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ORIGINAL RESEARCH ARTICLE

Environmental factors affecting *Trichoderma* spp. and their biocontrol potential in post-harvest disease management

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ABSTRACT

Trichoderma species are filamentous fungi inhabiting soil environments and employed in agriculture due to their capacity to enhance plant growth, disease resistance, and tolerance to adverse environmental conditions. This research aimed to examine the influence of NaCl concentration, temperature, pH, and photoperiodicity on the growth of four *Trichoderma* species, and to evaluate the potential of *Trichoderma* spp. as a biological control agent against five post-harvest fungal pathogens under laboratory conditions. Mycelial growth of *Trichoderma* species exhibited a negative correlation with salinity. Maximum growth rates (4.35-4.59 cm) were observed at control salinity (0 µM). Potato Dextrose Agar media consistently supported significantly ($p \leq 0.05$) higher mycelial growth (4.33-4.35 cm) than Malt Extract Agar, Carrot Agar, and Komada for all *Trichoderma* species. The optimal temperature for mycelial growth of *Trichoderma* spp. ranged from 25-30°C, with maximum growth rates of 4.12-4.40 cm. Temperatures below 20°C and above 35°C resulted in substantial growth reduction, demonstrating temperature's critical influence on mycelial development. Besides, the optimal pH for mycelial growth of *Trichoderma* spp. ranged from 5 to 7, with maximum growth rates of 4.12-4.43 cm. Mycelial growth of *Trichoderma* spp. was significantly enhanced under a 12-hour light/dark cycle (4.33-4.43 cm) compared to continuous light (2.24-2.79 cm) and continuous darkness (2.28-2.56 cm) conditions. Therefore, *Trichoderma* spp. treatments significantly inhibited the mycelial growth of five fungal pathogens compared to the control group, among them, *T. koningii* demonstrated the highest level of inhibition, ranging from 0.28 cm (*B. cinerea*) to 1.65 cm (*A. niger*).

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INTRODUCTION

Phytopathogenic fungi constitute a primary etiological factor in crop disease, resulting in substantial reductions in both crop yield and quality (Rhouma *et al.*, 2023a). These impacts pose significant risks to global food security and economic progress. Furthermore, numerous fungal pathogens produce mycotoxins during infection, which present severe hazards to human and animal health (Rhouma *et al.*, 2023b). The evolving global climate and agricultural practices are facilitating the emergence of fungal diseases in novel host organisms and geographical regions (Rhouma *et al.*, 2021). Research into alternative plant disease management strategies, such as biocontrol, has intensified recently (Matrood *et al.*, 2023). The application of antagonistic microorganisms to suppress plant pathogens has emerged as a more environmentally benign alternative to conventional chemical treatments (Rhouma *et al.*, 2023c; Rhouma *et al.*, 2024). Among these, *Trichoderma* spp. have been investigated as a biocontrol agent for various plant pathogens (Hajji-Hedfi *et al.*, 2023a,b). This genus has demonstrated potential as a

multifunctional agent capable of sustaining agricultural yields (Ghazi Mohammed *et al*., 2024). *Trichoderma* spp. are ubiquitous filamentous soil and rhizosphere fungi characterized by high survival rates, reproductive capacity, nutrient utilization efficiency, and plant growth-promoting attributes, which collectively contribute to their efficacy as biocontrol agents against various plant pathogens (Matrood & Rhouma, 2022; Matrood *et al*., 2022). The antifungal mechanisms of *Trichoderma* primarily involve the production of volatile and non-volatile inhibitory compounds, hydrolytic enzymes, and the induction of host plant resistance to suppress disease development (Matrood *et al*., 2021; Matrood & Rhouma, 2021a, b).

Effective application of antagonistic *Trichoderma* strains for biological control necessitates consideration of factors influencing their growth. Abiotic parameters, including salinity, temperature, pH, light, and darkness, have been shown to adversely affect the antagonistic capabilities of *Trichoderma* species against plant pathogens (Moreno-Ruiz *et al*., 2020; Carro-Huerga *et al*., 2021; Rimkus *et al*., 2023). Given the critical role of *Trichoderma* strains as biocontrol agents, they should exhibit superior stress tolerance compared to the target plant pathogens (Matrood *et al*., 2021 and 2022; Hajji-Hedfi *et al*., 2023b). Consequently, a comprehensive evaluation of these parameters' impact on *Trichoderma* growth is essential before their widespread application as biocontrol agents (Matrood & Rhouma, 2021a, b; Matrood & Rhouma, 2022). The present study aimed to investigate the influence of environmental factors on the growth of *Trichoderma* spp. and to evaluate their potential as biocontrol agents in post-harvest disease management.

MATERIALS AND METHODS

Fungal isolates

In this study, *Trichoderma* spp. (*T. atroviride*, *T. hamatum*, *T. koningii*, and *T. viride*) and phytopathogens (*Botrytis cinerea*, *Alternaria solani*, *Penicillium expansum*, *P. digitatum*, and *Aspergillus niger*) used in the present study were obtained from the Laboratory of Plant Protection, Iraq.

Effect of salinity and culture media on mycelial growth of *Trichoderma* spp.

During the study, PDA osmotic potential was adjusted by incorporating NaCl at concentrations of 0, 250, 500, 750, and 1000 µM into 250 ml Erlenmeyer flasks containing PDA before Petri dish (9 cm diameter) pouring. Four-millimeter diameter agar plugs excised from the colony margins of four-day-old *Trichoderma* spp. cultures were centrally positioned on each treated Petri dish. Colony diameter (cm) was measured after five days of incubation at 25°C. Each treatment was replicated three times, with five plates per replicate (Abu-Shanab *et al*., 2022). Four culture media - Malt Extract Agar (MA), Carrot Agar (CA), Komada, and Potato Dextrose Agar (PDA) - were evaluated for optimal mycelial growth of *Trichoderma* spp. Mycelial radial growth was measured at five days of incubation. Each treatment included three replicates of five plates and was incubated at 25°C (Rimkus *et al*., 2023).

Effect of temperature and pH on mycelial growth of *Trichoderma* spp.

The growth of *Trichoderma* spp. was assessed across a temperature range encompassing minimum, optimal, and maximum values. A 5 mm diameter disc of each fungal species (four-day-old culture) was inoculated centrally onto PDA plates. Mycelial growth was calculated after five days of incubation by measuring the average perpendicular diameter of each colony. Experiments were conducted at 15, 20, 25, 30, 35, and 40°C with three replications (five plates per replication) for each temperature condition (Carro-Huerga *et al*., 2021). To assess optimal pH for *Trichoderma* spp. growth, 100 ml of PDA medium was prepared in 250 ml Erlenmeyer flasks and adjusted to pH levels of 4, 5, 6, 7, and 8 using 0.1 N HCl or NaOH, measured by a digital pH meter. A 5 mm diameter disc of each fungal species (4-day-old culture) was inoculated centrally onto PDA plates. Mycelial growth was measured at five days of incubation, with three replicates of five plates each incubated at 25°C for each pH level (Wenjie *et al*., 2024).

Effect of light and darkness on mycelial growth of *Trichoderma* spp.

After five days of incubation in light, darkness, or a 12-hour light -dark cycle, the mycelial growth of *Trichoderma* spp. was evaluated to understand the impact of these light regimes. Mycelial growth was determined by averaging perpendicular colony diameters. Each treatment condition was replicated three times, with five plates per replicate, and the plates were incubated at 25°C (Moreno-Ruiz *et al*., 2020).

Antagonistic action of *Trichoderma* spp. toward post-harvest diseases

A dual culture assay on potato dextrose agar (PDA) plates was conducted to assess the antagonistic interaction between *Trichoderma* spp. and various phytopathogens (*B. cinerea*, *A. solani*, *P. expansum*, *P. digitatum*, and *A. niger*). Agar plugs (0.5 cm) were excised from four-day-old cultures of *Trichoderma* spp. and phytopathogens. These plugs were placed on opposite sides of a 9-cm diameter PDA plate, maintaining a distance of 2 cm from the plate edge towards the center for the *Trichoderma* sp. plug and a distance of 5 cm between the two plugs. A control plate included only a PDA plug on one side and the phytopathogen plug on the opposite side. Each treatment was replicated three times with five plates per replicate. All plates were incubated at 28±2°C for 5 days. After incubation, the radial growth of each phytopathogen was measured in centimeters (Rhouma *et al*., 2024).

Statistical analysis

Analysis of variance (ANOVA) was conducted using SPSS software (version 20) to compare the effects of different environmental conditions on the growth of *Trichoderma* spp. According to Tukey's multiple range test, differences were considered significant at the 5% ($p \leq 0.05$) level of significance.

RESULTS AND DISCUSSION

Effects of salinity on mycelial growth of *Trichoderma* spp.

In this study, mycelial growth responses of four *Trichoderma*

species to varying salinity levels are summarized in Table 1. Salinity levels were manipulated across five treatments: 0, 250, 500, 750, and 1000 µM. The data reveals a consistent negative correlation between salinity and mycelial growth for all *Trichoderma* species. At the control salinity of 0 µM, all species displayed their maximum growth rates, as indicated by the highest mean values varied from 4.35 cm (*T. hamatum*) to 4.59 (*T. viride*). As salinity increased, a significant reduction in mycelial growth was observed for all species, as evidenced by the decreasing mean values accompanied by different superscripts according to the ANOVA Test. This statistical analysis confirms that the observed differences in growth between salinity levels are highly unlikely to be due to chance. The consistently low p-values ($p<0.01$) for all *Trichoderma* species further strengthen the conclusion that salinity exerts a detrimental effect on their mycelial growth (Table 1). Our investigation into the impact of salinity on four *Trichoderma* species revealed a consistent and negative relationship between increasing salt concentrations (0-1000 µM) and fungal growth. All species displayed the highest growth rates at 0 µM salinity, with average mycelial diameters ranging from 4.35 cm for *T. hamatum* to 4.59 cm for *T. viride*. Salt stress constitutes a significant abiotic factor adversely affecting soil microbial ecosystem structure and function, consequently limiting crop productivity, as documented by Poosapati *et al.* (2021). Given its influence on *Trichoderma* isolate viability, soil salinity emerges as a critical environmental determinant in the selective process shaping microbial community composition, as reported by Rasheela *et al.* (2025). All *Trichoderma* species evaluated in this study exhibited growth across all tested NaCl concentrations, aligning with findings by Alwadai *et al.* (2022) who reported the NaCl tolerance of *Trichoderma* spp. up to 2%. However, in contrast to these results, no growth was observed for *T. asperellum* It-13 under any tested NaCl condition (Alwadai *et al.*, 2022). Microbial growth is intrinsically linked to water activity, influenced by the osmotic pressure exerted by the surrounding medium on cellular transmembrane exchanges. The observed salt stress tolerance of our *Trichoderma* isolates can be attributed to the development of extrusion systems (efflux pumps) in response to high salt concentrations, enabling the maintenance of intracellular sodium levels below toxic thresholds, as described by Sekmen Cetinel *et al.* (2021). A common physiological response of microorganisms to reduced water activity involves the accumulation of specific intracellular substances through enhanced synthetic metabolic pathways (Abu-Shanab *et al.*, 2022). These accumulated substances, known as neutral osmolites, function as osmoprotective agents (Othman *et al.*, 2022). Our media study findings corroborate those of Abu-Shanab *et al.* (2022), identifying PDA as the optimal culture medium for *Trichoderma* mycelial growth.

Effects of culture media on mycelial growth of *Trichoderma* spp.

A comparative analysis of the impact of four different culture media (MA, CA, Komada, and PDA) on the mycelial growth of four *Trichoderma* species is illustrated in Table 2. The data reveals a clear superiority of PDA over the other media in supporting mycelial growth for all *Trichoderma* species, which varied from 4.33 cm (*T. koningii*) to 4.35 cm (*T. atroviride*). The mean mycelial growth values on PDA were consistently higher than those on MA, CA, and Komada, and this difference was found to be statistically significant according to Tukey's Multiple Range Test ($p<0.01$). While there were some variations in growth among the *Trichoderma* species on MA, CA, and Komada, the overall trend indicates that these media provided suboptimal conditions for mycelial development compared to PDA (Table 2).

Effects of temperature on mycelial growth of *Trichoderma* spp.

Mycelial growth of four *Trichoderma* species under varying temperatures was investigated, and the outcomes are detailed in Table 3. Temperature treatments ranged from 15°C to 40°C in increments of 5°C. The data reveals a clear optimal temperature range for mycelial growth between 25°C (4.12-4.23 cm for *T. viride* and *T. atroviride*, respectively) and 30°C (4.23-4.40 cm for *T. viride* and *T. atroviride*, respectively) for all *Trichoderma* species. At these temperatures, the highest mean growth values were recorded, and statistical analysis using Tukey's Multiple Range Test confirmed significant differences in growth compared to other temperatures ($p \leq 0.01$). In contrast, temperatures below 20°C and above 35°C led to a substantial reduction in mycelial growth for all species. The consistently low p-values ($p<0.01$) for all *Trichoderma* species at these temperature extremes emphasize the detrimental impact of temperature outside the optimal range (Table 3).

Table 1. Effect selected salinity levels on mycelial growth of *Trichoderma* spp.

Salinity (µM)	*T. atroviride*	*T. hamatum*	*T. koningii*	*T. viride*
0	4.46a[a]	4.35a	4.45a	4.59a
250	3.45b	3.54b	3.67b	3.48b
500	2.85c	2.84c	2.72c	2.71c
750	2.29d	2.27d	2.24d	2.22c
1000	1.69e	1.45e	1.64e	1.48d
P-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Tukey's Multiple Range Test, values followed by different superscripts are significantly different at $P \leq 0.05$. [b]Probabilities associated with individual F tests.

Table 2. Effect of culture media selected culture media on mycelial growth of *Trichoderma* spp.

Culture media	*T. atroviride*	*T. hamatum*	*T. koningii*	*T. viride*
MA	3.18b[a]	3.38b	3.46b	3.25b
CA	3.07b	3.36b	3.18b	3.21b
Komada	3.23b	3.45b	3.22b	3.10b
PDA	4.35a	4.34a	4.33a	4.34a
P-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Tukey's Multiple Range Test, values followed by different superscripts are significantly different at $P \leq 0.05$. [b]Probabilities associated with individual F tests.

Table 3. Effect of selected temperatures on mycelial growth of *Trichoderma* spp.

Temperature (°C)	*T. atroviride*	*T. hamatum*	*T. koningii*	*T. viride*
15	1.92d[a]	1.57d	1.54d	1.33d
20	2.43c	2.49c	2.56c	2.32c
25	4.23a	4.20a	4.13a	4.12a
30	4.40a	4.37a	4.37a	4.23a
35	3.37b	3.27b	3.40b	3.20b
40	2.67c	2.86bc	2.47c	2.27c
P-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Tukey's Multiple Range Test, values followed by different superscripts are significantly different at $P \leq 0.05$. [b]Probabilities associated with individual F tests.

Our investigation into the impact of temperature on four *Trichoderma* species revealed an optimal growth range between 25°C and 30°C. Within this range, the highest growth rates were observed, with average mycelial diameters ranging from 4.12 cm (*T. viride*) to 4.40 cm (*T. atroviride*). Temperatures below 20°C and above 35°C significantly reduced fungal growth in all species. Optimal growth for the antagonistic *Trichoderma* isolates in this study occurred within the temperature range of 25-30°C. These findings align with Athinuwat *et al.* (2024) who reported species-specific minimum and maximum temperature thresholds for *Trichoderma* development. Lombardi *et al.* (2023) demonstrated the ability of nine *Trichoderma* isolates to grow between 12 and 37°C, with an optimal temperature of 25°C. Similarly, Carro-Huerga *et al.* (2021) reported an optimal growth temperature of 25-30°C for 10 *T. viride* isolates. The observed heat stress tolerance of isolates grown at 40°C is potentially attributable to the synthesis of heat shock proteins (HSPs), a known heat tolerance mechanism in various organisms (Boamah *et al.*, 2025). Additionally, increased production and accumulation of molecules like trehalose, mannose, and raffinose within cells and the culture medium may contribute to this tolerance. Mannose, constituting 10-15% of filamentous fungi mycelial dry weight, has been implicated in abiotic stress resistance, including extreme temperatures (Amira *et al.*, 2021; Saravanakumar *et al.*, 2021). Zhu *et al.* (2022) reported increased trehalose accumulation in the conidia of *Trichoderma* strains exposed to a 40°C heat shock for 90 min. The role of these sugars in the survival and stabilization of cellular structures and proteins under heat stress conditions has been found for *Trichoderma* spp. (Poosapati *et al.*, 2021).

Effects of pH on mycelial growth of *Trichoderma* spp.

The influence of pH on the mycelial growth of *Trichoderma* spp. is shown in Table 4. The data indicates an optimal pH range of 5 to 7 for mycelial growth across all *Trichoderma* species, which varied from 4.12 cm (*T. koningii* / pH = 5) to 4.43 cm (*T. hamatum* / pH = 7). Within this range, the highest mean growth values were recorded, and statistical analysis using Tukey's Multiple Range Test confirmed significant differences in growth compared to pH 4 and 8. At pH 4 (2.45-2.60 cm for *T. koningii* and *T. atroviride*, respectively), a moderate reduction in growth was observed for all species, while at pH 8 (2-2.22 cm for *T. viride* and *T. koningii*, respectively), a substantial inhibition of mycelial growth was evident. The consistently low p-values at pH 4 and 8 highlight the significant impact of pH on the growth of these fungi (Table 4).

Our experiment demonstrated that *Trichoderma* spp. exhibited optimal growth within the pH range of 5-7, with mean mycelial growth values ranging from 4.12 cm to 4.43 cm. Growth was moderately reduced at pH 4 (2.45-2.60 cm) and significantly inhibited at pH 8 (2-2.22 cm) for all species. Medium pH constitutes a critical parameter influencing *Trichoderma* fungal growth, affecting mineral availability, metabolic reaction rates, and enzymatic activity (Alwadai *et al.*, 2022). Our study demonstrated that *Trichoderma* species grow within a pH range of 4 to 8, with optimal growth between 5 and 7. This pH interval represents optimal conditions for mycelial growth. These findings align with those of Alwadai *et al.* (2022), who reported an optimal pH range of 4 to 6 for *T. harzianum*, *T. viride*, *T. asperellum*, and *T. koningiopsis*. These findings are corroborated by Lombardi *et al.* (2023) and Yáñez-Hernández *et al.* (2023), who reported *Trichoderma* species growth across a pH range of 2 to 7 with an optimal pH of 4. *Trichoderma* fungi prefer acidic soils with high organic matter content (Zúñiga-Silgado *et al.*, 2020). Investigating the influence of pH on antagonistic *Trichoderma* isolate mycelial growth is crucial due to its correlation with extracellular enzyme activity, which is implicated in nutrient competition and mycoparasitism within *Trichoderma* species (Abu-Shanab *et al.*, 2022).

Effects of light and darkness on mycelial growth of *Trichoderma* spp.

Three light regimes were tested: continuous light (24 hours), continuous darkness (24 hours), and a 12-hour light and 12-hour dark cycle. The data clearly shows that the alternating light -dark cycle significantly promoted mycelial growth for all *Trichoderma* species (4.33-4.43 cm for *T. hamatum* and *T. viride*, respectively) compared to the other two conditions ($p < 0.01$). While continuous light (2.24-2.79 cm for *T. hamatum* and *T. atroviride*, respectively) had a slightly negative impact on the growth of some species, it was generally less detrimental than continuous darkness (2.28-2.56 cm for *T. viride* and *T. koningii*, respectively) (Table 5).

Table 4. Effect of selected pH values on mycelial growth of *Trichoderma* spp.

pH	*T. atroviride*	*T. hamatum*	*T. koningii*	*T. viride*
4	2.60b[a]	2.58b	2.45b	2.59b
5	4.13a	4.42a	4.12a	4.31a
6	4.36a	4.39a	4.15a	4.22a
7	4.32a	4.43a	4.23a	4.33a
8	2.10c	2.20c	2.22b	2.00c
P-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Tukey's Multiple Range Test, values followed by different superscripts are significantly different at P ≤ 0.05. [b]Probabilities associated with individual F tests.

Table 5. Effect of light and darkness on mycelial growth of *Trichoderma* spp.

Light / darkness	*T. atroviride*	*T. hamatum*	*T. koningii*	*T. viride*
Light (24 h)	2.79b[a]	2.24b	2.42b	2.28b
Darkness (24 h)	2.37c	2.48b	2.56b	2.28b
Light (12 h) and darkness (12 h)	4.40a	4.33a	4.38a	4.43a
P-value[b]	<0.01	<0.01	<0.01	<0.01

[a]Tukey's Multiple Range Test, values followed by different superscripts are significantly different at P ≤ 0.05. [b]Probabilities associated with individual F tests.

Table 6. Effect of *Trichoderma* spp. on mycelial growth of selected fungal species after 5 days of incubation at 28±2 °C under laboratory conditions.

Treatments	*B. cinerea*	*A. solani*	*P. expansum*	*P. digitatum*	*A. niger*
T. atroviride	0.58b[a]	0.92b	1.53b	1.68b	1.72b
T. hamatum	0.32b	0.88b	1.50b	1.62b	1.66b
T. koningii	0.28b	0.69b	1.47b	1.59b	1.65b
T. viride	0.40b	0.80b	1.55b	1.48b	1.95b
Control	4.63a	4.83a	4.89a	4.93a	4.80a
P-value[b]	<0.01	<0.01	<0.01	<0.01	<0.01

[a]Tukey's Multiple Range Test, values followed by different superscripts are significantly different at P ≤ 0.05. [b]Probabilities associated with individual F tests.

Antagonistic action of *Trichoderma* spp. toward post-harvest diseases

The impact of various *Trichoderma* spp. treatments (*T. atroviride*, *T. hamatum*, *T. koningii*, and *T. viride*) on the mycelial growth of five fungal pathogens (*B. cinerea*, *A. solani*, *P. expansum*, *P. digitatum*, and *A. niger*) following a five-day incubation at 28°C is presented in Table 6. The mycelial growth was measured and compared to a control group. The results indicate that all *Trichoderma* spp. treatments significantly inhibited the mycelial growth of all five fungal pathogens compared to the control group (P < 0.01). Among the *Trichoderma* spp. treatments, *T. koningii* consistently showed the highest level of inhibition against all fungal pathogens with a value ranging from 0.28 cm (*B. cinerea*) to 1.65 cm (*A. niger*). Overall, the findings suggest that *Trichoderma* spp. have the potential to be used as a biological control agent against a range of fungal pathogens (Table 6). Biocontrol represents an ecologically sound strategy for mitigating plant diseases (Hajji-Hedfi *et al*., 2023a, b). *Trichoderma* spp. are a prominent genus employed in biocontrol due to its multifaceted interactions with plants and pathogens (Matrood *et al*., 2022). These interactions encompass antagonism towards fungal pathogens, plant growth promotion, induction of plant defense responses, and enhanced tolerance to environmental stresses (Matrood & Rhouma, 2021a, b). Nevertheless, the effectiveness of *Trichoderma* species as biocontrol agents is influenced by abiotic factors including salinity, temperature, pH, and light conditions, which can negatively impact their antagonistic capabilities (Carro-Huerga *et al*., 2021; Moreno-Ruiz *et al*., 2020; Abu-Shanab *et al*., 2022; Rimkus *et al*., 2023). To optimize the application of *Trichoderma* as biocontrol agents, a thorough assessment of these factors' influence on their growth is imperative (Matrood & Rhouma, 2021a, b; Matrood & Rhouma, 2022).

Trichoderma spp. are effective biological control agents for post-harvest diseases. Furthermore, all four *Trichoderma* spp. treatments (*T. atroviride*, *T. hamatum*, *T. koningii*, and *T. viride*) significantly suppressed the mycelial growth of five fungal pathogens (*B. cinerea*, *A. solani*, *P. expansum*, *P. digitatum*, and *A. niger*) compared to the control. Among the *Trichoderma* treatments, *T. koningii* consistently exhibited the strongest inhibitory effect, with mycelial growth inhibition ranging from 0.28 cm (*B. cinerea*) to 1.65 cm (*A. niger*). These fungi lead to significant crop losses (Hajji-Hedfi *et al*., 2023a, b). *Trichoderma* spp. employ multiple mechanisms to antagonize pathogens. They compete for resources, directly parasitize fungal hyphae, and produce antimicrobial compounds. Additionally, they induce systemic resistance in plants and secrete hydrolytic enzymes that degrade pathogen cell walls (Ferreira *et al*., 2020). Numerous studies have demonstrated the efficacy of *Trichoderma* spp. in controlling various post-harvest diseases. Their adoption offers environmental benefits, including reduced chemical use and potential mitigation of fungicide resistance (Moreno-Ruiz *et al*., 2020; Matrood & Rhouma, 2021a, b; Matrood & Rhouma, 2022). However, the efficacy of *Trichoderma* spp. can be influenced by factors like strain selection, environmental conditions, and pathogen inoculum levels. Careful strain selection and optimized application are essential for effective disease control (Carro-Huerga *et al*., 2021).

Conclusion

The major findings of this study clearly demonstrate the significant influence of key environmental factors – salinity, culture media, temperature, pH, and light – on the mycelial growth of *T. atroviride*, *T. hamatum*, *T. koningii*, and *T. viride*. Specifically, our results highlight that PDA medium consistently provided the most favorable culture medium, while optimal growth generally occurred within a temperature range of 25-30°C and a pH of 5-7. Furthermore, a 12-hour light/dark cycle proved conducive to robust mycelial development across the tested species. Crucially, this research also unequivocally establishes the strong biocontrol potential of all four *Trichoderma* species against five common fungal pathogens. Notably, *T. koningii* consistently exhibited the most potent inhibitory effects, suggesting its superior efficacy in suppressing the growth of these target pathogens *in vitro*. Taken together, these findings provide critical insights for optimizing *Trichoderma* cultivation and underscore their promise as effective biological control agents. Moving forward, it is essential to conduct field trials to validate the in vitro efficacy of these *Trichoderma* spp. under real-world agricultural conditions. Future research should also explore their integration into comprehensive pest management strategies to maximize their beneficial impact.

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DECLARATIONS

Author contribution statement: Conceptualization: A.A.A.M. and A.R.; Methodology: A.A.A.M.; Software and validation: A.A.A.M. and A.R.; Formal analysis and investigation: A.R.; Resources: A.A.A.M.; Data curation: A.A.A.M.; Writing—original draft preparation: A.R.; Writing—review and editing: A.R.; Visualization: AR; Supervision: A.A.A.M.; Project administration: A.A.A.M. and A.R.; Funding acquisition: A.A.A.M. All authors have read and agreed to the published version of the manuscript.

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