Phytopathologia Mediterranea
The international journal of the
Mediterranean Phytopathological Union

Firenze University Press
www.fupress.com/pm

Research Papers

Potential biocontrol of Botryosphaeria dieback pathogens using endophytic *Aspergillus*, *Penicillium* and *Trichoderma* species from grapevines

İzzet BÜLBÜL[1], Davut Soner AKGÜL[2,*]

[1] *Biological Control Research Institute, Ministry of Agriculture and Forestry, 01321, Yüreğir, Adana, Türkiye*
[2] *Department of Plant Protection, Agriculture Faculty, University of Çukurova, 01330, Balcalı, Adana, Türkiye*
*Corresponding author. E-mail:sakgul@cu.edu.tr

Summary. This research aimed to determine abilities of endophytic *Aspergillus*, *Penicillium*, and *Trichoderma* species to colonize grapevine cuttings, and to evaluate their performance for protecting pruning wounds from *Botryosphaeriaceae* fungi under greenhouse and field conditions. Colonization ability of five endophytic species (*Aspergillus allahabadi, A. flavipes, Penicillium canescens, P. fructuariae-cellae*, and *Trichoderma brevicompactum*) was assessed after introductions into cuttings using two inoculation methods: immersion in conidial suspensions or contact with dry conidia. Colonization by the inoculated fungi was subsequently confirmed through re-isolations, and species identities were verified by PCR and gene sequencing. After confirming colonization, biological activity of the endophytes was assessed against pathogens. Conidium suspensions were either sprayed onto host wounds or applied systemically to cuttings, and necrotic lengths in internal tissues were measured 4 months after inoculations. In a subsequent field experiment, conidium suspensions were applied by spraying onto pruning wounds, or placing mycelium/agar plugs, and extent of necroses was assessed 6 months after inoculations. *Penicillium* and *Trichoderma* species colonized internal tissues of grapevine plants, whereas *Aspergillus* species were not re-isolated. In greenhouse trials, the endophytes reduced lesion lengths caused by *Botryosphaeriaceae* species by 22 to 85 %. Under field conditions, and where applied to pruning wounds, these fungi gave 51 to 70% protection against *Lasiodiplodia brasiliensis* and *Neofusicoccum parvum*, with *Trichoderma brevicompactum* giving greatest efficacy. This study has shown that vineyard-derived *Penicillium* and *Trichoderma* endophytes can establish within grapevine tissues, and have potential to effectively suppress *Botryosphaeriaceae* pathogens of grapevines.

Keywords. *Botryosphaeriaceae*, Grapevine Trunk Diseases (GTDs), *Lasiodiplodia brasiliensis, Neofusicoccum parvum, Vitis vinifera*

OPEN ACCESS

Citation: Bülbül, İ. & Akgül, D. S. (2026). Potential biocontrol of Botryosphaeria dieback pathogens using endophytic *Aspergillus*, *Penicillium* and *Trichoderma* species from grapevines. *Phytopathologia Mediterranea* 65(2): 287-299. doi: 10.36253/phyto-17392

Accepted: July 25, 2026

Published: September 10, 2026

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: José R. Úrbez Torres, Agriculture and Agri-Food Canada, Summerland, British Columbia, Canada.

ORCID:
DSA: 0000-0002-9990-4194
İB: 0000-0003-0527-8610

INTRODUCTION

Türkiye is an important grape-producing country, and grapevine nurseries are important agricultural industries. According to the Ministry of Agriculture and Forestry, approx. three million grapevine plants (registered) are produced annually in Türkiye, and these plants are sold to establish new vineyards or replace uprooted vines dying from pests or diseases in their first years (Anonymous, 2025). During vineyard establishment, some young plants die due to abiotic factors such as unfavorable soil conditions, poor cutting quality, improper planting, or biotic factors (fungal pathogens, and nematode or insect pests).

Fungal pathogens (*Botryosphaeriaceae, Diaporthe, Eutypa* species, *Cylindrocarpon*-like fungi, *Phaeomoniella*, *Phaeoacremonium, Fomitiporia* species) associated with grapevine trunk diseases (GTDs), and some soilborne fungi (species of *Phytophthora, Fusarium, Rhizoctonia, Macrophomina,* or *Verticillium*) are among the leading causes of plant death in newly established vineyards (Gramaje and Armengol, 2011). These pathogens are disseminated latently by dormant vines or propagation material, spreading over large areas and causing economic losses.

Controlling latent infections is challenging, and various management methods are followed at nursery propagation stages to ensure healthy plant production. Some disinfectants (quaternary ammonium salts, hydroxyquinoline sulfate) and fungicides added to water-soaking tanks reduce fungal contamination of propagation materials (Gramaje *et al.,* 2009). Hot water treatments can also eradicate most latent infections, and combining hot water treatments with fungicides has been found to augment these effects (Görür and Akgül, 2019). Systemic fungicides are also applied via drip irrigation systems when grafted cuttings are planted in the soil or during root formation.

These treatments may protect plant roots for short periods, but they are not effective against latent pathogen infections in grapevine stems. In recent years, research has focused on biological control of fungal pathogens associated with grapevine trunk diseases in grapevine nurseries and in field vineyards. These efforts aimed to reduce pathogen spread and promote production of healthy plants. Some microorganisms with potential biocontrol activity can minimize plant diseases by competing with pathogens for nutrients, producing antibiotics, or parasitizing pathogens. They can also contribute to plant growth by facilitating nutrient uptake and by producing beneficial substances that favour plant growth (Pieterse *et al.,* 2014).

Endophytic fungi live with most vascular plants, and do not cause disease symptoms. They are also known to suppress plant pathogens through production of various secondary metabolites, and supporting plants against environmental stress factors (Busby *et al.,* 2016). Grapevine is also a host for endophytic microorganisms, and research using advanced technologies such as Next Generation Sequencing (NGS) and metatranscriptomics analysis have shown that grapevines are rich in bacterial and fungal microbiota. Some studies have indicated that up to 60 endophytic fungal genera are present in adult grapevines, of which *Alternaria, Aspergillus, Cladosporium, Clonostachys, Epicoccum, Penicillium* and *Trichoderma* predominate. Species in these genera have been studied for biocontrol of fungal plant pathogens, due to their high proliferation, abilities to colonize organic matter rapidly, and production of antimicrobial secondary metabolites (Thambugala *et al.,* 2020). Deyett and Rolshausen (2020) showed that approx. 28% of grapevine endomycota were acquired from the soil and plant environments, gradually colonizing grapevines from the time of planting. This indicates that local and natural endophytes have coevolved with grapevine, and these have superior potential for colonization and pathogen antagonism compared to other endophytic species. *Aspergillus* and *Penicillium* are genera that are found wherever organic matter is present, and are adapted to various harsh environmental conditions, including drought and high temperatures. Some species of these genera can live with plants as endophytes, while others can cause fruit bunch rots, plant mortality, and canker diseases (Bustamente *et al.,* 2024; El-Dawy *et al.,* 2024). Endophytic species of these genera can directly or indirectly inhibit the growth of other microorganisms, and contribute to plant health by secreting various metabolites, antibiotics, or mycotoxins (Grijseels *et al.,* 2017; Hagag *et al.,* 2022). In addition to these genera, the *Trichoderma* also includes species proven to have biocontrol activities against plant diseases, including GTDs (Berbegal *et al*., 2020; Silva-Valderrama *et al.,* 2021). Most of these studies used commercially produced *Trichoderma* isolates, and some studies examined disease-reducing performance of local endophytic species.

Very few studies have assessed endophytic *Penicillium* and *Aspergillus* species for biocontrol of GTDs. Since biological fungicides produced in different countries may not perform well when used against a particular disease in another country, results obtained using local isolates can sometimes be more effective (Pollard-Flamand *et al.,* 2022).

The hypothesis examined in the present study was that antagonist isolates selected from local fungal endophytes could successfully reduce diseases caused

by *Botryosphaeriaceae*, both at young vine stages and under field conditions, through applications to pruning wounds and as inoculations of grapevine cuttings. Fungal endophytes from different locations or vineyards were assessed for abilities to suppress fungal pathogens associated with GTDs.

The objectives of this study were: (i) to determine colonization performance of the endophytic *Aspergillus, Penicillium*, and *Trichoderma* species in detached grapevine canes; and (ii) to assess their prophylactic performances for reducing lesions caused by *Botryosphaeriaceae* species through protecting wounds on grapevine plants and adult vines, using greenhouse and field experiments.

MATERIALS AND METHODS

Fungus isolates

The *Botryosphaeriaceae* pathogens and endophytic *Aspergillus, Penicillium*, or *Trichoderma* isolates used in this study were obtained from a survey conducted during 2020-21 in vineyards, from the local cultivars Hatun Parmağı, Horozkarası, Kabarcık, Mahrabaşı, or Tarsus Beyazı, grown on their own roots. These grapevines were in the Eastern Mediterranean Region of Türkiye. Pure cultures obtained were deposited in the culture collection of the Mycology Laboratory at the Faculty of Agriculture, Çukurova University. The isolates were identified using DNA analyses, and virulence of *Botryosphaeriaceae* isolates was previously shown using pathogenicity tests. In a preliminary study, endophytic *Aspergillus, Penicillium*, or *Trichoderma* isolates were subjected to dual-culture tests, and those with prominent antagonistic properties were selected for use in the pot and field trials described below (Table 1).

Assessments of colonization of grapevine cuttings by antagonistic fungi

Ability of antagonistic species to colonize grapevine cuttings was tested using two different methods. In the first, mycelial agar discs (maintained in 30% glycerol and 70% sterile distilled water) of antagonistic *Aspergillus allahabadii* (*Aab*), *A. flavipes* (*Afp*), *Penicillium canescens* (*Pcn*), *P. fructuariae-cellae* (*Pfc*), or *Trichoderma brevicompactum (Tbr)* were taken from the fungal culture collection, and transferred to Petri dishes containing potato dextrose agar (PDA). To ensure high conidium production, the isolates were incubated at 25 °C under a 12 h light, 12 h dark cycle for 15 d.

Sterile distilled water was added to the mature cultures, and conidia were dislodged into the water using a sterile spatula. The resulting conidium suspensions were then each homogenized with 50 µL of polyoxyethylene sorbitan monolaurate (Tween-20™), the conidia were counted with a haemocytometer (Thoma Slide™) under a light microscope (Olympus, model BX-51), and the concentration was adjusted to 10^8 conidia·mL^{-1}. The basal regions (5 cm) of dormant canes (*Vitis vinifera* 'Cardinal', 30 cm in length, with 2-3 buds) were dipped into the conidium suspension, and the canes were kept in this suspension at room temperature for 24 h.

The canes were then planted into the plastic pots (1 L capacity) containing rooting medium (equal volumes of peat moss and perlite). To induce root and shoot formation, the pots were kept in a climate controlled room (28 °C, relative humidity = 85%, 12 h light, 12 h dark cycle) for 4 weeks. The plants were then transferred to a lath-house to grow naturally for 4 months. During the growth period, the dormant cuttings were irrigated weekly with a liquid nutrient solution (20% nitrogen, 20% phosphorus, 20% potassium, 0.02% copper, 0.05% iron, 0.02% manganese, and 0.02% zinc), and no pesticides were applied. Twenty cuttings were used for each antagonistic species, and each cutting was planted into a separate pot.

In the second method, pure and highly sporulating PDA cultures of the antagonistic fungi were used to inoculate dormant canes. The basal ends of the canes were bluntly cut with sterilized pruning shears, and the fresh wounds were directly blotted on the surfaces of the sporulated cultures to ensure conidium adherence. The canes were then planted pots, placed in the controlled climate room, and grown as described (above) for the first method.

To assess internal colonization by antagonistic fungal species, grapevine plants were uprooted from the pots 4 months after planting, their roots were removed with pruning shears, and their basal parts were thoroughly washed under tap water. Segments (2 cm in length) were then cut from points 10 cm from the plant base, and were then surface disinfected for 3 min using a 2.5% sodium hypochlorite solution containing >5% active chlorine (Hypo: Koruma A.Ş). The segments were then rinsed twice in sterile distilled water and blotted with sterile paper towels. The bark tissues were then removed, internal tissues were cut aseptically with sterile scissors, and 3–5 mm woody tissues were plated onto PDA amended with streptomycin sulfate (250 mg·L^{-1}).

Internal stem colonization by the respective inoculated fungi was determined as the percentage of fungus re-isolated. Twenty PDA Petri plates were used for each

antagonistic fungus, with each plate containing seven internal tissues from one cane segment. Re-isolation rates were assessed as proportions of tissue pieces yielding antagonistic species.

Verifying endophytic colonization by PCR and gene sequencing

The re-isolated species were examined morphologically and microscopically, and species identifications were confirmed using PCR and gene sequencing.

Genomic DNA was extracted from *Aspergillus, Penicillium,* and *Trichoderma* colonies using the CTAB (2%) protocol described by O'Donnell *et al.* (1998). ITS1, 5.8S, and ITS2 regions of the rDNA, beta-tubulin, and partial translation elongation factor (TEF 1-α) genes were amplified with PCR reactions using ITS4/ITS5, Bt2a/Bt2b, and EF688F/EF1251R primers (White *et al.,* 1990; Glass and Donaldson, 1995; Alves *et al.,* 2008). PCR reactions were carried out in a thermal cycler (SimpliAmp A24811™, Applied Biosystems) in 50 µL mixtures for each sample. Each reaction tube contained 25 µL of Thermo Scientific Master Mix (2X)™, 23 µL of PCR grade water (Lonza™), 0.5 µL of forward and reverse primers (10 pmol·µL^{-1}), and 1 µL of genomic DNA (approx. 100 ng·µL^{-1}). PCR conditions were 95 °C for 3 min (initial denaturation), followed by 35 cycles each of denaturation at 95 °C for 1 min, annealing at 52 °C (for ITS), 55 °C (for beta-tubulin), or 53 °C (for TEF1-α), for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. Success of PCR reactions was then confirmed using agarose gel electrophoresis (1.5% agarose, Invitrogen) and visualization of DNA bands under a UV illuminator. PCR products were sequenced by Macrogen Co. (South Korea), The sequences obtained were compared with those deposited in the NCBI GenBank database using the BLAST search (version 2.0; National Center for Biotechnology Information, United States National Institutes of Health).

Assessments of prophylactic capacity of antagonistic fungi against Botryosphaeriaece *pathogens in grapevine cuttings (pot experiments)*

The prophylactic capacities of endophytic fungi were assessed using two methods: (1) spraying conidium suspensions onto open wounds in grapevine wood tissue, or (2) systemically inoculating dormant cuttings with these suspensions.

For Method (1), dormant Cardinal grapevine cuttings, each 30 cm long with 2 to 3 buds, were washed under tap water, then superficially disinfected in a 2.5% sodium hypochlorite solution for 3 min, then rinsed twice with sterile distilled water. Endophytic fungal isolates (five species, one isolate each) were incubated on PDA at 24 °C in the dark for 10 d. Sterile distilled water plus one to two drops of polyoxyethylene sorbitan monolaurate (Tween 20™) were then added to heavily conidiating cultures, and the conidium suspensions were adjusted to 10^8 conidia mL^{-1} after counting conidia with a haemocytometer (Thoma Slide™). An internode on each cutting was wounded with a sterile 5 mm diam. cork-borer, the bark tissues were removed, and 25 µL of endophytic fungus conidium suspension was sprayed onto each wound using a fine droplet sprayer. These cuttings were then incubated at room temperature for 24 h, and mycelium/agar plugs of the *Botryosphaeriaceae* fungi (eight species, one isolate each; Table 1) were then inoculated into the wounds. For inoculation controls, no endophytic isolates were inoculated, but pathogens were inoculated into the wounds (Blundell and Eskalen, 2022). The inoculation points were each wrapped with parafilm to prevent desiccation of the inoculation agar plugs. The cuttings were then planted into plastic pots (1 L capacity) containing rooting medium (equal volumes of peat moss and perlite). The plants were grown in a controlled climate room (28 °C, 85% relative humidity, 12 h light:12-h dark cycle) for 4 weeks, to induce root and shoot formation. The pots were then transferred to the lath-house to grow naturally for 4 months.

For Method (2), endophytic isolates were inoculated into grapevine cuttings as described by Benlioğlu and Özakman (1998). Transparent sterile rubber tubing was attached to both ends of each dormant grapevine cuttings. One end of the cutting was linked, via tubing, to a Buchner funnel fitted into an Erlenmeyer flask, which was then connected to an air pump (Merck Millipore) operating under negative pressure (-0.4 bar). One mL of each endophyte conidium suspension (10^8 conidia·mL^{-1}) was then injected into the tubing at the opposite end using an automatic pipette, ensuring that the conidia entered each cutting. To facilitate endophyte colonization and prevent dehydration, the base sections of the cuttings were immersed in water in plastic containers, and were then incubated at 25 °C for 1 week. Internode sections of stems were then laterally drilled with an electric drill and 2 mm diam. bit, and mycelium agar pieces of the respective pathogens were inoculated into the holes (Pouzoulet *et al.,* 2013). The inoculation points were then wrapped with parafilm, and the cuttings were planted into the plastic pots (as described above). The cuttings were then grown in an climate controlled room (28 °C, 85% relative humidity, 12 h light:12 h dark) for 4 weeks to induce root

Table 1. Locations of source vineyards and grapevine cultivars, and details of the endophytic and pathogenic *Botryosphaeriaceae* fungi used in this study (with their respective NCBI GenBank sequence numbers).

Fungus species	Isolate code	Location (in Türkiye)	Grapevine cultivar	GenBank accession numbers*			
				ITS	BT	TEF	CAL
Endophytic species							
Aspergillus allahabadii	BMAE208IB	Tarsus, Mersin	Tarsus Beyazı	PP989656	PV864710	-	-
Aspergillus flavipes	BMAE161IB	Tarsus, Mersin	Tarsus Beyazı	PP989655	PV864709	-	-
Penicillium canescens	BMAE291IB	Tarsus, Mersin	Tarsus Beyazı	PP989657	-	-	PV864707
Penicillium fructuariae-cellae	CUZF39HIK	İslahiye, Gaziantep	Dımışkı	OM980658	-	-	PV791715
Trichoderma brevicompactum	BMAE96IB	Hassa, Hatay	Hatunparmağı	PP989654	-	PV864708	-
Botryosphaeriaceae species							
Botryosphaeria dothidea	BMAE155IB	Pazarcık, K. Maraş	Kabarcık	PP703088	-	PV795919	-
Diplodia olivarum	BMAE184IB	Toroslar, Mersin	Tarsus Beyazı	PP703095	-	PV855303	-
Diplodia seriata	BMAE68IB	Tarsus, Mersin	Tarsus Beyazı	PP703073	-	PP791972	-
Dothiorella omnivora	BMAE290IB	Saimbeyli, Adana	Mücennis	PP703108	-	PV855304	-
Lasiodiplodia brasiliensis	BMAE89IB	Hassa, Hatay	Horozkarası	PP703079	-	PV763370	-
Neofusicoccum australe	BMAE229IB	Tarsus, Mersin	Tarsus Beyazı	PP703105	-	PV855300	-
Neofusicoccum parvum	BMAE201IB	Tarsus, Mersin	Tarsus Beyazı	PP703101	-	PV795916	-
Neoscytalidium dimidiatum	BMAE103IB	Onikişubat, K. Maraş	Mahrabaşı	PP703081	-	PV832488	-

*ITS, internal transcribed spacer; BT, β-tubulin; TEF, translation elongation factor 1-α; CAL, calmodulin.

and shoot formation. The pots were then transferred to the lath-house to grow naturally for 4 months.

For both inoculation methods, six replicates per endophyte-pathogen combination (one plant per pot, each representing a replicate) were used, with completely randomized experimental design for each testing method. The inoculation experiments were each conducted twice.

To evaluate the prophylactic effects of the endophytic isolates, in Method (1) lesion lengths were measured in wood tissues by removing bark tissues. In Method (2) lesions were measured after excising the plant stems longitudinally.

Variance analyses of data obtained were carried out for lesion length data to reveal differences between means of the treatments, and the statistically similar groups were determined by Fisher's Least Significant Difference (LSD) test ($P \leq 0.05$) in all the experiments (Gomez and Gomez, 1984).

Assessments of prophylactic activity of antagonistic fungi against Lasiodiplodia brasiliensis *or* Neofusicoccum parvum *in field conditions*

Trials to assess performance of endophytic isolates under field conditions were carried out in 2023 and 2024, in a 7-year-old vineyard ('Cardinal' grapevines grafted on 1103 Paulsen rootstock) at the implementation area of Çukurova University Faculty of Agriculture.

All the endophytic fungi listed in Table 1 (five species, one isolate each) were used in field trials against the two most virulent pathogenic species (*L. brasiliensis* and *N. parvum*). To prepare inocula of the endophytic fungi, the isolates were grown on PDA at 25 °C under a 12 h light, 12 h dark cycle for 10 d. Conidium suspensions of the endophytic isolates were prepared as described above, and the suspension concentration in each suspension was adjusted to 10^8 conidia·mL^{-1}, after counting using a haemocytometer. The vines were each spur-pruned to three buds in March of 2023 or 2024. Immediately after spur pruning, the endophyte conidium suspensions were sprayed onto fresh pruning wounds. Pathogen inoculations (with either *L. brasiliensis* or *N. parvum*) were the applied 24 h later.

Two pathogen inoculation methods were used. For the first, mycelium agar plugs of *L. brasiliensis* or *N. parvum* obtained from 10-d-old PDA cultures were placed onto pruning wounds, and were sealed with parafilm. For the second method, pycniospore suspensions were used as inoculum. Because sufficient pycniospores could only be produced by *L. brasiliensis*, this method was evaluated only for that species. *Neofusicoccum parvum* was therefore tested only using mycelium agar plugs. Positive experimental control wounds received sterile distilled water instead of endophyte conidium suspensions, and were inoculated with the pathogens 24 h later. The experiment was arranged in a randomized complete block design, with four replicates. Each grapevine spur represented one replicate, and each vine car-

ried five spurs receiving different endophyte treatments. Six months after inoculation, spurs were collected, lesion lengths were measured, and pathogen re-isolations were carried out to confirm infections (Blundell and Eskalen, 2022; Akgül *et al.,* 2023). A total of 192 grapevines were used in the field experiments. The experiments were set up in separate vineyard blocks, with four replicates per block, and with randomized block designs. There were five spurs on each vine, and each spur formed one replicate. On each vine, separate endophyte fungal isolates were inoculated onto each spur (five endophyte species plus one pathogen species on five spurs).

Six months after inoculations (in September 2023 and September 2024), the spurs were cut to length 10–12 cm, and lesion lengths were measured from the treated and non-treated spurs. The re-isolation procedure was used to confirm that the lesions were caused by the particular inoculated pathogens. Efficacy of treatments was calculated according to the Abbott formula [(lesion length in non-treated control - lesion length in treated spur) / lesion length in non-treated control) ×100]. Variance analyses were carried out on these data to determine differences between means of lesion lengths, and statistically similar groups were determined by Fisher's Least Significant Difference (LSD) test ($P \leq 0.05$) in all experiments (Gomez and Gomez, 1984).

RESULTS

Abilities of antagonistic species to colonize grapevine cuttings

The re-isolation rates of endophytic fungal species inoculated into grapevine cuttings using two different methods, 10 cm from the basal ends of the cuttings, after 4 months, are shown in Table 2. *Penicillium* and *Trichoderma* species were re-isolated from inoculated tissues 10 cm from the inoculation point, whereas *Aspergillus* species were not detected. In general, the re-isolation percentages of endophytic species from internal tissues using the basal ends of the cuttings blotting-in-conidial-mass method have been greater than from the conidium suspension immersion method. The *Penicillium* and *Trichoderma* species were re-isolated from woody tissues located 10 cm above the inoculation points, whereas *Aspergillus* species were not detected. Among the assessed endophytes, *Trichoderma brevicompactum* gave the greatest re-isolation frequencies, from both of the inoculation methods and in both years, ranging from 22.8 to 34.2%. *Penicillium canescens* and *P. fructuariae-cellae* were recovered at lower frequencies, ranging from 4.2 to 12.8% for *P. canescens*, and from 5.7 to 11.4% for *P. fructuariae-cellae.* No re-isolations were obtained of *Aspergillus allahabadii* or *A. flavipes* from internal tissues sampled 10 cm from the basal ends of the cuttings. PCR amplification and gene sequencing confirmed that the re-isolated *Penicillium* or *Trichoderma* colonies corresponded to the species originally inoculated into the cuttings, showing 99% similarity in the respective BLAST analyses.

Prophylactic activity of antagonistic species against Botryosphaeriaceae *fungi in grapevine plants*

The effects of endophytic *Aspergillus, Penicillium*, or *Trichoderma* species on internal lesions caused by the eight *Botryosphaeriaceae* species were examined, focusing on their protective effects on external plant wounds. As well, the question of how much endophytes could reduce lesions caused by pathogens, if they had systemically colonized the cuttings earlier, was also assessed, four months after inoculations, in grapevine plantlets not treated with endophytes (experimental controls), *L. brasiliensis* and *N. parvum* caused the longest lesions, whereas *Diplodia olivarum* and *Neofusicoccum australe* produced shorter lesions. In comparison, *Botryosphaeria dothidea, Diplodia seriata, Dothiorella omnivora*, and *Neoscytalidium dimidiatum* caused shorter lesions than the previously fungi. All the endophytes applied to grapevine canes reduced ($P \geq 0.05$) mean internal lesion lengths compared to the experimental controls.

All the endophyte treatments reduced mean lesion lengths ($P \geq 0.05$) caused by the eight *Botryosphaeriaceae* species, compared with the pathogen-inoculated controls in both years (Figures 1 and 2). *Lasiodiplodia brasiliensis* and *Neofusicoccum parvum* produced the longest lesions in the control plants, whereas *Botryosphaeria dothidea*, *Diplodia seriata*, *Dothiorella omnivora*, and

Table 2. Mean re-isolation proportions for endophytic fungi from internal grapevine tissues sampled at 10 cm from the basal ends of grapevine rootstock cuttings.

Endophytic fungus species	Re-isolation proportions (%)			
	Dipping in conidial suspension		Blotting in conidial mass	
	2023	2024	2023	2024
Aspergillus allahabadii	-	-	-	-
A. flavipes	-	-	-	-
Penicillium canescens	11.4	4.2	12.8	7.1
P. fructuariae-cellae	8.5	5.7	11.4	8.5
Trichoderma brevicompactum	28.5	22.8	34.2	28.5

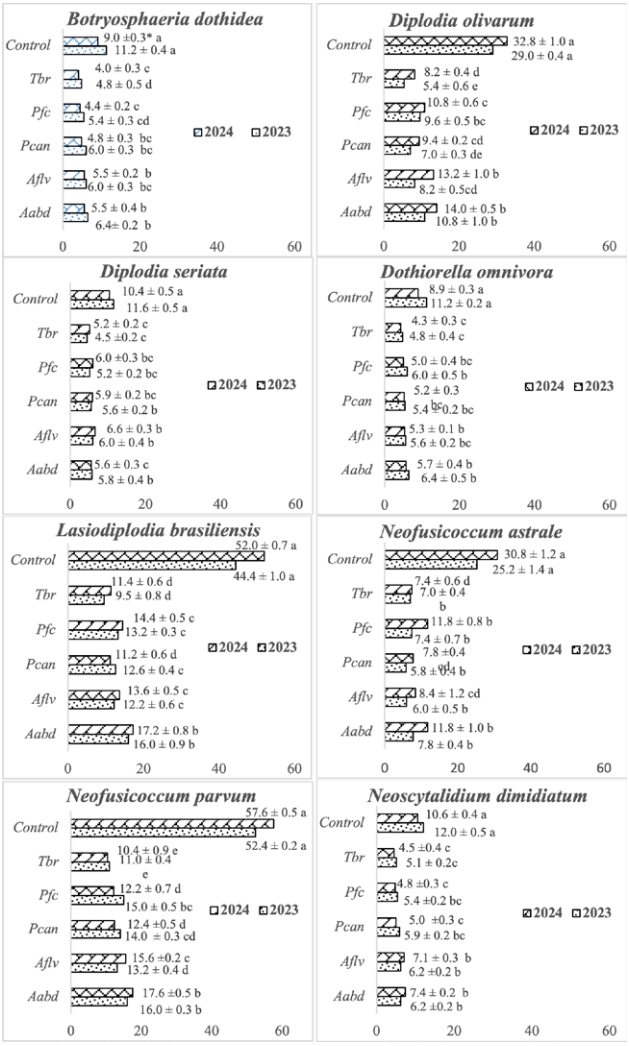

Figure 1. Mean lesion lengths (mm) caused by six *Botryosphaeriaceae* fungi in grapevine plants under greenhouse conditions, following applications of five endophytic fungi (by wound spraying). *Means (± standard errors) accompanied by different letters are different ($P \leq 0.05$), as indicated by Fisher's Least Significant Difference tests. *Aabd*, *Aspergillus allahabadi*; *Aflv*, *A. flavipes*); *Pcan*, *Penicillium canescens*; *Pfc*, *P. fructuariae-cellae*; *Tbr*, *Trichoderma brevicompactum*.

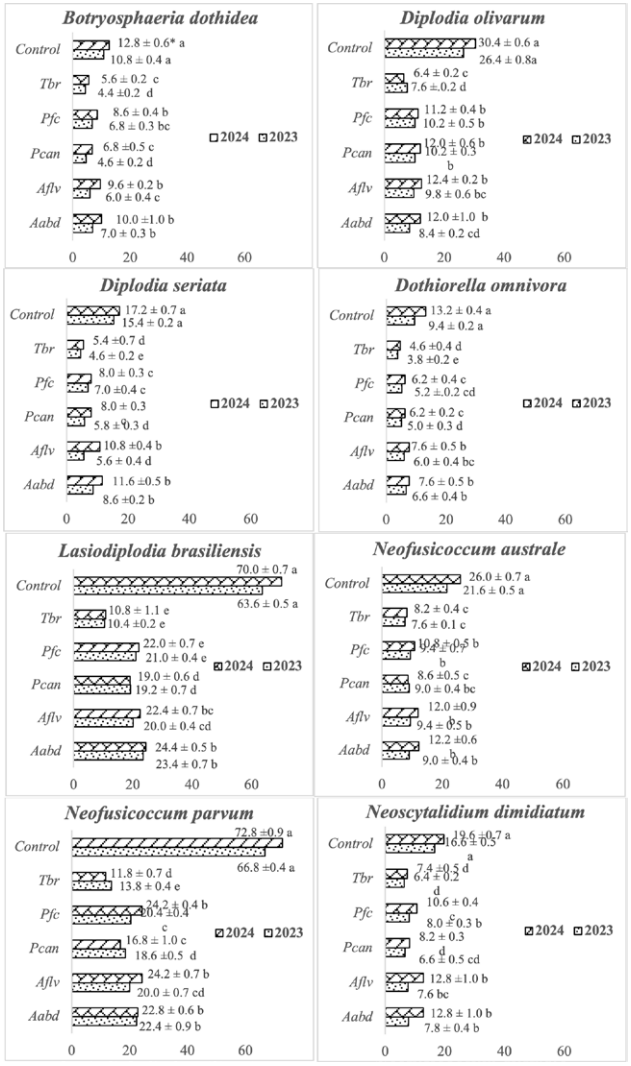

Figure 2. Mean lesion lengths (mm) caused by six *Botryosphaeriaceae* fungi in grapevine plants under greenhouse conditions, following applications of five endophytic fungi (by systemic inoculation). *Means (± standard errors) accompanied by different letters are different ($P \leq 0.05$), as indicated by Fisher's Least Significant Difference tests. *Aabd*, *Aspergillus allahabadi*; *Aflv*, *A. flavipes*); *Pcan*, *Penicillium canescens*; *Pfc*, *P. fructuariae-cellae*; *Tbr*, *Trichoderma brevicompactum*.

Neoscytalidium dimidiatum caused comparatively shorter lesions. Similar trends were observed in the wound-protection and the systemic-inoculation experiments. Depending on the pathogen, endophyte species, or inoculation method, lesion reduction ranged from means of 21.9% to 84.6%. The greatest reductions were obtained from *Trichoderma brevicompactum* against *L. brasiliensis* or *N. parvum* in the systemic inoculation experiments, whereas the lowest efficacy was observed for *Aspergillus allahabadii* against *B. dothidea*. Overall, *Trichoderma* treatments provided the greatest levels of protection, followed by *Penicillium* species, while *Aspergillus* species generally gave lower efficacy.

Effects of antagonists on lesion development caused by Lasiodiplodia brasiliensis *or* Neofusicoccum parvum *under field conditions*

The effects of endophytic fungi on lesion development caused by *L. brasiliensis* and *N. parvum* were evalu-

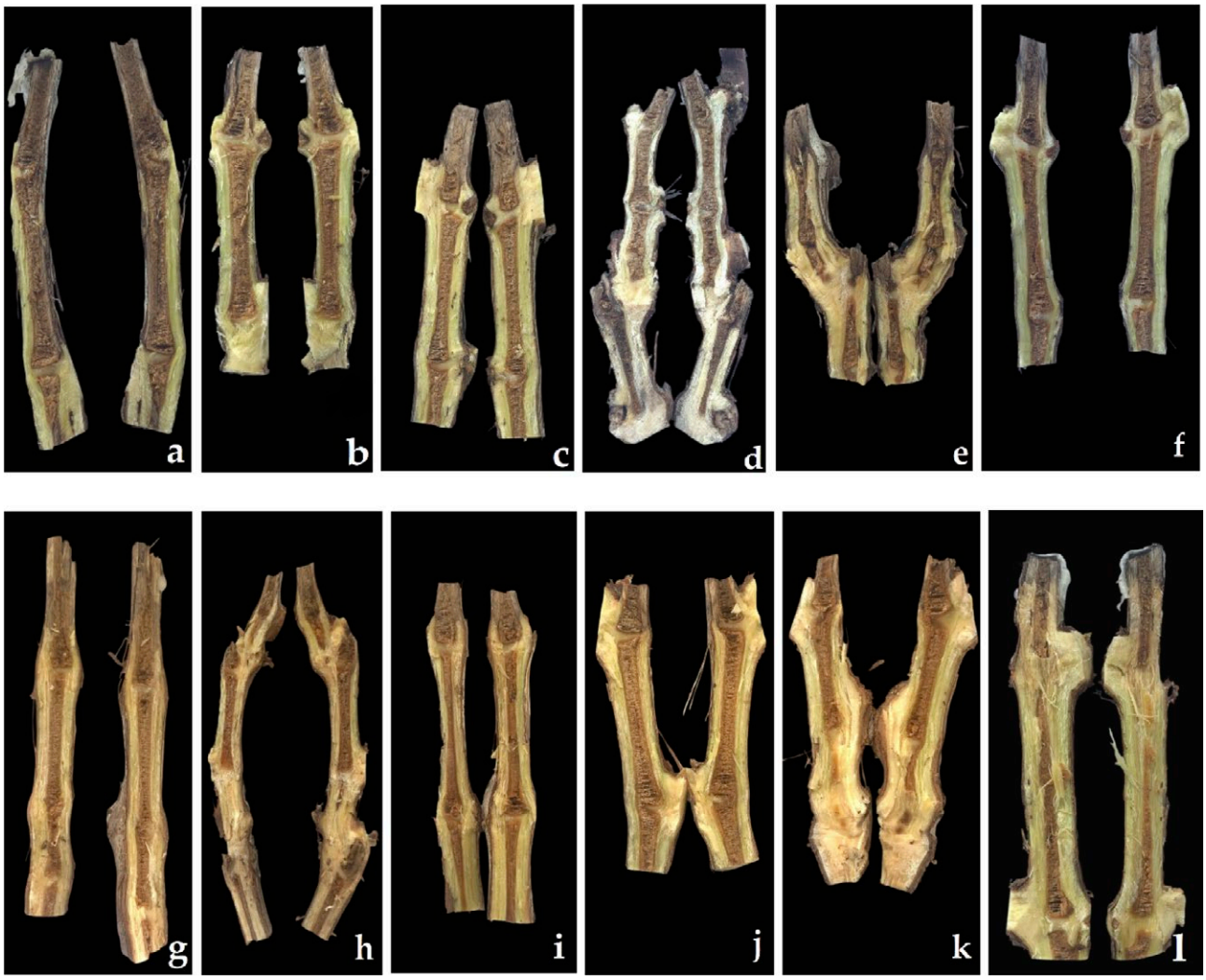

Figure 3. Internal lesions in 1-year-old woody shoots of grapevine ('Cardinal'), caused by *Botryosphaeriaceae* pathogens under field conditions, following prior applications of endophytic fungi. a to f; Inoculation with *L. brasiliensis*: a, control (water); b, *A. allahabadi*; c, *A. flavipes*; d, *P. canescens*; e, *P. fructuariae-cellae*; f) *T. brevicompactum.* g to l; Inoculation with *N. parvum*: g, control (water); h, *A. allahabadi*; i, *A. flavipes*; j, *P. canescens*; k, *P. fructuariae-cellae*; l, *T. brevicompactum.*

ated under field conditions in 2023 and 2024. Six months after inoculations, all *Aspergillus, Penicillium*, and *Trichoderma* treatments reduced internal lesion lengths ($P \geq 0.05$) compared with the pathogen-inoculated controls (Figure 3; Table 3). All treatments reduced lesion lengths compared with the pathogen-inoculated controls. Overall, biological efficacy ranged from 42.8% to 71.8% against *L. brasiliensis* and from 51.0% to 69.6% against *N. parvum*. Among the tested endophytes, *T. brevicompactum* consistently provided the greatest reductions in lesion development, whereas Aspergillus species generally showed lower efficacy. *Penicillium canescens* and *P. fructuariae-cellae* exhibited intermediate but consistent performance in both years. Similar trends were observed regardless of the inoculation method used.

DISCUSSION

This is the first study to evaluate local endophytic isolates against *Botryosphaeriaceae* pathogens in grapevines in Türkiye. To date, research has focused on species such as *T. asperellum, T. atroviride, T. harzianum*, and *T. viride*, or antagonistic *Bacillus* bacteria, for effects on grapevine trunk pathogens (Billar de Almeida *et al.,* 2020; Langa-Lomba *et al.,* 2023; Zanfano *et al.,* 2025). Little data on bioactivity of *T. brevicompactum* against these pathogens has been reported.

Aspergillus species can exist as pathogens or endophytes in agricultural plants, and they are most prevalent in hot, arid regions (Sibakwe *et al.,* 2017). Some endophytic *Aspergillus* species can protect plants to some

Table 3. Mean lesion lengths (± standard errors) and bio-control efficacy (%) from treatments with endophytic fungi against *L. brasiliensis (Lbra)* or *Neofusicoccum parvum (Np)* infections in field conditions

Endophyte	Pycniospore inoculations				Mycelium disc inoculations			
Treatment	Lesion length (mm)		Efficacy (%)		Lesion length (mm)		Efficacy (%)	
	2023	2024	2023	2024	2023	2024	2023	2024
Aab + Lbra	17.8 ± 0.9 b*	19.8 ± 1.4 b	47.0	42.8	15.5 ± 0.9 b	15.8 ± 0.6 b	51.6	53.0
Afp + Lbra	14.8 ± 0.3 c	17.3 ± 0.8 c	56.0	50.0	15.3 ± 0.9 b	15.0 ± 0.6 b	52.3	54.9
Pcn + Lbra	12.8 ± 0.5 d	12.5 ± 0.6 d	62.0	63.8	12.5 ± 0.3c	11.3 ± 1.0 c	60.9	66.1
Pfc + Lbra	12.0 ±0.4 de	12.8 ± 0.5 d	64.1	63.0	12.3 ± 0.9 c	11.5 ± 0.6 c	61.7	65.4
Tbr + Lbra	10.5 ± 0.5 e	11.3 ± 0.3 d	68.7	67.4	9.0 ± 0.7 d	10.0 ± 0.4 c	71.8	69.9
Control (*Lbra*)	33.5 ± 0.5 a	34.5 ± 0.6 a	-	-	32.0 ± 0.8 a	33.3 ± 1.3 a	-	-
Aab + Np	-	-	-	-	13.3 ± 0.3 b	14.3 ± 0.6 b	51.4	51.7
Afp + Np	-	-	-	-	12.0 ± 0.8 bc	11.3 ± 0.8 c	56.0	61.9
Pcn + Np	-	-	-	-	10.5 ± 0.6 cd	11.0 ± 0.4 c	61.5	67.8
Pfc + Np	-	-	-	-	11.3 ± 0.6 c	9.5 ± 0.6 cd	58.7	62.8
Tbr + Np	-	-	-	-	9.3 ± 0.5 d	9.0 ± 0.7 d	66.1	69.6
Control (*Np*)	-	-	-	-	27.3 ± 0.9 a	29.5 ± 0.5 a	-	-

*Means within columns are different ($P \leq 0.05$), based on Fisher's Least Significant Difference tests. *Aab*, *Aspergillus allahabadi*: *Aflv*, *A. flavipes*; *Pcn*, *Penicillium canescens*; *Pfc*, *P. fructuariae-cellae*; *Tbr*, *Trichoderma brevicompactum*.

extent against adverse environmental stresses, including high temperatures and drought, through the secondary metabolites they produce, and may these fungi can enhance plant growth (Ngo *et al.,* 2021; Niaz *et al.,* 2024). As endophytes, *Penicillium* species can produce a variety of metabolites that protect plants from stresses, encourage plant growth, and inhibit the development of plant diseases. Poveda *et al.* (2025) reported that *Trichoderma* species have antagonistic activity against plant diseases, and have beneficial effects comparable to endophytes. These results are supported by the fact that the *Aspergillus, Penicillium*, and *Trichoderma* isolates employed by Poveda *et al.* (2025) were from old vineyards (>40-years-old) growing in the hot, arid regions of southern Türkiye. Their prevalence under these challenging conditions, and their stability, demonstrate their potential as biological control agents, and indicate that they have adapted to the local environment. According to Baron and Rigobelo (2022), soil-borne fungi in plant rhizospheres disrupt root hair apical dominance, infect root cortices with hyphae, and then spread to endoderm cells, pericycles, and xylem and phloem tissues. This lends credence to the theory that the grapevines may have been infected over time by soil-inhabiting *Aspergillus, Penicillium*, and *Trichoderma*.

Although the re-isolation frequencies of *Penicillium* and *Trichoderma* species were moderate (4.2–34.2%), their recovery from woody tissues located 10 cm above the inoculation points demonstrated that these endophytes were could establish within grapevine tissues following inoculations. The present study was designed to assess the ability of selected local endophytes to colonize grapevine cuttings, rather than to optimize colonization under nursery production conditions. Colonization efficiency is likely to be influenced by factors such as inoculum formulation, application method, frequency of exposure, and environmental conditions during propagation. Previous studies have shown that *Trichoderma*-based biological control agents can establish in grapevine tissues, and provide long-term protection when applied during nursery propagation processes (Berbegal *et al*., 2020). Therefore, further research focusing on formulation development and nursery-scale applications may provide knowledge to help improve establishment of these local endophytic isolates. The present study results further highlight the potential of *Penicillium* and *Trichoderma* as biological control agents, demonstrating that they can successfully colonize grapevine cuttings when adequately formulated.

Aspergillus allahabadi has previously been detected in the rhizospheres of tomatoes and cabbage in northern Benin, as well as in internal tissues of these plants (Affokpon *et al.,* 2010). This fungus has also been found in root endodermis tissues of cinnamon plants in Thailand (Sadorn *et al.,* 2016), and in agricultural soils in India and South Korea (Mehrotra and Agnihotri, 1962; Tagele *et al.,* 2018). On the other hand, neither its rhizospheric nor endophytic forms have been documented in grapevines. Thus, the present study observation in Türkiye constitutes the first known detection of *A. allahabadi* in

grapevines. This species was isolated from a vine that was more than 40 years old, indicating, as with other plants, that it infects roots of grapevine plants before progressively colonizing internal tissues. *Aspergillus allahabadi* and *A. flavipes*, on the other hand, have slower infection and colonization processes than *Penicillium* and *Trichoderma* species, which can be occur more rapidly in these tissues. This was shown by their inabilities to be isolated from woody tissues 10 cm from the stem bases, even at 4 months after two separate inoculations. *Trichoderma brevicompactum* had a greater re-isolation rate than *Penicillium*, suggesting a greater ability to persist or grow in grapevine cuttings in the internal tissues within 4 months.

This study has also shown that these endophytes prevented internal lesions caused by *Botryosphaeriaceae* pathogens in grapevine stems, at rates of up to 84.6% when applied as sprays over open trunk wounds or systemically infiltrated into dormant grapevine cuttings. These results indicate that the endophytic isolates used in this study may have exerted their effects through competition for nutrients and for habitat, as well as through the secondary metabolites they produce. Sadorn *et al.* (2016) reported that *A. allahabadi* produced twelve different antimicrobial compounds, including allahabadolactones A and B. Ten of these compounds were found to inhibit growth of *Bacillus cereus*. Additionally, Nashat *et al. (*2024) investigated ability of *A. flavipes* isolated from grapevine trunks to prevent the *in vitro* mycelium growth of several grapevine trunk disease pathogens. Culture filtrates of *A. allahabadi* inhibited the mycelium growth of *Ilyonectria liriodendri* by 100%, of *Botrytis cinerea* by 49%, of *Macrophomina phaseolina* by 28%, and of *Neoscytalidium dimidiatum* by 3%.

Penicillium fructuariae-cellae was first discovered as a unique species on withered grape berries in the two Italian wine-producing regions of Soave and Valpolicella, and was classified as a weak pathogen (Lorenzini *et al.,* 2019). Its presence on grapevine trunks in Türkiye was later reported by Akgül and Kara (2022), who discovered that it had antagonistic properties that prevent growth of other fungi associated with GTDs. Building on these results, the present study has shown that *P. fructuariae-cellae* has potential to be an antagonistic fungus for use in grapevine wound protection, producing systemic infections and protecting grapevine wounds by 42 to 78.8%. Beyond grapevines, the study of Ren *et al.,* (2024) in China showed that applying culture filtrates of endophytic *P. fructuariae-cellae* isolates as sprays to seedling shoots decreased (by approx. 50-60%) internal lesions caused by *Diplodia sapinea*, a pathogen linked to shoot dieback of Mongolian pine. Through processes including nutrient and space competition, the treatment also had positive impact on Mongolian pine microbiota. Similarly, *P. canescens* isolated from grapevine roots reduced *in vitro* mycelium growth of pathogenic *Cytospora chrysosperma*, associated with GTDs, by 39% (using a dual culture assay) (Pazooki *et al*. (2026) in Iran. *Penicillium canescens* has been proposed as a possible biocontrol agent based on this effect, which is due to the synthesis of secondary metabolites (Toghueo and Boyom, 2020).

Biocontrol studies on grapevine plants and mature vines under field conditions using *Trichoderma* species have shown that fungi are consistently preferred over others, due to their significant activities. For example, in southern Italy (Urbez-Torres *et al.,* 2020), protective and tissue-colonizing properties of eight *Trichoderma* species isolated from grapevines against infections by *Diplodia seriata, N. parvum*, and *Eutypa lata* were examined. Of these, *T. atroviride, T. koningiopsis, and T. paratroviride* provided the strongest protection and host colonization abilities, while the other species showed comparatively lower performance. Contrary to present study results, *T. brevicompactum* was not detected in the southern Italian vineyards where isolations were carried out. These results indicate regional variability in presence and biocontrol effectiveness of different fungal species. In similar in Chile (Silva-Valderrama *et al.,* 2021), endophytic *Clonostachys rosea* and *Trichoderma* sp. obtained from grapevine rhizospheres and internal tissues inhibited *D. seriata, N. parvum*, and *Phaeomoniella chlamydospora* in grapevine cuttings. *Clonostachys rosea* gave stronger inhibitory effects than *Trichoderma* sp., likely due to a dual mechanism involving both hyperparasitism and antibiosis. These results show that performance of endophytic isolates can vary with regional and experimental conditions. Pollard-Flamand *et al. (*2023) conducted studies in Canada in greenhouse and field conditions, comparing how local isolates of *T. asperelloides*, *T. atroviride*, and *T. canadense* protected grapevine wounds after pruning, alongside a commercial product (*T. atroviride* 77-b) and a fungicide (tebuconazole + boric acid). The local *T. atroviride* and *T. asperelloides* together reduced lesions of *D. seriata* and *N. parvum* by 93 to 100%, and this combination gave better pathogen control than commercial treatments in glasshouse and field conditions.

A limitation of the present study was that pycniospore inoculations could not be assessed for *N. parvum* under field conditions. Despite repeated attempts using the methods of Amponsah *et al*. (2008), pycniospore production by the isolate used in the present study remained insufficient for inoculation experiments. Consequently, mycelium agar plugs were used as inoculum for *N. parvum*. Since *Botryosphaeriaceae* species naturally infect pruning wounds primarily through airborne

conidia, results obtained with mycelium inoculations should be interpreted with caution, although use of mycelium plugs is a common experimental approach for evaluating susceptibility of grapevine tissues and efficacy of pruning wound protectants under controlled conditions (e.g. Blundell and Eskalen, 2022). Future research should evaluate endophytic fungi for effects on naturally produced pathogen conidia under field conditions.

CONCLUSIONS

This study has demonstrated the biocontrol potential of vineyard-origin endophytic *Penicillium* and *Trichoderma* species from the Eastern Mediterranean Region (Türkiye) against *Botryosphaeriaceae* pathogens, in laboratory and field conditions. This is also the first report of *P. fructuariae-cellae* and *T. brevicompactum* applications against GTDs. While *Aspergillus* species showed lower performance than other candidate fungi, they still protected grapevine pruning wounds. Effective colonization and systemic establishment of *Penicillium* and *T. brevicompactum*, together with substantial wound protection, indicates that these local isolates merit further evaluation for nursery and field uses. Nevertheless, practical applications in vineyards require formulation development and further field performance assessments across different locations.

ACKNOWLEDGEMENTS

The authors thank the Rectorate of Çukurova University, the Turkish Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies, and the Directorate of the Biological Control Research Institute. Additionally, T. Tarık Erper, Suat Erdem, Utkucan Özbilen, and Mert Sönmez are acknowledge for their assistance in the laboratory and field. This study was financially supported by the Turkish Ministry of Agriculture and Forestry, in project TAGEM/BSAD/A/22/A2/P1/5333, and The Çukurova University Scientific Research Projects Department, in project FDK-2021-14133.

LITERATURE CITED

Affokpon D., Coyne L., Tossou C., Coosemans J., 2010. First report of *Aspergillus allahabadii* Mehrotra and Agnihotri in vegetable fields in Northern Benin (West-Africa). *Bulletin de la Recherche Agronomique du Bénin* 67: 20–25.

Akgül D.S., Kara H.İ., 2022. Gaziantep bağ alanlarındaki endofitik funguslar ve bunların fungal gövde patojenleriyle antagonistik ilişkileri. *Türkiye Biyolojik Mücadele Dergisi* 13: 48–66 (in Turkish). https://doi.org/10.31019/tbmd.1095497

Akgül D.S., Savaş N.G., Yildiz M., Bülbül I., Özarslandan M., 2023. Current status of grapevine trunk disease pathogens on asymptomatic nursery-produced grapevines in Türkiye. *Phytopathologia Mediterranea* 62:151–163. https://doi.org/10.36253/phyto-14148

Alves A., Crous P.W., Correia A., Phillips A.J.L., 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Diversity* 28:1–13.

Amponsah N., Jones E., Ridgway H., Jaspers M., 2008. Production of Botryosphaeria species conidia using grapevine green shoots. *New Zealand Plant Protection*, 61: 301–305. https://doi.org/10.30843/nzpp.2008.61.6840

Anonymous, 2025. General Directorate of Plant Production at Turkish Ministry of Agriculture and Forestry. https://www.tarimorman.gov.tr/BUGEM. Accessed 14.04.2026

Baron N.C., Rigobelo E.C., 2022. Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. *Mycology* 13:39–55. https://doi.org/10.1080/21501203.2021.1945699

Benlioğlu K., Özakman M., 1998. Bağ üretim materyalinde kök uru etmeni *Agrobacterium tumefaciens*'in saptanması (in Turkish). *Turkish Journal of Agriculture and Forestry* 22: 167–174.

Berbegal M., Ramón-Albalat A., León M., Armengol J. 2020. Evaluation of long-term protection from nursery to vineyard provided by *Trichoderma atroviride* SC1 against fungal grapevine trunk pathogens. *Pest Management Science* 76: 967–977. https://doi.org/10.1002/ps.5605

Billar de Almeida A., Concas J., Campos M.D., Materatski P., Varanda C., Patanita M., Felix M.D.R., 2020. Endophytic fungi as potential biological control agents against grapevine trunk diseases in Alentejo region. *Biology* 9: 420. https://doi.org/10.3390/biology9120420

Blundell R., Eskalen A., 2022. Evaluation of biological and chemical pruning wound protectants to control grapevine trunk disease pathogens. *Eutypa lata* and *Neofusicoccum parvum*. *Plant Health Progress* 23: 197–205. https://doi.org/10.1094/PHP-08-21-0113-RS

Busby P.E., Ridout M., Newcombe G., 2016. Fungal endophytes: modifiers of plant disease. *Plant Molecular Biology* 90: 645–655. https://doi.org/10.1007/s11103-015-0412-0

Bustamante M.I., Elfar K., Kuzmenko J., Zaninovich T., Arreguin M., Carachure C., Eskalen A., 2024. Reassessing the etiology of Aspergillus vine canker and summer bunch rot of table grapes in California. *Plant Disease* 108:941–950. https://doi.org/10.1094/PDIS-06-23-1137-RE

Deyett E., Rolshausen P.E., 2020. Endophytic microbial assemblage in grapevine. *FEMS Microbiology Ecology* 96: fiaal053. https://doi.org/10.1093/femsec/fiaa053

El-Dawy E.G., Hussein M.A., El-Nahas S., 2024. Description and management of *Aspergillus* section Nigri causing post-harvest bulbs rot of onion. *Scientific Reports* 14: 6076. https://doi.org/10.1038/s41598-024-53849-9

Glass N.L., Donaldson G.C., 1995. Development of primer sets designed for use with PCR to amplify conserved genes from filamentous ascomycetes. *Applied Environmental Microbiology* 61: 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Gomez K.A., Gomez A.A., 1984. *Statistical Procedures for Agricultural Research* (2nd ed.). Wiley, London, 680 pp.

Görür V., Akgül D.S., 2019. Fungicide suspensions combined with hot-water treatments affect endogenous *Neofusicoccum parvum* infections and endophytic fungi in dormant grapevine canes. *Phytopathologia Mediterranea* 58: 559–571. https://doi.org/10.14601/Phyto-10822.

Gramaje D., Aroca A., Raposo R., García-Jiménez J., Armengol J., 2009. Evaluation of fungicides to control Petri disease pathogens in the grapevine propagation process. *Crop Protection* 28: 1091–1097. https://doi.org/10.1016/j.cropro.2009.05.010

Gramaje D., Armengol J., 2011. Fungal trunk pathogens in the grapevine propagation process: potential inoculum sources, detection, identification, and management strategies. *Plant Disease* 95: 1040–1055. https://doi.org/10.1094/PDIS-01-11-0025

Grijseels S., Nielsen J.C., Nielsen J., Larsen T.O., Frisvad J.C., Nielsen K.F., Workman M., 2017. Physiological characterization of secondary metabolite producing *Penicillium* cell factories. *Fungal Biology and Biotechnology* 4: 8. https://doi.org/10.1186/s40694-017-0036-z

Hagag A., Abdelwahab M.F., Abd El-Kader A.M., Fouad M.A., 2022. The endophytic *Aspergillus* strains: a bountiful source of natural products. *Journal of Applied Microbiology* 132: 4150–4169. https://doi.org/10.1111/jam.15489

Langa-Lomba N., González-García V., Venturini-Crespo M.E., Casanova-Gascón J., Barriuso-Vargas J.J., Martín-Ramos P., 2023. Comparison of the efficacy of *Trichoderma* and *Bacillus* strains and commercial biocontrol products against grapevine Botryosphaeria dieback pathogens. *Agronomy* 13: 533. https://doi.org/10.3390/agronomy13020533

Lorenzini M., Cappello M.S., Perrone G., Logrieco A., Zapparoli G., 2019. New records of *Penicillium* and *Aspergillus* from withered grapes in Italy, and description of *Penicillium fructuariae-cellae* sp nov. *Phytopathologia Mediterranea* 58: 323–340. https://doi.org/10.14601/Phytopathol_Mediter-10619

Mehrotra B.S., Agnihotri V.P., 1962. Two new species of *Aspergillus* from India. *Mycologia* 54: 400–406.

Nashat L.H., Haleem R.A., Ali S.H., 2024. Molecular identification and antimicrobial potential of endophytic fungi against some grapevine pathogens. *PLoS One* 19: e0309041. https://doi.org/10.1371/journal.pone.0309041

Ngo M.T., Van Nguyen M., Han J.W., Kim B., Kim Y.K., Park M.S., Choi G.J., 2021. Biocontrol potential of *Aspergillus* species producing antimicrobial metabolites. *Frontiers in Microbiology* 12: 804333. https://doi.org/10.3389/fmicb.2021.804333

Niaz K., Rauf M., Arif M., Hamayun M., Gul H., Hashem A., Abd_Allah E.F., Wu Q.S., 2024. Drought-tolerant fungal microbes *Aspergillus oryzae* and *Aspergillus fumigatus* elevate physiohormonal and antioxidant responses of maize under drought stress. *Frontiers in Microbiology* 15: 1488639. https://doi.org/10.3389/fmicb.2024.1488639

O'Donnell K., Cigelnik E., Nirenberg H.I., 1998. Molecular systematics and phylogeography of the *Gibberella fujikuroi* species complex. *Mycologia* 90 (3): 465–493. https://doi.org/10.1080/00275514.1998.12026933

Pazooki S., Shekariesfahlan A., Maleki M., Naeimi S., 2024. Isolation and identification of endophytic and grapevine trunk diseases associated fungi with antagonistic potential against *Cytospora chrysosperma*. *Journal of Plant Pathology* 108: 99–110. https://doi.org/10.1007/s42161-024-01731-y

Pieterse C.M.J., Zamioudis C., Berendsen R.L., Weller D.M., Van Wees S.C.M., Bakker P. 2014. Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology* 52: 347–375. https://doi.org/10.1146/annurev-phyto-082712-102340

Pollard-Flamand J., Boulé J., Hart M., Úrbez-Torres J.R., 2022. Biocontrol activity of *Trichoderma* species isolated from grapevines in British Columbia against Botryosphaeria dieback fungal pathogens. *Journal of Fungi* 8: 409. https://doi.org/10.3390/jof8040409

Pollard-Flamand J., Boulé J., Hart M., Úrbez-Torres J.R., 2023. Biological control of Botryosphaeria dieback of grapevines in British Columbia, Canada. *Ameri-*

can Journal of Enology and Viticulture 74. https://doi.org/10.5344/ajev.2023.23052

Pouzoulet J., Mailhac N., Couderc C., Besson X., Dayde J., Jacques A., 2013. A method to detect and quantify *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* DNA in grapevine wood samples. *Applied Microbiology and Biotechnology* 97: 10163–10175. https://doi.org/10.1007/s00253-013-5299-6

Poveda J., Santamaria O., Martin-Garcia J., 2025. Fungal metabolites in plant protection. In: *Fungal Metabolites for Agricultural Applications: Biostimulation fnd Crop Protection by Fungal Biotechnology*. (Poveda J, Santamaría Ó, Martín-García J,. ed.) Springer, Cham. https://doi.org/10.1007/978-3-031-76587-2

Ren N., Wang L., You C., 2024. Diversity, community structure, and antagonism of endophytic fungi from Mongolian pine trees. *Journal of Fungi* 10: 212. https://doi.org/10.3390/jof10030212

Sadorn K., Saepua S., Boonyuen N., Laksanacharoen P., Rachtawee P., Prabpai S., 2016. Allahabadolactones A and B from the endophytic fungus *Aspergillus allahabadii*. *Tetrahedron* 72: 489–495. https://doi.org/10.1016/j.tet.2015.11.056

Sibakwe C.B., Kasambara-Donga T., Njoroge S.M.C., Msuku W.A.B., Mhango W.G., Brandenburg R.L., Jordan D.L., 2017. The role of drought stress on aflatoxin contamination in groundnuts (*Arachis hypogea* L.) and *Aspergillus flavus* population in the soil. *Modern Agricultural Science and Technology* 3(5-6): 22–29. https://doi.org/10.15341/mast(2375-9402)/03.03.2017/005

Silva-Valderrama I., Toapanta D., Miccono M.L.A., Lolas M., Díaz G.A., Cantu D., Castro A., 2021. Biocontrol potential of grapevine endophytic and rhizospheric fungi against trunk pathogens. *Frontiers in Microbiology* 11: 614620. https://doi.org/10.3389/fmicb.2020.614620

Tagele S.B., Adhikari M., Gurung S.K., Lee H.G., Kim S.W., Kim H.S., Lee Y.S., 2018. New records of *Aspergillus allahabadii* and *Penicillium sizovae* from crop field soil in Korea. *Mycobiology* 46: 297–304. https://doi.org/10.1080/12298093.2018.1538069

Thambugala K.M., Daranagama D.A., Phillips A.J.L., Kannangara S.D., Promputtha I., 2020. Fungi vs fungi in biocontrol: an overview of fungal antagonists applied against fungal plant pathogens. *Frontiers in Cellular and Infection Microbiology* 10: 604923. https://doi.org/10.3389/fcimb.2020.604923

Toghueo R.M.K., Boyom F.F., 2020. Endophytic *Penicillium* species and their agricultural, biotechnological, and pharmaceutical applications. 3 *Biotech* 10: 107. https://doi.org/10.1007/s13205-020-2081-1

Urbez-Torres J.R., Tomaselli E., Pollard-Flamand J., Boule J., Gerin D., Pollastro S., 2020. Characterization of *Trichoderma* isolates from southern Italy and their potential biocontrol activity against grapevine trunk disease fungi. *Phytopathologia. Mediterranea* 59: 425–440. https://doi.org/10.14601/Phyto-11273

White T.J., Bruns T., Lee S., Taylor J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: (Innis M.A. *et al*., ed.), *PCR protocols*. Academic Press, pp 315–322.

Zanfaño L., Carro-Huerga G., Rodríguez-González Á., Ramírez-Lozano D., Mayo-Prieto S., Gutiérrez S., Casquero P.A., 2025. Biosolutions from native *Trichoderma* strains against grapevine trunk diseases. *Agronomy* 15:1901. https://doi.org/10.3390/agronomy15081901