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Differential performance of *Bacillus* and *Trichoderma* against olive-associated
Colletotrichum and *Fusarium*: from *in vitro* antagonism to anthracnose suppression in
fruits

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Abstract

Brazilian olive growing is expanding but faces major phytosanitary challenges, particularly anthracnose caused by *Colletotrichum* spp. and Fusarium wilt caused by *Fusarium* spp. Disease management in olive orchards has traditionally relied on chemical fungicides, which may result in phytotoxicity, accumulation of heavy metals in the soil, environmental contamination, and selection of resistant pathogen populations. In this study, the antagonistic potential of *Bacillus* spp. and *Trichoderma* spp. isolates was evaluated through *in vitro* assays against *Colletotrichum acutatum*, *C. gloeosporioides*, *C. nymphaeae*, *Fusarium oxysporum*, and *F. solani*, as well as through bioassays on olive fruits (*Olea europaea* L., cv. Arbequina) inoculated with *C. acutatum* and *C. gloeosporioides*. *In vitro* assays showed that *Bacillus* sp. CRS3 inhibited the mycelial growth of *C. acutatum* by up to 46%, whereas *Trichoderma asperelloides* and *T. virens* were more effective against *F. oxysporum* and *F. solani*, reaching inhibition levels of 49% and 41%, respectively. In fruit bioassays, the efficacy of the antagonists was pathogen dependent. *Bacillus* sp. CRS3 and *B. velezensis* KC1 significantly reduced anthracnose severity, whereas *Trichoderma* isolates did not provide effective fruit protection. Overall, these results demonstrate that *Bacillus* spp., particularly isolates CRS3 and KC1, represent a promising alternative for the control of olive anthracnose, while *Trichoderma* spp. shows greater potential for the management of soilborne pathogens such as *Fusarium*. The integration of these antagonistic microorganisms into disease management programs may reduce reliance on chemical fungicides and contribute to more sustainable olive production systems.

Keywords: Olive fruit; Anthracnose; Fusarium wilt; Biological control; Sustainability

Introduction

The olive tree (*Olea europaea* L.) is a perennial crop of major agronomic, economic, and cultural importance throughout the Mediterranean Basin (Talhinhas et al. 2018; Fraga et al. 2020). In recent decades, increasing global demand for olive oil and table olives has driven the expansion of olive cultivation into new regions, supported by advances in climatic zoning, orchard mechanization, and plant breeding (Chacón-Ortiz et al. 2024; Martins et al. 2024). In Brazil, olive growing has expanded rapidly, particularly in the state of Rio Grande do Sul, where approximately 6,500 ha are currently planted, with about 5,000 ha already in commercial production (Secretaria da Agricultura, Pecuária, Produção Sustentável e Irrigação, 2024). However, expansion into non-traditional growing regions has been accompanied by technical and phytosanitary challenges that may limit sustainable olive production.

Olive trees are susceptible to several diseases affecting both aerial tissues and the root system. Emerging pathogens such as *Xylella fastidiosa* subsp. *pauca* have raised phytosanitary concerns and have already been detected in olive trees in Brazil (Coletta-Filho et al. 2016; Canale et al. 2025). Among fungal diseases affecting aerial tissues, anthracnose caused by *Colletotrichum* spp. is considered the most economically important fruit disease of olive worldwide.

Anthracnose, mainly associated with *Colletotrichum acutatum*, *C. gloeosporioides*, and *C. nymphaeae*, causes necrotic lesions on leaves, shoots, and fruits, often accompanied by characteristic orange sporulation, premature fruit drop, and substantial yield losses, particularly under humid conditions (Talhinhas et al. 2018; Moral et al. 2021). Even low levels of fruit infection may significantly impair olive oil quality by

increasing acidity and peroxide values and reducing oxidative stability through the degradation of phenolic compounds (Martins et al. 2019; Azevedo-Nogueira et al. 2020).

Important diseases are also caused by soilborne pathogens, including *Verticillium dahliae*, *Fusarium* spp., and *Phytophthora* spp., which may cause vascular wilt, root decay, and progressive plant decline (Trabelsi et al. 2017). *Fusarium oxysporum* and *F. solani* are associated with root and vascular wilt, causing leaf chlorosis, branch dieback, and eventual plant death (Trabelsi et al. 2017; Domingues et al. 2020). These fungi can persist in soil for long periods and colonize vascular tissues, resulting in severe physiological stress and plant decline (Bishop and Cooper 1983; Gordon 2017). Their occurrence in olive nurseries and orchards in southern Brazil reinforces the need for effective and sustainable management strategies (Oliveira et al. 2022).

Management of fungal diseases in olive orchards has traditionally relied on copper-based fungicides and, more recently, systemic or translaminar compounds such as triazoles and strobilurins (Sergeeva 2011; Buonauro et al. 2023). However, repeated use of a limited number of fungicide active ingredients may increase the risk of selecting resistant pathogen populations, compromising long-term disease management (Mikaberidze et al. 2014; Petrović et al. 2024). Intensive fungicide use may also result in phytotoxicity, accumulation of copper and other metals in soil, disruption of beneficial microbial communities, and environmental contamination (Van Zwieten et al. 2004; Ibarra-Laclette et al. 2022; Grzanka et al. 2024). In this context, biological control using beneficial microorganisms has emerged as a promising alternative to chemical fungicides, particularly for perennial crops such as olive (Álvarez et al. 2019; Zhang et al. 2020). Species belonging to the genera *Trichoderma* and *Bacillus* have received particular attention due to their broad antagonistic activity and ecological adaptability (Harman et al. 2004; Ongena and Jacques 2008).

Trichoderma species suppress plant pathogens through multiple mechanisms, including mycoparasitism, competition for nutrients and space, antibiosis, production of hydrolytic enzymes, and induction of plant defense responses (Harman et al. 2004; Poveda et al. 2019a, 2019b). Several studies have demonstrated the potential of *Trichoderma* species to suppress *Colletotrichum* and *Fusarium* and other fungal pathogens associated with olive (Amira et al. 2017; Carrero-Carrón et al. 2016; Marques et al. 2018)

Likewise, *Bacillus* species are important bacterial biocontrol agents due to their ability to produce antifungal metabolites, particularly cyclic lipopeptides such as iturins, fengycins, and surfactins (Caulier et al. 2019; Andrić et al. 2020). These compounds can disrupt fungal membranes, inhibit spore germination, and contribute to plant defense responses (Ongena and Jacques 2008; Abdelkhalek et al. 2020). *Bacillus velezensis*, *B. subtilis*, and *B. amyloliquefaciens* have shown antagonistic activity against *Fusarium* and *Colletotrichum* species in several crops, while their ability to form resistant endospores favors persistence and stability under variable environmental conditions (Shoda 2000; Reva et al. 2004; Xu et al. 2018; Walaszczyk et al. 2024).

Despite advances in microbial biological control, studies on olive diseases have largely focused on *in vitro* antagonism or soil and nursery applications, with fewer studies integrating laboratory screening with fruit protection assays. Furthermore, information on the performance of locally sourced *Bacillus* and *Trichoderma* isolates in emerging olive-growing regions of South America remains limited. Therefore, the present study aimed to evaluate the antagonistic potential of selected *Bacillus* and *Trichoderma* isolates against *Colletotrichum* and *Fusarium* species associated with olive diseases, integrating *in vitro* antagonism assays with detached fruit bioassays.

Materials and methods

Phytopathogenic and antagonistic isolates

Isolates of *Colletotrichum acutatum*, *C. nymphaeae*, *C. gloeosporioides*, *Fusarium solani*, and *F. oxysporum* were used in this study. These isolates had been previously purified and preserved in sterile distilled water and were obtained from studies conducted jointly by the Federal University of Rio Grande do Sul (UFRGS) and the Plant Pathology Laboratory of CEAGRO/DDPA, Porto Alegre, RS, Brazil (Finger et al. 2019; Oliveira et al. 2022). The *F. solani* isolate was obtained from the André Tosello Foundation, Campinas, SP, Brazil. Antagonistic isolates of *Bacillus* spp. and *Trichoderma* spp. were obtained from the microbial collections of CEAGRO and CEFLOP (DDPA/SEAPI-RS). Details regarding the isolates and their origins are presented in Table 1. Two commercial biofungicides were used as reference controls: Serenade® (based on *Bacillus subtilis*) and ICB Nutrisolo® (based on *Trichoderma* spp.).

Table 1 Phytopathogenic and antagonistic microorganisms used.

Species	Isolate	Source and GenBank accession number
<i>Colletotrichum acutatum</i>	OLI12	UFRGS / CEAGRO-DDPA
<i>Colletotrichum gloeosporioides</i>	CAK2	UFRGS / CEAGRO-DDPA
<i>Colletotrichum nymphaeae</i>	BRK3	UFRGS / CEAGRO-DDPA
<i>Fusarium oxysporum</i>	FUSGA4	UFRGS / CEAGRO-DDPA
<i>Fusarium solani</i>	7967	Fundação André Tosello
<i>Bacillus velezensis</i>	CRE4	CEAGRO-DDPA / OQ268070.1
<i>Bacillus velezensis</i>	KC1	CEAGRO-DDPA / OQ268068.1
<i>Bacillus</i> sp.	CRS3	CEAGRO-DDPA / PV954828
<i>Trichoderma asperelloides</i>	TA	DDPA/CEFLOR/SEAPI
<i>Trichoderma virens</i>	TV	DDPA/CEFLOR/SEAPI

***In vitro* antagonism assays**

Antagonistic activity of Bacillus spp. against phytopathogenic fungi

Direct antagonism assays between *Bacillus* spp. and phytopathogenic fungi were conducted on potato dextrose agar (PDA) commercial medium (Kasvi), prepared according to the manufacturer's instructions. Bacterial suspensions were obtained from pure cultures grown on tryptone soy agar (TSA) commercial medium (Oxoid), prepared according to the manufacturer's instructions and incubated at 28 ± 1 °C for 24 h. Cell density was adjusted using a spectrophotometer to an optical density of $OD_{600} = 0.4$, corresponding to approximately 10^9 CFU mL⁻¹, equivalent to the concentration used for commercial biofungicide Serenade®, which served as a reference.

In each Petri dish containing solidified PDA, 5 µL of the bacterial suspension were streaked along one edge of the plate. On the opposite edge, a 5-mm-diameter agar plug was placed, excised from the actively growing margin of seven-day-old colonies of *C. acutatum*, *C. gloeosporioides*, *C. nymphaeae*, *F. oxysporum*, and *F. solani*, previously cultured on PDA at 25 ± 2 °C.

Plates were arranged in a completely randomized design with four replicates per treatment and incubated at 25 ± 2 °C under a 12 h light/12 h dark photoperiod. Evaluations were performed at 7 and 14 days after assay establishment by measuring the mean diameter of pathogen mycelial growth. The percentage of inhibition was calculated relative to the untreated control (without *Bacillus*).

Antagonistic activity of Trichoderma spp. against phytopathogenic fungi

Antagonism assays between *Trichoderma* spp. and phytopathogenic fungi were also performed on PDA medium. Agar plugs (5 mm in diameter) were obtained from the margins of seven-day-old cultures of *Trichoderma virens* and *T. asperelloides* grown on

PDA at 25 ± 2 °C. Each antagonist plug was placed on one side of the plate, approximately 5 mm from the plate edge. On the opposite side, an agar plug of the same size was placed, taken from seven-day-old cultures of *C. acutatum*, *C. gloeosporioides*, *C. nymphaeae*, *F. oxysporum*, or *F. solani*.

Control treatments consisted of plates containing only the phytopathogen, without the antagonist. For comparative evaluation of the commercial biofungicide ICB Nutrisolo Trichoderma®, 20 µL of the product, applied at the recommended dose, was evenly distributed on 20 mL of solidified PDA medium. Plates were incubated at 25 ± 2 °C under a 12 h light/12 h dark photoperiod in a completely randomized design with four replicates per treatment. Evaluations were performed seven days after assay establishment by measuring mycelial growth along two perpendicular axes. The percentage of mycelial growth inhibition was then calculated.

Calculation of mycelial growth inhibition

Based on the values obtained from the antagonism assays, the percentage of mycelial growth inhibition (PMGI) was calculated using the formula proposed by Menten et al. (1976):

$$\text{PMGI} = [(\text{CR}_{\text{control}} - \text{CR}_{\text{treatment}}) / \text{CR}_{\text{control}}] \times 100,$$

where $\text{CR}_{\text{control}}$ represents the radial growth of the pathogen in the control treatment, and $\text{CR}_{\text{treatment}}$ represents the radial growth observed in the presence of the antagonist.

Statistical analysis

In vitro assays were conducted in a completely randomized design with four replicates per treatment, each replicate consisting of one Petri dish. Data were subjected to analysis of variance (ANOVA), and mean values were compared using Tukey's test at a

significance level of $\alpha = 0.05$. Statistical analyses were performed using RStudio software (R Core Team, 2015).

Bioassay on olive fruits

Fruit collection and preparation

Healthy olive fruits (cv. Arbequina) were collected in February 2025 at the Agronomic Experimental Station of UFRGS, from an area with no history of fungicide application. Fruits were surface disinfested by immersion in 2.5% sodium hypochlorite for 10 min, followed by 70% ethanol for 3 min, and rinsed with sterile distilled water. To standardize infection and ensure pathogen penetration, a small wound was made at the centre of each fruit using a sterile needle, as fruits collected under field conditions do not always present complete epidermal integrity.

Preparation of suspensions

Bacterial suspensions of *Bacillus* spp. were prepared by transferring 20 μ L of inoculum preserved in mineral water to Petri dishes containing TSA, followed by incubation at 28 °C for 24 h. Resulting colonies were scraped using a sterile Drigalski loop in phosphate buffer containing Tween 20, and the suspension was adjusted in a spectrophotometer to OD₆₀₀ = 0.4, corresponding to approximately 10⁹ CFU mL⁻¹. *Trichoderma* spp. isolates were cultured on PDA for seven days at 26 $\pm$ 1 °C under a 12 h photoperiod. Colonies were scraped to obtain conidial suspensions, which were filtered through double-layer gauze and adjusted to a concentration of 1 $\times$ 10⁷ spores mL⁻¹.

For *C. gloeosporioides* and *C. acutatum*, isolates were cultured on oat agar medium prepared with 30 g of oat flakes, 15 g of agar, and 1 L of distilled water, and incubated for seven days at 25 $\pm$ 2 °C under a 12h photoperiod to stimulate sporulation. Conidial

suspension was filtered through double-layer gauze and adjusted using a Neubauer chamber to 1.5×10^5 conidia mL⁻¹, which was used for fruit inoculation by immersion.

Two pathogenic isolates (*C. gloeosporioides* and *C. acutatum*), two fungal antagonists (*T. virens* and *T. asperelloides*), and two bacterial antagonists (*B. velezensis* KC1 and *Bacillus* sp. CRS3) were evaluated.

Treatments and experimental design

Fruits were immersed for 20 min in suspensions of *Bacillus* or *Trichoderma*. After 24 h, fruits were inoculated by immersion in a conidial suspension (1.5×10^5 conidia mL⁻¹). The negative control consisted of fruits immersed in sterile water only. After treatment, fruits were placed in sterile plastic boxes and incubated at 25 °C under high relative humidity and a 10/14 h (light/dark) photoperiod for nine days. The treatments evaluated are described in Table 2.

Table 2 Treatments applied to evaluate the efficacy of antagonists against *Colletotrichum* spp. in olive fruits

Treatment	Pathogen	Biocontrol agent
T1	<i>C. acutatum</i>	KC1
T2	<i>C. acutatum</i>	CRS3
T3	<i>C. acutatum</i>	<i>T. virens</i>
T4	<i>C. acutatum</i>	<i>T. asperelloides</i>
T5	<i>C. acutatum</i>	Inoculated control
T6	<i>C. gloeosporioides</i>	KC1
T7	<i>C. gloeosporioides</i>	CRS3
T8	<i>C. gloeosporioides</i>	<i>T. virens</i>
T9	<i>C. gloeosporioides</i>	<i>T. asperelloides</i>
T10	<i>C. gloeosporioides</i>	Inoculated control
T11	-	Non inoculated control

Disease severity assessment

Disease severity was assessed every three days from the appearance of the first visible symptoms. A disease severity scale was adopted as follows: 0 = no symptoms; 1 = small, localized punctiform or circular lesions; 2 = coalescent lesions without acervuli; 3 = coalescent lesions with acervuli; and 4 = fully affected fruit.

Disease severity was quantified using the Disease Severity Index (DSI), calculated according to the formula:

$$DSI = \left(\sum (n_i \times i) / (N \times 4) \right) \times 100$$

where n_i is the number of fruits in severity class i ; i is the severity class value (0 - 4); N is the total number of fruits evaluated, and 4 is the maximum severity class.

Each treatment consisted of 15 olive fruits, evenly distributed among three plastic containers, each containing five fruits. Each container was considered an experimental unit, resulting in three replicates per treatment.

Statistical analysis

Fruit bioassays were conducted in a completely randomized design using a 2x5 factorial arrangement, with two *Colletotrichum* species (*C. acutatum* and *C. gloeosporioides*) and five antagonist treatments (including the untreated control). Each treatment combination consisted of three experimental replicates, with each replicate represented by one plastic container containing five fruits, resulting in a total of 15 fruits per treatment combination.

Disease severity data were subjected to analysis of variance (ANOVA), considering the effects of *Colletotrichum* species, antagonist treatment, and their interaction. When significant effects were detected, means were compared using Tukey's test at a

significance level of $\alpha = 0.05$. Statistical analyses were performed using R software (R Core Team, 2015).

Results

Antagonistic activity of *Bacillus* spp. against phytopathogenic fungi.

All *Bacillus* isolates significantly reduced the mycelial growth of *Colletotrichum* spp. and *Fusarium* spp. ($p < 0.001$), with inhibition levels varying among antagonist isolates and target pathogens. At 7 days after incubation, isolates CRE4 and KC1 inhibited the growth of *C. acutatum* by 10.4% and 9.5%, respectively, whereas KC1 (21.2%) and CRS3 (14.4%) showed greater inhibition against *C. gloeosporioides*. For *F. oxysporum*, mycelial growth inhibition reached 12.1% with CRS3 and 8.6% with KC1. Negative inhibition values were observed for KC1 against *C. nymphaeae* (−2.5%), indicating stimulation of fungal growth under these conditions (Table 3).

Table 3 Percentage of mycelial growth inhibition (PMGI) of *Colletotrichum* spp. and *Fusarium* spp.

Pathogens	<i>Bacillus</i> isolates						Serenade	
	CRE4		CRS3		KC1			
	7 d	14 d	7 d	14 d	7 d	14 d	7 d	14 d
<i>C. acutatum</i>	10.4 abB	28.8 aA	8.8 abB	46.5 aB	9.5 bB	26.3 aA	26.6 aA	43.1 aB
<i>C. gloeosporioides</i>	12.9 aB	22.9 bB	14.4 aB	21.7 bB	21.2 aAB	20.9 bB	24.8 abA	40.0 aC
<i>C. nymphaeae</i>	7.8 abA	30.9 aA	10.6 abA	32.1 cC	-2.5 cB	14.4 cC	16.9 abcA	42.8 aC
<i>F. oxysporum</i>	7.4 abA	18.6 cC	12.1 abA	24.9 aB	8.6 bA	25.4 aB	15.0 bcA	30.7 bC
<i>F. solani</i>	2.3 bA	13.7 dD	3.1 bA	15.6 dD	5.2 bcA	6.7 dD	7.3 cA	32.2 bC

Means followed by the same lowercase letter within a column and the same uppercase letter within a row do not differ according to Tukey's test ($p < 0.05$) when compared at the same time point (7 or 14 days). Lowercase letters compare pathogens within each antagonist treatment and incubation period; uppercase letters compare antagonist

treatments within each pathogen and incubation period. Means followed by the same letter do not differ significantly according to Tukey's test ($p < 0.05$).

At 14 days after incubation, inhibition levels increased substantially for most treatments. The isolate CRS3 suppressed *C. acutatum* growth by up to 46.5%, whereas CRE4 and KC1 showed inhibition levels of 28.8% and 26.3%, respectively. The commercial biofungicide Serenade® consistently exhibited higher inhibition rates, exceeding 40% against all *Colletotrichum* isolates. For *F. oxysporum* and *F. solani*, inhibition levels were lower than those observed for *Colletotrichum* spp., although still significantly different from the untreated control (Table 3).

Antagonistic activity of *Trichoderma* spp. against phytopathogenic fungi.

The isolates *Trichoderma virens* and *T. asperelloides* consistently reduced the mycelial growth of all phytopathogens evaluated, confirming their antagonistic potential under *in vitro* conditions. Inhibition percentages ranged from 33.1% to 49.3%, with the highest reductions observed against *Fusarium* species. For *F. oxysporum*, mycelial growth was reduced by 49.3% with *T. virens* and 49.1% with *T. asperelloides*, whereas inhibition against *F. solani* reached 40.1% and 41.6%, respectively (Table 4).

High inhibitory activity was also observed against *Colletotrichum* species. *T. virens* reduced the growth of *C. gloeosporioides* by 43.1% and *C. nymphaeae* by 37.6%, while *T. asperelloides* promoted reductions of 47.2% and 38.9%, respectively. For *C. acutatum*, both *Trichoderma* isolates showed similar inhibition levels, close to 33% (Table 4). Overall, these results indicate that *Trichoderma* spp. were effective in suppressing the *in vitro* growth of the different phytopathogens tested.

Table 4 Percentage of mycelial growth inhibition (PMGI) of *Colletotrichum* spp. and *Fusarium* spp. seven days after exposure to two *Trichoderma* species and the commercial product ICB® on PDA medium (25 ± 2 °C)

Pathogen	<i>Trichoderma</i> species		ICB®
	<i>T. virens</i>	<i>T. asperelloides</i>	
<i>C. acutatum</i>	33.7 bB	33.1 bB	56.9 bA
<i>C. gloeosporioides</i>	43.1 abA	47.2 aA	51.0 bcA
<i>C. nymphaeae</i>	37.6 bB	38.9 abB	54.0 bcA
<i>F. oxysporum</i>	49.3 aB	49.1 aB	74.2 aA
<i>F. solani</i>	40.1 abA	41.6 abA	45.0 cA

Means followed by the same lowercase letter within a column and the same uppercase letter within a row do not differ according to Tukey's test ($p < 0.05$). CV (%) = 11.85%

Evaluation of the efficacy of antagonistic microorganisms in protecting olive fruits against anthracnose

The efficacy of the antagonistic microorganisms was evaluated based on their ability to reduce anthracnose severity in olive fruits inoculated with *C. acutatum* and *C. gloeosporioides*. At three days after inoculation, no significant interaction among the evaluated factors was detected, although fruits inoculated with *C. gloeosporioides* exhibited more severe symptoms than those inoculated with *C. acutatum*. At this early stage, punctiform and circular lesions predominated, corresponding to severity levels 1 and 2 on the disease severity scale.

At six days after inoculation, disease severity increased, with the occurrence of coalescent lesions and the presence of acervuli on some fruits, corresponding to severity levels 2 and 3. The *Bacillus* isolates (CRS3 and KC1) showed superior performance, significantly reducing disease severity compared with the untreated control and with

Trichoderma treatments (Table 5; Fig. 1). Fruits treated with KC1 and CRS3 exhibited restricted necrosis and slower disease progression, whereas untreated fruits showed generalized rot and tissue collapse.

Table 5 Anthracnose severity (%) in olive fruits of cv. Arbequina at 6 days after inoculation with *Colletotrichum* spp. and treated with *Bacillus* and *Trichoderma* isolates.

Pathogens	Antagonistic isolates				
	Control	CRS3	KC1	TA	TV
<i>C. acutatum</i>	56.7 aA	30.0 bBC	23.3 bC	51.7 aAB	45.0 aABC
<i>C. gloeosporioides</i>	55.0 aA	66.7 aA	65.0 aA	68.3 aA	63.3 aA

TA= *Trichoderma asperelloides*, TV= *Trichoderma virens*, CRS3= *Bacillus* sp. and KC1= *B. velezensis*. Means followed by the same lowercase letter within a column and the same uppercase letter within a row do not differ according to Tukey's test ($p < 0.05$). CV (%) = 11.51

At nine days after inoculation, symptom progression was evident in the control treatments, with a predominance of fruits classified at severity level 4, characterized by extensive necrosis and fruit decay.

Although no significant interaction among factors was detected at this stage, a significant main effect of antagonist treatments was observed. In treatments with *T. virens* against *C. acutatum*, visible colonization by the antagonist was recorded, accompanied by partial suppression of disease development. In contrast, fruits treated with CRS3 and inoculated with *C. gloeosporioides* showed partial containment of symptom progression, although acervuli formation was still observed (Fig. 2). When data from both *Colletotrichum* species were considered, antagonist treatment significantly affected disease severity. CRS3 and *T. virens* resulted in the lowest mean severity values, at 73.3% and 72.5%, respectively, compared with 94.2% in the untreated control (Table 6).



Fig. 1 Anthracnose symptoms on olive fruits inoculated with *C. acutatum* and treated with different *Bacillus* sp. strains at 6 days after inoculation. (a) KC1 × CA; (b) CRS3 × CA; (c) Control (without *Bacillus*)

Table 6 Anthracnose severity in olive fruits at 9 days after inoculation with *Colletotrichum* spp. treated with different *Bacillus* spp. strains and two *Trichoderma* species

Treatments	Severity (%)
Inoculated Control	94.17 a
<i>Trichoderma asperelloides</i>	78.33 ab
<i>Bacillus velezensis</i> (KC1)	75.83 ab
<i>Bacillus</i> sp. (CRS3)	73.33 b
<i>Trichoderma virens</i>	72.50 b

Means followed by the same lowercase letter do not differ according to Tukey's test at the 5% probability level. Values represent means across *C. acutatum* and *C. gloeosporioides*, because the pathogen $\times$ antagonist interaction was not significant.



Fig. 2 Anthracnose symptoms on olive fruits inoculated with *Colletotrichum* spp. and subjected to different biological treatments at 9 days after inoculation. (a) *Trichoderma virens* $\times$ *C. acutatum*; (b) Control with *C. acutatum*; (c) *Bacillus* sp. CRS3 $\times$ *C. gloeosporioides*; (d) Control with *C. gloeosporioides*. Red arrows indicate depressed necrotic lesions associated with anthracnose symptoms

Discussion

The present study evaluated the antagonistic potential of selected *Bacillus* and *Trichoderma* isolates against *Colletotrichum* and *Fusarium* species affecting olive trees, integrating *in vitro* antagonism assays with detached fruit bioassays. Overall, the results provide consistent evidence that the evaluated *Bacillus* and *Trichoderma* isolates exhibit antagonistic activity against the target phytopathogens and demonstrate potential for the management of anthracnose and fusariosis in olive production systems.

The relationship between *in vitro* antagonism and fruit protection was not uniform across the microorganisms evaluated, indicating that laboratory screening alone may not fully predict the performance of antagonists on olive fruits. It should be noted that commercial products were evaluated exclusively under *in vitro* conditions, whereas fruit assays were conducted only with selected isolates. Nevertheless, *in vitro* antagonism assays represent a useful first step for screening microbial antagonists, although subsequent validation under conditions that more closely represent the host environment is essential.

Under *in vitro* conditions, *Bacillus velezensis* KC1 and *Bacillus* sp. CRS3 showed high inhibitory activity against *C. acutatum* and *C. gloeosporioides*, an effect that was maintained in the detached olive fruit assays (cv. Arbequina). Both isolates significantly reduced anthracnose severity, indicating that the antagonistic mechanisms expressed under controlled conditions remain functional on the fruit surface.

While inhibition of halos and reduced mycelial growth were observed on agar plates, treated fruits exhibited restricted necrotic lesions and delayed symptom progression. These observations indicate that *Bacillus* activity does not rely solely on direct physical contact but also involves diffusible and persistent mechanisms that remain effective under

heterogeneous physicochemical conditions. Such conditions include natural barriers such as the cuticle, epidermis, and the epiphytic microbiota, as discussed by Vorholt (2012).

The inhibition of *Colletotrichum* spp. observed both *in vitro* and *in vivo* is consistent with previous studies highlighting the extensive biosynthetic capacity of *Bacillus* spp. to produce antifungal cyclic lipopeptides, including iturins/bacillomycins, fengycins, and surfactins (Caulier et al. 2019; Xu et al. 2020). These metabolites act synergistically to disrupt fungal membranes, inhibit conidial germination, and suppress appressorium formation. In addition, *Bacillus* spp. release volatile organic compounds (VOCs), such as acetoin and 2,3-butanediol, which can inhibit pathogen growth at a distance and activate plant defence responses, inducing a physiological “priming” state (Kloepper et al. 2004; Ryu et al. 2004; Grahovac et al. 2023).

Genomic studies have shown that *B. velezensis* harbors a conserved core of biosynthetic gene clusters encoding these metabolites, including surfactin, fengycin, iturins/bacillomycins, bacillibactin, bacilysin, difficidin, and bacillaene, among others (Xu et al. 2020; Liu et al. 2024; Zheng et al. 2024). Recent evidence integrating genomic, metabolomic, and field-based assays with *B. velezensis* strains confirms that these mechanisms remain functional under real environmental conditions, reinforcing the effectiveness of biocontrol strategies against phytopathogenic fungi in perennial crops (Tam et al. 2023).

The combined action of these metabolites, together with competition for nutrients, production of lytic enzymes (chitinases and glucanases), and exclusion of colonization sites, likely explains the suppression observed both in *Colletotrichum* growth and in disease symptom development on treated fruits.

Beyond direct antibiosis, *Bacillus* spp. are also recognized for their ability to induce induced systemic resistance (ISR), involving the activation of defense-related genes such

as PR-1 and PAL, as reported for *B. velezensis* (Abdelkhalek et al. 2020) and other *Bacillus* species (Kloepper et al. 2004). Although full activation of systemic defense mechanisms depends on the integrity of the plant system, an aspect limited in detached fruits, it is plausible that localized defense responses contributed to the reduced anthracnose progression observed in fruits treated with CRS3 and KC1. In addition, the ability to form highly resistant endospores confers an adaptive advantage, allowing survival on dry fruit surfaces under ultraviolet radiation and temperature fluctuations (McKenney et al. 2013).

In Mediterranean countries, where anthracnose is endemic and causes severe yield and quality losses, applications of *B. subtilis* during pre-flowering have shown efficacy comparable to copper-based fungicides in reducing latent infections (Nigro et al. 2018). These findings corroborate the potential of *Bacillus* spp. as sustainable tools within integrated olive disease management programs, in agreement with the consistent *in vitro* and fruit assay performance demonstrated by the *Bacillus* isolates evaluated in this study.

In contrast to the consistent performance of *Bacillus*, *Trichoderma* isolates showed variable efficacy across the conducted assays. *T. virens* and *T. asperelloides* exhibited strong antagonism against *Fusarium* spp. under *in vitro* conditions, with inhibition levels within ranges reported in previous studies using these species (Modrzewska et al. 2022; Yao et al. 2023; Sehim et al. 2023). However, in detached fruit assays, *T. virens* showed only superficial colonization and limited suppression of *C. acutatum* lesions at nine days after inoculation, without significant reductions in disease severity.

These results reflect the distinct ecology of *Trichoderma*, which is naturally adapted to the rhizosphere. On aerial surfaces, *Trichoderma* faces adverse conditions such as desiccation, ultraviolet radiation, limited substrate availability, and intense competition with the epiphytic microbiota, all of which may reduce its viability and antagonistic

activity (Woo et al. 2014; Vindas-Reyes et al. 2024). Similar studies in strawberry and apple have shown that *T. harzianum* can colonize flowers and reduce infections by *Colletotrichum* and *Botrytis* but may present variable effectiveness under field conditions (Freeman et al. 2004).

Thus, the superficial colonization observed in this study does not indicate tissue damage or detrimental effects to the host but rather reflects an experimental scenario under controlled conditions. Under field and postharvest environments, abiotic factors and epiphytic microbial communities are expected to further modulate *Trichoderma* growth, reinforcing the need for protective formulations and validation under more realistic application regimes. This interpretation highlights two practical implications: (i) the need to optimize formulations to protect antagonist viability under abiotic stress, and (ii) the importance of field validation considering spray volume, coverage, and application intervals, to better align biological responses with operational conditions.

Despite limited efficacy on fruits, the strong *in vitro* performance of *Trichoderma* isolates against *Fusarium oxysporum* and *F. solani* indicates their potential utility in managing soilborne pathogens and in nursery substrate treatments for olive production, as previously reported by Harman et al. (2004). In such contexts, *Trichoderma* acts through mycoparasitism, production of lytic enzymes, and induction of systemic resistance, effectively suppressing root rot diseases in seedlings (Woo et al. 2014).

Innovative formulation strategies have been proposed to overcome the limitations of *Trichoderma* in foliar applications. Microencapsulation using natural polymers such as alginate, chitosan, and gums has been shown to extend conidial viability under radiation and desiccation stress, enabling gradual release of the antagonist in the field (Vindas-Reyes et al. 2024). In parallel, the combined use of *Bacillus* and *Trichoderma* in microbial

consortia may broaden the spectrum of action and enhance biocontrol resilience (Minchev et al. 2021; Koskey et al. 2021).

Such integrated strategies may be adapted to critical phenological stages of olive production, with *Bacillus* applications during pre-flowering and early fruit ripening, when the risk of *Colletotrichum* infection is highest, and *Trichoderma* applications directed to soil or nursery substrates to reduce pathogen inoculum pressure.

The integration of *Bacillus* and *Trichoderma* into olive disease management programs represents a sustainable alternative to the continuous use of copper-based and systemic fungicides. Beyond reducing selection pressure for resistant pathogen populations, these antagonists contribute to nutrient cycling, soil health, and the maintenance of beneficial microbial communities (Minchev et al. 2021).

To our knowledge, this study represents one of the first Brazilian reports evaluating *Bacillus* and *Trichoderma* isolates for the protection of olive fruits against anthracnose, integrating *in vitro* and *in vivo* assays. Future research should prioritize continued evaluation of these antagonists in plant-microbe interactions under controlled conditions and in the field, both individually and in consortia, as well as validation across different phenological stages and environmental conditions. Through this progressive and multidisciplinary approach, the potential observed under laboratory conditions may be translated into technically viable solutions for olive orchards, reducing the dependence on chemical fungicides and strengthening the principles of low-impact and sustainable agriculture.

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Data availability The supporting information associated with this article can be obtained from the corresponding author upon making a reasonable request.

Declarations

512 **Ethical approval** This research fully complies with Ethical Standards applicable for this
513 journal and the relevant national and international ethics related rules and professional
514 codes of conduct.

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516 **Competing interests** The authors declare that they have no conflict of interest.

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References:

- Abdelkhalek A, Behiry SI, Al-Askar AA (2020) *Bacillus velezensis* PEA1 inhibits *Fusarium oxysporum* growth and induces systemic resistance to cucumber mosaic virus. *Agronomy* 10:1312. <https://doi.org/10.3390/agronomy10091312>
- Álvarez B, Couanon W, Olivares J, Nigro F (2019) Biocontrol agents and cropping practices to control olive diseases. In: EIP-AGRI Focus Group “Pests and Diseases of the Olive Tree”. European Commission, Brussels. Available at: <https://www.researchgate.net/publication/343638974> (accessed 4 June 2025)
- Amira MB, Lopez D, Mohamed AT, Khouaja A, Chaar H, Fumanal B, Gousset-Dupont A, Bonhomme L, Label P, Goupil P (2017) Beneficial effect of *Trichoderma harzianum* strain Ths97 in biocontrolling *Fusarium solani* causal agent of root rot disease in olive trees. *Biol Control* 110:70–78. <https://doi.org/10.1016/j.biocontrol.2017.04.008>
- Andrić S, Meyer T, Ongena M (2020) *Bacillus* responses to plant-associated fungal and bacterial communities. *Front Microbiol* 11:1350. <https://doi.org/10.3389/fmicb.2020.01350>
- Azevedo-Nogueira F, Martins-Lopes P, Gomes S (2020) Current understanding of *Olea europaea*–*Colletotrichum acutatum* interactions in the context of identification and quantification methods: A review. *Crop Prot* 132:105131. <https://doi.org/10.1016/j.cropro.2020.105131>
- Bishop CD, Cooper RM (1983) An ultrastructural study of vascular colonization in tomato plants infected with *Fusarium oxysporum* f. sp. *lycopersici*. *Physiol Plant Pathol* 23:347–357. [https://doi.org/10.1016/0048-4059\(83\)90041-5](https://doi.org/10.1016/0048-4059(83)90041-5)

- Buonaurio R, Almadi L, Famiani F, Moretti C, Agosteo GE, Schena L (2023) Olive leaf spot caused by *Venturia oleaginea*: An updated review. *Front Plant Sci* 13:1061136. <https://doi.org/10.3389/fpls.2022.1061136>
- Canale MC, Cardoza Y, Ortiz PC, Mituti T, Fernandes J, Sabião RR, Barbé S, Brugnara EC (2025) First report of *Xylella fastidiosa* subsp. *pauca* in *Olea europaea* in Santa Catarina, Southern Brazil. *J Plant Pathol* 107:1311–1312. <https://doi.org/10.1007/s42161-025-01894-2>
- Carrero-Carrón I, Trapero-Casas JL, Olivares-García C, Monte E, Hermosa R, Jiménez-Díaz RM (2016) *Trichoderma asperellum* is effective for biocontrol of *Verticillium* wilt in olive caused by *Verticillium dahliae*. *Crop Prot* 88:45–52. <https://doi.org/10.1016/j.cropro.2016.05.009>
- Caulier S, Nannan C, Gillis A, Licciardi F, Bragard C, Mahillon J (2019) Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Front Microbiol* 10:302. <https://doi.org/10.3389/fmicb.2019.00302>
- Chacón-Ortiz A, Maia LC, Oliveira AC, Perruolo G, Pegoraro C (2024) Olive trees in the world: Past and present with future perspectives. *Rev Ciênc Agroveterinárias* 23:1–16. <https://doi.org/10.5965/223811712312024143>
- Coletta-Filho HD, Francisco C, Lopes J, Oliveira A, Silva LF (2016) First report of olive leaf scorch in Brazil associated with *Xylella fastidiosa* subsp. *pauca*. *Phytopathol Mediterr* 55:130–135. https://doi.org/10.14601/Phytopathol_Mediterr-17259
- Domingues RJ, Tófoli JG, Zanota S, Harakava R (2020) First report of *Fusarium solani* causing root rot on olive in Brazil. *Plant Dis* 104:1974. <https://doi.org/10.1094/PDIS-09-19-1921-PDN>
- Finger G, Wegner BR, Zanini F, Lanna-Filho R, Kersck AP, Bertolini E, Rotta de Oliveira AM (2019) Species of *Colletotrichum* causing olive anthracnose in South Brazil.

In: Proc IOBC-WPRS Working Group “Integrated Protection of Olive Crops”,
Florence, pp 71–74

Fraga H, Malheiro AC, Moutinho-Pereira J, Santos JA (2020) Mediterranean olive
orchards under climate change: Impacts and adaptation strategies. *Agronomy*
10:56. <https://doi.org/10.3390/agronomy10010056>

Freeman S, Minz D, Kolesnik I, Barbul O, Zveibil A, Maymon M, Nitzani Y, Kirshner
B, Rav-David D, Dag A, Shafir S, Elad Y (2004) *Trichoderma* biocontrol of
Colletotrichum acutatum and *Botrytis cinerea*. *Eur J Plant Pathol* 110:361–370.
<https://doi.org/10.1023/B:EJPP.0000021057.93305.d9>

Gordon TR (2017) *Fusarium oxysporum* and the Fusarium wilt syndrome. *Annu Rev
Phytopathol* 55:23–39. <https://doi.org/10.1146/annurev-phyto-080615-095919>

Grahovac J, Pajčin I, Vlajkov V (2023) *Bacillus* VOCs in the context of biological
control. *Antibiotics* 12:581. <https://doi.org/10.3390/antibiotics12030581>

Grzanka M, Sobiech Ł, Filipczak A, Danielewicz J, Jajor E, Horoszkiewicz J, Korbas M
(2024) The efficacy of plant pathogen control by complexed forms of copper.
Agriculture 14:139. <https://doi.org/10.3390/agriculture14010139>

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species—
opportunistic, avirulent plant symbionts. *Nat Rev Microbiol* 2:43–56.
<https://doi.org/10.1038/nrmicro797>

Ibarra-Laclette E, Blaz J, Pérez-Torres CA, Villafán E, Lamelas A, Rosas-Saito G, Ibarra-
Juárez LA, García-Ávila CdJ, Martínez-Enriquez AI, Pariona N (2022) Antifungal
effect of copper nanoparticles against *Fusarium kuroshium*. *J Fungi* 8:347.
<https://doi.org/10.3390/jof8040347>

- Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94:1259–1266. <https://doi.org/10.1094/PHYTO.2004.94.11.1259>
- Koskey G, Mburu SW, Awino R, Njeru EM, Maingi JM (2021) Potential use of beneficial microorganisms for soil amelioration and phytopathogen biocontrol. *Front Sustain Food Syst* 5:606308. <https://doi.org/10.3389/fsufs.2021.606308>
- Liu Y, Yin C, Zhu M, Zhan Y, Lin M, Yan Y (2024) Comparative genomic analysis of *Bacillus velezensis* BRI3 reveals genes associated with antagonism. *Genes* 15:1588. <https://doi.org/10.3390/genes15121588>
- Marques E, Martins I, Mello SCM (2018) Antifungal potential of crude extracts of *Trichoderma* spp. *Biota Neotrop* 18:e20170418. <https://doi.org/10.1590/1676-0611-BN-2017-0418>
- Martins F, Pereira JA, Baptista P (2019) Olive anthracnose and its management by fungal endophytes. In: Varma A, Tripathi S, Prasad R (eds) *Plant–microbe interface*. Springer, Cham, pp 253–269. https://doi.org/10.1007/978-3-030-19831-2_11
- Martins S, Pereira S, Dinis LT, Brito C (2024) Enhancing olive cultivation resilience under climate change. *Horticulturae* 10:1066. <https://doi.org/10.3390/horticulturae10101066>
- McKenney PT, Driks A, Eichenberger P (2013) The *Bacillus subtilis* endospore: Assembly and functions. *Nat Rev Microbiol* 11:33–44. <https://doi.org/10.1038/nrmicro2921>
- Menten JOM, Machado CC, Minussi F, Castro C, Kimati H (1976) Efeito de fungicidas no crescimento micelial de *Macrophomina phaseolina*. *Fitopatol Bras* 1:57–66

612 Mikaberidze A, McDonald BA, Bonhoeffer S (2014) Can high-risk fungicides be used
613 without selecting resistance? *Phytopathology* 104:324–331.
614 <https://doi.org/10.1094/PHYTO-07-13-0204-R>

615 Minchev ZI, Kostenko O, Soler R, Pozo MJ (2021) Microbial consortia for biocontrol of
616 plant diseases. *Front Plant Sci* 12:756368.
617 <https://doi.org/10.3389/fpls.2021.756368>

618 Modrzewska M, Błaszczuk L, Stępień Ł, Urbaniak M, Waśkiewicz A, Yoshinari T, Bryła
619 M (2022) *Trichoderma* versus *Fusarium*: Inhibition of pathogen growth. *Molecules*
620 27:8146. <https://doi.org/10.3390/molecules27238146>

621 Moral J, De Oliveira R, Trapero A (2021) Diversity of *Colletotrichum* species associated
622 with olive anthracnose. *J. Fungi* 7:325. <https://doi.org/10.3390/jof7050325>

623 Nigro S, Antelmi I, Labarile L, Sion V, Pentimone I (2018) Biological control of olive
624 anthracnose. *Acta Hort* 1199:439–444.
625 <https://doi.org/10.17660/ActaHortic.2018.1199.70>

626 Oliveira AMR, Finger G, Ritt ABB, Büttow MV, Betencourt MR (2022) Caracterização
627 de olivais no Rio Grande do Sul. Circular técnica 14. SEAPDR/DDPA, Porto
628 Alegre. Available at:
629 [https://www.agricultura.rs.gov.br/upload/arquivos/202211/16175358-circular-](https://www.agricultura.rs.gov.br/upload/arquivos/202211/16175358-circular-oliva-vfinal-111122.pdf)
630 [oliva-vfinal-111122.pdf](https://www.agricultura.rs.gov.br/upload/arquivos/202211/16175358-circular-oliva-vfinal-111122.pdf) (accessed 5 February 2023)

631 Ongena M, Jacques P (2008) *Bacillus* lipopeptides: Versatile weapons for plant disease
632 biocontrol. *Trends Microbiol* 16:115–125.
633 <https://doi.org/10.1016/j.tim.2007.12.009>

634 Petrović E, Vrandečić K, Ćosić J, Godena S (2024) Chemical control of olive fungal
635 diseases. *Poljoprivreda* 30:44–53. <https://doi.org/10.18047/poljo.30.1.6>

- Poveda J, Hermosa R, Monte E, Nicolás C (2019a) ThKEL1 protein in *Trichoderma harzianum* and plant defense. *Front Plant Sci* 10:1478. <https://doi.org/10.3389/fpls.2019.01478>
- Poveda J, Hermosa R, Monte E, Nicolás C (2019b) *Trichoderma harzianum* enhances plant productivity. *Sci Rep* 9:48269. <https://doi.org/10.1038/s41598-019-48269-z>
- R Core Team (2015) R: A language and environment for statistical computing. R Foundation, Vienna. Available at: <https://cran.r-project.org/> (accessed 9 May 2025)
- Reva ON, Dixelius C, Meijer J, Priest FG (2004) Industrial applications of *Bacillus subtilis*. *Microbiology* 150:341–350. <https://doi.org/10.1099/mic.0.26847-0>
- Ryu CM, Farag MA, Hu CH, Reddy MS, Wei HX, Paré PW, Kloepper JW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol* 134:1017–1026. <https://doi.org/10.1104/pp.103.026583>
- Secretaria da Agricultura, Pecuária, Produção Sustentável e Irrigação (2024) Câmara setorial das oliveiras apresenta números da safra 2023/2024. Governo do RS, Porto Alegre. Available at: <https://www.estado.rs.gov.br/camara-setorial-das-oliveiras-apresenta-numeros-da-safra-2023-2024> (accessed 13 February 2025)
- Sehim AE, Hawedy OA, Altammar KA, Alhumaidi MS, Elghaffar RY (2023) *Trichoderma asperellum* suppresses *Fusarium oxysporum*. *Front Microbiol* 14:1140378. <https://doi.org/10.3389/fmicb.2023.1140378>
- Sergeeva V (2011) Anthracnose in olives: Symptoms and management. In: *Proceedings of the International Congress on Olive Biotechnology*, Perpignan, pp 269–274
- Shoda M (2000) Bacterial control of plant diseases. *J Biosci Bioeng* 89:515–521. [https://doi.org/10.1016/S1389-1723\(00\)80006-2](https://doi.org/10.1016/S1389-1723(00)80006-2)
- Talhinhas P, Loureiro A, Oliveira H (2018) Overview of olive anthracnose. *Plant Pathol* 67:925–941. <https://doi.org/10.1111/ppa.12812>

661 Tam LTT, Jähne J, Luong PT, et al (2023) *Bacillus velezensis* strains enhance crop yield
662 in field trials. Front Plant Sci 14:1194887.
663 <https://doi.org/10.3389/fpls.2023.1194887>

664 Trabelsi R, Riahi H, Mhadhbi H (2017) Response of olive tree to soilborne fungi. J Plant
665 Dis Prot 124:153–162. <https://doi.org/10.1007/s41348-016-0062-8>

666 Van Zwieten L, Rust J, Kingston T, Merrington G, Morris S (2004) Influence of copper
667 fungicides on earthworms. Sci Total Environ 329:29–41.
668 [https://doi.org/10.1016/S0048-9697\(04\)00125-1](https://doi.org/10.1016/S0048-9697(04)00125-1)

669 Vindas-Reyes E, Chacón-Cerdas R, Rivera-Méndez W (2024) *Trichoderma* production
670 and encapsulation methods. AgriEngineering 6:2366–2384.
671 <https://doi.org/10.3390/agriengineering6030138>

672 Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10:828–840.
673 <https://doi.org/10.1038/nrmicro2910>

674 Walaszczyk E, Lisiecki P, Cybulska J (2024) *Bacillus* lipopeptides and volatile
675 metabolites. Appl Microbiol Biotechnol 108:893–909.
676 <https://doi.org/10.1007/s00253-024-13014-3>

677 Woo SL, Ruocco M, Vinale F, Nigro M, Marra R, Lombardi N, Pascale A, Lanzuise S,
678 Manganiello G, Lorito M (2014) *Trichoderma*-based products in agriculture. Open
679 Mycol J 8:71–126. <https://doi.org/10.2174/1874437001408010071>

680 Xu P, Xie S, Liu W, Jin P, Wei D, Yaseen DG, Wang Y, Miao W (2020) Comparative
681 genomics of *Bacillus velezensis*. Front Microbiol 11:594079.
682 <https://doi.org/10.3389/fmicb.2020.594079>

683 Xu S, Wei L, Zhang Y (2018) Lipopeptides produced by *Bacillus subtilis* suppress fungal
684 pathogens. Sci Rep 8:15210. <https://doi.org/10.1038/s41598-018-33635-2>

685 Yao X, Guo H, Zhang K, Zhao M, Ruan J, Chen J (2023) *Trichoderma* in biological
686 control of plant diseases. *Front Microbiol* 14:1160551.
687 <https://doi.org/10.3389/fmicb.2023.1160551>

688 Zhang H, Li Y, Zhu J (2020) Biological control as an alternative to synthetic fungicides.
689 *Crit Rev Microbiol* 46:450–462. <https://doi.org/10.1080/1040841X.2020.1794793>

690 Zheng Y, Liu T, Wang Z, Wang X, Wang H, Li Y, Zheng W, Wei S, Leng Y, Li J, Yang
691 Y, Liu Y, Li Z, Wang Q, Tian Y (2024) Whole-genome sequencing of *Bacillus*
692 *velezensis* BN. *Front Microbiol*. <https://doi.org/10.3389/fmicb.2024.1498653>