles and thrive as soilborne, saprophytic and endophytic organisms, with potential implications for ecological dynamics and agricultural practices. Antagonistic isolates of *C. rosea* are used as biological control measures against various significant foliar plant diseases, including gray mold (*Botrytis cinerea*), gray leaf spot in maize (*Cercospora zeae-maydis)*,[8] leaf blotch in barley (*Bipolaris sorokiniana),*[9] leaf spots (*Alternaria* spp.) and various other pathogens.[5] While engaging in antagonistic mechanisms, isolates of *C. rosea* generate and release antimicrobial compounds and cell wall-degrading enzymes.[10,11] These fungi can also strongly tolerate pathogenic toxins and chemical fungicides, probably due to the presence and activity of numerous membrane-associated transporters.[12]

The effectiveness of *C. rosea* strains in terms of biocontrol activity has been widely established, as indicated by their ability to generate a broad spectrum of metabolic compounds. These include enzymatically active proteins, small molecules linked with fungal or plant cell walls, and various secondary metabolites. Moreover, these metabolites can stimulate the defensive systems of plants, supporting their resilience against infections.[13] Therefore, this study was performed to evaluate the effects of *C. rosea* on the development of leaf spots on tomatoes caused by *Alternaria* spp. under laboratory conditions. This study also involved metabolomic analyses to identify key metabolites and pathways of *C. rosea* involved in pathogen inhibition.

## MATERIALS AND METHODS

### Isolation, identification and characterization of *Alternaria* spp.

Tomato leaves showing characteristic early blight infections were collected from several major tomato cultivation areas in Tamil Nadu, India. After confirming the presence of fungal spores, isolation was performed and *Alternaria* spp. were isolated from infected leaves using the tissue segment method. The pathogen was identified by morphological methods and sequencing of the ITS region. The virulence of these strains was subsequently tested and confirmed through Koch's postulates. The most virulent isolate in pathogenicity studies was selected for assessing antagonistic activity. Following purification on PDA media, fungal cultures were stored at 4°C for subsequent studies.

### Collection of biocontrol organisms

Five strains of *C. rosea*, were designated as TNAU CR01 (Accession No.: OK147890),

TNAU: CR02 (Accession No.: ON926972), TNAU: CR03 (Accession No.: ON926975), TNAU: CR04 (Accession No.: ON926968) and TNAU CR05 (Accession No.: ON926986), which were characterized by Gowrisri *et al*. were acquired from Culture Collection Centre, Department of Plant Pathology, TNAU, Coimbatore, Tamil Nadu, India. These isolates were then grown on PDA media at 27°C for seven days, and the actively growing colonies were stored at 4°C for future experiments.

**Effect of *Clonostachys rosea* on the growth of *Alternaria* spp. *in vitro***

**Dual culture technique**

The ability of *C. rosea* to suppress pathogen growth was recorded *in vitro* using a dual culture technique. A 6 mm mycelial disc was excised from the rapidly growing edges of 12-day-old cultures of *C. rosea* using a sterilized cork borer. The mycelial disc was inoculated onto a sterilized PDA medium placed 1 cm away from the edge of the Petri dish. Concurrently, the fungal mycelial disc of the pathogen was placed at the far end of the Petri dish, opposite the *C. rosea* disc. Petri plates solely grown with the pathogen were used as controls. The treatments were replicated four times and the plates were kept at 28 ± 2°C. The inhibition caused by *C. rosea* was assessed by calculating the percentage (%) of mycelial growth inhibition using the formula described by Rapilly (1968).[14]

$$\% I = [(C-T)/C] \times 100$$

Here,

I= percentage inhibition of pathogen by antagonist

C= radial growth of the pathogen in the control (mm)

T= radial growth of the pathogen in the treatment (mm)

**Scanning electron microscopy**

We performed SEM to observe hyphal interactions between *C. rosea* and *Alternaria* spp. First, fungal disks (5 mm) of both microorganisms were grown on PDA for 8-10 days. These cultures were observed under a light microscope to look at the early contact stage, after which 1 cm agar blocks containing the labeled mycelial interactions were excised for SEM sample preparation. Control blocks were collected from the two peripheries of the blocks comprising the antagonist and the pathogen and these blocks were fixed with osmium tetroxide. Images were captured using a FAI QUANTA 250 Model SEM at 15 kV in the Department of Nanotechnology, TNAU, Coimbatore, Tamil Nadu, India. Through SEM imaging, the mycelial growth of *C. rosea* over *Alternaria* spp. was documented.

**Preparation of crude culture filtrates of *Clonostachys rosea***

To prepare crude culture filtrates, a 5 mm disc from a 12-day culture of *C. rosea* grown on PDA medium was added to Erlenmeyer flasks containing 100 mL of potato dextrose broth (PDB). The conical flasks containing culture broth were then kept at 28 ± 2°C for 15 days. The fungal extract was acquired through filtration using Whatman filter paper No. 4. After filtration, the fungal filtrate was stored at 4°C for further use.

**Poisoned food technique**

To evaluate the *in vitro* inhibition of *Alternaria* spp. mycelium, the filtrates of *C. rosea* were mixed with PDA medium. PDA plates were augmented with culture filtrate (CF) from *C. rosea*, which consisted of 20 ml of PDA per Petri dish at various percentages (20%, 30%, 40% and 50%). Next, the plates were inoculated with a 5 mm mycelial disc from the periphery of a seven day-old *Alternaria* spp. culture. Each treatment was repeated four times. For comparison, control plates devoid of culture filtrate suspension were also included. The plates were incubated at 28°C for 7-10 days until the pathogen reached the periphery of the control plate. The colony diameter of each plate was subsequently recorded and compared to that of the control.

**Metabolomic analysis of antagonist, pathogen and their interactions**

Crude culture metabolites from the potent strain of *C. rosea.* TNAU CR04 which presented the greatest reduction in mycelial development of the pathogen was selected from dual culture to determine the non-volatile organic compounds or metabolites responsible for suppressing *Alternaria* spp. PDB broth was inoculated with the respective antagonist and pathogen to determine their interaction. The control broth was also maintained with only the antagonist or pathogen. Cultures were incubated at 28°C for 12-15 days, followed

by filtration through two layers of filter paper. An equal volume of ethyl acetate was added in to the extracted culture filtrate, and the mixture was incubated overnight in a shaker at 150 rpm. The ethyl acetate fraction was then separated using a separating funnel. The collected upper phase was evaporated under a rotary evaporator and dried. The dried residue was dissolved in 1 mL of HPLC-grade methanol and subjected to GC-MS analysis using a Perkin Elmer Clarus SQ 8C gas chromatograph-mass spectrometry equipment. The primary compounds were detected using of a computer-based method, and their mass spectrum was checked using the NIST library. Principal component analysis (PCA) was performed and a heat map was constructed for metabolite distribution patterns using R software. Metabolite set enrichment analysis and network-based analysis were conducted out using Metaboanalyst 6.0 and Cytoscape 3.3, respectively.

**Statistical analysis**

The data were statistically analyzed using R software version 2.14.1, where an ANOVA was conducted and the mean values were compared by Duncan Multiple Range Test.

## RESULTS

**Isolation, morphological and molecular identification of the pathogen**

*Alternaria* spp. were isolated from diseased tomato leaves collected from various regions of Tamil Nadu, and their pure culture was maintained on PDA media. The characterization of these isolates revealed predominantly gray or brown colonies with consistent growth patterns (Figure 1a). On the underside of the same medium, the colonies displayed central black coloration surrounded by a hazy yellowish-brown rim and white margins (Figure 1b). Microscopic examination revealed the presence of solitary conidia, varying from straight to slightly flexuous, muriform or ellipsoidal with a tapered beak (Figure 1c). These conidia were generally pale and occasionally branched. The pathogenic fungal isolates were confirmed by ITS sequence analysis. A 531 bp band was amplified with ITS primers,

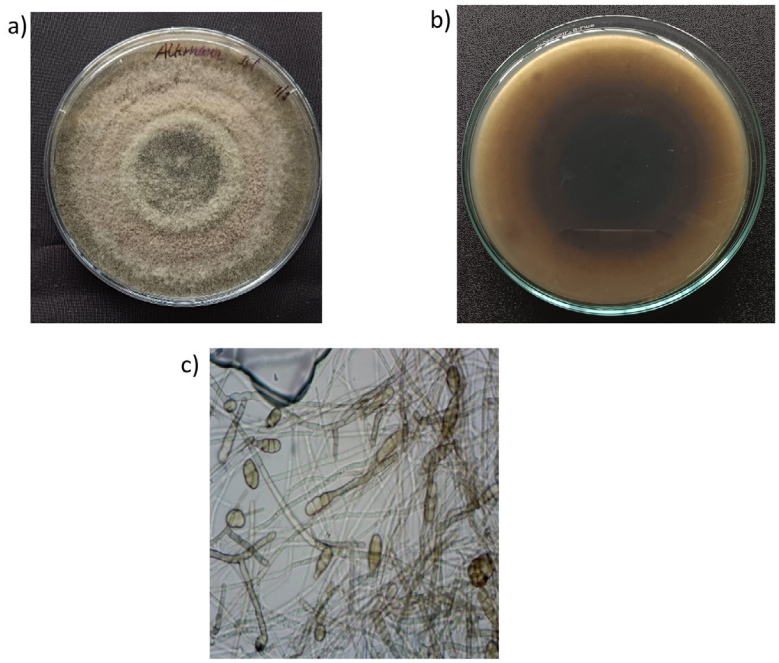


**Figure 1.** (a) Pure culture of *A. alternata* (CBE Alt 1) grown on PDA medium, displaying characteristic concentric rings; (b) Production of dark pigmentation by the pure culture of *A. alternata* on the back side of the PDA medium; (c) Microscopic view of *A. alternata* showing septate hyphae and muriform conidia

**Table 1.** Antagonistic effect of *C. rosea* on the mycelial growth of *A. alternata*

| Isolate | Radial mycelial growth (mm) | Mycelial inhibition % |
|---|---|---|
| TNAU CR01 | 42.300[d] | 53.18[d] (46.82) |
| TNAU CR02 | 44.667[e] | 50.33[e] (45.18) |
| TNAU CR03 | 37.967[c] | 57.78[c] (49.47) |
| TNAU CR04 | 23.267[a] | 77.22[a] (59.48) |
| TNAU CR05 | 27.900[b] | 69.00[b] (56.17) |
| Control | 90.00[f] | 0.00[f] (0.28) |
| CD (p = 0.05) | 1.775 | 0.879 |
| SED | 0.814 | 0.403 |

**Table 2.** Inhibition rate of culture filtrates of *Clonostachys rosea* TNAU CR04 against *Alternaria alternata* by poisoned food technique

| Culture filtrate concen. (%) | Radial mycelial growth (mm) | Mycelial inhibition % |
|---|---|---|
| 20% | 27.12[d] | 71.89[c] (57.982) |
| 30% | 19.73[c] | 78.11[b] (62.104) |
| 40% | 13.46[b] | 85.11[a] (67.36) |
| 50% | 11.90[a] | 86.78a (68.72) |
| Control | 90.00[f] | 0.00[d] (0.286) |
| CD (p = 0.05) | 1.345 | 2.372 |
| SED | 0.604 | 1.31 |

and sequence alignment was performed using the Basic Local Alignment Search Tool (BLAST) to assess the phylogenetic relationships of the strains. The fungal strains showed 100% identity with both *A. alternata* and *A. solani*. The ITS sequences of *A. alternata* and *A. solani* were submitted to GenBank under the accession numbers PP763705, PP763571, PP760124, PP767753, PP767412 and PP784700. In pathogenicity studies, the strain *A. alternata* (CBE Alt1- PP767412) was found to be more virulent, leading to its selection for further antagonistic assays.

### Efficacy of *Clonostachys rosea* isolates on *Alternaria* spp. *in vitro* (dual culture)

A dual culture assay was performed to determine the ability of *C. rosea* to suppress *A. alternata* CBE Alt1 growth *in vitro*. Five antagonistic isolates were tested against the early blight pathogen *A. alternata*. All *C. rosea* isolates

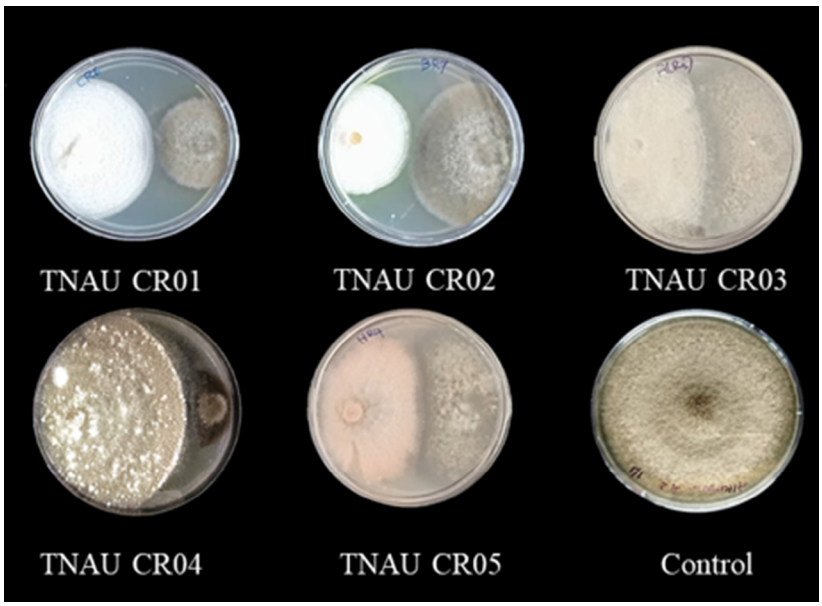


**Figure 2.** *In vitro* antagonistic potential of *C. rosea* isolates against *A. alternata* in dual-culture assay

inhibited the growth on *A. alternata*, with the *C. rosea* isolate TNAU CR04 showing the highest inhibition rate of 77.22%, followed by TNAU CR05, TNAU CR03 and TNAU CR01 with inhibition rates of 69.00%, 57.78% and 53.18% respectively. The lowest inhibitory effect was recorded for the *C. rosea* isolate TNAU CR02 (50.33%) (Table 1 and Figure 2). Antifungal testing confirmed that *C. rosea* can hinder the growth of *A. alternata*, with TNAU CR04 being the most effective.

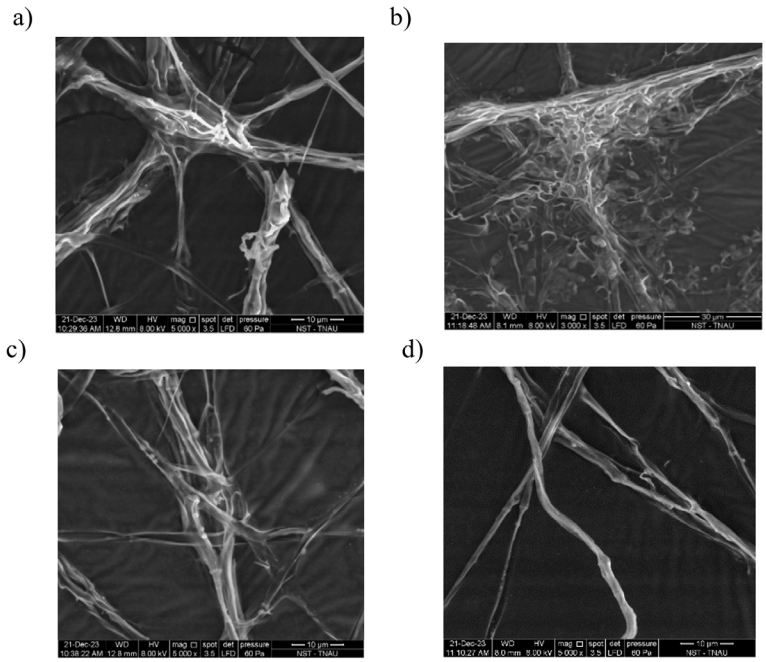


**Figure 3.** SEM analysis of mycoparasitic *C. rosea* TNAU CR04 interacting with hyphae of *A. alternata* (a) Mycelia of *C. rosea* growing over *A. alternata* (b) Mycelial shrinkage and disintegration of mycelia of *A. alternata* (c) Conidiogenous cells with conidia of *C. rosea* emerging from hyphae of *A. alternata* (d) Control- hyphae of *A. alternata*

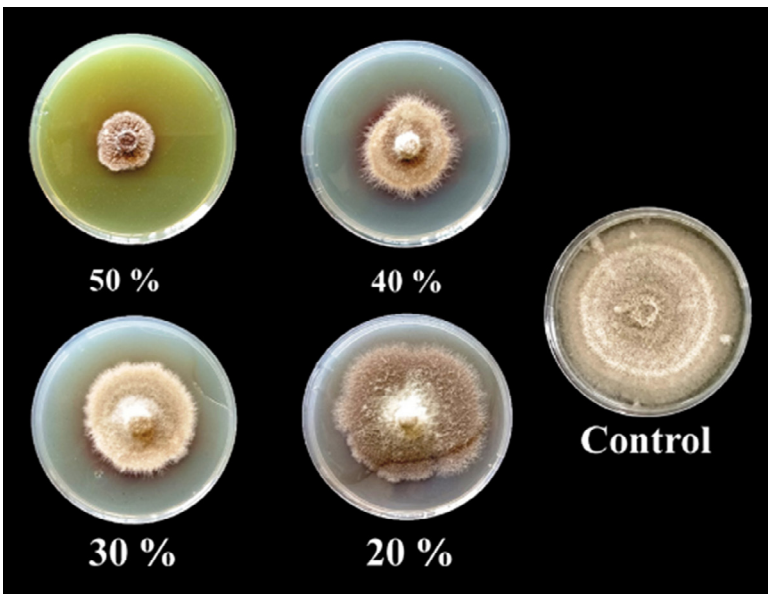


**Figure 4.** *In vitro* efficacy of *C. rosea* TNAU CR04 culture filtrates on mycelial growth inhibition of *A. alternata*

Consequently, TNAU CR04 was selected for further *in vitro* and *in vivo* studies.

**Scanning electron microscopy**

The mycoparasitic behavior of *C. rosea* TNAU CR04 toward *A. alternata* in dual culture was investigated via SEM. For observation, mycelial samples were scraped from the interaction zone of the dual plate. The hyphae of the antagonistic fungus *C. rosea* formed dense coils and tightly encircled the hyphae of *A. alternata*. This encircling led to a wrinkled appearance or collapse of the *A. alternata* hyphae. The hyphae of pathogen and the mycoparasitic hyphae were attached longitudinally. We also recorded several other structures, including hyphal depressions, hooks, small contact branches and coils in the interaction region (Figure 3).

**Mycelial growth inhibition by culture filtrates of *Clonostachys rosea* TNAU CR04**

**Poisoned food technique**

The effective *C. rosea* isolate TNAU CR04 was also studied to determine the potential of its non-volatile compounds. The percentage inhibition of the pathogenic isolate (CBE Alt1- PP767412) was calculated and compared to their full growth on control plates. Evaluation of these non-volatile components at different concentrations revealed different inhibitory activities against growth of pathogenic mycelia. TNAU CR04 at a 50% concentration of the culture filtrate exhibited the most potent inhibition against *Alternaria* spp., with an inhibition rate of 86.78% followed by the 40% concentration (85.11%). The culture filtrates at a concentration of 20% presented the lowest inhibition of about 71.89% (Table 2 and Figure 4).

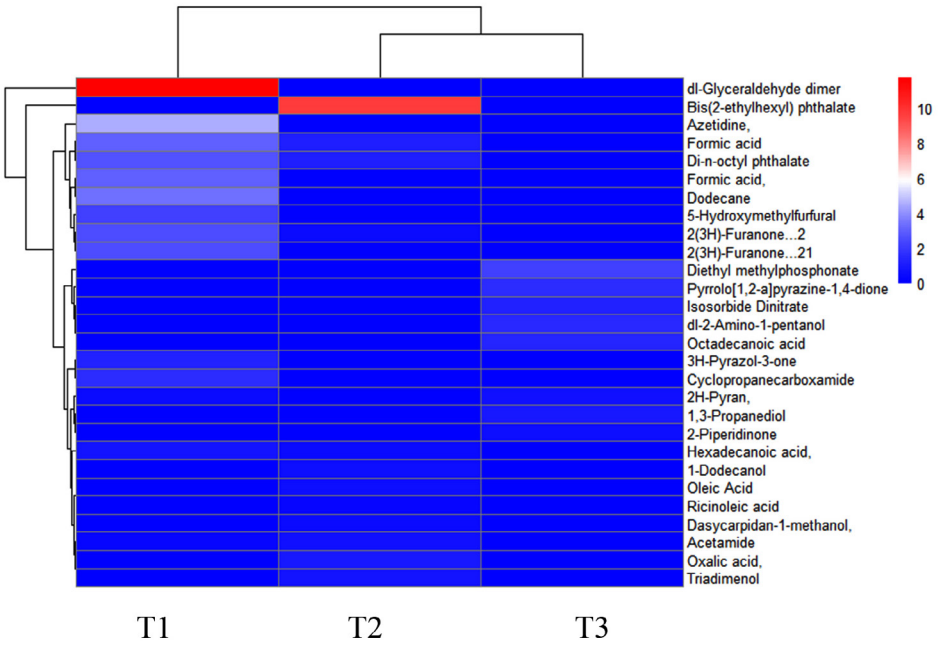


**Figure 5.** The heat map illustrates the secondary metabolites released by *C. rosea* TNAU CR04 and their interaction with *A. alternata*. The scale on the right ranges from 0 to 10. Blue represents low values (near 0), indicating low or no abundance of the compound in the treatment. Red represents high values (up to 10), indicating high abundance of the compound. The gradation from blue (low) to red (high) visually encodes the relative intensity of the measured compounds. T1- *C. rosea* + *A. alternata*, T2- *A. alternata* and T3- *C. rosea*

**Profiling of secondary metabolites of *Clonostachys rosea* TNAU CR04 and its interaction with *Alternaria alternata***

GC-MS analysis of non-volatile secondary metabolic compounds in *C. rosea* TNAU CR04 revealed the presence of formic acid, 2(3H)-furanone, Di-n-octyl phthalate and 5-hexadecenoic acid. These compounds were also detected during the interaction between *A. alternata* and *C. rosea*. However, non-volatile metabolites including dl-glyceraldehyde dimer, 5-hydroxymethylfurfural, acetidine and cyclopropanecarboxamide were exclusively detected during the association between *C. rosea* and *A. alternata*, and not when *C. rosea* was present alone. The heat map (Figure 5) delineated the presence or absence of compounds, as well as their upregulation and downregulation, highlighting interactions among them. The gradation from blue (low) to red (high) in the heat map visually represented the relative intensity of the measured compounds. Metabolite enrichment analysis revealed that specific pathways were upregulated during the interaction between antagonist and pathogen. When grown alone (Figure 6a), *C. rosea* prioritizes phenylalanine metabolism, a critical pathway for producing phenylpropanoids, which can enhance its biocontrol potential through the synthesis of antifungal compounds. During the interaction with *A. alternata* (Figure 6b), *C. rosea* exhibited a broader metabolic reconfiguration. The significant enrichment in butanoate and glycerolipid metabolism indicated a shift toward lipid and membrane-related processes, which may be critical for maintaining cellular integrity under the stress of fungal antagonism. Pathway maps with network diagrams were used to assess the relevance of the key pathways, providing insights into the significance of the results. The compounds present in the TCA cycle and the synthesis of terpenes are shown in Figure 7. Key enzymes such as citrate synthase, aconitase and malate dehydrogenase indicate active energy metabolism through the TCA cycle, whereas enzymes in the terpene biosynthesis pathway suggest the production of terpenoids, possibly for antifungal defense. Additionally, the presence of ATP-producing enzymes and redox-related enzymes such as NADH dehydrogenase, indicates the role of energy production and redox reactions in

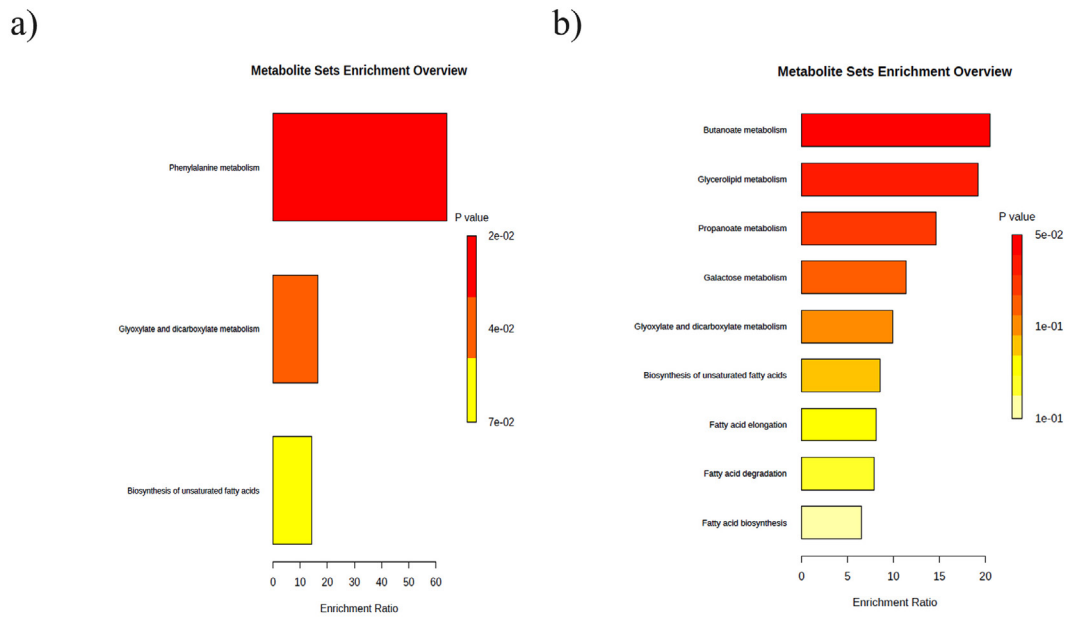


**Figure 6.** KEGG enrichment classification of non-volatile secondary metabolites for various treatments (a) KEGG enrichment classification of metabolites of *C. rosea* TNAU CR04 alone (b) KEGG enrichment classification of metabolites produced during the interaction between *C. rosea* TNAU CR04 and *A. alternata*

supporting this metabolic interaction. Overall, the analysis highlighted the metabolic coordination of *C. rosea* in utilizing both primary and secondary metabolic pathways during its interaction with *A. alternata*, likely contributing to its biocontrol efficacy.

## DISCUSSION

Tomato plants are frequently afflicted by early blight caused by *Alternaria* spp., which is a widespread leaf disease, that greatly threatens the health of plants. This disease reduces the size and number of fruits and causes economic losses in yield of up to 79%.[15,16] Fungicides serve as common chemical interventions used to effectively combat early blight disease. However, the utilization of chemicals to combat fungal infections in plants may not always be favorable due to associated risks. As an alternative approach, biocontrol agents comprising various microorganisms are preferred. These microorganisms are sustainable and have positive environmental effects, making them suitable for disease control in plants. *Clonostachys rosea*, known as a mycoparasite, that can antagonize various plant pathogens, such as *Alternaria* spp., *Bipolaris sorokiniana*, *Botrytis cinerea*, *Fusarium culmorum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum, Pythium* spp., and *Verticillium dahliae* as reported in studies by Yu and Sutton.,[17] Jensen *et al*.,[5] Huang,[18] Knudsen *et al*.,[19] McQuilken *et al*.,[20] Rodriguez *et al*.[11] Chatterton and Punja.[10] and Keinath *et al*.[21] Although many *Clonostachys* strains have been identified, only a small subset of fungi has been used in metabolomic profiling studies. Additionally, in this study we systematically investigated the inhibitory effect of *C. rosea* and its metabolites on the growth of *Alternaria* spp., via a combination of *in vitro* assays conducted in a laboratory environment.

The isolation and characterization of *Alternaria* spp. from diseased tomato leaves highlighted the prevalence and importance of this pathogen in agricultural settings. The observed colony morphology and growth patterns are consistent with previous descriptions of *Alternaria* species. The inhibitory effects observed under *in vitro* dual culture conditions across different *C. rosea* isolates, with isolate TNAU CR04 exhibiting the highest efficacy, revealed the promising biocontrol capabilities of this fungus. These results matched those of previous studies demonstrating the antagonistic activity of *C. rosea* toward plant pathogens, including seed-borne *Alternaria* spp. and *Sclerotinia sclerotiorum*.[5,11] SEM images demonstrated the mycoparasitic behavior of *C. rosea* toward *A. alternata*, with *C. rosea* forming dense coils around *A. alternata* hyphae, leading to their collapse. These findings highlighted the ability of *C. rosea* to effectively control organism towards plant pathogens.[22] This observation was also consistent with SEM observations of the association between *C. rosea* and *B. cinerea*, which revealed the penetration of conidia and germ tubes directly into *B. cinerea*. This penetration occurred without the formation of an appressorium, ultimately leading to cytoplasmic disintegration.[8] We also assessed the inhibitory effects of secondary metabolites released by *C. rosea* isolates against pathogens. The isolate TNAU CR04 demonstrated the greatest inhibition rate of 77.22% at a 50% concentration of culture filtrate. This finding indicated that *C. rosea* acts as an antagonistic fungus by secreting inhibitory compounds.[23,24] Han *et al*.[25] and Saraiva *et al*.[26] reported *C. rosea* generates diverse secondary metabolic compounds with strong antagonistic activity against plant pathogens. *Clonostachys* fungi produce copious amounts of various metabolites, including nitrogenous compounds, polyketide derivatives and terpenes. These metabolites exhibit a wide array of biological mechanisms including germicidal, insecticidal, nematicidal and cytocidal properties.[27-29] The identification and characterization of non-volatile organic metabolites in *C. rosea* and their interactions with *A. alternata* offer valuable insights into the biochemical dynamics of fungal interactions. GC-MS analysis revealed a spectrum of compounds, including formic acid, 2(3H)-furanone, Di-n-octyl phthalate and 5-hexadecenoic acid in *C. rosea*. During its interaction with *A. alternata*, additional compounds such as dl-glyceraldehyde dimer, 5-hydroxymethylfurfural, acetidine and cyclopropanecarboxamide were detected, indicating a shift in chemical composition.

A heat map showed significant metabolic shifts in *C. rosea* when interacting with *A. alternata*. KEGG analysis revealed that metabolites were enriched during the interaction between

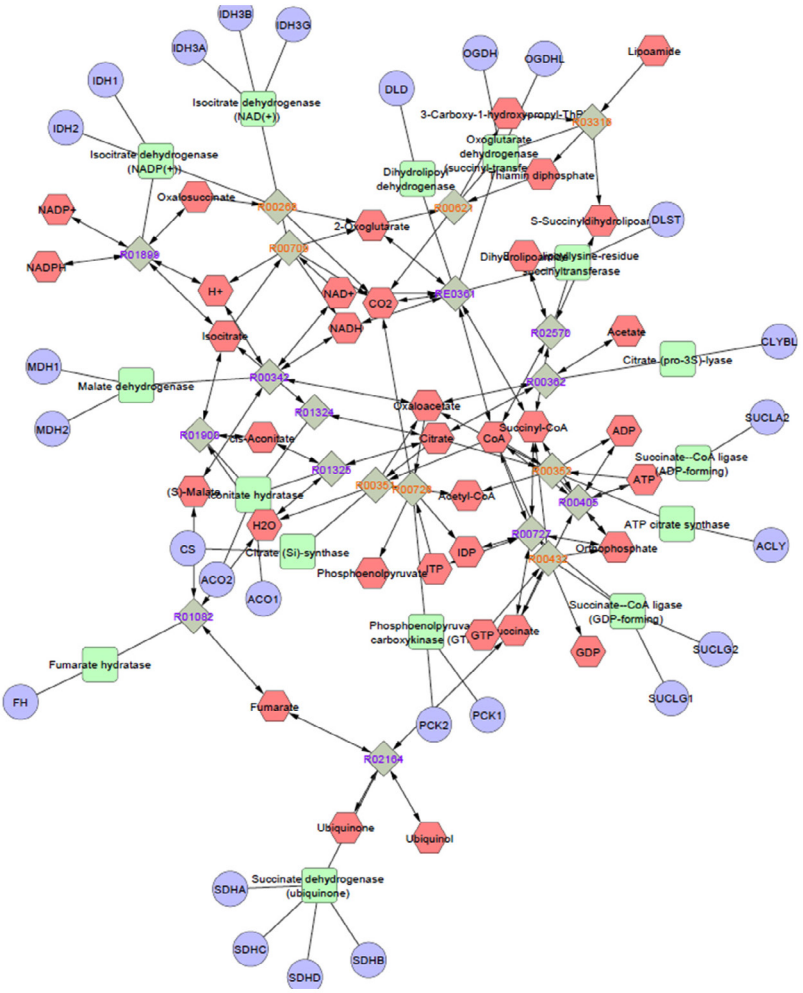


**Figure 7.** Pathway enrichment analysis. Various pathways involved in the interaction between the secondary metabolites of *C. rosea* TNAU CR04 and *A. alternata* was identified using Metscape in Cytoscape 3.3. Enzymes that participate in the biosynthesis of the TCA cycle and terpenes are indicated in green

*C. rosea* and *A. alternata* that participate in fatty acid-mediated and lipid-mediated signaling contribute to defense responses. These findings align to those of previous studies highlighting the metabolic shifts that occur in biocontrol fungi during pathogen antagonism.[30-32]

Pathway analysis depicted that metabolites from *C. rosea* play a crucial roles in enhancing plant immunity by regulating hormone signaling, influencing the tricarboxylic acid (TCA) cycle and stimulating the biosynthesis of defense-related metabolites. Similar results were found for other biocontrol agents such as *Trichoderma* spp., where energy metabolism is upregulated during antagonistic interactions to sustain the production of antifungal compounds and maintain cellular homeostasis.[33] Manganiello *et al.*[32] reported that *Trichoderma harzianum* stimulates the TCA cycle and the hexose monophosphate (HMP) pathway to increase tomato growth by increasing the activities of succinate dehydrogenase and glucose-6-phosphate dehydrogenase. In *C. rosea*, terpenoid production during interaction with *A. alternata* may increase its ability to suppress the growth of

this pathogen. Similar findings have been reported in other studies, where terpenoid production was linked to the biocontrol efficacy of fungal antagonists.[34] These findings can help understand fungal interactions and may aid in development of innovative biocontrol strategies.[25,35,36] We further determined the molecular mechanisms underlying the antagonistic efficacy of *C. rosea* and its culture filtrates, including the identification of key bioactive compounds and biosynthetic genes and elucidated of their modes of action and the interaction dynamics between *C. rosea* and *Alternaria* spp.

## CONCLUSION

To summarize, this study highlighted the promising potential of *C. rosea* as an effective antagonistic agent against plant pathogens using *Alternaria* spp. as a model system. By adopting a comprehensive approach involving *in vitro* assays, metabolomic profiling and detailed characterization of culture filtrates, we obtained significant insights into the inhibitory mechanisms of *C. rosea* against *Alternaria* spp. Our findings highlighted the multifaceted mode of action exhibited by *C. rosea*, including the production of inhibitory metabolites. In the future, further identification and characterization of the bioactive compounds, optimization of production methods and field trials to validate their efficacy in real-world agricultural settings needs to be performed. Overall, this study provided deeper insights into the antagonistic potential of *C. rosea* and laid the groundwork for its application in integrated disease management strategies, thus our findings may help to enhance crop productivity and sustainability.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

VS and SH conceptualized the study and performed validation. RK performed project administration, supervision and applied methodology. AA performed investigation and experiments. US and NS collected resources and performed data interpretation. US, NS and AA performed analysis. VS and SH reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

## FUNDING

None.

## DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

## ETHICS STATEMENT

Not applicable.

## REFERENCES

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