# Biological control of grapevine gray mold: *in vitro* and *in vivo* evaluation of the antagonistic activity of indigenous *Trichoderma harzianum* strains (Mascara, Algeria)

Control biológico del moho gris de la vid: evaluación *in vitro* e *in vivo* de la actividad antagónica de cepas indígenas de *Trichoderma harzianum* (Mascara, Argelia)

Controle biológico da podridão cinzenta da videira: avaliação *in vitro* e *in vivo* da atividade antagônica de cepas indígenas de *Trichoderma harzianum* (Mascara, Argélia)

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## Abstract

Gray mold, caused by the necrotrophic fungus *Botrytis cinerea*, was responsible for significant economic losses in grapevine (*Vitis vinifera* L.) production worldwide, including Algeria, which highlighted the need for sustainable control alternatives. The objective of this study was to evaluate the antagonistic potential of an indigenous *Trichoderma harzianum* strain against local *B. cinerea* isolates from Mascara, Algeria. A highly virulent *B. cinerea* isolate (BC3) was collected from infected grapevine leaves and identified through morphological and molecular analyses, while the antagonistic *T. harzianum* isolate (T5) was obtained from the rhizosphere of healthy vines. *In vitro* dual-culture assays showed that *T. harzianum* significantly inhibited the mycelial growth of *B. cinerea*, with direct inhibition of 80.50 % and indirect inhibition of 72.87 %. *In vivo* experiments further confirmed its efficacy, reducing disease incidence by 56.25 % and enhancing plant growth, increasing height from 60 to 96 cm with notable improvement in vegetative biomass. These findings suggested that the indigenous *T. harzianum* T5 strain was a promising and effective biocontrol agent for the sustainable management of gray mold in vineyard.

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## Resumen

El moho gris, causado por el hongo necrotrófico *Botrytis cinerea*, ha sido responsable de perdidas económicas significativas en la producción de vid (*Vitis vinifera* L.) en todo el mundo, incluyendo Argelia, lo que resalta la necesidad de alternativas de control sostenibles. El objetivo de este estudio fue evaluar el potencial antagónico de una cepa autóctona de *Trichoderma harzianum* contra aislados locales de *B. cinerea* de Mascara, Argelia. Un aislado altamente virulento de *B. cinerea* (BC3) se recolectó de hojas de vid infectadas y se identificó mediante análisis morfológicos y moleculares, mientras que el aislado antagonista de *T. harzianum* (T5) se obtuvo de la rizosfera de vides sanas. Los ensayos de cultivo dual *in vitro* mostraron que *T. harzianum* inhibió significativamente el crecimiento micelial de *B. cinerea*, con una inhibición directa del 80,50 % y una inhibición indirecta del 72,87 %. Experimentos *in vivo* confirmaron aún más su eficacia, reduciendo la incidencia de la enfermedad en un 56,25 % y acelerando el crecimiento de las plantas, aumentando su altura de 60 a 96 cm, con una notable mejora en la biomasa vegetativa. Estos resultados sugieren que la cepa nativa *T. harzianum* T5 es un agente de biocontrol prometedor y eficaz para el manejo sostenible de la podredumbre gris en viñedos.


**Palabras clave**: *Botrytis cinerea, Vitis vinifera,* antagonismo, promoción de crecimiento vegetal.


## Resumo

O mofo cinzento, causado pelo fungo necrotrófico *Botrytis cinerea*, foi responsável por perdas econômicas significativas na produção de videira (*Vitis vinifera* L.) em todo o mundo, incluindo a Argélia, o que destacou a necessidade de alternativas de controle sustentáveis. O objetivo deste estudo foi avaliar o potencial antagônico de uma cepa indígena de *Trichoderma harzianum* contra isolados locais de *B. cinerea* de Mascara, Argélia. Um isolado altamente virulento de *B. cinerea* (BC3) foi coletado de folhas de videira infectadas e identificado por meio de análises morfológicas e moleculares, enquanto o isolado antagonista de *T. harzianum* (T5) foi obtido da rizosfera de videiras saudáveis. Ensaios de dupla cultura *in vitro* mostraram que *T. harzianum* inibiu significativamente o crescimento micelial de *B. cinerea*, com inibição direta de 80,50 % e inibição indireta de 72,87 %. Experimentos *in vivo* confirmaram ainda mais sua eficácia, reduzindo a incidência da doença em 56,25 % e acelerando o crescimento das plantas, aumentando a altura de 60 para 96 cm, com notável melhora na biomassa vegetativa. Esses resultados sugerem que a cepa nativa *T. harzianum* T5 é um agente de biocontrole promissor e eficaz para o manejo sustentável do mofo cinzento em vinhedos.


**Palavras-chave:** *Botrytis cinerea, Vitis vinifera,* antagonismo, promoção de crescimento vegetal.

## Introduction

The grapevine (*V. vinifera*) is one of the world's most economically important perennial crops, grown across 7.3 million hectares globally supporting an industry valued at over € 300 billion annually (OIV, 2022). In Algeria, grapevine farming holds significant historical and cultural value, especially in regions such as Mascara, which is a major viticultural area in the northwest. Despite the potential of Algerian viticulture, the sector faces ongoing challenges, particularly from fungal diseases including gray mold, caused by *Botrytis cinerea* Pers. This necrotrophic pathogen can cause pre and post harvest losses of up to 50-80 % during severe outbreaks (Rhouma *et al*., 2023).

*B. cinerea* is notoriously difficult to control due to its genetic adaptability, its ability to form persistent sclerotia and its rapid development of resistance to fungicides (Shao *et al*., 2021). Furthermore, the environmental and the health risks associated with synthetic fungicides have spurred growing interest in sustainable alternatives, particularly biological control agents (Ayilara *et al.,* 2023). *Trichoderma* spp. are well-established biocontrol agents against *B. cinerea*, acting through multiple mechanisms such as competition, mycoparasitism, antibiosis, and induction of systemic resistance (ISR) in plants (Harman *et al*., 2004; Lorito *et al*., 2010).

These fungi, commonly found in soils and adaptable to diverse environments, colonize the roots of both monocots and dicots, enhancing disease resistance (Harman *et al*., 2004). They also produce extracellular enzymes like cellulases and chitinases with industrial relevance, and promote plant growth, nutrient assimilation, and stress tolerance (Lorito *et al*., 2010). Their use in agriculture contributes to disease control while reducing reliance on agrochemicals, supporting sustainable farming practices (Wang *et al*., 2022).The objective of this study was to identify the *Botrytis* species responsible for gray mold in grapevine in the Mascara region of Algeria, to isolate and molecularly characterize an indigenous *T. harzianum* strain from the vine rhizosphere, and to evaluate its antagonistic potential against *B. cinerea* through *in vitro* and *in vivo* assays. The ultimate aim was to establish a basis for the development of a sustainable biological control strategy to reduce gray mold incidence and promote grapevine growth.

## Materials and methods

### Fungal material

#### Pathogen

Samples of grapevine organs, such as clusters, leaves, and stems, showing typical gray mold symptoms, were collected from 20 vineyards in the Mascara region (Tighennif, Ghriss, El Bordj, and Maoussa) in northwest Algeria.

These infected tissues were used to isolate *B. cinerea*. Small tissue pieces (5-10 mm) were cut from the edge of the infected areas and surface sterilized by immersing them in 2.0 % sodium hypochlorite (NaOCl) for 2 minutes, followed by three washes with sterile distilled water (30 seconds each) (Leslie and Summerell 2006). After that, the samples were aseptically dried on sterile filter paper. To avoid bacterial contamination, four to five sterile tissue pieces were added to potato dextrose agar (PDA) plates that were treated with streptomycin (50 $mg.L^{-1}$). For five to seven days, the plates were incubated at 25 ± 2 °C in the dark. After incubation, a single spore from each isolate was transferred onto fresh PDA plates. Monosporic isolates were stored at 4 °C in 20 % (v/v) glycerol, and the isolates were morphologically characterized to confirm *B. cinerea* identification.

#### Antagonist

During 2022-2023 season, a soil sample was collected from the rhizosphere of a healthy grapevine in the Mascara region (Tighennif). *Trichoderma* isolates were obtained using the soil dilution method: 1 g of soil was mixed with 9 mL of sterile water, serially diluted ($10^{-1}$ to $10^{-7}$) and 0.1 mL from selected dilutions was plated on PDA. After 3 days at 25 °C, colonies showing *Trichoderma*-like morphology were purified and stored at 4 °C for later use (Kouadri *et al*., 2023a).

**Plant material**

In this experiment, we used the Sultanina variety of *V. vinifera*, which is known for its high susceptibility to grapevine gray mold.

**Molecular Identification and phylogenetic analysis**

DNA was extracted using the NucleoSpin® Food kit (Macherey-Nagel, Germany). DNA quality and quantity were assessed by measuring 260/280 and 260/230 absorbance ratios with a NanoDrop® 2000. The ITS and TEF1-α regions were PCR-amplified using an Applied Biosystems® GeneAmp® PCR System 9700 thermocycler. ITS1/ITS4 primers targeted the ITS region, while EF-728F/EF-2 primers amplified TEF1-α. For *B. cinerea*, identification relied on ITS sequencing alone, whereas the *Trichoderma* isolate was identified using both ITS and TEF1-α sequences. PCR reactions (25 µL) contained 2 µL genomic DNA, 1X Solis BioDyne® Taq buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer, and 1 U Solis BioDyne® Taq polymerase. Thermal cycling for ITS1/ITS4 included: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 55 s; and final extension at 72 °C for 7 min. For EF-728F/EF-2, the same conditions applied except annealing at 54 °C. PCR products were stained with RedSafe® dye (Intron, South Korea) and visualized on 1.5 % agarose gels under UV light using a Bio-Rad® Gel Doc™ System. Sequences were analyzed via BLAST for isolate identification (White *et al*., 1990; Carbone and Kohn, 1999).

***Trichoderma* isolates' antagonistic effects on *Botrytis cinerea* mycelia development *in vitro***

**Dual culture**

The dual culture method was used to evaluate the *Trichoderma* isolate *in vitro* antagonistic activity against *B. cinerea*. On PDA plates (90 mm), a 6 mm disc of 7-day-old *B. cinerea* was placed on one side, and a 6 mm disc of *Trichoderma* was placed 3 cm apart, on the other side plates were incubated at 25 °C for 10 days. Controls contained only *B. cinerea* in the center (Bekkar *et al*., 2016). The inhibition of mycelial growth was calculated using this formula:

$$\% \text{ Inhibition} = \left[ \frac{(\text{R Control} - \text{R Tets})}{\text{R Control}} \right] \times 100$$

Where:

R control is the pathogen's maximal radial growth in the control.

R test = the pathogen's radial development when the antagonist is present.

After 2-6 days of incubation, microscopic observations at the interaction interface were conducted to evaluate another variable: the mode of action of *Trichoderma* on *B. cinerea* mycelium.

**Inhibition of fungal growth by *Trichoderma* volatile metabolites**

The impact of volatile compounds produced by *Trichoderma* isolate on *B. cinerea* growth was evaluated by placing a 6 mm *Trichoderma* mycelial disc at the center of a PDA plate, with another PDA plate containing a 6 mm *B. cinerea* disc inverted and placed above it. The two plates were sealed together with Parafilm to trap volatiles and incubated at 25 °C for 10 days. *B. cinerea* growth was measured every 48 hours. Control plates contained only *B. cinerea* and were sealed similarly (Kouadri *et al*., 2023a). Growth inhibition was calculated using the method described previously for the dual culture assay. The experiments were repeated three times.

***In vivo* testing of *Trichoderma* antagonism against *Botrytis cinerea* on grapevine leaves**

**Inoculum preparation**

The *B. cinerea* isolate (BC3), chosen for its high virulence, and the *Trichoderma* isolate (T5), identified as the most potent antagonist were both cultured on PDA at 25 °C for seven days. Spores were harvested by gently rubbing the surface of the cultures and suspending them in sterile distilled water. The *B. cinerea* conidial suspension was adjusted to $10^6$ conidia.mL$^{-1}$ to induce gray mold symptoms (Lian *et al*., 2018), while the *Trichoderma* suspension was diluted to $10^8$ conidia.mL$^{-1}$ for protective purposes inoculation (Maruyama *et al*., 2020).

**Field experience and procedure for treating grapevine**

A field trial was carried out in 2024 in the Tighennif region (Mascara), within the Technical Institute of Fruit Arboriculture and Viticulture (ITAF), in order to evaluate the *in vivo* efficacy of the *T. harzianum* isolate (T5) against *B. cinerea* on vines. The sensitive grape variety 'Sultanina' was used to facilitate artificial inoculation. The experimental design included four treatments (table 2), each replicated five times, for a total of 20 plants. The *Trichoderma* treatment was applied only once at the beginning of the experiment, using two complementary methods (application to the soil at the base of the plants to promote root colonization and irrigation with a spore suspension to ensure homogeneous diffusion in the rhizosphere). These two application methods were part of the same treatment and were not analyzed separately.

**Disease assessment**

The quantitative disease scoring method involves assessing disease incidence by calculating the percentage of diseased plants or leaves relative to the total number observed. It uses the following formula (Kouadri *et al.,* 2023b):

$$DI = \left[ \frac{\text{Number of diseased plants or leaves}}{\text{Total number of plants or leaves diseased}} \right] \times 100$$

This method allows for accurate quantification of disease frequency in the field, providing an incidence index expressed as a percentage.

**Statistical analysis**

Data collected from the *in vitro* and *in vivo* experiments were analyzed by analysis of variance (ANOVA) in SPSS (Statistical Package for Social Sciences). Comparisons of means between treatments were performed using the Tukey test, with a significance level set at $p<0.05$. The *in vitro* test included three replicates per treatment, while the *in vivo* field trial was conducted with four treatments, each replicated five times, for a total of 20 plants.

**Table 1: Primers used for molecular characterization of *Trichoderma* sp.**

| Non | Séquence 5'-3' | Tm | Taille fragment | Sources |
|---|---|---|---|---|
| ITS1 | TCCGTAGGTGAACCTGCGG | 55 | 700 bp | White *et al*. (1990) |
| ITS4 | TCCTCCGCTTATTGATATGC | 55 | | |
| EF-728F | CATYGAGAAGTTCGAGAAGG | 54 | 700bp | Carbone and Kohn (1999) |
| EF-2 | GGARGTACCAGTSATCATGTT | 54 | | |

**Tabla 2. Description of the treatments y application methods in order to evaluate the *in vivo* efficacy of the *Trichoderma harzianum* isolate (T5) against *Botrytis cinerea* on vines.**

| Treatment | Description | Application method | Fungi involved | Notes |
|---|---|---|---|---|
| T1 | Uninoculated control | Roots irrigated with sterile distilled water - Leaves sprayed with sterile distilled water | None(control) | No fungal inoculation |
| T2 | Pathogen only | Leaves sprayed with a conidial suspension of *B. cinerea* | *Botrytis cinerea* only | No *Trichoderma* |
| T3 | *Trichoderma* only | Roots irrigated with a conidial suspension of *Trichoderma* T5 (soil + irrigation combined) | *Trichoderma* T5 only | No *B. cinerea* |
| T4 | Combined treatment | Roots irrigated with *Trichoderma* T5 (soil + irrigation) Six weeks later, leaves sprayed with *B. cinerea* conidial suspension | *Trichoderma* T5+ *B. cinerea* | Plants kept under high humidity (e. g, covered with plastic) for 48 h post-inoculation |

## Results and discussion

**Cultural and morphological characteristics**

**Pathogen**

Four isolates of *Botrytis* sp. were isolated from diseased grapevine plants in total Initially, the colonies grown on PDA were white but turned gray as they matured. The fungus produced conidia that are ovoid or round, hyaline, and measured between 11 and 15 µm. These conidia were clustered at the tips of branched conidiophores. Additionally, *B. cinerea* formed sclerotia, which are black, irregularly shaped structures ranging from 1 to 5 mm in diameter. The mycelium consisted of septate, grayish or olive-colored cylindrical hyphae, which can sometimes appear vesicular at the median septum. These morphological traits were typical of *B.* species (Yan-gang *et al.,* 2019). In the present study, one isolate (BC3) has been selected as the most virulent isolate (unpublished data).

*Botrytis cinerea* plays a dual and significant role in agriculture and viticulture, highlighting its importance. This fungus is primarily known as the pathogen responsible for "grey mold," a disease that affects a wide range of crops, causing substantial economic losses due to degradation of plant tissues, necrosis of stems and leaves, and fruit rot both before and after harvest. Thus, this pathogen represents both a major phyto-pathological threat and a crucial factor in producing high-quality wine products, illustrating the complexity of its ecological and economic impact (Rhouma *et al*., 2023).

**Antagonist**

A *Trichoderma* isolate (T5) was obtained from grapevine rhizosphere soil using the serial dilution method. This isolate was selected for further experiments based on its strong antagonistic activity against *B. cinerea* in preliminary dual culture assays (data not shown). This isolate exhibited typical characteristic of *Trichoderma* sp. Colonies grown on PDA usually formed one or two concentric rings with production of green conidia. The mycelium was sparse, smooth, and watery white at first, but it eventually became a fluffy aerial mycelium. The pyramid-shaped conidiophores have many branches, most of which are arranged in threes or fours. The phialides are flask-shaped, usually short and wide in the middle, measuring about 4-6 µm in length. The conidia are globose to sub globose, smooth, with an average diameter of 2-3 µm and exhibit a pale green color. According to studies reported by Okoth *et al*. (2007), *T. harzianum* was the most frequently isolated species from 60 soil samples collected from various locations. Isolation by dilution plating on PDA medium is a commonly used method to obtain pure strains of *Trichoderma* from rhizosphere soils. This method makes it possible to choose strains that may have antagonistic action against phytopathogenic fungi in addition to morphologically characterizing the isolates. Moreover, this method facilitates the study of *Trichoderma* biodiversity across different agricultural ecosystems, supporting their future application in biological control.

**Molecular characterization and phylogenetic analysis**

The ITS sequence of BC3 showed 99.80 % similarity with *B. cinerea* sequences available in GenBank (OP415635.1, KU992698.1, OP415636.1). For the *Trichoderma* isolate (T5), both the ITS and TEF1-α regions were successfully amplified and analyzed. A BLAST search of ITS sequences revealed a 100 % match with *T. harzianum* sequences (OQ789696.1, MN518418.1), while the TEF1-α sequence showed 99.66 % similarity with *T. harzianum* sequences (OQ108506.1, MK050521.1). The sequences have been deposited in the NCBI GenBank under accession numbers PV290870.1 (*B. cinerea*), and PV290866 (ITS) and PV297897 (TEF1-α) for *T. harzianum*.

Previous studies have also used ITS and TEF1-α sequencing to identify *Trichoderma* isolates, confirming the reliability of these molecular markers for species-level identification. For example, Gorman *et al*. (2023) and Druzhinina *et al*. (2010) reported high similarity (>99 %) of ITS and TEF1-α sequences for *T. harzianum* isolates, which is consistent with the findings for isolate T5 in this study. This supports the accuracy of the molecular identification. This supports the accuracy of the molecular identification and aligns with global research confirming *T. harzianum* as a common and effective antagonist against phytopathogens. Such comparisons strengthen the discussion and situate the results within the broader scientific context.

**Antagonistic activity of *Trichoderma harzianum* against *Botritys cinerea***

***In vitro* antagonistic activity of *Trichoderma harzianum* on *Botrytis cinerea* in dual culture**

The dual culture method demonstrated that *T. harzianum* (T5) significantly inhibited the growth of *B. cinerea* (BC3), achieving a growth inhibition rate of 80.5 % (table 3). After ten days on PDA, *T. harzianum* aggressively colonized and sporulated over *B. cinerea* colonies, showcasing strong mycoparasitic activity.

Microscopic observations of the interaction zone revealed hyphal coiling, where *Trichoderma* hyphae wrapped around those of *B. cinerea*, as well as appressorium formation facilitating penetration into the pathogen's mycelium. Severe mycelial degradation was evident, including hyphal lysis, vacuolization, vesicle formation, cytoplasmic leakage, and coagulation.

Interestingly, *T. harzianum* also stimulated early sclerotia production in *B. cinerea* by day three, underscoring its antagonistic effect. This rapid inhibition is mainly due to competition for nutrients and space. *Trichoderma* isolates typically grow faster than *Botrytis*, colonizing the culture medium within four days, consistent with findings Yao *et al.* (2023).

A key antagonistic mechanism observed is mycoparasitism (figure 1), where *T. harzianum* hyphae encircle and parasitize *B. cinerea* hyphae, leading to their lysis (Kuzmanovska *et al*., 2018). Enzymatic degradation of mycelial walls by β (1,3)-glucanases, chitinases, and proteases further weakens the pathogen (Dos Santos *et al*., 2014), along with vacuolization of the pathogen's cytoplasm.

**Inhibition of *Botrytis cinerea* mycelial growth by volatile metabolites of *Trichoderma harzianum***

The volatile compound assay further confirmed the antifungal activity of *T. harzianum*, showing a 72.87 % inhibition of *B. cinerea* growth after seven days compared to the control (table 3).

Widely recognized as an effective biocontrol agent, *T. harzianum* exhibited rapid and potent inhibition of *B. cinerea* growth, with visible effects occurring within two days post-inoculation.

The antifungal effect involves the production of volatile and non-volatile metabolites such as harzianic acid, peptaibols, and gliovirin, which inhibit mycelial growth and spore germination (Khan *et al*., 2020). These volatile compounds play a key role in biocontrol, as supported by recent studies (Saddek *et al*., 2023).

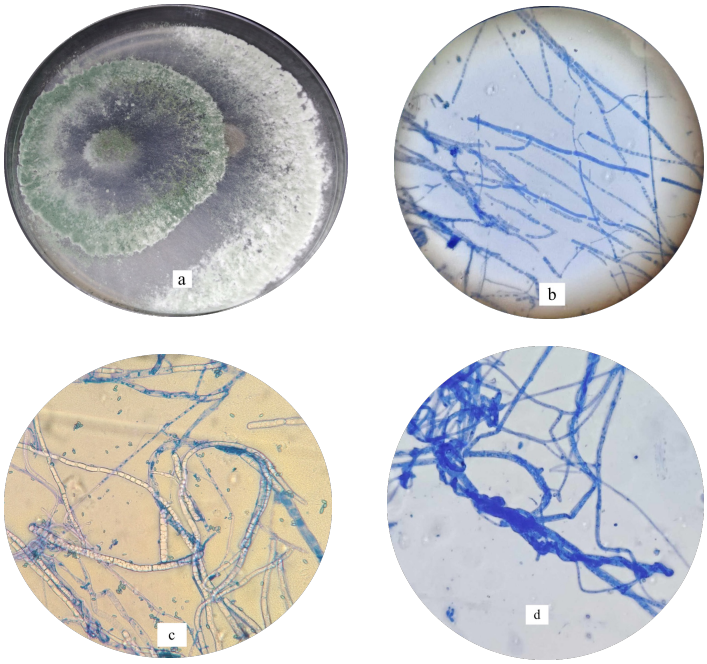


**Figure 1. Direct *in vitro* confrontation between *T. harzianum* (T5) and *B. cinerea* (BC3).** (a) macroscopic observations of the confrontation, (b) the degradation of pathogenic hyphae by lysis, (c) the formation of vacuoles within pathogenic hyphae, (d) the characteristic coiling of antagonistic hyphae around host structures.

These findings, which showed up to 80.5 % inhibition of *B. cinerea* growth in dual culture and 72.87 % inhibition by volatile compounds, align with previous studies by Bendahmane *et al*. (2012) and Mokhtar and Dehimate (2012), confirming *T. harzianum*'s strong potential for sustainable management of gray mold disease in grapevines.

**Table 3. *In vitro* effect of *Trichoderma harzianum* on the mycelia growth of four *Botrytis cinerea* isolates.**

| *B. cinerea* | Radial growth inhibition (%) | |
|---|---|---|
| | Dual culture | Volatile compounds |
| T5-BC01 | 55.04±1.59 b | 51.87±0.43c |
| T5-BC02 | 54.27±3.03b | 50.70±4.40c |
| T5-BC03 | 80.50±7.08a | 72.87±2.78b |
| T5-BC04 | 74.97±2.23a | 63.71±1.86a |
| p≤0.05 | 0.000076 | 0.000027 |

The statistical significance as evaluated by ANOVA and the Tukey test ($p< 0.05$) is shown by various letters for the data displayed (mean ± SD).

**Effect of *Trichoderma harzianum* on vegetative growth and protection of prapevine plants**

**Growth promotion and disease suppression by *Trichoderma harzianum***

Table 4 shows that treating grapevine roots with *T. harzianum* spores significantly enhanced plant growth compared to controls. Inoculated plants exhibited greater height, more leaves, and increased biomass, likely due to improved nutrient uptake, production of growth regulators, and detoxification of harmful soil substances. In the present study, complete protection by the T5 strain of *T. harzianum* was observed in the treatment where grapevine roots were irrigated with T5 prior to *B. cinerea* inoculation (treatment 4). This is supported by the results in table 4, which show a significant reduction in disease incidence from 96 % in infected-only plants to 42 % in treated plants. Additionally, leaf lesions were reduced to 1.49 % in treated vines compared to 5.80-9.09 % in the pathogen-only treatment, demonstrating the effectiveness of T5 in limiting disease spread.

These results confirm that *T. harzianum* effectively promotes grapevine growth and protects against *B. cinerea* infection, highlighting its potential as a biocontrol agent. This stimulation was primarily reflected in enhanced axial growth and increased biomass production. In the present study, the inoculated *Trichoderma* strain significantly reduced the percentage of leaf lesions in grapevines compared to the controls. This reduction (56.25 %) in disease symptoms likely contributed, at least in part, to the observed enhancement in plant growth. Finally, root treatment of grapevine plants with *T. harzianum* significantly reduced disease incidence plants.

The reduction of foliar disease, despite the application of *T. harzianum* to the roots, can be explained by the induction of systemic resistance. As previously discussed, *Trichoderma* colonizes the root epidermis and outer cortical layers, releasing bioactive molecules that trigger defense responses throughout the plant. This systemic effect enhances resistance in aerial tissues such as leaves, consistent with the findings of Kthiri *et al*. (2020), who reported increased enzymatic activity and activation of defense systems following *T. harzianum* treatment.

These results corroborate previous work showing that *Trichoderma* can promote plant growth through hormone production, improved nutrient uptake, and the reduction of abiotic and biotic stresses (Harman *et al*., 2021).

However, the present study was conducted under semi-commercial field conditions, within an experimental vineyard of the ITAF station. Therefore, while our results highlight the potential of *T. harzianum* as a component of integrated pest management (IPM) strategies, further validation under field conditions is required.

If confirmed in the field, *T. harzianum* could complement or replace chemical fungicides for controlling *B. cinerea*, thereby reducing the environmental impact of chemical treatments. Its adaptability to diverse environments and broad antagonistic activity against plant pathogens also make it an attractive candidate for biological control programs, including in Algeria, where local isolates have shownpromising effects.

**Table 4. Comparative analysis of the impacts of *Trichoderma harzianum* based treatments on leaf health, vine height growth, and disease incidence in grapevine plants infected with *Botrytis cinerea* (BC3) (T5: *Trichoderma harzianum*).**

| Treatments | Number of healthy leaves | Number of infected leaves | Height (cm) | Disease Incidence (%) |
|---|---|---|---|---|
| Control | 180±0 | 11±0b | 60±0a | 5.75 |
| Vine +BC3 | 13±2c | 34±4.58a | 50.67±5.86c | 96 |
| Vine + T5+BC3 | 109.67±9.50b | 30.33±0.58a | 65.00±3.00b | 42 |
| Vine + T5 | 198.33±10.41a | 3±0b | 96.00±3.61a | 1.49 |

ANOVA and the Tukey test ($p<0.05$) were used to establish statistical significance. The data displayed (mean ± standard deviation) with different letters.


**Figure 2. *In vivo* evaluation of *Trichoderma harzianum* (T5) against *Botrytis cinerea* (BC3) under field conditions. (a) Control (no treatment), (b) Grapevine + BC3, (c) Grapevine + BC3 + T5, (d) Grapevine + T5 only.**

## Conclusion

*Trichoderma harzianum* isolate T5 showed strong antifungal activity against *Botrytis cinerea*, reducing mycelial growth by 80.5 % *in vitro* and disease symptoms by 56.25 % *in vivo*. Its biocontrol effect is based on mycoparasitism and antifungal metabolite production, which also promote grapevine growth. As the first report of such an isolate in Algerian vineyards, this study highlights its dual benefit for plant health and pathogen control. T5 holds promise for integration into eco-friendly IPM programs in viticulture, pending validation through field trials.

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