

Article

Characterization of a *Trichoderma asperellum* Strain's Ecophysiological Traits and Compatibility with Fungicides and Insecticides for Integrated Crop Protection

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Abstract

The growing demand for sustainable crop protection has intensified interest in biological control agents. This study characterized a *Trichoderma asperellum* strain (IDE-10) to assess its suitability for integrated pest and disease management. Key ecophysiological traits, including temperature, pH, and ultraviolet (UV) tolerance, were evaluated, along with compatibility with commonly used fungicides and insecticides. The strain exhibited optimal growth at 28.28 °C and sustained high growth rates up to 35 °C, with a maximum threshold of 39.21 °C, indicating strong adaptation to warm climates. Sporulation was enhanced between 25 and 35 °C, with no growth at 5 °C, and soil viability remained stable from 7 to 21 °C. The IDE-10 strain demonstrated broad pH tolerance (3–7) and high resistance to UV-A and UV-B radiation, distinguishing it from commercial strains. Insecticides had no significant adverse effects, while fungicide compatibility varied, with IDE-10 being compatible with metalaxyl- and copper-based formulations. These findings highlight the robustness and adaptability of *T. asperellum* IDE-10, supporting its potential as a biocontrol agent for sustainable crop protection strategies, particularly in Mediterranean agricultural systems.

Keywords: biological control; pests; temperature; UV radiation; pH

1. Introduction

In the current global context marked by continuous population growth and climate change impacts, ensuring high crop yields requires the implementation of sustainable and efficient agricultural strategies [1]. Agricultural losses caused by insect pests and plant pathogens are estimated to be 20–30% of potential yields and can reach up to 40% in some crops [2,3]. Intensive reliance on synthetic fungicides has led to concerns regarding human health, environmental contamination, and the rapid development of fungicide resistance. In response to these limitations, recent studies have highlighted beneficial microorganisms, including bacteria and fungi associated with roots and foliage, as central alternatives that can be used as biopesticides to suppress insect pests and plant pathogens [4,5].

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Numerous soil-dwelling and plant-associated microorganisms function as effective biocontrol agents or biopesticides. In particular, fungi represent a diverse group of microorganisms with numerous applications in biotechnology and industry; in agriculture, fungal mycopesticides have emerged as significant alternatives to chemical pesticides [6]. Approximately 90% of fungal biocontrol agents targeting phytopathogenic microorganisms are *Trichoderma* strains [7–9]. *Trichoderma* spp. possesses several characteristics that explain their effectiveness: a high capacity for mass production, stress resistance, efficient rhizosphere-nutrient exploitation, aggressiveness against phytopathogenic fungi, plant growth-promoting effects, ability to induce plant defense mechanisms, and the production of secondary metabolites [10], enzymes [11], and proteins [12]. The potential of *Trichoderma* spp. as a biocontrol agent in the management of plant diseases was first identified in the early 1920s [13] and since then, their efficacy in controlling numerous diseases has been documented [14]. Various *Trichoderma* species present in the soil microbiome produce enzymes that degrade specific pathogenic fungi or generate toxic compounds that restrict their development. The antagonistic properties of *Trichoderma* spp. involve multiple mechanisms, including mycoparasitism and indirect action as plant endophytes through activation of systemic responses, attraction of natural enemies, or parasitism of insect-associated symbiotic microorganisms [15–17].

Among *Trichoderma* spp., *T. asperellum* has been increasingly attracting research attention due to its biocontrol activity against a broad range of fungal and oomycete plant pathogens [18]. Numerous studies have shown that treating host plants with *T. asperellum* can suppress diseases caused by *Phytophthora ramorum* [19], *P. infestans* [20], *P. cinnamomi* [21], *Fusarium oxysporum* [22,23], *Verticillium* sp. [24], *Sclerotium cepivorum* [25], and *Colletotrichum gloeosporioides* [24,26]. Disease suppression has been associated with both competitive interactions and mycoparasitism through the secretion of lytic enzymes [27]. The *T. asperellum* IDE-10 strain was previously tested in preliminary experiments at Ideagro, which demonstrated its ability to control some pathogens, such as *Venturia oleaginea* (data not published).

The use of *Trichoderma* spp. as a bioinsecticide has increased in recent years. Beyond classical mycoparasitism, several *Trichoderma* strains display direct and indirect activity against insect pests, reflecting an evolutionary history linked to entomoparasitic ancestors [28]. *Trichoderma* spp. act directly as entomopathogens through parasitism and the production of insecticidal secondary metabolites, antifeedant compounds, and repellent metabolites [29–31], or by secreting cell wall-degrading enzymes that affect insect cuticle and peritrophic matrices [32]. As a biocontrol agent against pests, *T. asperellum* is currently recognized as an emerging entomopathogen with high efficacy against a wide range of arthropods of agricultural relevance [33]. In agricultural systems, *T. asperellum* can cause rapid mortality of herbivorous mites and insects (e.g., *Ostrinia furnacalis*, *Bemisia tabaci*, and *Xylosandrus germanus*), which is linked to both the production of volatile compounds such as 6-pentyl- α -pyrone and to the disruption of insect gut integrity and microbiota [34–37]. Some preliminary trials with the *T. asperellum* IDE-10 strain showed promising results in relation to its use as an entomopathogen [38,39].

To determine if a *Trichoderma* strain is a suitable candidate for the development of a biological control product, several aspects must be considered, including the type of propagule produced, the optimal conditions for generating infective propagules, and the formulation strategy. These factors ultimately determine the ability of the agent to control the target pest and to persist under the environmental conditions prevailing in the cropping system. *Trichoderma* spp. generates three propagule types: hyphae, chlamydospores, and conidia [40]. Although hyphae are the primary propagules, they are not resilient to drying and rapidly lose viability upon dehydration. Chlamydospores and conidia therefore constitute the active components in most *Trichoderma*-based products

[41,42]. Propagules can be produced through liquid or solid-state fermentation, where reducing substrate costs while maximizing yield is crucial. During inoculum production, culture medium pH is a key factor influencing mycelial growth, biomass accumulation, and conidial production; in general, pH values between 4.5 and 7.5 favor biomass formation and sporulation in several *Trichoderma* species, including *T. asperellum* [43–45]. In addition, thermal stress is one of the main factors affecting conidial survival during inoculum production, drying, and storage, and thus must be carefully controlled to ensure an adequate shelf life and field performance of the final formulation [46,47]. The choice of formulation method often depends on the intended use. Common application methods for *Trichoderma* spp. spores include pre-planting seed or propagation material treatments, incorporation into the soil during planting or transplanting, application via irrigation, or root drench or dip techniques. The highest colonization success rates are observed when spores are directly applied to seeds and roots [48]. However, in their role as a pest and disease control agent, *Trichoderma*-based products are also being increasingly applied to foliage. In this context, ultraviolet (UV) radiation is regarded as one of the most detrimental environmental factors for fungal propagules, causing rapid declines in conidial germination and viability on exposed plant surfaces [49]. Studies on antagonistic fungi, including *Trichoderma* spp., have likewise reported strong reductions in conidial survival under UV exposure, underscoring the need to select strains and application strategies that mitigate this constraint [50]. For *Trichoderma*-based products, recent formulation research has further highlighted the role of carrier substances and encapsulation technologies in enhancing propagule protection, viability, and shelf life, including under light and radiation stress [51].

Integrated pest and disease management (IPM) promotes the combined use of all available control methods to achieve effective and environmentally sound crop protection [52]. Within this framework, *Trichoderma*-based products are being increasingly incorporated as key biopesticidal tools, but in most production systems, they are applied alongside fungicides and insecticides rather than as stand-alone interventions. Consequently, their successful deployment requires that the selected *Trichoderma* strain be compatible with the agrochemicals commonly used on the target crop, since several active ingredients can markedly inhibit fungal growth and sporulation [53]. Recent studies in Mediterranean agroecosystems have further demonstrated the value of locally adapted *Trichoderma* strains in managing diseases in citrus [54], solanaceous crops [55], and vegetable systems [56], underscoring the need to characterize the ecophysiological fitness of candidate strains under the specific thermal, UV, and agrochemical conditions prevalent in these regions.

Thus, the main objective of this study was to characterize a *Trichoderma asperellum* strain with proven efficacy in preliminary assays, by determining its ecophysiological requirements (temperature and pH) for growth and conidial viability, its tolerance to UV radiation, and its compatibility with fungicides and insecticides commonly used in integrated pest and disease management programs.

2. Materials and Methods

2.1. *Trichoderma asperellum* IDE-10 Strain

The *T. asperellum* strain (IDE-10) used in this study was obtained from the collection of the Ideagro S.L.U. company (Lorquí, Spain); it was originally isolated from an irrigation water sample. Molecular identification of strain IDE-10 was previously performed using PCR amplification of the internal transcribed spacer (ITS) region of the 18S rRNA gene with the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The resulting sequence was compared against the NCBI GenBank database using the BLASTn algorithm, yielding 100% sequence identity

with reference sequences of *T. asperellum*, thus confirming the taxonomic assignment of the strain. The ITS sequence of *T. asperellum* IDE-10 has been deposited in the GenBank database under accession number PZ713279. The strain was cultured on Potato Dextrose Agar (PDA), Sabouraud chloramphenicol agar, and potato dextrose broth as required by the experimental design. For short-term storage, *Trichoderma* was maintained under refrigeration in Petri plates containing purified, sporulating mycelia. For medium-term preservation, cultures were stored at $-20\text{ }^{\circ}\text{C}$ in 10–15% glycerol, whereas for long-term preservation, after obtaining the restricted reservoir of the strain, the culture was freeze-dried and stored.

2.2. Characterization of Strain

To characterize the IDE-10 strain, a series of laboratory assays were conducted to evaluate the effects of temperature on growth and germination in plates and in soil, as well as the effects of ultraviolet radiation and pH.

2.2.1. Effect of Temperature on *T. asperellum* In Vitro Viability

Fungal cultures were incubated at 5, 15, 25, and $35\text{ }^{\circ}\text{C}$. Circular plugs with a diameter of 8 mm were excised from non-sporulating mycelia of 3-day-old cultures using a cork borer, and a single plug was placed in an inverted position in the center of a new Petri plate (45 mm diameter) containing PDA medium. The ten replicates used in the thermal profiling assay were prepared from a single sporulating colony of *T. asperellum* IDE-10, ensuring uniform inoculum origin and minimizing within-strain variability, and were prepared for each temperature treatment. The radial growth was measured at 1, 2, 3, 7, and 13 days. Only the first three days were used to model growth, as growth rates at the different temperatures converged thereafter. Radial growth was evaluated daily over a 3-day period and was modeled with a generalized beta function. The effect of temperature on the fungal growth rates (V) was evaluated using a nonlinear model β according to [57]:

$$V(T) = TY_{opt} \times \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^{T_B \times \left(\frac{T_{opt} - T_{min}}{T_{max} - T_{opt}} \right)} \times \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right)^{T_B} \quad (1)$$

where $V(T)$ is the dependent variable (fungal growth in mm per day), and T is the independent variable (incubation temperature). T_{min} , T_{max} , and T_{opt} are, respectively, the lowest, highest, and optimal temperatures for fungal growth. TY_{opt} is the fungal growth at the optimal temperature (T_{opt}). T_B is the shape parameter that influences the temperature range around T_{opt} in which the curve remains near TY_{opt} . T_{min} was fixed at $5\text{ }^{\circ}\text{C}$. Consequently, a better fit was achieved, and the standard error estimation of the other parameters was also improved. The nonlinear model β and the estimation of parameters along with their standard errors were performed using five values of V obtained (mm/day) from five repetitions at each temperature. T_{max} , T_{opt} , TY_{opt} , and T_B values were estimated using the Newton method.

Sporulation was evaluated during fungal growth in Petri dishes containing PDA culture medium. Sporulation was evaluated at 1, 2, 3, 7, and 13 days and was given a value based on the percentage: 1 (<25% sporulation), 2 (26–50% sporulation), 3 (51–75% sporulation), 4 (76–90% sporulation), and 5 (100% sporulation).

2.2.2. Effect of Temperature on Strain Viability in Sterile Soil Microcosm

The experiment was conducted in soil from the Ideagro experimental station. First, 10 g of soil was transferred to borosilicate bottles, with three bottles per treatment (3 repetitions), which were autoclaved at $121\text{ }^{\circ}\text{C}$ for 60 min on three consecutive days. Then, 1 mL of fungal suspension (1×10^8 conidia mL^{-1}) was added to each bottle and incubated

at 7, 14, and 21 °C for 7 days. After incubation, the number of colony-forming units (CFU) of *T. asperellum* per g of soil was determined.

2.2.3. Effect of Ultraviolet Radiation on Strain Viability

The IDE-10 strain was grown in PDA medium in darkness at 25 °C for 10 days. The conidia were collected and used to make a 2.3×10^7 conidia mL⁻¹ suspension. Sterile plates containing the fungal suspension, with four repetitions per treatment (1 plate per repetition), were exposed to UV-A and UV-B radiation for 0, 0.5, 3, and 24 h using a 15 W tube (LuckyHerp, Changzhou, China) at a distance of 20 cm. Afterwards, serial dilutions were performed ($1:10^{-1}$ to $1:10^{-7}$ conidia mL⁻¹) and 500 µL of dilution was plated and incubated in darkness at 25 °C for 3 days. The effect was assessed by measuring the CFU mL⁻¹ in each plate.

2.2.4. Effect of Medium pH on Strain Viability

To determine the optimal pH for isolate growth, sterile peptone–water solutions with a pH ranging from 3 to 9 were prepared. The pH was adjusted using 1 N NaOH and 1 N HCl, and the solutions were sterilized at 121 °C for 20 min. Then, 100 mL of the sterile solutions was homogenized for 1 h with 1 mL of the strain (1×10^8 CFU mL⁻¹) in sterile cylindrical polypropylene containers at room temperature in darkness. Three repetitions were performed (1 container per repetition). pH solutions were prepared without buffering agents in order to replicate the conditions typically encountered during field application. Afterwards, the treatments were incubated in darkness at 25 °C for 24 h. The viability of the strain was determined by measuring the CFU mL⁻¹ of each pH treatment.

2.3. Compatibility of the Strain with Fungicides

The fungicides selected for this study are among the most widely used active ingredients in Mediterranean horticultural and permanent crops (e.g., solanaceous vegetables, cucurbits, grapevine, olive, and citrus). They represent contrasting modes of action, including multi-site contact inhibitors (copper oxychloride, metiram, and cupric calcium sulfate), systemic fungicides (metalaxyl and fosetyl-Al), DMI fungicides (difenoconazole), and SDHI + QoI mixtures (boscalid + pyraclostrobin), and cover the main chemical families routinely applied in IPM programs in southern Spain, where co-application with biocontrol agents is becoming increasingly common. The maximum authorized dose (MAD) of each fungicide was used in the current work; the fertigation solutions are presented in Table 1. To prepare the experimental doses, 1 mL of the fungal suspension (1×10^8 CFU mL⁻¹) was added. The mixtures were homogenized for 1 h at 150 rpm in sterile cylindrical polypropylene containers. Three repetitions were performed (1 container per repetition). The data are expressed as the percentage of inhibition with respect to the control.

Table 1. Fungicides used in the compatibility assay.

Active Ingredient	Commercial Name	MAD	Experimental Dose (mL or mg L ⁻¹)	Mode of Action
Copper Oxychloride 50% (WP) P/P	Curenox 50	0.5%	5	Multi-site contact activity
Fosetyl-Al 80% w/w	Puntal	0.3%	3	Host plant defense induction
Difenoconazole 25% [EC] w/v	Lexor-25	0.08%	0.8	Targets sterol biosynthesis in membranes
Metiram 70% WG	Polyram DF	0.20%	2	Multi-site contact activity
Metalaxyl 25%	Armetil 25 WP	1.52 kg/ha	1.52	Targets nucleic acid metabolism
Pyraclostrobin 6.7% + Boscalid 26.7%	Signum	1.5 kg/ha	1.5	Targets respiration
Cupric Calcium Sulfate 20%	Caldo Manica 20 WG	0.62%	6.2	Multi-site contact activity

2.4. Compatibility of the Strain with Insecticides

The insecticides selected for the compatibility assay represent the main chemical classes used against key arthropod pests in Mediterranean crops (e.g., citrus, grapevine, and vegetables) (Table 2). Their inclusion reflects the realistic co-application scenarios faced by growers integrating *Trichoderma*-based products into IPM programs. To prepare the experimental doses, 50 mL of *T. asperellum* conidial suspension (1×10^7 CFU mL⁻¹) was added to sterile cylindrical polypropylene containers with sterile distilled water. Three repetitions were performed (1 container per repetition). An appropriate insecticide amount was added to achieve the required dose and the mixture was incubated for 1 h at 150 rpm under RT laboratory conditions (23 °C) in darkness. Afterward, serial dilutions were performed ($1:10^{-1}$ to $1:10^{-7}$ conidia mL⁻¹) and 100 µL of dilution was plated and incubated in darkness at 25 °C for 3 days. The effect was assessed by measuring the CFU mL⁻¹ in each plate. The data are expressed as the percentage variation with respect to the control.

Table 2. Insecticides used in the compatibility assay.

Active Ingredient	Commercial Name	Recommended Dose (L ha ⁻¹)–(kg ha ⁻¹)	Experimental Dose (mL L ⁻¹)	Mode of Action
Abamectin 1.8%	Apache EW	1	1	Allosteric modulation of the glutamate-dependent chloride channel
Hexythiazox 10% [WP] p/p	Zepo	1	1	Mite growth inhibition by targeting CHS1
Fenpiroximate 5.12% [SC] p/v	FlashUM	0.13%	1.3	Electron transport inhibition by targeting mitochondrial complex I
Sulfur 80% [WP] p/p	SOFREVAL 80 WG	2	2	Unknown or uncertain mode of action
Paraffin 20% [EC] p/v	Laincoil	15	15	Non-specific mechanical and physical disruptor

2.5. Statistical Analysis

First, the normality and homoscedasticity of the data were checked using the Shapiro–Wilk and Levene’s tests, respectively. Statistical analyses were performed using IBM SPSS Statistics v.26 (IBM Corp., Armonk, NY, USA). Analysis of variance (ANOVA) was used to compare the means of the different treatment groups when the data were normal and homoscedastic, using different post hoc tests depending on the data characteristics. LSD or Tukey’s b tests were used for homoscedastic data, while T2 Tamhane tests were used for heteroscedastic data. When the data did not have a normal distribution (non-parametric data), the Kruskal–Wallis test was used for multiple-median comparisons, and the Mann–Whitney U test was used for paired comparisons. The JMP student edition version 18 software (JMP Statistical Discovery LLC, Cary, NC, USA) was used to develop the thermal growth model.

3. Results

3.1. Characterization of the Strain

3.1.1. Effect of Temperature on *T. asperellum* In Vitro Viability

Fungal growth was significantly influenced by temperature (after 7 days; χ^2 : 0.000) (Figure 1a). The *T. asperellum* IDE-10 strain was unable to grow at 5 °C, but at 15 °C, fungal growth began on day 1, reaching approximately 35 mm by day 7, and attaining a maximum of 45 mm at day 13. The growth at 25 °C and 35 °C did not show a significant difference; these temperatures had the greatest influence on fungal growth, supporting rapid and extensive fungal development, with maximum radial growth (45 mm) reached by day 3 and maintained through to day 13.

Figure 1b illustrates the sporulation value (1–5 scale) of the *T. asperellum* IDE-10 strain at 5, 15, 25, and 35 °C over a 13-day period. *T. asperellum* IDE-10 was unable to sporulate at 5 °C throughout the evaluation period. However, at 15 °C, sporulation initiated slowly, reaching less than 25% sporulation (value of 1) by day 3 and gradually increasing to approximately 50% sporulation (value of 2) by day 13. Statistical analysis revealed significant differences between 15 °C and the optimal temperatures (25 °C and 35 °C), indicating that 15 °C markedly restricts the sporulation capacity of the fungus. In contrast, both 25 °C and 35 °C supported rapid and robust sporulation, with sporulation increasing steadily and reaching 100% (value of 5) by day 13.

As most of the plates incubated at 25 °C and 35 °C reached maximum growth by the third day, the fungal growth model was constructed based solely on the first three days of incubation. The radial measurements from the 1st to the 3rd day fit a linear model (Figure 2). The fungal isolate grew at 15, 25, and 35 °C, but not at 5 °C. The fixed regression lines indicate that the maximum growth rate was reached at 28.28 °C (T_{opt}), with an optimum fungal growth rate (TY_{opt}) of 13.84 mm/day (Table 3). The maximum temperature for fungal growth (T_{max}) was 39.21 °C (Table 3).

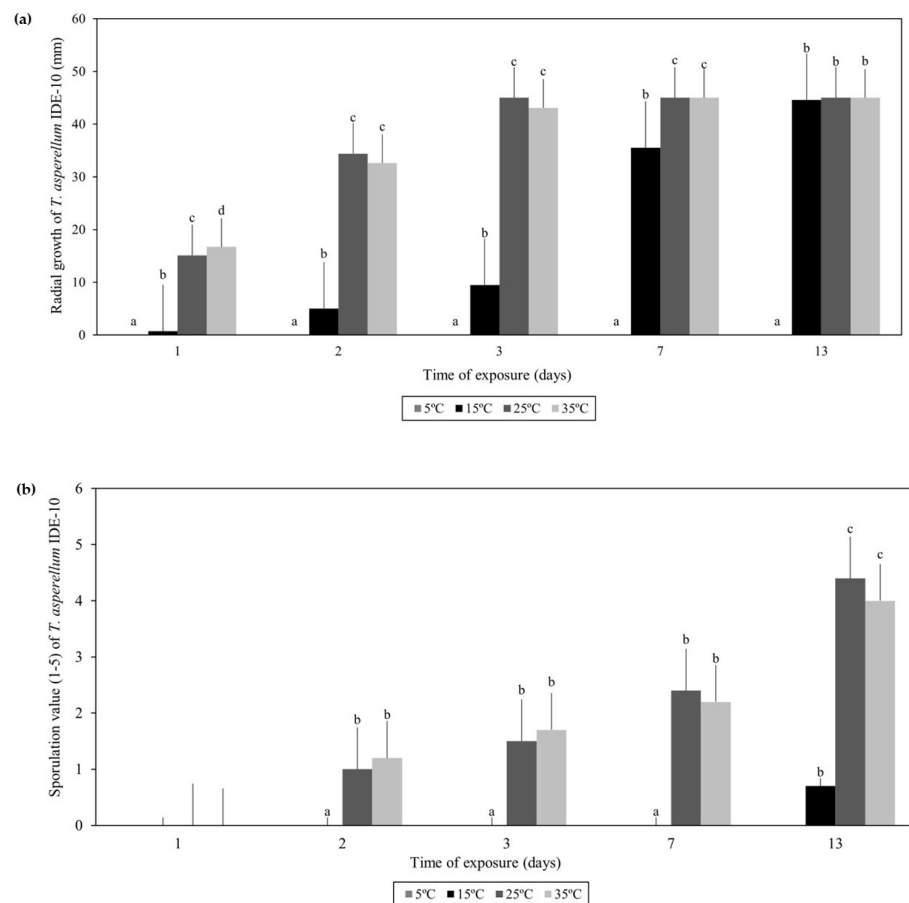


Figure 1. (a) Radial growth and (b) sporulation values (1–5 scale) of *T. asperellum* IDE-10 strain grown at different temperatures for 13 days. Values are the mean $\pm$ SE ($n = 10$). Different letters indicate statistical differences ($p < 0.05$, Kruskal–Wallis test with post hoc Mann–Whitney U) between temperatures at the same time point.

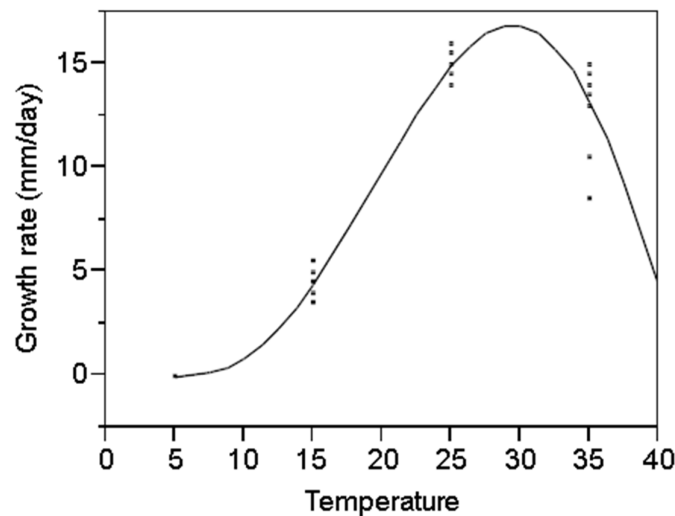


Figure 2. Model prediction for the effect of temperature on the growth rate of *T. asperellum* IDE-10. The line represents fitted curves obtained using the generalized β function, which was modified according to [57].

Table 3. Estimated parameters ($\pm$ SE) of the generalized β function (modified according to [57]), which was fitted to the vegetative growth data of *T. asperellum* IDE-10.

Strain	T_{opt}	T_{max}	TY_{opt}	T_B
IDE-10	28.28 ± 0.23	39.21 ± 0.24	13.84 ± 0.23	0.93 ± 0.08

The generalized β function is given by $V(T) = TY_{opt} \times [(T - T_{min})/(T_{opt} - T_{min})]^{T_B} \times [(T_{opt} - T_{min})/(T_{max} - T_{opt})]^{T_B} / [(T_{max} - T)/(T_{max} - T_{opt})]^{T_B}$, where $V(T)$ is the fungal growth in mm per day (dependent variable) and T is the incubation temperature (independent variable). T_{min} , T_{max} , and T_{opt} are, respectively, the lowest, highest, and optimal temperatures for fungal growth. TY_{opt} is the fungal growth at the optimal temperature T_{opt} and T_B is the shape parameter.

3.1.2. Effect of Different Factors (Temperature of Soil Microcosm, Ultraviolet Radiation Exposure, and pH Level) on Strain Viability

There were no significant differences in *T. asperellum* IDE-10 growth among the three soil microcosm temperatures evaluated (7, 14, and 21 °C) ($F = 2.571$; $p = 0.156$). Despite that, the highest CFU g^{-1} concentration was found in the soil microcosm incubated at 14 °C (7.19×10^5 CFU g^{-1} soil), followed by that incubated at 7 °C (4.96×10^5 CFU g^{-1} soil) and at 21 °C (3.15×10^5 CFU g^{-1} soil) (Figure 3a).

No statistically significant differences in viability were observed between the UV radiation exposure durations evaluated (0, 0.5, 3, and 24 h). The CFU mL^{-1} concentration remained relatively stable throughout the experiment ($F = 1.933$; $p = 0.178$). Despite that, the highest CFU mL^{-1} was recorded after 3 h of exposure (1.79×10^7 CFU g^{-1} soil), followed by 0 (1.35×10^7 CFU g^{-1} soil) and 0.5 h (1.46×10^7 CFU g^{-1} soil) and, finally, 24 h (9.88×10^6 CFU g^{-1} soil) (Figure 3b).

No significant differences were observed in viability at the pH levels evaluated (pH 3, 4, 5, 6, 7, 8, and 9) (χ^2 : 4.411). The CFU concentration remained relatively high in the pH range of 3 to 7, with values close to 2.03×10^6 and 3.44×10^6 CFU g^{-1} of soil. pH 3 and 7 showed the highest CFU concentrations (3.14×10^6 and 3.44×10^6 CFU g^{-1} , respectively), while pH 8 and 9 showed the lowest concentrations (1.0×10^6 and 1.08×10^6 CFU g^{-1} , respectively) (Figure 3c).

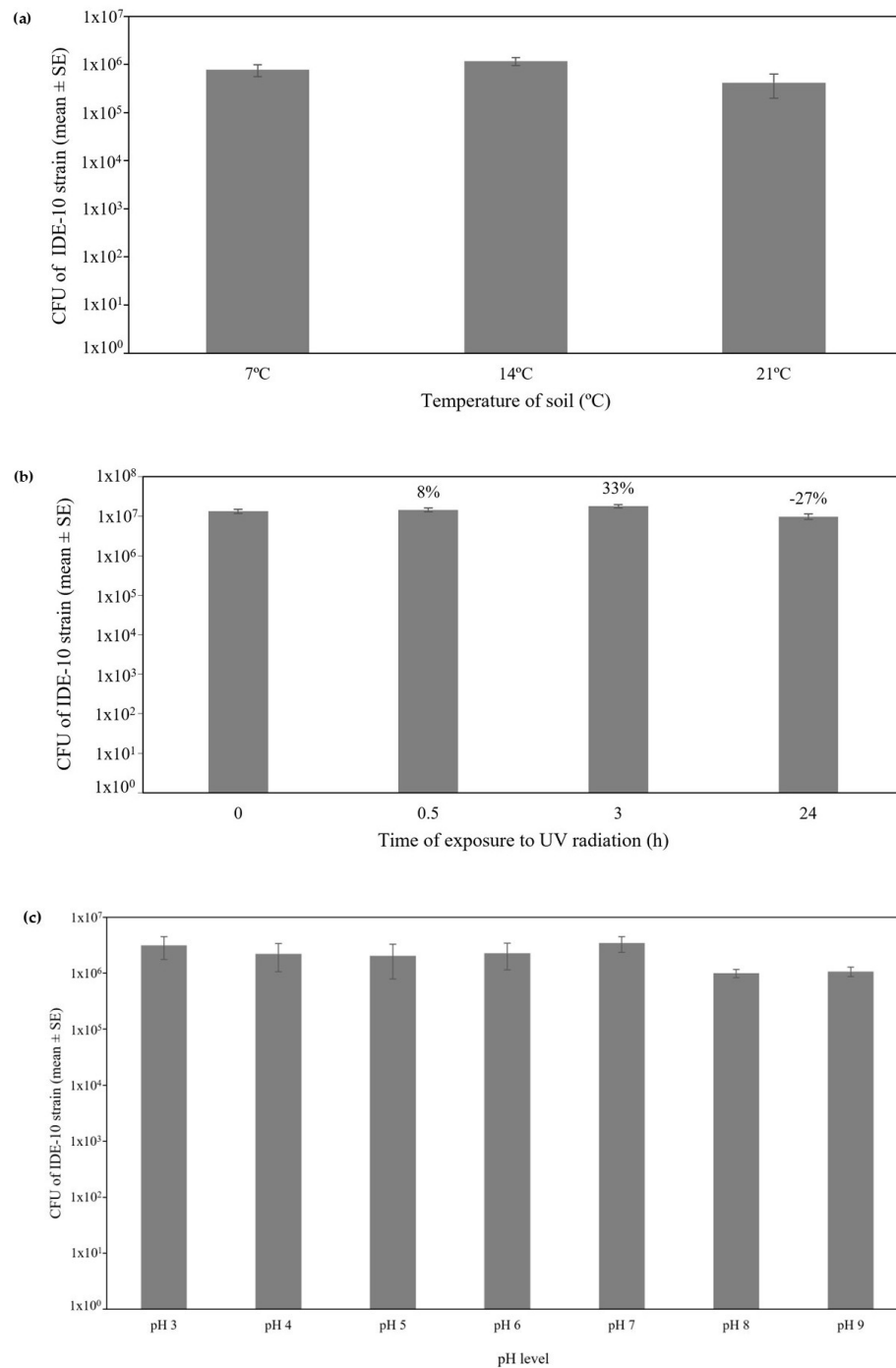


Figure 3. Effect of different factors on *T. asperellum* IDE-10 strain viability: (a) soil temperature; (b) ultraviolet radiation exposure; and (c) pH. Values are the mean $\pm$ SE. No significant differences were observed among the different temperatures, UV-B radiation exposure durations, or pH levels.

3.2. Compatibility of the Strain with Fungicides and Insecticides

The evaluation of the impact of various fungicide treatments on the viability of the *T. asperellum* IDE-10 strain revealed significant differences ($\chi^2 = 16.104$; $p = 0.024$). As shown in Figure 4a, the control group, which received no treatment, maintained a CFU concentration of approximately 9.28×10^4 CFU mL⁻¹. This level of viability was statistically similar to the Caldo Manica 20 WG (1.07×10^5 CFU mL⁻¹), Armetil 25 WP (8.69×10^4 CFU mL⁻¹), and Lexor-

25 (6.49×10^4 CFU mL⁻¹) treatments, suggesting that these fungicides do not have a significant detrimental effect on the *T. asperellum* IDE-10 strain under the evaluated conditions.

On the other hand, the Curennox 50 (4.54×10^4 CFU mL⁻¹), Puntal (6.43×10^4 CFU mL⁻¹), Polyram DF (5.59×10^4 CFU mL⁻¹), and Signum (5.40×10^4 CFU mL⁻¹) treatment groups showed significantly lower CFU concentrations than the control group, implying a reduction in the viability of the strain. These findings are crucial for the selection of fungicides compatible with biological control strategies involving *T. asperellum* IDE-10, highlighting the importance of evaluating the selectivity of plant protection products to preserve the efficacy of biocontrol agents.

No significant differences in the viability of the *T. asperellum* IDE-10 strain were detected between the different insecticide treatments evaluated (control, abamectin, hexythiazox, fenpyroximate, sulfur, and paraffin) ($F = 0.828$; $p = 0.553$). In general, the CFU concentration remained high and fairly stable in all cases, with values ranging from approximately 1.26×10^7 to 2.05×10^7 CFU mL⁻¹.

The paraffin treatment showed the highest concentration, close to 2.05×10^7 CFU g⁻¹ of soil, followed by abamectin and hexythiazox, both with values around 1.78×10^7 CFU g⁻¹ of soil. The control, fenpyroximate, and sulfur groups showed slightly lower concentrations, with 1.28×10^7 , 1.48×10^7 , and 1.26×10^7 CFU mL⁻¹, respectively (Figure 4b).

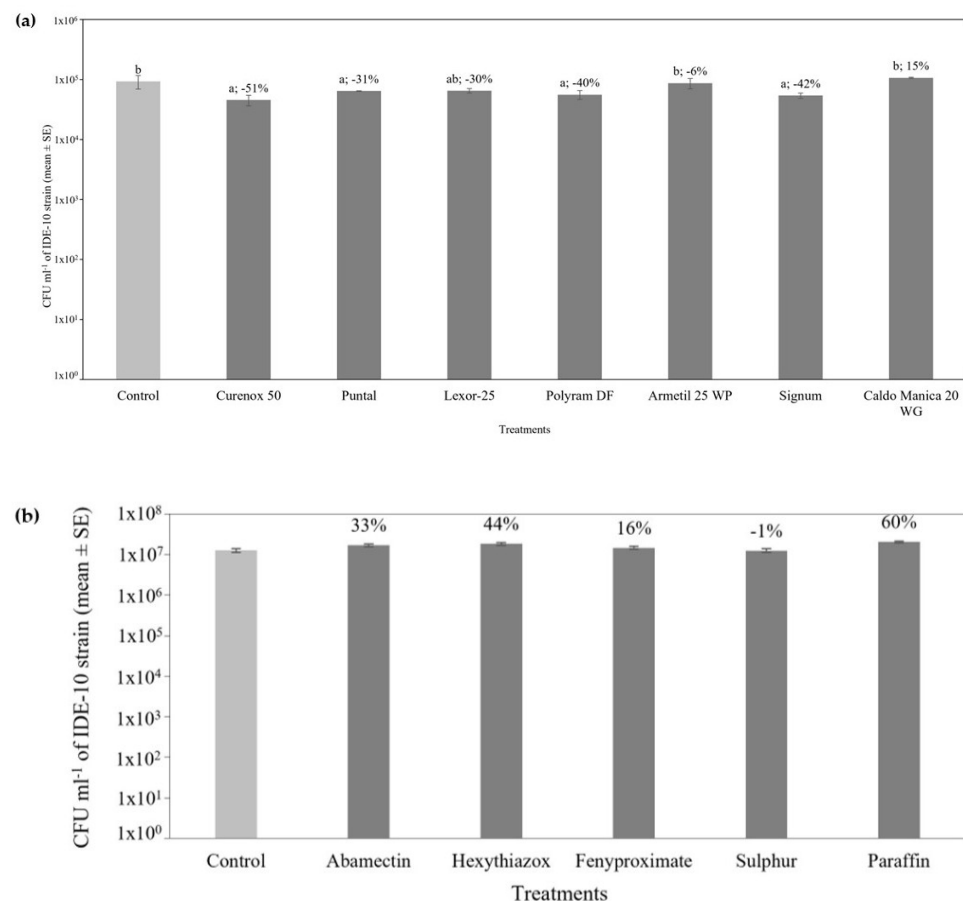


Figure 4. Compatibility of *T. asperellum* IDE-10 strain with fungicides and insecticides. Values are mean $\pm$ SE (CFU mL⁻¹). Percentage change relative to the control group was included inside each bar. (a) With fungicides, significant differences were detected. Different letters indicate statistical differences ($\chi^2 = 16.104$; $p = 0.024$, Kruskal–Wallis test). (b) With insecticides, no significant differences were observed ($F = 0.828$; $p = 0.553$, one-way ANOVA).

4. Discussion

The characterization of the *Trichoderma asperellum* IDE-10 strain in this study revealed its ecophysiological traits and compatibility with agrochemicals, which position it as a promising biological control agent in agricultural systems, particularly under the challenging environmental conditions typical of the Mediterranean region. The results regarding its tolerance to different temperatures, pH levels, and ultraviolet radiation, and its compatibility with fungicides and insecticides provide essential insights into its potential application within integrated pest management programs.

Regarding the effect of temperature, the *T. asperellum* IDE-10 strain showed optimal growth at 28.28 °C (T_{opt}) and a maximum growth temperature (T_{max}) of 39.21 °C. This thermal range is consistent with the expected adaptations for a microorganism with potential application in warm areas. Previous studies on *T. asperellum* have reported optimal growth temperatures in a similar range, generally between 25 °C and 30 °C [58]. The ability of the IDE-10 strain to maintain robust growth at 35 °C and its relatively high T_{max} suggest a competitive advantage in warm climates, which is particularly relevant for agriculture in southern Spain, where summer temperatures can be high. Some studies even indicate that high temperatures, such as 36 °C, can enhance the germination rate of certain *T. asperellum* strains [59], which could be a factor to explore in future research with *T. asperellum* IDE-10. From a biosafety perspective, although mycelial growth was not experimentally evaluated at 37 °C in this study, the fitted beta model predicts a substantially reduced growth rate at this temperature compared to the optimum, which is consistent with the classification of *T. asperellum* as a biosafety level 1 (BSL-1) organism with low pathogenic risk to immuno-competent humans. Nevertheless, dedicated experimental validation at 37 °C is recommended as part of a full safety evaluation for commercial registration of IDE-10-based products. Moreover, the marked tolerance to elevated temperatures makes this strain a strong candidate for the development of commercial formulations, as thermal stability is a key parameter in product design and manufacturing [40].

The viability of the strain under different soil conditions and its pH tolerance determine its establishment and persistence when used as a biocontrol agent. The *T. asperellum* IDE-10 strain demonstrated remarkable stability over a wide pH range (3 to 7), with a slight decrease under more alkaline conditions (pH 8–9). This plasticity is fundamental, as agricultural soils can vary considerably in pH. While *Trichoderma* spp. is often associated with acidic soils, the existence of strains adapted to alkaline conditions has been documented [60], which is a desirable characteristic for their use in neutral to slightly alkaline soils. Our results are consistent with the optimal range reported for commercial *Trichoderma* isolates, typically between pH 5 and 7 [53]. Notably, IDE-10 remains active under more acidic conditions than those commonly evaluated (pH 4.1–8.6 for *Trichoderma* spp. and 5.5–7.5 for *T. viride* isolates), suggesting an extended functional window towards low-pH environments that may be advantageous in acidic soils [45,53]. The ability of *T. asperellum* IDE-10 to survive at soil temperatures of 7, 14, and 21 °C without significant loss of performance complements previous findings for *T. asperellum* MSCL 309, which focused on long-term viability in peat at 15 and 30 °C [58], and indicates that IDE-10 has an enhanced capacity to remain active under cooler soil conditions.

Although IDE-10 was originally isolated from irrigation water collected in a tomato cropping system, this does not by itself prove the strain's rhizosphere competence or root colonization ability. The isolation of *Trichoderma* spp. from irrigation water and aquatic environments is well documented, and water systems can act as natural dispersal routes connecting soil and rhizosphere compartments [55,56]. However, persistence in a water matrix is ecologically distinct from active rhizosphere colonization, which requires a competitive saprophytic ability, root surface attachment, and tolerance to root exudate

chemistry [61,62]. Our data indicate that IDE-10 has a favorable ecophysiological profile, including broad pH tolerance and thermal stability in soil, that may support its establishment and persistence in rhizosphere environments. These characteristics are considered key prerequisites for rhizosphere competence in *Trichoderma* spp. Nevertheless, dedicated root colonization assays and rhizosphere competence studies under controlled and field conditions will be required to confirm this potential.

A critical aspect for the efficacy of foliar-applied biological control agents is their resistance to ultraviolet radiation. The *T. asperellum* IDE-10 strain exhibited high tolerance to UV exposure, maintaining viability even after 24 h. Sametz-Baron, Berrocal, Amit, Herrera-Estrella, and Horwitz [50] reported a strong UVC sensitivity for *T. harzianum* conidia, where germination was severely inhibited and survival largely depended on subsequent photoreactivation by visible/UVA light. The high UV tolerance of the IDE-10 strain suggests that it possesses more effective intrinsic protective mechanisms against UV-induced damage, potentially enhancing its persistence and efficacy when applied on exposed surfaces. This finding is of great importance, as UV radiation is a primary abiotic factor limiting the persistence of many biopesticides in the field [63]. The intrinsic UV tolerance observed in vitro suggests that IDE-10 may require less protective encapsulation than UV-sensitive strains; however, field persistence studies under real agronomic conditions are needed to confirm this hypothesis [64,65].

The majority of *T. asperellum* strains described in the literature display an optimal growth temperature (T_{opt}) between 25 and 30 °C and a maximum temperature (T_{max}) in the range of 35–37 °C, beyond which growth ceases [66,67]. The modeled T_{max} of IDE-10 near 39 °C exceeds this range, suggesting a thermal tolerance profile comparable to the most thermotolerant *T. asperellum* strains reported to date, such as TaDOR7316 [67]. Commercially registered strains such as *T. atroviride* T11, *T. asperellum* T25 and TV1, and *T. viride* ICC080 have been characterized under temperate climate conditions, with growth temperatures ranging from 10 to 35 °C and an optimal pH range of 4.5–6.0. In contrast, strain IDE-10 was predicted to grow at temperatures up to 39.21 °C and exhibited vigorous growth across a pH range of 3–7, representing a broader ecophysiological window than those of the aforementioned commercial strains. Furthermore, neither T11, T25, nor TV1 has published data on UV radiation tolerance [68,69], whereas IDE-10 maintained complete conidial viability after 24 h of continuous UV exposure, a key advantage for foliar applications in high-radiation Mediterranean environments.

The ecophysiological profile of IDE-10 has direct implications for the selection of suitable formulation strategies. Its thermal stability and broad pH tolerance (pH 3–7) suggest compatibility with wettable powder (WP) and water-dispersible granule (WG) formulations, which are the most common commercial formats for *Trichoderma*-based products and typically require conidial survival across a wide range of storage and application conditions [51]. Published shelf-life data for registered *T. asperellum* WP formulations indicate viability maintenance for up to 2 years at 4 °C and 6–8 months at 15–25 °C [51], values that serve as benchmarks against which IDE-10 stability data should be evaluated. The high intrinsic UV tolerance of the IDE-10 strain further reduces the need for costly protective additives such as UV-absorbing photoprotectants or microencapsulation technologies, substantially reducing production costs [70]. Oil-based formulations have also been shown to provide superior UV protection for *Trichoderma* conidia compared to aqueous suspensions, improving post-UV viability from 8–12% (water) to 56–63% (vegetable oil emulsion) [51], suggesting that oil-dispersion formats could further enhance the field persistence of IDE-10 without requiring encapsulation.

Compatibility with fungicides and insecticides is a fundamental pillar for integrating biological control agents into integrated pest management programs. The results showed that the *T. asperellum* IDE-10 strain is compatible with fungicides such as Armetil 25 WP

(metalaxyl), Lector-25, and Caldo Manica 20 WG (copper), allowing for co-application or sequential application without significant loss of viability, which is consistent with previous reports of low inhibition of *T. asperellum* by metalaxyl-based formulations such as Ridomil Gold and tolerance to mancozeb at agronomic doses [71]. However, other fungicides such as Curenox 50 (copper), Puntal, Polyram DF, and Signum showed an inhibitory effect, which is in line with studies showing that several active ingredients (e.g., carbendazim, propiconazole, and fludioxonil) can strongly suppress the mycelial growth or conidial viability of *Trichoderma* spp., and that compatibility is highly dependent on the specific formulation and dose of each product [53]. Several physicochemical factors could explain the differential response of IDE-10 to Curenox 50 (copper oxychloride) versus Caldo Manica 20 WG (cupric calcium sulfate): (i) fungitoxicity is primarily determined by free Cu^{2+} activity in solution and the bioavailability of cupric ions (Cu^{2+}) differs markedly between formulations, being considerably lower in sparingly soluble oxychlorides than in more soluble copper sulfate-based products [72]; (ii) the pH of the spray solution influences copper speciation, with lower pH values increasing Cu^{2+} solubility and thus fungitoxicity [73]; and (iii) adjuvants, wetting agents, and carrier substances present in each commercial formulation alter the physical contact between the active ingredient and fungal conidia, modulating the effective dose reaching the conidial surface [74,75]. It is encouraging to observe the IDE-10 strain's high compatibility with all evaluated insecticides (abamectin, hexythiazox, fenpyroximate, sulfur, and paraffin), which is consistent with the literature generally reporting lower toxicity of insecticides to *Trichoderma* compared to fungicides [71,76]. This characteristic is vital for the development of biological control strategies that can coexist with the use of insecticides in agricultural pest management.

The compatibility data obtained for IDE-10 have direct practical implications for its integration into IPM programs in Mediterranean cropping systems. The results confirm that metalaxyl, difenoconazole, and cupric calcium sulfate can be tank-mixed or applied sequentially with IDE-10 without significant loss of conidial viability, facilitating their co-use during critical disease windows in horticultural and permanent crops. Conversely, fungicides that show inhibitory effects should preferably be applied sequentially rather than as tank mixes, with an appropriate interval between the chemical and biological applications to allow for recovery of conidial populations [77]. This sequential strategy has been validated in field trials where the combination of *Trichoderma* spp. with low doses of incompatible fungicides, applied separately, achieved greater disease suppression than either treatment alone [78,79], suggesting that the two approaches can be complementary rather than mutually exclusive when application timing is carefully managed. The full compatibility with all five insecticides tested (abamectin, hexythiazox, fenpyroximate, sulfur, and paraffin oil) further simplifies the integration of IDE-10 into spray programs targeting key Mediterranean arthropod pests, as tank mixes with these products pose no apparent risk to biocontrol efficacy [78].

Despite the promising ecophysiological profile of IDE-10 demonstrated under controlled laboratory conditions, several limitations of this study must be acknowledged. First, all assays were conducted in vitro, and the translation of these results to field conditions, where multiple stresses act simultaneously, soil microbiome competition is present, and agrochemical exposure occurs in complex mixtures, remains to be validated. Second, the compatibility assays were performed at the maximum authorized doses, which represent worst-case scenarios; sub-lethal field concentrations may result in higher compatibility than those reported here. Third, formal demonstration of the rhizosphere competence and root colonization ability of IDE-10 has not been conducted, which is a prerequisite for deployment as a soil-applied biocontrol agent. Future research should therefore prioritize (i) field efficacy trials in representative Mediterranean cropping

systems under the thermal and UV conditions characterized here; (ii) investigation of the molecular mechanisms underlying the thermal and UV tolerance of IDE-10; and (iii) rhizosphere competence assays and root colonization studies to confirm the capacity of IDE-10 to establish and persist in agricultural soils.

5. Conclusions

The *Trichoderma asperellum* IDE-10 strain was shown to be a robust and adaptable biological control agent, with ecophysiological and compatibility characteristics that make it highly promising for use in agriculture. Its optimal growth at elevated temperatures, broad pH tolerance, and remarkable resistance to UV radiation are key attributes that favor its establishment and persistence in agricultural environments, especially in regions with warm climates and high insolation. Compatibility with a significant range of insecticides and specific fungicides opens the door to its effective integration into integrated pest management programs, maximizing the efficacy of biological control without compromising other agricultural practices. Future research should focus on evaluating this strain's field efficacy against specific insect and plant pathogens and on developing formulations that optimize its application and persistence under the agroclimatic conditions of the region.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
CFU	Colony-forming unit
IPM	Integrated pest and disease management
MAD	Maximum authorized dose
PDA	Potato dextrose agar
UV	Ultraviolet

References

1. Ingram, J. A food systems approach to researching food security and its interactions with global environmental change. *Food Secur.* **2011**, *3*, 417–431. <https://doi.org/10.1007/s12571-011-0149-9>.
2. Savary, S.; Willocquet, L.; Pethybridge, S.J.; Esker, P.; McRoberts, N.; Nelson, A. The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* **2019**, *3*, 430–439. <https://doi.org/10.1038/s41559-018-0793-y>.

3. Kashyap, A.S.; Manzar, N.; Sharma, P.K. Unravelling the wheat leaf blight pathobiome: A shift beyond the one pathogen–one disease paradigm. *J. Plant Pathol.* **2026**, *108*, 343–357. <https://doi.org/10.1007/s42161-025-02045-3>.
4. Ab Rahman, S.F.S.; Singh, E.; Pieterse, C.M.; Schenk, P.M. Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci.* **2018**, *267*, 102–111. <https://doi.org/10.1016/j.plantsci.2017.11.012>.
5. Adkar-Purushothama, C.R.; Chettimada, A.; Murali, T.S.; Muthusamy, A.; Bouarab, K.; Perreault, J.-P. Non-chemical control of fungal pathogens in crops: A one-health perspective on strategies, mechanisms, and future directions. *Front. Plant Sci.* **2025**, *16*, 1746521. <https://doi.org/10.3389/fpls.2025.1746521>.
6. Manivel, S.B.; Rajkumar, G.S. Mycopesticides: Fungal based pesticides for sustainable agriculture. In *Fungi and Their Role in Sustainable Development: Current Perspectives*; Springer, Singapore, 2018; pp. 183–203.
7. Hermosa, R.; Viterbo, A.; Chet, I.; Monte, E. Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology* **2012**, *158*, 17–25. <https://doi.org/10.1099/mic.0.052274-0>.
8. Kashyap, A.S.; Manzar, N. Molecular characterization and biocontrol potential of rhizospheric *Trichoderma* and *Bacillus* spp. from Indo-Gangetic plains with botanical applications against *Bipolaris sorokiniana* in wheat. *Physiol. Mol. Plant Pathol.* **2025**, *136*, 102567. <https://doi.org/10.1016/j.pmpp.2025.102567>.
9. Kashyap, A.S.; Kannoja, P.; Manzar, N.; Srivastava, D.; Malaviya, D.; Singh, U.B.; Sharma, P.K. Development of a *Trichoderma*–*Bacillus* biofilm inoculant for plant growth promotion and biocontrol of *Sclerotium* and *Fusarium* in chickpea. *Discov. Sustain.* **2026**, *7*, 301. <https://doi.org/10.1007/s43621-025-02356-6>.
10. Abbey, J.A.; Percival, D.; Abbey, L.; Asiedu, S.K.; Prithiviraj, B.; Schilder, A. Biofungicides as alternative to synthetic fungicide control of grey mould (*Botrytis cinerea*)—Prospects and challenges. *Biocontrol Sci. Technol.* **2019**, *29*, 207–228. <https://doi.org/10.1080/09583157.2018.1548574>.
11. Elad, Y. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Prot.* **2000**, *19*, 709–714. [https://doi.org/10.1016/S0261-2194\(00\)00094-6](https://doi.org/10.1016/S0261-2194(00)00094-6).
12. Yadav, M.; Dubey, M.K.; Upadhyay, R.S. Systemic resistance in chilli pepper against anthracnose (caused by *Colletotrichum truncatum*) induced by *Trichoderma harzianum*, *Trichoderma asperellum* and *Paenibacillus dendritiformis*. *J. Fungi* **2021**, *7*, 307. <https://doi.org/10.3390/jof7040307>.
13. Tjamos, E.C.; Papavizas, G.C.; Cook, R.J. *Biological Control of Plant Diseases: Progress and Challenges for the Future*; Plenum Press: New York, NY, USA, 1922; p. 222.
14. Tyśkiewicz, R.; Nowak, A.; Ozimek, E.; Jaroszuk-Ściśel, J. *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci.* **2022**, *23*, 2329. <https://doi.org/10.3390/ijms23042329>.
15. Contreras-Cornejo, H.A.; Viveros-Bremauntz, F.; Del-Val, E.; Macías-Rodríguez, L.; López-Carmona, D.A.; Alarcón, A.; González-Esquivel, C.E.; Larsen, J. Alterations of foliar arthropod communities in a maize agroecosystem induced by the root-associated fungus *Trichoderma harzianum*. *J. Pest Sci.* **2021**, *94*, 363–374. <https://doi.org/10.1007/s10340-020-01261-3>.
16. Kushiyevev, R.; Tuncer, C.; Erper, I.; Özer, G. The utility of *Trichoderma* spp. isolates to control of *Xylosandrus germanus* Blandford (Coleoptera: Curculionidae: Scolytinae). *J. Plant Dis. Prot.* **2020**, *128*, 153–160. <https://doi.org/10.1007/s41348-020-00375-1>.
17. Lopez, E.; Orduz, S. *Metarhizium anisopliae* and *Trichoderma viride* for control of nests of the fungus-growing ant, *Atta cephalotes*. *Biol. Control* **2003**, *27*, 194–200. [https://doi.org/10.1016/S1049-9644\(03\)00005-7](https://doi.org/10.1016/S1049-9644(03)00005-7).
18. Manzar, N.; Kashyap, A.S.; Goutam, R.S.; Rajawat, M.V.S.; Sharma, P.K.; Sharma, S.K.; Singh, H.V. *Trichoderma*: Advent of versatile biocontrol agent, its secrets and insights into mechanism of biocontrol potential. *Sustainability* **2022**, *14*, 12786. <https://doi.org/10.3390/su141912786>.
19. Widmer, T.L. Screening *Trichoderma* species for biological control activity against *Phytophthora ramorum* in soil. *Biol. Control* **2014**, *79*, 43–48. <https://doi.org/10.1016/j.biocontrol.2014.08.003>.
20. Kariuki, W.; Mungai, N.; Otaye, D.; Thuita, M.; Muema, E.; Korir, H.; Masso, C. Antagonistic effects of biocontrol agents against *Phytophthora infestans* and growth stimulation in tomatoes. *Afr. Crop Sci. J.* **2020**, *28*, 55–70. <https://doi.org/10.4314/acscj.v28i1.5S>.
21. Andrade-Hoyos, P.; Silva-Rojas, H.V.; Romero-Arenas, O. Endophytic *Trichoderma* species isolated from *Persea americana* and *Cinnamomum verum* roots reduce symptoms caused by *Phytophthora cinnamomi* in avocado. *Plants* **2020**, *9*, 1220. <https://doi.org/10.3390/plants9091220>.
22. Díaz-Gutiérrez, C.; Arroyave, C.; Llugany, M.; Poschenrieder, C.; Martos, S.; Peláez, C. *Trichoderma asperellum* as a preventive and curative agent to control *Fusarium* wilt in *Stevia rebaudiana*. *Biol. Control* **2021**, *155*, 104537. <https://doi.org/10.1016/j.biocontrol.2021.104537>.

23. Win, T.T.; Bo, B.; Malec, P.; Khan, S.; Fu, P. Newly isolated strain of *Trichoderma asperellum* from disease suppressive soil is a potential bio-control agent to suppress *Fusarium* soil borne fungal phytopathogens. *J. Plant Pathol.* **2021**, *103*, 549–561. <https://doi.org/10.1007/s42161-021-00780-x>.
24. Yu, Z.; Liu, Z.; Zhang, Y.; Wang, Z. The disease resistance potential of *Trichoderma asperellum* T-Pa2 isolated from *Phellodendron amurense* rhizosphere soil. *J. For. Res.* **2022**, *33*, 321–331. <https://doi.org/10.1007/s11676-021-01332-w>.
25. Rivera-Méndez, W.; Obregón, M.; Morán-Diez, M.E.; Hermosa, R.; Monte, E. *Trichoderma asperellum* biocontrol activity and induction of systemic defenses against *Sclerotium cepivorum* in onion plants under tropical climate conditions. *Biol. Control* **2020**, *141*, 104145. <https://doi.org/10.1016/j.biocontrol.2019.104145>.
26. De la Cruz-Quiroz, R.; Roussos, S.; Rodríguez-Herrera, R.; Hernandez-Castillo, D.; Aguilar, C.N. Growth inhibition of *Colletotrichum gloeosporioides* and *Phytophthora capsici* by native Mexican *Trichoderma* strains. *Karbala Int. J. Mod. Sci.* **2018**, *4*, 237–243. <https://doi.org/10.1016/j.kijoms.2018.03.002>.
27. Wang, C.; Zeng, Z.-Q.; Zhuang, W.-Y. Comparative molecular evolution of chitinases in ascomycota with emphasis on mycoparasitism lifestyle. *Microb. Genom.* **2021**, *7*, 000646. <https://doi.org/10.1099/mgen.0.000646>.
28. Monte, E. The sophisticated evolution of *Trichoderma* to control insect pests. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2301971120. <https://doi.org/10.1073/pnas.2301971120>.
29. Anwar, W.; Subhani, M.N.; Haider, M.S.; Shahid, A.A.; Mushatq, H.; Rehman, M.Z.; Hameed, U.; Javed, S. First record of *Trichoderma longibrachiatum* as entomopathogenic fungi against *Bemisia tabaci* in Pakistan. *Pak. J. Phytopathol.* **2016**, *28*, 287–294.
30. Kaushik, N.; Díaz, C.E.; Chhipa, H.; Julio, L.F.; Andrés, M.F.; González-Coloma, A. Chemical composition of an aphid antifeedant extract from an endophytic fungus, *Trichoderma* sp. EF1671. *Microorganisms* **2020**, *8*, 420. <https://doi.org/10.3390/microorganisms8030420>.
31. Nawaz, A.; Gogi, M.D.; Naveed, M.; Arshad, M.; Sufyan, M.; Binyameen, M.; Islam, S.U.; Waseem, M.; Ayyub, M.B.; Arif, M.J.; et al. In vivo and in vitro assessment of *Trichoderma* species and *Bacillus thuringiensis* integration to mitigate insect pests of brinjal (*Solanum melongena* L.). *Egypt. J. Biol. Pest Control* **2020**, *30*, 60. <https://doi.org/10.1186/s41938-020-00258-5>.
32. da Silveira, A.A.; Andrade, J.S.P.; Guisisoni, A.C.P.; da Costa, A.C.; de Carvalho e Silva, A.; da Silva, H.G.; Brito, P.; de Souza, G.R.L.; Fernandes, K.F. Larvicidal potential of cell wall degrading enzymes from *Trichoderma asperellum* against *Aedes aegypti* (Diptera: Culicidae). *Biotechnol. Prog.* **2021**, *37*, e3182. <https://doi.org/10.1002/btpr.3182>.
33. Podder, D.; Ghosh, S.K. A new application of *Trichoderma asperellum* as an anopheline larvicide for eco friendly management in medical science. *Sci. Rep.* **2019**, *9*, 1108. <https://doi.org/10.1038/s41598-018-37108-2>.
34. Batool, R.; Umer, M.J.; Wang, Y.; He, K.; Zhang, T.; Bai, S.; Zhi, Y.; Chen, J.; Wang, Z. Synergistic effect of *Beauveria bassiana* and *Trichoderma asperellum* to induce maize (*Zea mays* L.) defense against the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera, Crambidae) and larval immune response. *Int. J. Mol. Sci.* **2020**, *21*, 8215. <https://doi.org/10.3390/ijms21218215>.
35. Di Lelio, I.; Forni, G.; Magoga, G.; Brunetti, M.; Bruno, D.; Becchimanzi, A.; De Luca, M.G.; Sinno, M.; Barra, E.; Bonelli, M. A soil fungus confers plant resistance against a phytophagous insect by disrupting the symbiotic role of its gut microbiota. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2216922120. <https://doi.org/10.1073/pnas.2216922120>.
36. Jafarbeigi, F.; Samih, M.; Alaei, H.; Shirani, H. Induced tomato resistance against *Bemisia tabaci* triggered by salicylic acid, β -aminobutyric acid, and *Trichoderma*. *Neotrop. Entomol.* **2020**, *49*, 456–467. <https://doi.org/10.1007/s13744-020-00771-0>.
37. Sholla, S.; Kottb, M.R. Bioactivity of *Trichoderma* (6-Pentyl α -pyrone) against *Tetranychus urticae* Koch (Acari: Tetranychidae). *Egypt. Acad. J. Biol. Sci.* **2017**, *10*, 29–34.
38. Montes-Moreno, D.; López-Herreros, M.; Valdés, R.; Palazón, P.; Garrido-Jurado, I. *Eficacia de Una Cepa de Trichoderma Spp. (IDE-10) Frente a Tetranychus urticae En Cítricos*; Jornadas de Ciencias Hortícolas: Cartagena, Spain, 2024.
39. Montes-Moreno, D.; Garrido-Jurado, I.; López-Herreros, M.; Valdés, R.; Palazón, P. Potential of *Trichoderma* spp. (IDE-10) as a biological control agent against *Polyphagotarsonemus latus* in tomato crop under greenhouse. In Proceedings of the XIII Congreso Nacional de Entomología Aplicada, Gijón, Spain, 7–11 October 2024.
40. Papavizas, G. *Trichoderma* and *Gliocladium*: Biology, ecology, and potential for biocontrol. *Annu. Rev. Phytopathol.* **1985**, *23*, 23–54.
41. Harman, G.; Jin, X.; Stasz, T.; Peruzzotti, G.; Leopold, A.; Taylor, A. Production of conidial biomass of *Trichoderma harzianum* for biological control. *Biol. Control* **1991**, *1*, 23–28.
42. Jin, X.; Harman, G.; Taylor, A. Conidial biomass and desiccation tolerance of *Trichoderma harzianum* produced at different medium water potentials. *Biol. Control* **1991**, *1*, 237–243.
43. Ajam, M.R.; Devi, S.; Sinha, B.; Singh, L.; Singh, N.; Singh, K.I.; Khwairakpam, N.; Chanu, M.V.; Yaiphabee, O.; Soibam, N. Growth promotion of native *Trichoderma asperellum* on pH, temperature and sugar concentration. *Pharma Innov. J.* **2023**, *12*, 21–33.

44. Mamo, Z.; Alemu, T. Evaluation and optimization of agro-industrial wastes for conidial production of *Trichoderma* isolates under solid state fermentation. *J. Appl. Biosci.* **2012**, *54*, 3870–3879.
45. Maurya, M.; Srivastava, M. Harshita Effect of various pH levels on the growth and sporulation of *Trichoderma viride* isolates and assessing their antagonistic activity against soil-borne pathogens. *J. Pure Appl. Microbiol.* **2024**, *18*, 2516–2527. <https://doi.org/10.22207/JPAM.18.4.23>.
46. Fernández-Sandoval, M.; Ortiz-García, M.; Galindo, E.; Serrano-Carreón, L. Cellular damage during drying and storage of *Trichoderma harzianum* spores. *Process Biochem.* **2012**, *47*, 186–194. <https://doi.org/10.1016/j.procbio.2011.10.006>.
47. Martinius, M. Viability and Temperature Effect to conidia germination of *Trichoderma* spp. indigenous banana rhizosphere in West Sumatera Indonesia. *Res. J. Pharm. Biol. Chem. Sci.* **2017**. Available online: [https://rjpbcs.com/pdf/2017_8\(3\)/\[41\].pdf](https://rjpbcs.com/pdf/2017_8(3)/[41].pdf) (accessed on 7 May 2026).
48. Woo, S.L.; Ruocco, M.; Vinale, F.; Nigro, M.; Marra, R.; Lombardi, N.; Pascale, A.; Lanzuise, S.; Manganiello, G.; Lorito, M. *Trichoderma*-based products and their widespread use in agriculture. *Open Mycol. J.* **2014**, *8*, 71–126.
49. Fernandes, É.K.; Rangel, D.E.; Braga, G.U.; Roberts, D.W. Tolerance of entomopathogenic fungi to ultraviolet radiation: A review on screening of strains and their formulation. *Curr. Genet.* **2015**, *61*, 427–440. <https://doi.org/10.1007/s00294-015-0492-z>.
50. Sametz-Baron, L.; Berrocal, T.; G.M.; Amit, R.; Herrera-Estrella, A.; Horwitz, B.A. Photoreactivation of UV-inactivated spores of *Trichoderma harzianum*. *Photochem. Photobiol.* **1997**, *65*, 849–854. <https://doi.org/10.1111/j.1751-1097.1997.tb01933>.
51. Martinez, Y.; Ribera, J.; Schwarze, F.W.; De France, K. Biotechnological development of *Trichoderma*-based formulations for biological control. *Appl. Microbiol. Biotechnol.* **2023**, *107*, 5595–5612. <https://doi.org/10.1007/s00253-023-12687-x>.
52. Dent, D. Principles of integrated pest. In *Integrated Pest Management*; Chapman & Hall, London, UK, 1995; 8–46.
53. Alves Cavalcante, A.L.; Paiva Negreiros, A.M.; de Assis Melo, N.J.; Queiroz Santos, F.J.; Azevêdo Soares Silva, C.S.; Lima Pinto, P.S.; Khan, S.; Mendes Sales, I.M.; Sales Júnior, R. Adaptability and sensitivity of *Trichoderma* spp. isolates to environmental factors and fungicides. *Microorganisms* **2025**, *13*, 1689. <https://doi.org/10.3390/microorganisms13071689>.
54. Leonardi, G.R.; Polizzi, G.; Vitale, A.; Aiello, D. Efficacy of biological control agents and resistance inducer for control of mal secco disease. *Plants* **2023**, *12*, 1735. <https://doi.org/10.3390/plants12091735>.
55. Napolitano, A.; Senatore, M.; Coluccia, S.; Palomba, F.; Castaldo, M.; Spasiano, T.; Avino, A.G.; Vitale, A.; Bonfante, A.; Sacco, A.; et al. Development and evaluation of a *Trichoderma*-based bioformulation for enhancing sustainable potato cultivation. *Horticulturae* **2024**, *10*, 664. <https://doi.org/10.3390/horticulturae10070664>.
56. D’Errico, G.; Greco, N.; Vinale, F.; Marra, R.; Stillitano, V.; Davino, S.W.; Woo, S.L.; D’Addabbo, T. Synergistic effects of *Trichoderma harzianum*, 1,3 dichloropropene and organic matter in controlling the root-nnot nematode *Meloidogyne incognita* on tomato. *Plants* **2022**, *11*, 2890. <https://doi.org/10.3390/plants11212890>.
57. Bassanezi, R.B.; Amorim, L.; Bergamin Filho, A.; Hau, B. Effects of bean line pattern mosaic virus on the monocyclic components of rust and angular leaf spot of *Phaseolus bean* at different temperatures. *Plant Pathol.* **1998**, *47*, 289–298.
58. Rimkus, A.; Namina, A.; Dzierkale, M.T.; Grigs, O.; Senkovs, M.; Larsson, S. Impact of growth conditions on the viability of *Trichoderma asperellum* during storage. *Microorganisms* **2023**, *11*, 1084. <https://doi.org/10.3390/microorganisms11041084>.
59. Qiu, Z.; Wu, X.; Zhang, J.; Huang, C. High temperature enhances the ability of *Trichoderma asperellum* to infect *Pleurotus ostreatus* mycelia. *PLoS ONE* **2017**, *12*, e0187055. <https://doi.org/10.1371/journal.pone.0187055>.
60. Cabral-Miramontes, J.P.; Olmedo-Monfil, V.; Lara-Banda, M.; Zúñiga-Romo, E.R.; Aréchiga-Carvajal, E.T. Promotion of plant growth in arid zones by selected *Trichoderma* spp. strains with adaptation plasticity to alkaline pH. *Biology* **2022**, *11*, 1206. <https://doi.org/10.3390/biology11081206>.
61. Cecchi, G.; Manzi, D.; Di Piazza, S.; Sacchi, R.; Zotti, M.; Picco, A.M. Fungi in rice field open irrigation system: Ecological implications and biosecurity. *Curr. Microbiol.* **2023**, *80*, 316. <https://doi.org/10.1007/s00284-023-03427-5>.
62. Fernandes Sousa, T.; Gwinner, R.; Santos da Silva, I.J.; dos Santos Castro, G.; Vasconcelos da Costa, G.; de Filippi, M.C.C.; Athayde Sobrinho, C.; Yamagishi, M.E.B.; Eiji Hanada, R.; Muniz, A.W.; et al. Isolation and characterization of *Trichoderma* from Amazonian white-water river sediments with descriptions of five new species and new records from Brazil. *Front. Microbiol.* **2026**, *17*, 1778622. <https://doi.org/10.3389/fmicb.2026.1778622>.
63. Qi, Q.; Fan, C.; Wu, H.; Sun, L.; Cao, C. Preparation of *Trichoderma asperellum* microcapsules and biocontrol of cucumber powdery mildew. *Microbiol. Spectr.* **2023**, *11*, e0508422. <https://doi.org/10.1128/spectrum.05084-22>.
64. Elshahawy, I.; Saied, N.; Abd-El-Kareem, F.; Abd-Elgawad, M. Enhanced activity of *Trichoderma asperellum* introduced in solarized soil and its implications on the integrated control of strawberry-black root rot. *Heliyon* **2024**, *10*, e36795. <https://doi.org/10.1016/j.heliyon.2024.e36795>.

65. Rossouw, S.; Mathulwe, L.L.; Stokwe, N.F.; Malan, A.P. Effect of visible light and ultraviolet light on the pathogenicity of entomopathogenic fungi to false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) larvae. *Afr. Entomol.* **2023**, *31*, 1–8. <https://doi.org/10.17159/2254-8854/2023/a13141>.
66. Begoude, B.A.D.; Lahlali, R.; Friel, D.; Tondje, P.R.; Jijakli, M.H. Response surface methodology study of the combined effects of temperature, pH, and a_w on the growth rate of *Trichoderma asperellum*. *J. Appl. Microbiol.* **2007**, *103*, 845–854. <https://doi.org/10.1111/j.1365-2672.2007.03305.x>.
67. Poosapati, S.; Ravulapalli, P.D.; Tippirishetty, N.; Vishwanathaswamy, D.K.; Chunduri, S. Selection of high temperature and salinity tolerant *Trichoderma* isolates with antagonistic activity against *Sclerotium rolfsii*. *SpringerPlus* **2014**, *3*, 641. <https://doi.org/10.1186/2193-1801-3-641>.
68. Sanco, D.G.; European Commission; Health and Consumer Protection Directorate-General. *Review Report for the Active Substance Trichoderma atroviride (Formerly T. harzianum) T-11. Finalised in the Standing Committee on the Food Chain and Animal Health at Its Meeting on 11 July 2008 in View of the Inclusion of Trichoderma atroviride (Formerly T. harzianum) T-11 in Annex I of Directive 91/414/EEC. SANCO/1841/08—Rev. 6*; European Commission: Brussels, Belgium, 2014.
69. Sanco, D.G.; European Commission; Health and Consumer Protection Directorate-General. *Review Report for the Three Active Substances Trichoderma gamsii ICC080 (Formerly Trichoderma viride Strain ICC080), Trichoderma asperellum T25* (Formerly Identified as Trichoderma viride Strain T-25) and Trichoderma asperellum TV1 (Formerly Identified as Trichoderma viride Strain TV1). Finalised in the Standing Committee on the Food Chain and Animal Health at Its Meeting on 11 July 2008 in View of the Inclusion of Trichoderma gamsii ICC080 (Formerly Trichoderma viride Strain ICC080), Trichoderma asperellum T25 (Formerly Identified as Trichoderma viride Strain T-25) and Trichoderma asperellum TV1 (Formerly Identified as Trichoderma viride Strain TV1) in Annex I of Directive 91/414/EEC. SANCO/1868/08—Rev. 6*; European Commission: Brussels, Belgium, 2014.
70. Arias-Chavarría, L.D.; Batista-Menezes, D.; Orozco-Cayasso, S.; Vargas-Martínez, A.; Vega-Baudrit, J.R.; Montes de Oca-Vásquez, G. Evaluation of the viability of microencapsulated *Trichoderma longibrachiatum* conidia as a strategy to prolong the shelf life of the fungus as a biological control agent. *Front. Microbiol.* **2025**, *12*, 1473217. <https://doi.org/10.3389/fchem.2024.1473217>. Erratum in *Front. Chem.* **2025**, *13*, 1562696. <https://doi.org/10.3389/fchem.2025.1562696>.
71. Kumar, T.V.; Veena, S.S.; Karthikeyan, S.; Sreekumar, J. Compatibility of *Trichoderma asperellum* with fungicides, insecticides, inorganic fertilizers and bio-pesticides. *J. Root Crops* **2017**, *43*, 68–75.
72. Borkow, G.; Gabbay, J. Copper as a biocidal tool. *Curr. Medic. Chem.* **2005**, *12*, 2163–2175.
73. Chaignon, V.; Sanchez-Neira, I.; Herrmann, P.; Jaillard, B.; Hinsinger, P. Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Environ. Pollut.* **2003**, *123*, 229–238. [https://doi.org/10.1016/S0269-7491\(02\)00374-3](https://doi.org/10.1016/S0269-7491(02)00374-3).
74. Widmer, T.L. Compatibility of *Trichoderma asperellum* isolates to selected soil fungicides. *Crop Prot.* **2019**, *120*, 91–96. <https://doi.org/10.1016/j.cropro.2019.02.017>.
75. Hollomon, D.W. Fungicide resistance: 40 years on and still a major problem. In *Fungicide Resistance in Plant Pathogens: Principles and a Guide to Practical Management*; Springer: Tokyo, Japan, 2015; pp. 3–11.
76. Kumari, N.; Yadav, D.L.; Bochalya, R.K.; Sharma, S.; Meena, C.B.; Gautam, C. Compatibility of *Trichoderma asperellum* with different fungicides. *J. Adv. Microbiol.* **2025**, *25*, 1–7. <https://doi.org/10.9734/jamb/2025/v25i8970>.
77. Mayo-Prieto, S.; Squarizoni, A.; Carro-Huerga, G.; Porteous-Álvarez, A.J.; Gutiérrez, S.; Casquero, P.A. Organic and conventional bean pesticides in development of autochthonous *Trichoderma* strains. *J. Fungi* **2022**, *8*, 603. <https://doi.org/10.3390/jof8060603>.
78. Ruano-Rosa, D.; Arjona-Girona, I.; López-Herrera, C.J. Integrated control of avocado white root rot combining low concentrations of fluazinam and *Trichoderma* spp. *Crop Prot.* **2018**, *112*, 363–370. <https://doi.org/10.1016/j.cropro.2017.06.024>.
79. Abd-El-Khair, H.; Elshahawy, I.E.; Haggag, H.K. Field application of *Trichoderma* spp. combined with thiophanate-methyl for controlling *Fusarium solani* and *Fusarium oxysporum* in dry bean. *Bull. Natl. Res. Cent.* **2019**, *43*, 19. <https://doi.org/10.1186/s42269-019-0062-5>.

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