

RESEARCH ARTICLE

Characteristics of *Trichoderma* strains from the IAFB Collection of Industrial Microorganisms in terms of their possible practical applications

Barbara Sokołowska,^{ID} Karolina Mróz

Prof. Wacław Dąbrowski Institute
of Agricultural and Food Biotechnology –
State Research Institute,
Department of Microbiology,
36 Rakowiecka Str.,
02-532 Warsaw, Poland

Correspondence

barbara.sokolowska@ibprs.pl

Prof. Wacław Dąbrowski Institute
of Agricultural and Food Biotechnology –
State Research Institute,
Department of Microbiology,
36 Rakowiecka Str.,
02-532 Warsaw, Poland

Abstract

Trichoderma spp. are widely recognized as multifunctional biological control agents whose efficacy is based on a combination of enzymatic activity, direct antagonism, and secondary metabolite production. This study evaluated the biocontrol potential of *Trichoderma* strains from the IAFB Collection by analyzing extracellular enzymatic activity, direct antagonism against phytopathogens, and volatile organic compound (VOC) - mediated inhibition. Plate assays revealed a strong and strain-dependent production of cellulases, chitinases, and lipases. All 14 strains exhibited cellulolytic activity, while chitinolytic and lipolytic activities were detected in 10 and 12 strains, respectively. Dual-culture assays against 13 plant pathogenic fungi demonstrated broad-spectrum antagonism, with the inhibition of radial growth ranging from 5.9% to 80%. *T. longibrachiatum* KKP 532, *T. atroviride* KKP 3765, and *Trichoderma* sp. KKP 3537 showed particularly strong and consistent inhibition across multiple pathogens. Volatile-mediated inhibition was generally weaker but detectable, reaching up to 38% inhibition and thus confirming the contribution of VOCs to indirect antagonism. Overall, the results demonstrate a multifactorial mode of action combining enzymatic degradation, rapid competitive growth, mycoparasitism, and volatile metabolite production. The studied strains represent promising candidates for further development as environmentally friendly biocontrol agents for sustainable plant protection, as well as for use in the industry and biotechnology.

KEYWORDS

Trichoderma; enzymatic activity; anti-fungal activity; volatile compounds

Introduction

Plant diseases caused by fungal pathogens remain as one of the most significant constraints on global crop production, leading to substantial economic losses, reduced yields, and compromised food quality (Dean et al. 2012). Species such as *Fusarium*, *Rhizoctonia*, *Botrytis*, *Sclerotinia*, and *Alternaria* infect a wide range of economically important crops, often resulting in severe pre- and post-harvest losses (Strange and Scott 2005; Redda et al. 2021). Although chemical fungicides have long been the primary means of managing these pathogens, their extensive use raises concerns

related to environmental contamination, human health risks, the development of fungicide-resistant strains, and the disruption of beneficial microbial communities (Brent and Hollomon 2007; Lucas et al. 2015). These challenges highlight an urgent need for sustainable and eco-friendly disease management strategies.

Among microbial antagonists, fungi of the genus *Trichoderma* have emerged as some of the most effective and widely studied biological control agents against plant-pathogenic fungi. *Trich-*

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oderma species are ubiquitous soil inhabitants known for their rapid growth, metabolic versatility, and competitive ability in the rhizosphere (Harman et al. 2004a; Modrzeska et al. 2022; Tyśkiewicz et al. 2022). Their antagonistic activity is multifaceted, involving mycoparasitism, antibiosis, competition for nutrients and space, production of lytic enzymes, induction of systemic resistance, and plant growth promotion (Viterbo et al. 2002; Howell 2003; Druzhinina et al. 2011; Grzegorzczuk et al. 2015; Tyśkiewicz et al. 2022). These mechanisms enable *Trichoderma* spp. to suppress a broad spectrum of economically important fungal pathogens, making them valuable in integrated disease management programs.

Furthermore, *Trichoderma* strains are generally regarded as environmentally safe (status GRAS-Generally Recognized as Safe) and are used globally in commercial biocontrol formulations (Woo et al. 2014). However, their biocontrol efficacy can vary depending on the species and strain characteristics, pathogen identity, environmental conditions, and formulation technology (Mukherjee et al. 2013). Therefore, the selection and characterization of highly effective *Trichoderma* isolates remain as essential steps in developing reliable, robust biocontrol agents.

Although strains of the *Trichoderma* genus are primarily used as biological control agents, they exhibit many features that enable alternative applications. These include the synthesis of pigments - used in the food, cosmetics, and textile industries - and the synthesis of fragrance compounds. Furthermore, enzymes produced by *Trichoderma* spp. are used in brewing processes (β -glucanases), fruit maceration in fruit juice production (pectinases, cellulases, hemicellulases), as a feed additive in livestock farming (xylanases), and in the biofuel industry as a lignocellulose degradation agent (cellulases) (Troiano et al. 2020). Cellulases produced by *Trichoderma* are utilized in the textile industry to soften and condition fabrics, as well as to manufacture high-quality laundry detergents. Enzymes obtained from this genus are also used in the pulp and paper industry to modify fiber properties and reduce lignin content (Błaszczuk et al. 2014).

Potential industrial applications of lipases from *Trichoderma* include food additives (aroma modification), fine chemicals (ester synthesis), detergents (fat hydrolysis), wastewater treatment (decomposition and removal of oleaginous substances), leather processing (degreasing of animal hides), and the pharmaceutical and medical fields (manufacturing medicines and digestive enzymes for diagnostics) (Ülker et al. 2011).

Trichoderma spp. exhibit resistance to many agrochemicals and toxicants, such as heavy metals, organometallic compounds, cyanide, and tannery effluents (Harman et al. 2004b). Thus, *Trichoderma* spp. play an increasingly important role in the bioremediation of toxic pollutants.

This study aims to evaluate antagonistic interactions between selected *Trichoderma* strains from IAFB (Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology - State Research Institute) Collection of Microorganisms (KKP) and key phytopathogenic fungi using in vitro assays. By assessing growth inhibition, competitive interactions, and morphological traits, we seek to identify promising isolates and contribute to the devel-

opment of effective, eco-friendly strategies for managing fungal plant diseases.

Our research hypothesis posits that the high biocontrol efficacy of selected strains from the IAFB collection results from a synergistic combination of enzymatic activity, the synthesis of volatile metabolites, and aggressive competitive growth.

Materials and Methods

Cultures of *Trichoderma*

Fourteen strains of *Trichoderma* from the IAFB Collection of Industrial Microorganisms (KKP) were investigated in the present study (Table 1). All of the strains were cultured on potato dextrose agar medium, PDA (Merck, Darmstadt) at 25°C. After 7 days, 5 mm mycelial discs (taken from the edge of fungal colonies using sterile tips) were used for tests.

Table 1. *Trichoderma* strains used in the study

Strain Name	Isolation Source	GenBank accession number (ITS region)
<i>Trichoderma longibrachiatum</i> KKP 532	unknown	ON725019
<i>Trichoderma harzianum</i> KKP 534	unknown	ON763219
<i>Trichoderma harzianum</i> KKP 535	unknown	ON763218
<i>Trichoderma reesei</i> KKP 569	unknown	ON763222
<i>Trichoderma reesei</i> KKP 570	unknown	ON724227
<i>Trichoderma</i> sp. KKP 786	unknown	ON763221
<i>Trichoderma reesei</i> KKP 790	unknown	ON725015
<i>Trichoderma</i> sp. KKP 793	unknown	ON763220
<i>Trichoderma reesei</i> KKP 794	unknown	ON763224
<i>Trichoderma</i> sp. KKP 3053	sugar beet juice, Poland	ON763227
<i>Trichoderma</i> sp. KKP 3537	isotonic drink with multifruit flavor and vitamins, Poland	ON763225
<i>Trichoderma harzianum</i> KKP 3764	soil	ON774471
<i>Trichoderma atroviride</i> KKP 3765	soil	ON763226
<i>Trichoderma</i> sp. KKP 3832	soil	ON763223

Cultures of fungal plant pathogens

Ten strains of plant pathogens from the IAFB Collection of Industrial Microorganisms and three from DSMZ (German Collection of Microorganisms and Cell Cultures – GmbH) were used in this research (Table 2). All of the strains were cultured on potato dextrose agar medium, PDA (Merck, Darmstadt) at 25°C. After 7 days, 5 mm mycelial discs (taken from the edge of fungal colonies using sterile tips) were used for tests.

Assessment of extracellular enzyme production

The hydrolytic activities of chitinase, cellulase, and lipase were detected on specific solid media. A 5 mm mycelial disc of a seven-day pre-culture of *Trichoderma* was located in the middle of the plate. *Trichoderma* strains were grown on the media containing the enzyme substrates, and the zone of degraded substrate (halos) formed around the colony was measured after 3 days of incubation, using a ruler. The colony diameter was included. For all enzyme activity tests, the bioassays were done in 3 replicates.

Table 2. Fungal plant pathogen strains used in the study

Strain Name	Isolation Source	GenBank accession number (ITS region)
<i>Alternaria alternata</i> KKP 3368	beverage (collagen water), Poland	PX651339
<i>Botrytis cinerea</i> KKP 3292	beverage production environment, Poland	OK617302
<i>Fusarium oxysporum</i> KKP 458	unknown	PX482405
<i>Fusarium graminearum</i> DSM 1096	<i>Zea mays</i> (Maize/Corn), USA	-
<i>Fusarium poae</i> DSM 62376	<i>Avena sativa</i> (Oat), kernel, Germany	-
<i>Fusarium oxysporum</i> KKP 3492	milk processing environment, Poland	ON461480
<i>Fusarium culmorum</i> DSM 1094	<i>Triticum aestivum</i> (common wheat), USA	-
<i>Fusarium oxysporum</i> KKP 761	unknown	PX482406
<i>Fusarium oxysporum</i> KKP 851	barley malt, Poland	PX975897
<i>Fusarium oxysporum</i> KKP 760	unknown	PX488345
<i>Fusarium avenaceum</i> KKP 756	stored grain, Poland	PX488968
<i>Fusarium oxysporum</i> KKP 762	unknown	PX600307

Cellulase activity was determined by using plate medium containing: 10 g/L carboxymethylcellulose – CMC (Acros Organics, Poland), 3.0 g/L NaNO₃ (POCH, Poland), 1.0 g/L K₂HPO₄ (Chempur, Poland), 0.5 g/L MgSO₄ (Chempur, Poland), 0.5 g/L KCl (Chempur, Poland), 10 mg/L FeSO₄ × 7H₂O (Chempur, Poland), 20 g/L agar (Oxoid, England), pH 5.0 adjusted by 1N H₂SO₄ (Merck, Germany), and autoclaved at 121°C for 15 min. (Florencio et al. 2012). The inoculated plates were incubated at 28°C and thereafter stained with Congo red dye solution – 2.5 g/L (Pol-Aura, Poland) for 15 min; the solution was discarded and the cultures washed with 1M NaCl (POCH, Poland) for 15 min.

Chitinase activity at 25°C was performed according to the method described by Agrawal and Kotasthane (2012). The detection medium was prepared using: 4.5 g/L of colloidal chitin (prepared from chitin (Pol-Aura, Poland) according to Joe and Sarojini (2017). 3.0 g/L (NH₄)₂SO₄ (POCH, Poland), 0.3 g/L MgSO₄ (Chempur, Poland), 2.0 g/L K₂HPO₄ (Chempur, Poland), 1 g/L citric acid monohydrate (POCH, Poland), 15 g/L agar (Oxoid, England), 0.15 g/L bromocresol purple (Merck, Germany), 200 µL of tween 80 (Merck, Germany), pH 4.7 adjusted by 1N H₂SO₄ (Merck, Germany), and autoclaved at 121°C for 15 min.

Lipase activity was conducted according to the method by Singh et al. (2006). *Trichoderma* strains were grown at 25°C on chromogenic substrate plates containing 0.1 g/L phenol red (Acros Organics, Poland), 10 g/L lipidic substrate (olive oil Goya, Spain), 1.1 g/L CaCl₂ (Merck, Germany) 20 g/L agar (Oxoid, England), with the pH adjusted to 7.4 using 0.1 N NaOH (Merck, Germany), and autoclaved at 121°C for 15 min.

Screening of *Trichoderma* strains for antagonistic activity

A Dual-culture technique was used to conduct the antagonistic test (El Komy et al. 2015; Redda et al. 2018). The *Trichoderma* and

the pathogen strains to be tested were cultured separately on PDA (Merck, Darmstadt) for 7 days at 25°C. After 7 days, 5 mm mycelial disc (taken from the edge of the fungal colonies) of each species to be tested were transferred to PDA plates using sterile tips. The mycelial discs of *Trichoderma* species and pathogens were placed opposite each other on a PDA surface. PDA plates inoculated with the pathogens were included as controls. The antagonistic tests were conducted in triplicate. All culture plates were incubated at 25°C, and observations were made after 3, 5, and 7 days. The radial growth of the pathogen strains was measured after 7 days using a ruler, including the colony itself in the measurement. The percent inhibition of average radial growth was calculated in relation to growth of the controls as follows:

$$I\% = (C-T)/C \times 100 \text{ (El Komy et al. 2014)}$$

Where, I=Percent inhibition of pathogen growth, C=radial growth of pathogen (mm) in control. T=radial growth of pathogen (mm) in the presence of *Trichoderma* isolates.

Evaluation of the effect of volatile compounds

The effect of volatile compounds produced by *Trichoderma* on pathogens was evaluated on two-sections of Petri plate, that prevented direct contact of the tested strains. A 5 mm mycelial disc of seven days' pre-culture of *Trichoderma* was inoculated on the outer edge of one section of a PDA plate (Merck, Darmstadt). The second section of the Petri dish was inoculated on the opposite side with a 5 mm disc of pathogen. The plates were sealed with parafilm tape. All of the culture plates were incubated at 25°C, and observations were made after 3 days. The PDA two-sections plates inoculated with the pathogens without *Trichoderma* strains were included as controls. The percent inhibition was calculated as described above.

Statistical analysis

The software TIBCO Statistica™, version 13.5 (TIBCO Software Inc, Palo Alto, CA, USA) was used to perform the statistical analysis. A One-way analysis of variance (ANOVA) and Duncan's test were applied to assess the significance of the differences ($p < 0.05$) between the mean values of enzymatic activity for the individual *Trichoderma* strains, performed separately for each type of enzymatic activity (cellulolytic, chitinolytic, and lipolytic). Similarly, one-way ANOVA and Duncan's test were performed to evaluate the differences between the mean values of the inhibition of the growth of thirteen plant pathogens by *Trichoderma* strains. The data are presented in tables as mean ± standard deviation (SD), with superscripts indicating statistically significant groupings.

Results and Discussion

Enzymatic activity of *Trichoderma* strains

The production of extracellular hydrolytic enzymes is one of the key mechanisms underlying the ecological fitness and biocontrol potential of *Trichoderma* spp. These fungi are well known for secreting a wide spectrum of cell wall-degrading enzymes that enable the penetration of plant and fungal tissues, nutrient acquisition, and mycoparasitic interactions. Among the most relevant enzymes are cellulases, chitinases, and lipases, which participate in the degradation of cellulose, chitin, and lipid components of host or pathogen structures (Cherkupally et al. 2017; Hernandez-Melchor et al. 2021).

Enzymatic activities of the tested *Trichoderma* strains were evaluated using plate assays based on the formation of substrate degradation halos surrounding colonies (Figure 1-3, Table 3). The diameter of the halo zone is commonly used as a semi-quantitative indicator of extracellular enzyme secretion. Considerable variability in enzymatic profiles was observed among the *Trichoderma* strains from the IAFB Collection (Table 3), highlighting substantial intra-genus diversity in metabolic potential.

Table 3. Enzymatic activity of *Trichoderma* strains

Strain Name	Halo-enzyme zone [mm]		
	Cellulase	Chitinase	Lipase
<i>Trichoderma longibrachiatum</i> KKP 532	85.0±0.0 ^f	55.0±5.0 ^g	nd
<i>Trichoderma harzianum</i> KKP 534	85.0±0.0 ^f	64.0±1.0 ^h	71.0±1.0 ⁱ
<i>Trichoderma harzianum</i> KKP 535	21.0±1.0 ^b	43.0±1.0 ^{cd}	66.0±1.0 ^e
<i>Trichoderma reesei</i> KKP 569	17.0±1.0 ^a	nd	13.0±1.0 ^a
<i>Trichoderma reesei</i> KKP 570	21.0±1.0 ^b	nd	14.0±1.0 ^{ab}
<i>Trichoderma</i> sp. KKP 786	62.0±1.0 ^e	53.0±3.0 ^{fg}	nd
<i>Trichoderma reesei</i> KKP 790	18.0±1.0 ^a	nd	45.0±3.0 ^e
<i>Trichoderma</i> sp. KKP 793	52.7±7.5 ^d	35.0±5.0 ^{ab}	16.0±1.0 ^b
<i>Trichoderma reesei</i> KKP 794	41.0±1.0 ^c	nd	69.0±1.0 ^{hi}
<i>Trichoderma</i> sp. KKP 3053	85.0±0.0 ^f	72.0±2.0 ⁱ	61.0±1.0 ^f
<i>Trichoderma</i> sp. KKP 3537	57.0±1.0 ^d	45.0±1.0 ^{de}	36.0±1.0 ^d
<i>Trichoderma harzianum</i> KKP 3764	55.0±3.0 ^d	33.0±1.0 ^a	38.0±2.0 ^d
<i>Trichoderma atroviride</i> KKP 3765	55.0±1.0 ^d	39.0±1.0 ^{bc}	29.0±1.0 ^c
<i>Trichoderma</i> sp. KKP 3832	85.0±0.0 ^f	49.0±1.0 ^{ef}	67.0±1.0 ^{gh}

nd - The activity was not detected. a-i - Mean values in columns with different lowercase letters are significantly different at $p < 0.05$ (Duncan test), separately for each type of enzymatic activity. Data are presented as mean $\pm$ standard deviation SD (n=3).

All of the examined strains exhibited cellulolytic activity on CMC medium, confirming that cellulose degradation is a conserved trait within the analyzed *Trichoderma* collection. The diameter of the cellulose degradation zones ranged from 17 to 85 mm (Table 3). The highest cellulase activity (85 mm) was recorded for *T. longibrachiatum* KKP 532, *T. harzianum* KKP 534, *Trichoderma* sp. KKP 3053, and *Trichoderma* sp. KKP 3832, indicating a strong capacity for extracellular cellulase production (Table 3).

The results obtained in this study are slightly higher than those in earlier reports describing cellulase production by *Trichoderma*. Florencio et al. (2012) reported halo diameters ranging from 9.2 to 36.1 mm among 14 *Trichoderma* species using a comparable assay, whereas Bedine Boat et al. (2019) observed larger degradation zones (50.0–63.8 mm), with *T. harzianum* strains showing the highest activity. In a similar study conducted by Hernandez-Melchor et al. (2022), a halo diameter of 27.0–62.0 mm was achieved for 16 *Trichoderma* strains.

Cellulolytic fungi of the genus *Trichoderma* are considered to be the most effective organisms for degrading cellulosic biomass. The high cellulolytic activity of the three *Trichoderma* strains from the IAFB collection further highlights their potential for application in industry and biotechnology.



Figure 1. Cellulolytic activity of *Trichoderma* strains on medium with CMC stained with Congo red. From the top: *Trichoderma longibrachiatum* strain KKP 532, which has the highest activity, followed by *Trichoderma atroviride* KKP 3765 and *Trichoderma reesei* KKP 570 strains.

Chitinase production is a hallmark of *Trichoderma* mycoparasitism, as chitin constitutes a major structural component of fungal cell walls. 10 of the 14 tested strains exhibited detectable chitinolytic activity (Table 3). The largest halo zones were observed for *T. harzianum* KKP 534 (64 mm) and *Trichoderma* sp. KKP 3053 (72 mm), indicating particularly strong chitinase secretion (Table 3).

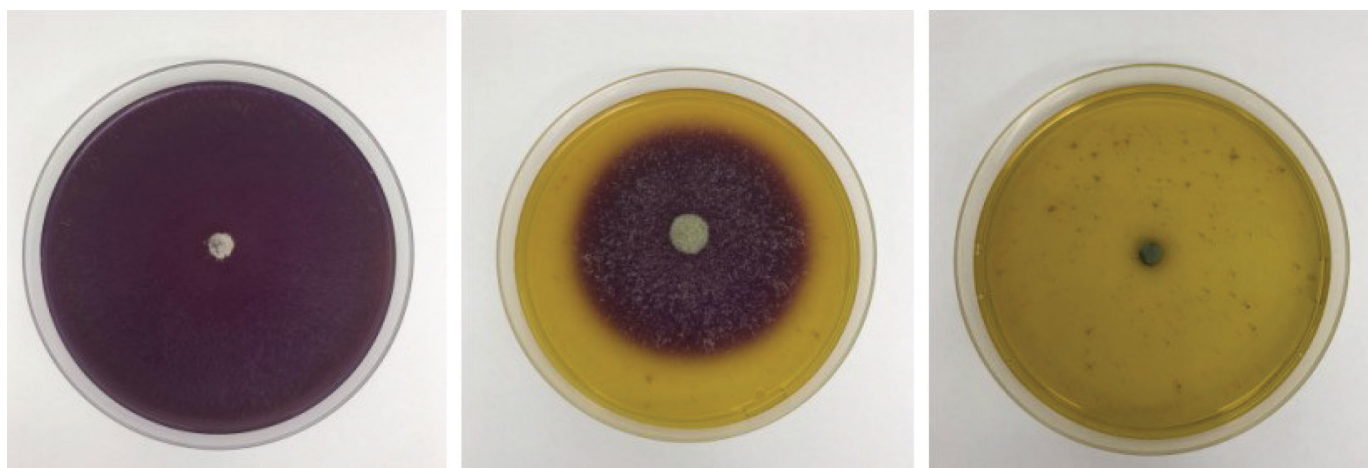


Figure 2. Chitinolytic activity of *Trichoderma* strains on medium with colloidal chitin and bromocresol purple. From the left: *Trichoderma* strain sp. KKP 3053, which has the highest activity, followed by *Trichoderma harzianum* KKP 535 and *Trichoderma reesei* KKP 570 strains.

These findings align with previous studies demonstrating that *Trichoderma* chitinases play a central role in the degradation of pathogenic fungi and in the suppression of plant diseases (Gruber and Seidl-Seiboth 2012). Comparable halo diameters (28.3–79.0 mm) were reported by Bedine Boat et al. (2019), again with *T. harzianum* strains displaying the highest activity.

Lipases contribute to the degradation of lipid components of biological membranes and may facilitate the colonization of host tissues as well as antagonistic interactions with pathogens. In this research, 12 of the 14 tested strains exhibited lipolytic activity, although with considerable variability (Table 3).

The strongest lipase producers were *T. harzianum* KKP 534 (71 mm), *T. reesei* KKP 794 (69 mm), and *Trichoderma* sp. KKP 3832 (67 mm). These results indicate that lipase production is widespread but at the same time a strain-dependent trait within the genus (Table 3). Comparable halo diameters (36.3–60.7 mm) were reported by Bedine Boat et al. (2019), who identified *T. afroharzianum* as the most active lipase producer.

Although numerous publications address the enzymatic activity of *Trichoderma*, they often employ different methods - such as liquid media or quantitative activity measurements - making a direct comparison of the results impossible (Asad et al. 2015; Cherkupally et al. 2017; Marecik et al. 2018).

The majority of the tested strains produced extracellular cellulases, chitinases, and lipases of industrial importance. Since the present study was semi-quantitative, quantitative analyses involving the optimization of culture conditions are necessary to fully assess the enzyme production potential of these strains. Ten *Trichoderma* strains with the highest enzymatic activity were selected for the antagonistic testing.

Direct antagonistic activity of *Trichoderma* strains against plant pathogens

In vitro antagonism assays constitute an essential first step in the screening of microbial biocontrol agents. Although laboratory tests cannot fully predict strain performance under greenhouse or field conditions, they provide valuable information on antagonistic potential, help identify promising candidates, and enable

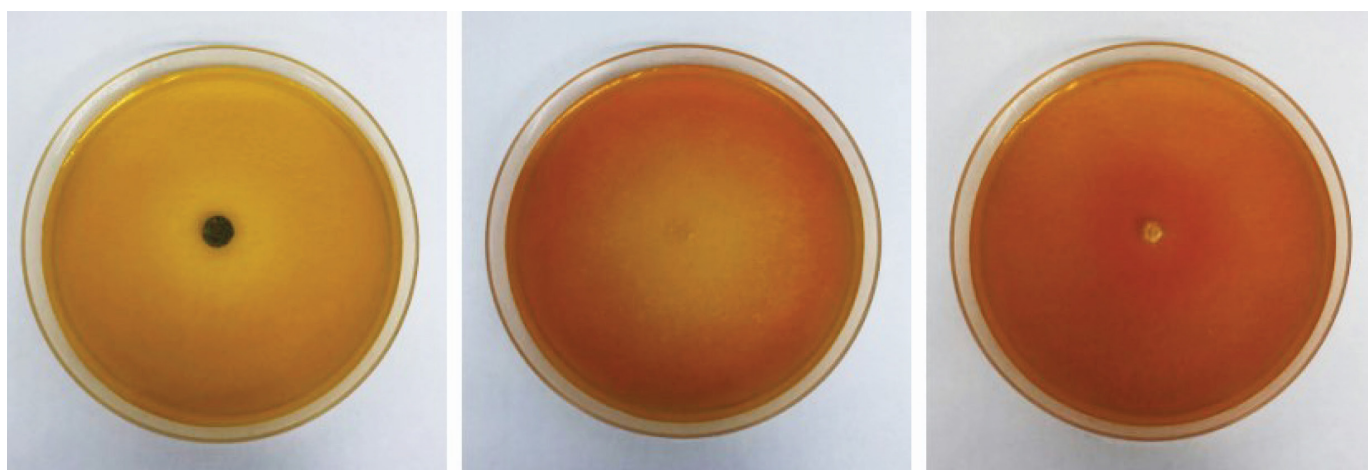


Figure 3. Lipolytic activity of *Trichoderma* strains on medium with olive oil and phenol red. From the left: *Trichoderma harzianum* strain KKP 534, which has the highest activity, followed by *Trichoderma atroviride* KKP 3765 and *Trichoderma longibrachiatum* KKP 532 strains.

the comparative evaluation of strain - pathogen interactions prior to more complex experiments.

In the present work, the antagonistic activity of ten *Trichoderma* strains from the IAFB Collection was evaluated against thirteen phytopathogenic fungi using the dual-culture method (Figure 4, Table 4). This widely applied assay allows the assessment of direct interactions between antagonists and pathogens, including competition for space and nutrients, antibiosis, and mycoparasitism.

The dual-culture assays demonstrated clear inhibitory effects of *Trichoderma* on all of the tested pathogens (Table 4). The percentage inhibition of radial growth ranged from 5.9% (*T. harzianum* KKP 535 vs. *Fusarium culmorum* DSM 1094) to 80% (*T. longibrachiatum* KKP 532 vs. *Botrytis cinerea* KKP 451). Several strains exhibited broad-spectrum antagonistic activity, frequently exceeding 50% inhibition across multiple pathogens. Particularly strong and consistent antagonistic performance was observed for: *T. longibrachiatum* KKP 532, *T. atroviride* KKP 3765 and *Trichoderma* sp. KKP 3537. These strains inhibited eleven of the thirteen tested pathogens, including species of *Fusarium*, *Alternaria*, and *Botrytis*. Such broad-spectrum activity indicates a combination of efficient competitive growth, secretion of antifungal metabolites, and mycoparasitic capabilities.

Trichoderma strains exhibiting the highest degree of direct antagonism towards phytopathogens also displayed enzymatic activity, although it was not the strongest. No direct correlation was found between these activities.

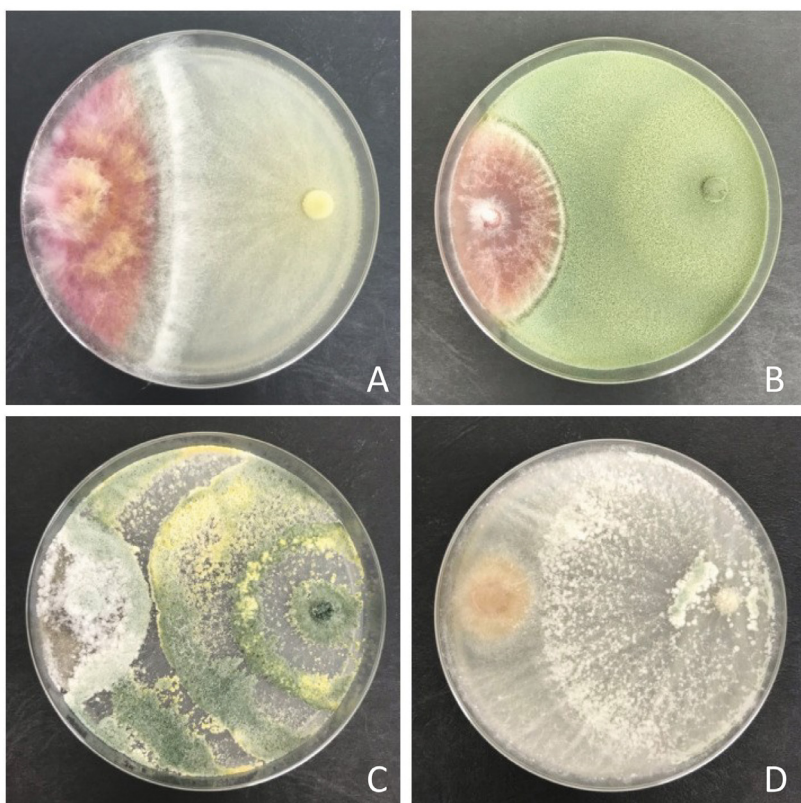
It is also worth noting that, among the panel of pathogens, the *Botrytis cinerea* KKP 3292 and *Fusarium culmorum* DSM 1094 strains were the least inhibited by *Trichoderma*. The estimated mycelial growth inhibition of *Botrytis cinerea* KKP 3292 by *Tricho-*

derma ranged from 20.8% to 52.0%, and that for *Fusarium culmorum* DSM 1094 from 5.9% to 47.5%.

In contrast, *Botrytis cinerea* KKP 451 was the most heavily inhibited, with growth inhibition reaching 46.8% to 80.2% (Table 4).

The inhibitory activity observed for the tested strains is consistent with the extensive literature documenting the antagonistic potential of *Trichoderma*. El Komy et al. (2014) reported inhibition of *Fusarium oxysporum* f. sp. *lycopersici* by *T. asperellum* strains ranging from 25.6% to 81.5%, which corresponds well with the range obtained in this study. Similarly, Meena et al. (2017) demonstrated high inhibition rates of several pathogens by *T. harzianum* and *T. viride*, with maximum inhibition recorded against *Alternaria alternata* (up to 86.2%) and substantial suppression of *Fusarium* species. A study by Błaszczuk et al. (2017) demonstrated that all 24 evaluated *Trichoderma* strains were capable of inhibiting the mycelial growth of at least four out of five *Fusarium* species by the fourth day post-co-inoculation. Specifically, the estimated growth inhibition of *F. avenaceum* and *F. culmorum* by *Trichoderma* ranged from 62% to 80% and from 27% to 80%, respectively. In contrast, lower inhibition rates were observed for *F. graminearum* (1%–64%) and *F. temperatum* (1%–60%). Redda et al. (2018) also reported a strong inhibition of *F. oxysporum* (10.0–70.7%) and *B. cinerea* (44.0–83.0%) by several *Trichoderma* species, including *T. longibrachiatum*. More recently, Tran et al. (2026) showed that *T. harzianum* D-LD inhibited *Fusarium oxysporum* f. sp. *cubense* TR4 by 87.37%, further confirming the strong antifungal potential of this genus.

Taken together, these comparisons indicate that the antagonistic activity observed for the IAFB strains falls within the upper range of values reported for effective *Trichoderma* biocontrol candidates.



During the dual-culture assays, *Trichoderma* colonies consistently exhibited faster radial growth than the tested pathogens. Rapid colonization of the growth medium is an important competitive trait that contributes to pathogen suppression by: depletion of readily available nutrients, occupation of ecological niches and physical space, and early establishment of the antagonist prior to pathogen expansion.

Figure 4. Antagonistic effects of some *Trichoderma* species against fungal plant pathogens by the dual culture technique. A) *Trichoderma harzianum* KKP 535 and *Fusarium graminearum* DSM 1096; B) *Trichoderma reesei* KKP 794 and *Fusarium oxysporum* KKP 760; C) *Trichoderma harzianum* KKP 3764 and *Alternaria alternata* KKP 3368; D) *Trichoderma* sp. KKP 3053 and *Botrytis cinerea* KKP 451 (incubation, 7 days at 25°C).

The accelerated growth of *Trichoderma* observed in this study supports the hypothesis that multiple biocontrol mechanisms act synergistically. Besides competition, direct mycoparasitism likely plays a major role, involving hyphal contact, coiling around pathogen hyphae, and the secretion of lytic enzymes. These mechanisms are frequently accompanied by the production of secondary metabolites with antifungal activity.

Antagonistic activity via volatile organic compounds (VOCs)

Continuous research on *Trichoderma* spp. has revealed that their biocontrol activity is strongly associated with the production of diverse secondary metabolites. These compounds contribute to pathogen suppression, plant growth promotion, and the modulation of microbial communities in the rhizosphere. The metabolome of *Trichoderma* includes a broad spectrum of bioactive molecules such as 6-pentyl- α -pyrone, gliotoxin, viridin, gliovirin, glisoprenin, heptelidic acid, koniginins, anthraquinones, trichodermamides, peptaibols, polyketides, terpenoids, trichothecenes, azaphilones, harzialactones, and numerous amino acid-derived metabolites (Reino et al. 2008; Salwan et al. 2019).

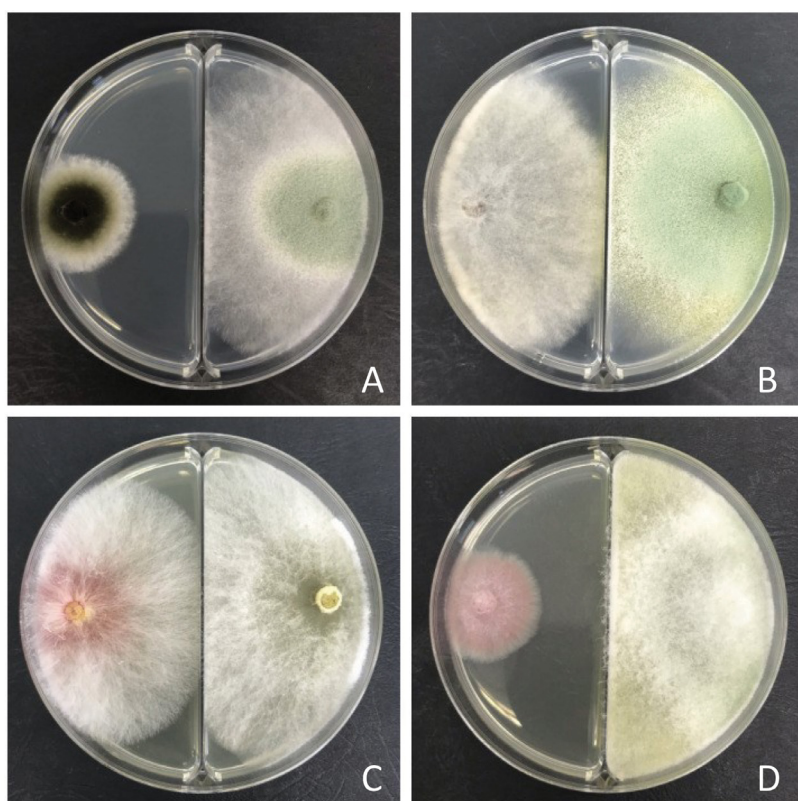
These metabolites are commonly divided into volatile organic compounds (VOCs) and non-volatile metabolites. While non-volatile compounds primarily act locally through direct contact or diffusion in the surrounding medium, VOCs represent an important long-distance communication and inhibition mechanism.

VOCs are low-molecular-weight, carbon-based compounds that readily evaporate and diffuse through air, soil pores, and plant tissues. This property allows them to influence organisms without physical contact, making them particularly important in complex soil ecosystems.

The volatile metabolome of *Trichoderma* is comprised of hundreds of compounds, including alcohols, aldehydes, esters, ketones, aromatic compounds, amines, thiols, and terpenes. Frequently reported VOCs include 6-pentyl- α -pyrone, α -farnesene, limonene, farnesol, benzoic acid, β -bisabolene, β -sesquiphellandrene, calamenene, cadinene, and β -himachalene (Salwan et al. 2019). VOCs play several ecological roles, including: inhibition of pathogen growth at a distance, alteration of pathogen physiology and sporulation, induction of plant systemic resistance, and the modulation of rhizosphere microbial communities (Lee et al. 2016; Nieto-Jacobo et al. 2017; Salwan et al. 2019).

The inhibitory effects of VOCs produced by ten *Trichoderma* strains from the IAFB Collection were evaluated against thirteen phytopathogenic fungi (Figure 5, Table 5). Compared with direct dual-culture interactions, VOC-mediated inhibition was generally weaker and more variable, yet still detectable against multiple pathogens. For several pathogen - strain combinations, no inhibition was observed, highlighting the strong strain specificity of volatile metabolite production. The highest inhibition level reached 37.3% for *Trichoderma* sp. KKP 3832 against *Botrytis cinerea* KKP 451. Notably, *T. longibrachiatum* KKP 532 and *T. harzianum* KKP 3764 exhibited the most consistent activity, with inhibition exceeding 20% for four of the thirteen tested pathogens.

The degree of effectiveness of VOCs varies according to the nature, quality and quantity of inhibitory substances secreted by the antagonistic strains. The generally lower inhibition values compared with dual-culture assays are expected, as VOC-mediated effects occur without direct mycelial contact or enzymatic degradation of pathogen cell walls. Nevertheless, VOCs represent an important complementary mechanism contributing to the overall biocontrol potential of *Trichoderma*.



The VOC-mediated inhibition observed in this work is consistent with previous reports. Raut et al. (2014) demonstrated the inhibition of *Fusarium graminearum* by volatile metabolites of *Trichoderma* strains ranging from 39.5% to 45.0%. In the present study, the inhibition of *F. graminearum* ranged from 0% to 24.8%, which falls within the lower range of published data and may reflect differences in culture conditions, incubation time, and strain diversity. Similarly, Meena et al. (2017) reported that VOCs from *T. harzianum* inhibited *Alternaria alternata* by 40%, *A. brassicae* by 35.38%, and *Fusarium solani* by 17.14%. Comparable inhibition levels were observed for *T. viride*.

Figure 5. Antagonistic effects of some *Trichoderma* species against fungal plant pathogens by volatile compounds (two-section culture) A) *Trichoderma* sp. KKP 786 and *Alternaria alternata* KKP 3368; B) *Trichoderma reesei* KKP 794 and *Botrytis cinerea* KKP 3292; C) *Trichoderma* sp. KKP 3832 and *Fusarium culmorum* DSM 1094; D) *Trichoderma longibrachiatum* KKP 532 and *Fusarium oxysporum* KKP 762 (incubation, 3 days at 25°C).

Trichoderma strains	Inhibition of fungal plant pathogen strains [%]												
	Alternaria alternata KKP 3368	Botrytis cinerea KKP 3292	Fusarium oxysporum KKP 458	Fusarium graminearum DSM 1096	Fusarium poae DSM 62376	Fusarium oxysporum KKP 3492	Fusarium culmorum DSM 1094	Fusarium oxysporum KKP 761	Fusarium oxysporum KKP 851	Fusarium oxysporum KKP 760	Fusarium avenaceum KKP 756	Fusarium oxysporum KKP 762	Botrytis cinerea KKP 451
Trichoderma longibrachiatum KKP 532	64.9 ±1.5 ^c CDE	52.0 ±3.6 ^e AB	63.8 ±1.6 ^f CDE	52.9 ±0.1 ^d B	62.4 ±2.4 ^e CDE	67.1 ±2.4 ^e E	47.5 ±4.9 ^d A	66.3 ±1.4 ^f E	60.0 ±0.1 ^e CD	59.0 ±1.6 ^g C	60.8 ±4.5 ^e CD	60.8 ±1.7 ^f CD	80.2 ±4.1 ^g F
Trichoderma harzianum KKP 534	55.3 ±0.1 ^{bc} G	36.5 ±4.7 ^{cd} B	47.6 ±3.3 ^{bc} CDE	52.9 ±4.7 ^d FG	51.4 ±2.7 ^e EFG	55.3 ±0.1 ^e G	17.6 ±0.1 ^b A	55.3 ±0.1 ^d G	50.0 ±2.5 ^e DEF	45.7 ±0.1 ^{cd} CD	44.1 ±2.9 ^b C	45.1 ±1.7 ^{bc} C	69.4 ±1.6 ^{de} H
Trichoderma harzianum KKP 535	48.2 ±4.0 ^a G	20.8 ±1.4 ^a C	37.1 ±0.1 ^a E	16.1 ±3.6 ^a B	37.3 ±1.4 ^a E	42.7 ±2.7 ^a F	5.9 ±3.4 ^a A	37.3 ±1.4 ^a E	28.3 ±2.9 ^a D	28.6 ±0.1 ^a D	25.5 ±1.7 ^a D	27.5 ±1.7 ^a D	46.8 ±1.6 ^a G
Trichoderma sp. KKP 786	52.6 ±4.6 ^b G	36.5 ±2.4 ^{cd} B	45.7 ±2.9 ^b DEF	50.6 ±4.1 ^{cd} FG	50.6 ±2.4 ^{bc} FG	49.8 ±1.4 ^b EFG	17.6 ±0.1 ^b A	48.2 ±2.4 ^b DEFG	40.0 ±4.3 ^b BC	43.8 ±1.6 ^c CD	39.2 ±3.4 ^b BC	45.1 ±1.7 ^{bc} DE	64.9 ±2.7 ^{cd} H
Trichoderma reesei KKP 794	48.2 ±1.5 ^a EF	24.7 ±4.7 ^{ab} B	51.4 ±2.9 ^{de} EF	12.9 ±3.3 ^a A	47.5 ±1.4 ^b DE	52.9 ±2.4 ^{bc} FG	17.6 ±4.7 ^b A	51.4 ±2.7 ^e EF	42.5 ±2.5 ^b CD	41.0 ±1.6 ^b C	41.2 ±2.9 ^b C	43.1 ±1.7 ^b CD	57.7 ±3.1 ^b G
Trichoderma sp. KKP 3053	51.8 ±1.5 ^{ab} BCD	41.2 ±0.1 ^d A	54.3 ±0.1 ^e D	42.7 ±7.2 ^b A	63.9 ±1.4 ^e E	64.7 ±2.4 ^e E	39.6 ±2.7 ^{cd} A	62.4 ±0.1 ^e E	53.3 ±1.4 ^{cd} CD	48.6 ±0.1 ^e B	50.0 ±0.1 ^c BCD	49.0 ±1.7 ^d BC	72.1 ±1.6 ^{ef} F
Trichoderma sp. KKP 3537	60.5 ±2.6 ^d EF	33.3 ±2.7 ^a A	51.4 ±0.1 ^{de} C	51.4 ±2.7 ^{cd} C	53.7 ±1.4 ^{cd} C	60.8 ±0.1 ^d DEF	39.6 ±9.5 ^{cd} B	56.9 ±2.7 ^d CDE	54.2 ±1.4 ^d CD	57.1 ±0.1 ^g CDE	54.9 ±1.7 ^{cd} CDE	55.9 ±0.1 ^e CDE	64.9 ±2.7 ^{cd} F
Trichoderma harzianum KKP 3764	57.0 ±1.5 ^{cd} F	30.2 ±1.4 ^{bc} A	50.5 ±1.6 ^{cd} D	44.3 ±4.9 ^{bc} C	56.9 ±1.4 ^d F	60.8 ±1.4 ^d F	36.5 ±4.7 ^e B	57.6 ±2.4 ^d F	59.2 ±1.4 ^e F	51.4 ±0.1 ^f DE	50.0 ±2.9 ^e D	55.9 ±2.9 ^e EF	60.4 ±4.1 ^{bc} F
Trichoderma atroviride KKP 3765	60.5 ±0.1 ^d EF	34.9 ±4.9 ^{cd} A	52.4 ±1.6 ^{de} B	63.1 ±2.7 ^e F	60.8 ±3.6 ^e EF	60.8 ±1.4 ^d EF	38.0 ±2.7 ^a A	55.3 ±0.1 ^d BCD	55.0 ±0.1 ^d BCD	53.2 ±3.3 ^{bc} BC	57.8 ±3.4 ^{de} CDE	56.9 ±1.7 ^e BCDE	59.5 ±2.7 ^b DEF
Trichoderma sp. KKP 3832	59.6 ±1.5 ^d G	36.5 ±4.1 ^{cd} B	52.4 ±1.6 ^{de} DEF	49.8 ±2.7 ^{cd} CDE	47.5 ±1.4 ^b C	55.3 ±4.1 ^c FG	24.7 ±4.7 ^b A	54.5 ±1.4 ^d EF	50.0 ±0.1 ^c CDE	46.7 ±1.6 ^{de} C	50.0 ±2.9 ^c CDE	48.0 ±1.7 ^{cd} CD	76.6 ±1.6 ^g H

0-19.99	20-39.99	40-49.99	50-59.9	60-69.9	70-79.9	≥80
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Table 4. Percent inhibition of thirteen plant pathogens growth by *Trichoderma* strains.
A-G Mean values in rows with different capital letters are significantly different at p < 0.05 (Duncan's test), separately for each *Trichoderma* strain. a-g Mean values in columns with different lowercase letters are significantly different at p < 0.05 (Duncan's test), separately for each plant pathogen strain. Data are presented as mean ± standard deviation (n=3).

Trichoderma strains	Inhibition of fungal plant pathogen strains [%]												
	Alternaria alternata KKP 3368	Botrytis cinerea KKP 3292	Fusarium oxysporum KKP 458	Fusarium graminearum DSM 1096	Fusarium poae DSM 62376	Fusarium oxysporum KKP 3492	Fusarium culmorum DSM 1094	Fusarium oxysporum KKP 761	Fusarium oxysporum KKP 851	Fusarium oxysporum KKP 760	Fusarium avenaceum KKP 756	Fusarium oxysporum KKP 762	Botrytis cinerea KKP 451
Trichoderma longibrachiatum KKP 532	8.3 ±2.8 ^a A	0.0 ±0.0	16.7 ±3.6 ^b BC	24.6 ±2.5 ^c DE	21.0 ±2.1 ^{cde} CD	21.7 ±2.1 ^c D	0.0 ±0.0	15.0 ±4.0 ^{ab} B	16.7 ±1.0 ^b BC	6.7 ±0.1 ^a A	0.0 ±0.0	6.3 ±0.1 ^a A	27.5 ±3.4 ^e E
Trichoderma harzianum KKP 534	13.0 ±3.2 ^{ab} BC	0.0 ±0.0	8.3 ±3.6 ^a AB	0.0 ±0.0	11.1 ±3.7 ^a ABC	16.3 ±2.3 ^a C	0.0 ±0.0	16.7 ±2.1 ^{abc} C	13.0 ±3.2 ^{ab} BC	0.0 ±0.0	6.3 ±0.1 ^a A	6.3 ±0.1 ^a A	35.5 ±5.9 ^d D
Trichoderma harzianum KKP 535	14.8 ±3.2 ^b CD	0.0 ±0.0	10.4 ±3.6 ^a ABC	10.9 ±3.3 ^a BC	17.3 ±2.1 ^{bc} DE	20.0 ±1.0 ^{ab} E	0.0 ±0.0	16.7 ±2.1 ^{abc} DE	13.6 ±2.8 ^{ab} CD	6.7 ±0.1 ^a AB	6.3 ±3.1 ^a A	0.0 ±0.0	27.5 ±3.4 ^e F
Trichoderma sp. KKP 786	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	11.6 ±2.5 ^a AB	21.0 ±2.1 ^{cde} CD	21.7 ±2.9 ^{bc} D	0.0 ±0.0	15.0 ±1.0 ^{ab} BC	11.1 ±5.6 ^{ab} AB	0.0 ±0.0	6.3 ±0.1 ^a A	14.6 ±3.6 ^b BC	17.5 ±5.9 ^{ab} BCD
Trichoderma reesei KKP 794	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	21.0 ±2.1 ^{cde} D	16.7 ±2.1 ^a CD	0.0 ±0.0	20.0 ±4.0 ^{cd} CD	11.1 ±0.1 ^{ab} AB	0.0 ±0.0	9.4 ±0.1 ^b A	11.6 ±1.6 ^b AB	15.7 ±3.4 ^a BC
Trichoderma sp. KKP 3053	0.0 ±0.0	0.0 ±0.0	10.4 ±3.6 ^a A	0.0 ±0.0	18.5 ±0.1 ^{cd} BC	20.0 ±4.0 ^{ab} C	0.0 ±0.0	13.3 ±2.1 ^a AB	9.3 ±3.2 ^a A	0.0 ±0.0	0.0 ±0.0	10.4 ±3.6 ^{ab} A	25.5 ±3.4 ^c D
Trichoderma sp. KKP 3537	13.8 ±2.8 ^b B	0.0 ±0.0	6.3 ±0.1 ^a A	0.0 ±0.0	28.4 ±2.1 ^{ef} E	23.3 ±2.9 ^{bc} D	0.0 ±0.0	18.3 ±2.1 ^{bc} C	9.3 ±3.2 ^a A	0.0 ±0.0	0.0 ±0.0	10.4 ±3.6 ^{ab} A	23.4 ±0.1 ^{bc} D
Trichoderma harzianum KKP 3764	16.7 ±1.0 ^b C	0.0 ±0.0	0.0 ±0.0	23.2 ±2.5 ^{bc} DE	13.6 ±2.1 ^{ab} BC	21.7 ±4.0 ^{bc} D	0.0 ±0.0	23.3 ±5.8 ^d DE	9.3 ±3.2 ^a AB	0.0 ±0.0	6.3 ±0.1 ^a A	10.4 ±3.6 ^{ab} AB	27.5 ±3.4 ^e E
Trichoderma atroviride KKP 3765	13.8 ±2.8 ^b BC	0.0 ±0.0	6.3 ±0.1 ^a A	15.9 ±5.0 ^a C	24.7 ±2.1 ^{ef} D	16.7 ±2.1 ^a C	0.0 ±0.0	15.0 ±1.0 ^{ab} BC	13.0 ±3.2 ^{ab} BC	0.0 ±0.0	0.0 ±0.0	10.4 ±3.6 ^{ab} AB	11.8 ±0.1 ^a BC
Trichoderma sp. KKP 3832	14.8 ±3.2 ^b B	0.0 ±0.0	6.3 ±0.1 ^a A	17.3 ±4.4 ^{ab} B	22.2 ±3.7 ^{de} C	25.0 ±1.0 ^c C	0.0 ±0.0	15.0 ±1.0 ^{ab} B	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	12.5 ±0.1 ^b B	37.3 ±3.4 ^d D

0-9.99	10-19.99	20-29.99	30-39.99
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Table 5. Percent inhibition of thirteen plant pathogens growth by volatile compounds of *Trichoderma* strains.
A-F Mean values in rows with different capital letters are significantly different at p < 0.05 (Duncan's test), separately for each *Trichoderma* strain. a-f Mean values in columns with different lowercase letters are significantly different at p < 0.05 (Duncan's test), separately for each plant pathogen strain. Data are presented as mean ± standard deviation (n=3).

Bedine Boat et al. (2019) reported VOC-mediated inhibition of *Fusarium oxysporum* ranging from 14.4% to 41.8%, whereas in this work the inhibition of five *F. oxysporum* strains ranged from 0% to 23.3%.

Taken together, these results confirm that VOC-mediated antagonism is highly strain-dependent and strongly influenced by experimental conditions, yet remains a consistent and important component of *Trichoderma* biocontrol activity

Conclusions

This study provides a comprehensive evaluation of the biocontrol potential of *Trichoderma* strains from the IAFB Collection based on their enzymatic activity, direct antagonism, and volatile-mediated effects.

The majority of strains produced extracellular cellulases, chitinases, and lipases, confirming their capacity to degrade plant and fungal structural components. Consequently, further research should focus on the isolation, production, and characterization of extracellular enzymes from these promising *Trichoderma* strains.

All of the tested strains inhibited the growth of 13 phytopathogenic fungi in dual-culture assays. The most promising candidates being - *T. longibrachiatum* KKP 532, *T. atroviride* KKP 3765, and *Trichoderma* sp. KKP 3537 - exhibited strong and consistent inhibition against multiple pathogens.

VOC-mediated inhibition, although generally weaker than direct antagonism, confirmed the ability of *Trichoderma* strains to suppress pathogens without physical contact, thereby expanding their ecological range of activity.

The combined effects of extracellular enzymes, competitive growth, mycoparasitism, antifungal metabolites, and VOC production demonstrate that *Trichoderma* biocontrol activity relies on synergistic mechanisms.

The next crucial step will be the validation of these promising candidates under greenhouse and field conditions, alongside the metabolomic characterization of their key bioactive compounds.

Abbreviations

IAFB – Institute of Agricultural and Food Biotechnology

KKP – Culture Collection of Industrial Microorganisms

VOCs – Volatile organic compounds

Source of Funding

Innovation Incubator 4.0 project – “*Trichoderma* filamentous fungi strains as a source of cellulolytic enzymes, a biological control agent and plant growth stimulator”.

Acknowledgements

The results presented in this article are taken from Karolina Mróz's Master's Thesis. The thesis, entitled “Isolation, identification and analysis of biocontrol mechanisms of *Trichoderma* spp.” was completed at the IAFB under the supervision of B. Sokołowska and the guidance of K. Dręzek from the Warsaw University of Technology.

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