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The comparative effect of the *Trichoderma atroviride* and biopesticide Biocont in suppressing date palm leaf spot disease caused by *Alternaria alternata*

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Abstract

Objective

Biological control of phytopathogenic fungi is a hopeful plan, exclusively applying high-potency bioagents. This investigation aimed to compare the effectiveness of *Trichoderma atroviride* and the biopesticide Biocont in preventing the growth of *Alternaria alternata*, the causal agent of leaf spot disease in date palms, with *Trichoderma harzianum* as a comparative bioagent.

Materials and Methods

The pathogenicity of *A. alternata* was evaluated on leaves of three date palm cultivars (Barhi, Halawi, Sayer). The antagonistic effects of *T. atroviride* and *T. harzianum* against *A. alternata* were experimented at different temperatures applying the dual culture method. The percentage of growth prevention was calculated for the biological agents and the pathogenic fungus after 7 days of co-inoculation. The impression of temperature on the antagonistic interactions was evaluated, calculating the radial growth of *A. alternata* at 20°C and 30°C.

Results

A. alternata created leaf spot disease, with symptoms described by blackish-gray spots spreading across the leaf surface. The highest reduction in *A. alternata* growth was observed at 30°C, where *T. atroviride* restricted pathogen growth to 1.23 cm. The lowest reduction occurred at 20°C with *T. harzianum*, where *A. alternata* growth reached 3.03 cm. The highest prevention rate of *A.*

alternata (81.31%) was achieved at 30°C in the presence of *T. atroviride*, while the lowest prevention rate (39.33%) was document at 20°C with *T. harzianum*. This investigation affirmed that *Trichoderma* species generate secondary metabolites with antagonistic effects against plant pathogens. Additionally, these metabolites can activate resistance mechanisms in plants, enhancing protection against diseases.

Conclusions

The results demonstrate that *Trichoderma* species synthesize diverse secondary metabolites effective in suppressing plant pathogens. *T. atroviride* exhibited superior prevention of *A. alternata* at 30°C compared to *T. harzianum*, which showed the lowest prevention at 20°C. These results support the selection of *T. atroviride* as an effective biological control agent for reducing date palm leaf spot disease. The investigation suggests potential for developing *T. atroviride*-based biocontrol agents tailored to the environmental situations of date palm cultivation.

Keywords: Biological control, date palm, plant resistance, secondary metabolites

Paper Type: Research Paper.

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Introduction

Date palm trees (*Phoenix dactylifera* L.) are affected by a broad range of agricultural pests and pathogens—estimated at over 280 species, containing fungi, bacteria, phytoplasmas, insects, mites, rodents, and birds (Ahmed, 2011). In Iraq, the number of date palms has decreased meaningfully over the past two decades, both in quantity and productivity. For instance, the total number of date palms has decreased from almost 33 million in the 1970s to around 17.3 million today. In Basra Governorate alone, numbers have dropped from almost 12 million through the

1980s and 1990s to just over two million. This drastic decrease is primarily attributed to overlook of palm groves, scarcity and salinity of irrigation water, and a rising groundwater table, situations that have facilitated the emergence of numerous pathogens and pests, many of which were previously attended of secondary importance. Among these threats, fungal pathogens are of particular concern, as they contribute to reduced growth and productivity in date palms (Djerbi, 1983). The presence of pathogenic fungi on leaves and shoots negatively impacts the photosynthetic area, leading to reduced growth, diminished flowering, and lower yields. Al-Rokibah (1998) identified different *Alternaria* species as causative agents of leaf spot disease in Saudi Arabia. In Iraq, *A. radicina* was announced for the first time as a pathogen responsible for date palm leaf spot disease (Ahmed, 2011). Modern agriculture increasingly prioritizes the production of safe, sustainable food through the applying of environmentally friendly biological agents, aiming to reduce the applying of synthetic chemicals and their related environmental impacts. In this context, biological control agents offer a hopeful alternative for managing pests and enhancing plant growth. Among them, fungi of the genus *Trichoderma* have garnered meaningful attention. These biofungicides have been broadly applied in the field against a variety of plant pathogens, containing *Pythium* spp., *Botrytis cinerea*, *Fusarium* spp., and *Rhizoctonia solani* (Kubicek and Harman, 1998; Mohiddin et al., 2010). *Trichoderma* spp. are widespread soil fungi commonly found in the rhizosphere, where they colonize plant roots and receive nutrients from root exudates. In return, they confer protection against both biotic and abiotic stresses (Harman, 2006; Woo and Lorito, 2007). Their interaction with plants involves complex signaling processes mediated by compounds secreted by the fungus, containing low-molecular-weight metabolites, peptides, and proteins. These molecules are distinguished by the host plant, triggering defense responses that enhance resistance to disease (Benítez et al., 2004; Hermosa et al., 2013; Herrera-Téllez et al., 2019). As biological control agents, *Trichoderma* spp. exhibit multiple modes of action, containing competition for nutrients and space, antagonism, mycoparasitism, and the induction of plant defense mechanisms (Harman et al., 2004; Shoresh et al., 2010; Druzhinina et al., 2011; Waghunde et al., 2016). These capabilities make *Trichoderma* one of the most hopeful and extensively studied genera in biological control systems (Vinale et al., 2008; Degenkolb et al., 2015). In addition to pathogen suppression, many *Trichoderma* species also promote plant growth (Harman et al., 2004; Qi and Zhao, 2013; Waghunde et al., 2016). The effectiveness of *Trichoderma*-based biocontrol strategies depends on selecting strains that are well-adapted to local environmental situations, containing temperature, humidity, soil microbial communities, and nutrient availability (Howell, 2003). Therefore, isolating and selecting native *Trichoderma* strains from local soils or plant residues is generally preferred. The extensive presence of *Trichoderma* in the rhizosphere and phyllosphere contributes to the

suppression of plant diseases in both open fields and greenhouse settings (Sharifi Tehrani and Nazari, 2004). For example, *T. harzianum* extracted from soil was shown to control leaf drop in rubber trees created by *Phytophthora palmivora* (Promwee et al., 2017). *Trichoderma* acts through both direct mechanisms, like parasitism of pathogens, nutrient competition, and antibiotic production and indirect mechanisms, containing the induction of systemic resistance (ISR) in the host plant, which further enhances its defense capacity (Zin and Badaluddin, 2020). As a broadly distributed genus, *Trichoderma* is capable of colonizing diverse ecological niches like soil, plant surfaces, and even the atmosphere. It has proven effective in mitigating numerous plant diseases and is thus a cornerstone in sustainable agriculture (Haouhach et al., 2020; Zheng et al., 2021; Wang et al., 2022). The current investigation aims to compare the effectiveness of *Trichoderma atroviride* and the commercial biopesticide Biocont in preventing the growth of *Alternaria alternata*, the causative agent of leaf spot disease in date palms.

Materials and Methods

Fungal isolates and purification: Isolates of *Trichoderma atroviride* and *Alternaria alternata* were achieved from the Palm Diseases Laboratory at the Date Palm Research Center, University of Basra, Iraq. These isolates were originally extracted and described in a previous investigation (Jassim & Ahmed, 2024). To ensure the purity of the fungal cultures, both *T. atroviride* and *A. alternata* were sub-cultured onto fresh potato dextrose agar (PDA; HiMedia, India) plates and incubated at 25 ± 2°C until uniform colony growth was achieved. The isolates were periodically transferred to fresh PDA plates to maintain viability and purity. The biocontrol agent applied in this investigation, Biocont®, is a commercial biopesticide formulation containing the biotrophic fungus *Trichoderma harzianum*. The product was achieved from Ain Al-Masa Natural Agriculture Company (Hashemite Kingdom of Jordan). Since Biocont® is a proprietary formulation and not extracted in this investigation, only *T. harzianum* was recovered and cultured on PDA under aseptic situations to be applied in subsequent experiments.

Purification of *Alternaria alternata:* For consistency, *A. alternata* was re-purified from existing cultures maintained at the Palm Diseases Laboratory. The isolate had been previously identified and preserved from earlier research. Fresh PDA plates were inoculated with the stored isolate and incubated at 25 ± 2°C. Pure colonies were selected and applied for subsequent pathogenicity and growth experiments.

Pathogenicity assay of *A. alternata* on date palm cultivars: To evaluate the pathogenicity of *A. alternata*, detached leaf assays were managed on fronds from three date palm (*Phoenix dactylifera* L.) cultivars: Sayer, Halawi, and Barhi. Three healthy, similarly sized fronds were

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gathered from each cultivar. The fronds were washed thoroughly under running tap water to remove surface debris and dust, then surface-sterilized by immersion in 70% ethanol for 1 minute. They were subsequently rinsed three times with sterile distilled water to eliminate any residual alcohol. A 1 cm incision was made on one side of each frond applying a sterile scalpel, leaving the opposite side intact to serve as the unwounded control. The wounds were made 11 cm above the basal attachment point of the frond. Each wounded and unwounded side was inoculated applying a sterile cotton swab dipped in a freshly grown culture of *A. alternata* (7 days old on PDA). The swab was gently rubbed over the designated area on the frond to ensure contact with fungal spores. After inoculation, each frond was placed vertically into a sterile experiment tube containing 20 mL of sterile distilled water. The mouth of each tube was sealed with sterile cotton and covered with aluminum foil to prevent contamination. The tubes were incubated at 25 ± 2°C for 30 days. Disease progression was monitored regularly, and symptom development was document. Infection was affirmed by the presence of visible fungal growth and lesion expansion greater than 1 cm from the original inoculation site. The experiment included three replicates per cultivar. Control treatments consisted of fronds treated with sterile PDA medium instead of fungal inoculum, also in triplicate (Ahmed, 2011).

Effect of temperature on radial growth of *T. atroviride* and *T. harzianum:* To evaluate the impact of temperature on fungal growth, PDA medium was prepared and autoclaved at 121°C for 15 minutes. After cooling to ~50°C, the medium was amended with chloramphenicol (250 mg/L) to suppress bacterial contamination and adjusted to pH 7. The media was poured into sterile 9 cm diameter Petri dishes. A 0.5 cm diameter mycelial disc from the edge of an actively growing 7-day-old colony of either *T. atroviride* or *T. harzianum* was placed at the center of each Petri dish applying a sterile cork borer. Plates were incubated at three different temperatures: 20°C, 25°C, and 30°C. Fungal radial growth was calculated once the mycelia reached the edge of the Petri dish by calculating the average of two perpendicular diameters. Each treatment was replicated three times.

Effect of temperature on radial growth of *A. alternata:* The effect of temperature on the growth of *A. alternata* was evaluated applying the same procedure described above. A 0.5 cm mycelial disc from a 7-day-old colony of *A. alternata* was inoculated in the center of PDA plates. The plates were incubated at 20°C, 25°C, and 30°C, and colony diameters were document applying the same measurement method. Each treatment included three replicates.

Antagonistic activity of *T. atroviride* and *T. harzianum* against *A. alternata* at different temperatures: The dual culture technique was applied to investigate the antagonistic potential of *T. atroviride* and *T. harzianum* against *A. alternata* at different temperatures (20, 25, and 30°C). PDA medium was poured into 9 cm Petri dishes and permited to solidify. Each dish was bisected

visually. A 0.5 cm disc of a 7-day-old *A. alternata* colony was inoculated on one half of the dish. On the opposite half, a 0.5 cm disc of either *T. atroviride* or *T. harzianum* (7-day-old cultures) was placed. The plates were incubated under the specified temperatures. Control treatments were included by inoculating plates with *A. alternata* alone or with each *Trichoderma* isolate alone. Each treatment and control were replicated three times. The extent of antagonistic interaction was calculated 7 days after inoculation by comparing the radial growth of *A. alternata* in the presence and absence of the antagonists. The percentage of prevention was calculated applying the modified Abbott's formula as described by Krzyśko-Łupicka et al. (2019):

Inhibition (%) = $[(R_1 – R_2) / R_1] \times 100$

Where, R_1 = radial growth of *A. alternata* in the control (without antagonist) and R_2 = radial growth of *A. alternata* in the presence of *Trichoderma*

Statistical analysis: All experiments were managed applying a completely randomized design (CRD). For most experiments, a single-factor design was applied. However, the antagonistic interaction experiment (effect of *T. atroviride* and *T. harzianum* on *A. alternata* under different temperatures) was analyzed as a two-factor factorial experiment within a CRD framework. Statistical analysis of variance (ANOVA) was carried out, and treatment means were compared applying the Least Significant Difference (LSD) test at a significance level of $p \leq 0.01$ (Al-Rawi and Khalaf Allah, 1980).

Results and discussion

Pathogenicity of *Alternaria alternata* on leaves of different date palm cultivars: The pathogenicity experiment affirmed the capability of *Alternaria alternata* to infect date palm leaves, with symptom expression varying across cultivars. Infected fronds exhibited blackish-grey lesions that extended beyond the initial site of fungal inoculation. The lesion margins were surrounded by chlorotic (yellowed) halos, which were more pronounced in some cultivars than others. Notably, no symptoms appeared in the control (non-inoculated) treatment. Initial symptoms included gray discoloration at the inoculation site, which gradually expanded as the infection progressed. The affected tissue eventually became necrotic and torn, with the surrounding area turning yellow—denoting a progressing infection. These artificially induced symptoms closely resembled those observed under natural field situations. It is important to note that symptoms developed only on the wounded portions of the fronds, as no visible symptoms were observed on the unwounded areas (Figure 1). Figure 2 shows the colony morphology of *A. alternata* grown on potato dextrose agar (PDA) medium and the characteristic conidia observed under light microscopy.

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Figure 1. Symptoms of *Alternaria alternata*-induced leaf spot on date palm compared with the uninfected control

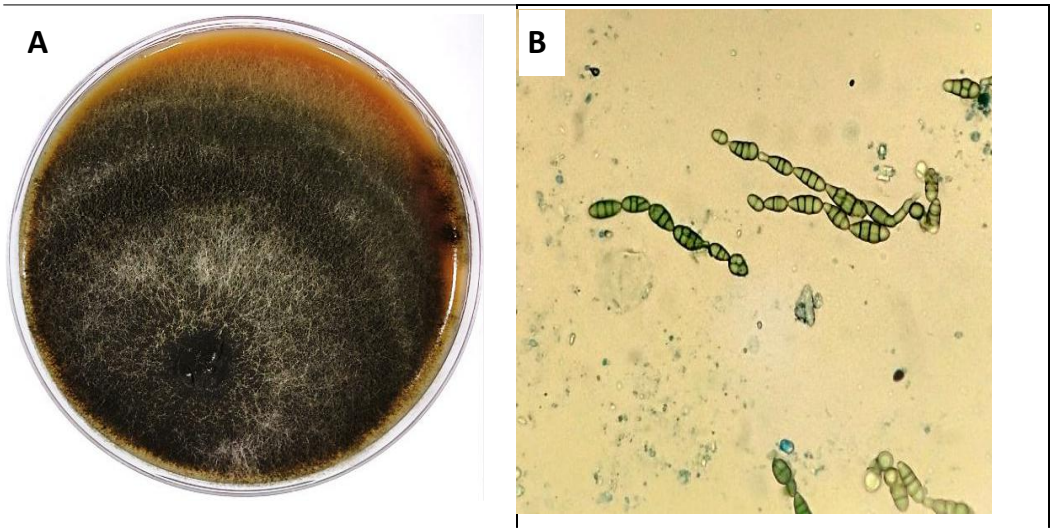

Figure 2. a) Fungal colony growth of *A. alternata* on PDA medium; b) Conidia of *A. alternata* under light microscopy

Previous results by Ahmed (2011) demonstrated that *A. alternata* exhibits moderate cellulolytic activity, producing a hydrolysis zone (halo) of 3–4 mm, and strong phenol oxidase activity, with halo diameters reaching 4–6 mm. These enzymatic capabilities enhance the fungus's capability to penetrate host tissues, facilitating disease progression and symptom development.

Response of different cultivars to *A. alternata* inoculation: Table 1 shows the variation in infection severity among different date palm cultivars following artificial inoculation. The 'Sayer' cultivar exhibited the highest lesion development, with an average infection zone of 3.5 cm, followed by 'Halawi' (3.3 cm), while 'Barhi' showed the lowest infection rate (2.2 cm). The visual symptoms were consistent across cultivars, beginning as gray to blackish discoloration, followed by tissue tearing and chlorosis surrounding the lesion area. The differential susceptibility is likely attributed to the compositional differences among the cultivars. Previous investigations (Ayika et al., 2024) have indicated that cultivars rich in cellulose and carbohydrates (e.g., 'Zahdi' and

'Halawi') are more susceptible to fungal invasion, whereas those with higher levels of protein and calcium, like 'Barhi', exhibit greater resistance.

Table 1. Effect of *A. alternata* on the infection rate of different date palm cultivars

Cultivar	Lesion Length (rate of development of infection with the *A. alternata*) (cm)
Sayer	3.5*
Halawi	3.3
Barhi	2.2
R.L.S.D (0.01)	0.21

*Each value shows the mean of three replicates.

Effect of temperature on the radial growth of *Trichoderma* spp. and *A. alternata:* Figures 3–5 show the effect of temperature on the mycelial growth of *T. atroviride*, *T. harzianum*, and *A. alternata*. Both *Trichoderma* species demonstrated optimal radial growth at 30°C, achieving maximum colony diameters of 9.0 cm. At 20°C, growth was meaningfully reduced—7.7 cm for *T. atroviride* and 7.5 cm for *T. harzianum*. Similarly, *A. alternata* exhibited its highest radial growth at 30°C (9.0 cm), with a slight decrease at 25°C (8.8 cm), and the lowest growth observed at 20°C (7.6 cm). These results align with prior reports by Gawai and Mangnalikar (2018), who found optimal growth for *A. alternata* at 30–35°C, and meaningful decrease above or below this range. Temperature affects fungal physiology, exclusively enzyme activity required for growth. Maheshwari (2005) highlighted that growth prevention and failure of spore germination in *Aspergillus nidulans* at 44°C may be due to gene mutations regulating thermal tolerance. Fungal adaptation to stress involves calcium signaling and pathways mediated by Protein Kinase C (Pkc) and Calcineurin A (CnaA), which are vital for cell wall integrity and stress response (Colabardini et al., 2014).

Antagonistic activity of *Trichoderma* spp. against *A. alternata* at different temperatures: The antagonistic potential of *T. atroviride* and *T. harzianum* against *A. alternata* was evaluated at 20°C, 25°C, and 30°C. As shown in Table 2 and Figure 6, the greatest prevention was document at 30°C. At this temperature, *T. atroviride* reduced the radial growth of *A. alternata* to 1.23 cm, compared to 1.63 cm with *T. harzianum*. Conversely, the lowest prevention occurred at 20°C with *T. harzianum* (3.03 cm). Trichoderma spp. are well-documented for their thermotolerance, with some strains staying active above 40°C, unlike many pathogenic fungi which are prevented at such temperatures (Hoitink et al., 1997; Ahmed, 2011). This makes *Trichoderma* exclusively valuable for biocontrol in arid and semi-arid regions.

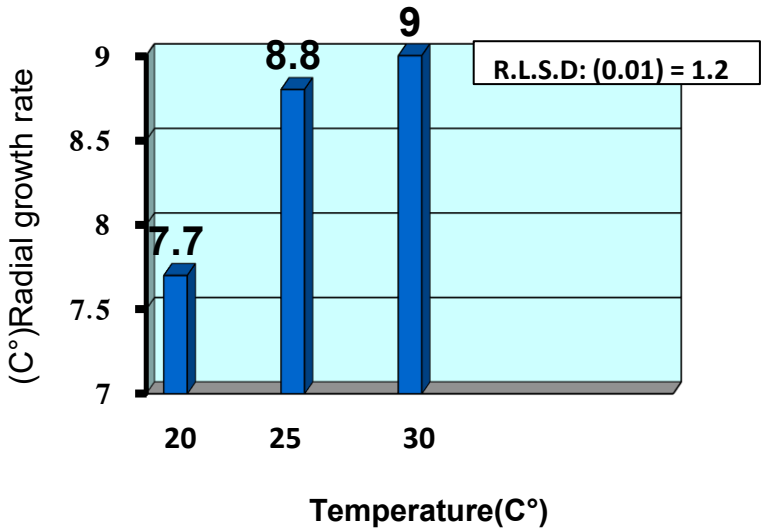

Figure 3. Radial growth of *T. atroviride* at different temperatures

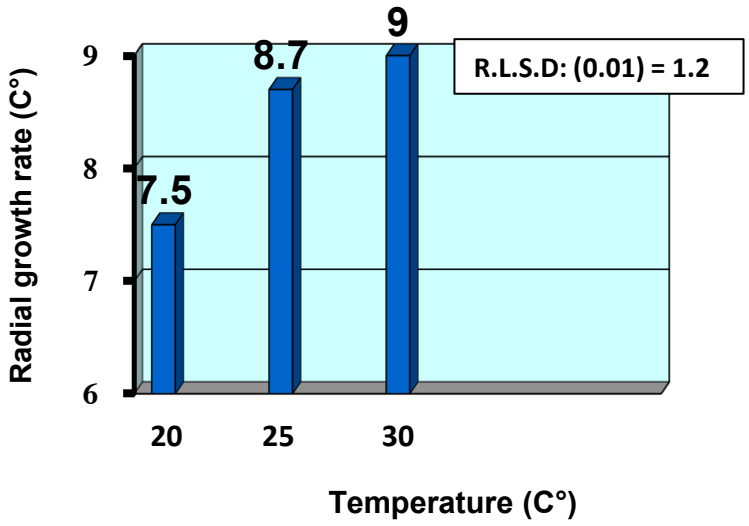

Figure 4. Radial growth of *T. harzianum* at different temperatures

Inhibition percentage of *A. alternata* growth by *Trichoderma* spp.: Table 3 summarizes the prevention percentage of *A. alternata* by *T. atroviride* and *T. harzianum*. The most effective treatment was *T. atroviride* at 30°C, which achieved an prevention rate of 81.31%. In contrast, the lowest prevention was observed with *T. harzianum* at 20°C (39.33%). Trichoderma species exhibit multiple mechanisms of antagonism, containing mycoparasitism, competition for nutrients and space, secretion of hydrolytic enzymes, and production of antifungal secondary metabolites. These metabolites can also activate plant defense pathways (Alfiky & Weisskopf, 2021; Manzar et al., 2022), making Trichoderma a dual-function biocontrol and plant growth-promoting agent.

Conclusions: *Alternaria alternata* is a meaningful pathogen responsible for leaf spot disease in date palms. This investigation demonstrated variation in susceptibility among cultivars, with 'Barhi' showing the most resistance. Among biocontrol agents experimented, *T. atroviride* exhibited superior antagonistic activity, exclusively at 30°C, suggesting its potential as a biopesticide under arid and semi-arid climatic situations. The results support the development and deployment of *Trichoderma*-based biocontrol strategies as a sustainable alternative to chemical fungicides.

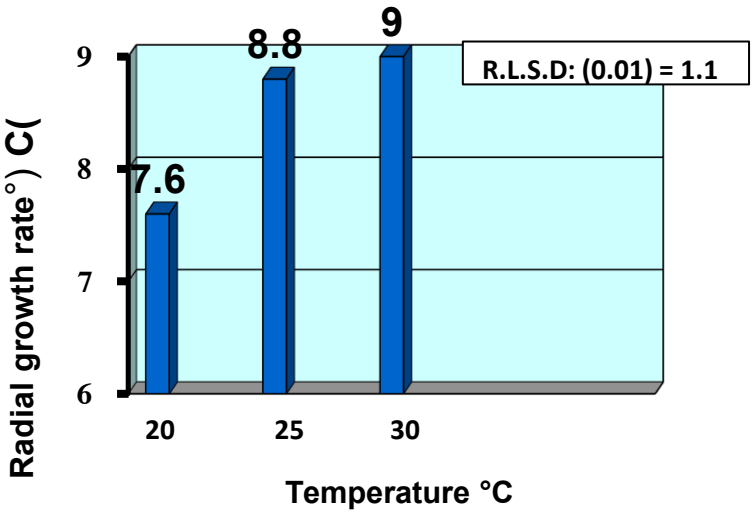

Figure 5. Radial growth of *A. alternata* at different temperatures

Table 2. Radial growth of *A. alternata* in the presence of biocontrol agents at different temperatures

Temp (°C)	A. alternata + T. atroviride	A. alternata + T. harzianum	Mean Growth (cm)
20	2.76	3.03	2.90
25	2.03	2.43	2.23
30	1.23	1.63	1.43
Mean	2.01	2.36	
RLSD (0.01)	Biotic = 0.08, Temp = 0.10, Interaction = 0.14		

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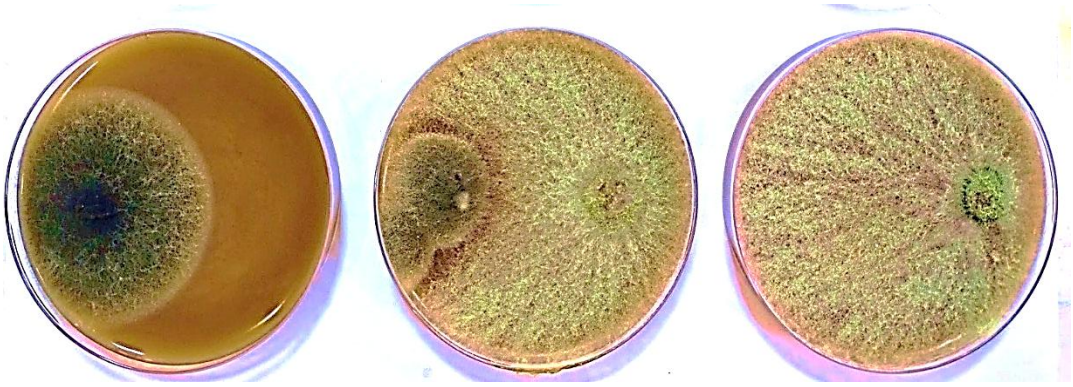
A.alternata *A.alternata + T.atroviride* *T.atroviride*

Figure 6. Inhibition of *A. alternata* by *T. atroviride* at different temperatures

Table 3. Inhibition rate (%) of *A. alternata* by biocontrol agents at different temperatures

Temp (°C)	T. atroviride (%)	T. harzianum (%)	Mean Inhibition (%)
20	44.67	39.33	42.00
25	68.23	61.98	65.10
30	81.31	75.25	78.28
Mean	64.73	58.85	
RLSD (0.01)	Biotic = 1.41, Temp = 1.73, Interaction = 2.45		



A.alternata *A.alternata + T.harzianum* *T. harzianum*

Figure 7. Inhibition of *A. alternata* by *T. harzianum* at different temperatures

Author contributions

NHM conceptualized, supervised, and wrote-reviewed and edited. ANA designed the research plan, procured necessary materials, managed all experimental procedures, and carried out computational analyses of the resulting data. ANA also prepared the figures and drafted the initial manuscript. All authors contributed critical feedback through the revision process, refining earlier drafts, and reviewed and approved the final manuscript for submission, ensuring its scientific accuracy and integrity.

Data Availability

The datasets generated and analyzed in this investigation are available from the corresponding author upon reasonable request, subject to any applicable ethical or institutional restrictions.

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Ethical Considerations

No human or animal subjects were involved in this investigation.

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Conflict of Interest

The authors declare no potential conflicts of interest that could impress the results or interpretation of this investigation. There are no financial, personal, or professional relationships that could be perceived as biasing the research.

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تأثیر مقایسه‌ای *Trichoderma atroviride* و آفت‌کش زیستی بیوکنت در سرکوب بیماری لکه برگی نخل خرما ناشی از *Alternaria alternata*

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چکیده

هدف: کنترل زیستی قارچ‌های بیماری‌زای گیاهی، به‌ویژه با استفاده از عوامل زیستی با پتانسیل بالا یک استراتژی نویدبخش است. این مطالعه با هدف مقایسه کارایی *Trichoderma atroviride* و آفت‌کش زیستی بیوکنت در مهار رشد *Alternaria alternata*، عامل بیماری لکه برگی در نخل‌های خرما، با استفاده از *Trichoderma harzianum* به‌عنوان یک عامل زیستی مقایسه‌ای انجام شد.

مواد و روش‌ها: بیماری‌زایی *A. alternata* بر روی برگ‌های سه رقم نخل خرما (برهی، هلاوی، سایر) ارزیابی شد. اثرات آنتاگونیستی *T. atroviride* و *T. harzianum* علیه *A. alternata* در دماهای مختلف با استفاده از روش کشت دوگانه آزمایش شد. درصد مهار رشد برای عوامل زیستی و قارچ بیماری‌زا پس از ۷ روز هم‌تلقیح محاسبه شد. تأثیر دما بر تعاملات آنتاگونیستی ارزیابی شد و رشد شعاعی *A. alternata* در دماهای ۲۰ و ۳۰ درجه سانتی‌گراد اندازه‌گیری شد.

نتایج: *A. alternata* باعث بیماری لکه برگی شد که علائم آن با لکه‌های خاکستری مایل به سیاه در سطح برگ مشخص بود. بیشترین کاهش رشد *A. alternata* در دمای ۳۰ درجه سانتی‌گراد مشاهده شد، جایی که *T. atroviride* رشد پاتوژن را به ۱/۲۳ سانتی‌متر محدود کرد. کمترین کاهش در دمای ۲۰ درجه سانتی‌گراد با *T. harzianum* رخ داد، جایی که رشد *A. alternata* به ۳/۰۳ سانتی‌متر رسید. بالاترین نرخ مهار (۸۱/۳۱ درصد) *A. alternata* در دمای ۳۰ درجه سانتی‌گراد در حضور *T. atroviride* به‌دست آمد، در حالی که کمترین نرخ مهار (۳۹/۳۳ درصد) در دمای ۲۰ درجه سانتی‌گراد با *T. harzianum* ثبت شد. این مطالعه

تأیید کرد که گونه‌های *Trichoderma* متابولیت‌های ثانویه‌ای با اثرات آنتاگونیستی علیه پاتوژن‌های گیاهی تولید می‌کنند. علاوه بر این، این متابولیت‌ها می‌توانند مکانیسم‌های مقاومت در گیاهان را فعال کنند و حفاظت در برابر بیماری‌ها را تقویت کنند.

نتیجه‌گیری: یافته‌ها نشان می‌دهند که گونه‌های *Trichoderma* متابولیت‌های ثانویه متنوعی تولید می‌کنند که در سرکوب پاتوژن‌های گیاهی مؤثر هستند. *T. atroviride* در دمای ۳۰ درجه سانتی‌گراد مهار برتری نسبت به *A. alternata* در مقایسه با *T. harzianum* نشان داد که کمترین مهار را در دمای ۲۰ درجه سانتی‌گراد داشت. این نتایج از انتخاب *T. atroviride* به‌عنوان یک عامل کنترل زیستی مؤثر برای کاهش بیماری لکه برگی نخل خرما حمایت می‌کند. این مطالعه پتانسیل توسعه عوامل کنترل زیستی مبتنی بر *T. atroviride* را که با شرایط محیطی کشت نخل خرما سازگار هستند، پیشنهاد می‌کند.

کلمات کلیدی: کنترل زیستی، نخل خرما، مقاومت گیاهی، متابولیت‌های ثانویه

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