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Biological activity of some Romanian and Turkish *Trichoderma* Pers. strains

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ABSTRACT
Disease infections caused by invasive fungi and bacteria, are some of the major causes of agricultural losses and food contamination. Human pathogenic infections are diminishing the quality of life, and in severe cases, they can trigger morbidity or even mortality. In the agricultural field, preventive treatments should be provided to control plant diseases, while in the healthcare system, for suppressing human pathogens infections, curative measures can also be applied, but with enormous costs in many parts of the world. To diminish these shortcomings, three *Trichoderma* spp. strains were screened in this study for antimicrobial and antibiofilm activities. The antifungal effect of these strains was evaluated against eight common plant pathogenic fungi: *Alternaria* sp. Nees, *Botrytis cinerea* Pers., *Fusarium culmorum* (Wm.G.Sm.) Sacc., *F. graminearum* Schwabe, *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *Macrophomina phaseolina* (Tassi) Goid. and *Sclerotinia sclerotiorum* (Lib.) de Bary. Tested *Trichoderma* spp. strains reduced the pathogenic growth with at least 50% inhibition; however, they were most effective against *B. cinerea*, with up to

92.1 ± 2.0% inhibition. The antibacterial activity of the crude extract obtained from liquid cultures of tested *Trichoderma* spp. strains was also screened against six opportunistic human pathogens and food contaminants: *Enterococcus faecalis* (Andrewes & Horder) Schleifer & Kilpper-Bälz, *Escherichia coli* (Migula) Castellani & Chalmers, *Listeria monocytogenes* (Murray *et al.*) Pirie, *Pseudomonas aeruginosa* (Schroeter) Migula, *Salmonella typhimurium* (Loeffler) Castellani & Chalmers and *Staphylococcus aureus* Rosenbach. Antibacterial activity was determined by disc diffusion assay, and tested *Trichoderma* spp. strains revealed particularly high inhibitory activity against gram positive bacteria. Moreover, the antibiofilm activity of these three biocontrol strains was also screened by XTT Assay kit. Turkish *Trichoderma* sp. Ş4 strain showed significant inhibitory activity against the tested pathogens.

KEY WORDS
Antimicrobial activity, antibiofilm, biocontrol, *Trichoderma*.

RÉSUMÉ

Activité biologique de certaines souches roumaines et turques de Trichoderma *Pers.*

Les infections causées par des champignons et des bactéries envahissants comptent parmi les principales causes de pertes agricoles et de contamination des aliments. Les infections fongiques humaines diminuent la qualité de vie et, dans les cas graves, elles peuvent entraîner une morbidité, voire une mortalité. Dans le domaine agricole, des traitements préventifs doivent être fournis pour contrôler les maladies des plantes, tandis que dans le système de santé, pour supprimer les infections par des agents pathogènes humains, des mesures curatives peuvent également être appliquées, mais avec des coûts importants dans n'importe quelle partie du monde. Pour pallier ces carences, trois souches de *Trichoderma* spp. ont été étudiées pour leurs activités antimicrobiennes et antibiofilm. L'effet antifongique de ces souches a été évalué contre huit champignons phytopathogènes communs : *Alternaria* sp. Nees, *Botrytis cinerea* Pers., *Fusarium culmorum* (Wm.G.Sm.) Sacc., *F. graminearum* Schwabe, *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *Macrophomina phaseolina* (Tassi) Goid. et *Sclerotinia sclerotiorum* (Lib.) de Bary. Les souches testées de *Trichoderma* spp. ont réduit la croissance des pathogènes d'au moins 50 %, et elles ont été le plus efficace contre *B. cinerea*, avec jusqu'à 92,1 ± 2,0 % d'inhibition. L'activité antibactérienne de l'extrait brut de cultures liquides du *Trichoderma* spp. a été aussi examinée contre six pathogènes humains opportunistes et contaminants alimentaires : *Enterococcus faecalis* (Andrewes & Horder) Schleifer & Kilpper-Bälz, *Escherichia coli* (Migula) Castellani & Chalmers, *Listeria monocytogenes* (Murray *et al.*) Pirie, *Pseudomonas aeruginosa* (Schroeter) Migula, *Salmonella typhimurium* (Loeffler) Castellani & Chalmers et *Staphylococcus aureus* Rosenbach. L'activité antibactérienne a été déterminée par la méthode du disque de diffusion. Les souches testées de *Trichoderma* spp. se sont révélé avoir une activité inhibitrice particulièrement élevée contre les bactéries Gram positives. De plus, l'activité antibiofilm de ces trois souches de biocontrôle a également été examinée par le kit XTT Assay. La souche turque *Trichoderma* sp. Ş4 a montré une activité inhibitrice significative contre les agents pathogènes testés.

MOTS CLÉS
Activité antimicrobienne, antibiofilm, biocontrôle, *Trichoderma*.

INTRODUCTION

Trichoderma Pers. (Hypocreales, Ascomycota) is a genus of filamentous fungi that have a symbiotic relationship with plants, and can also grow as saprobes (Ferreira & Musumeci 2021). These fungi are found in a variety of habitats, including soil, water, decaying wood, agricultural areas, plants, air, and dust (Bai *et al.* 2023). The genus is comprised of around 466 different species, identified based on their morphological features. *Trichoderma* species are well known for their beneficial properties such as their ability to produce enzymes for commercial use, promote plant growth, and control plant diseases (Waghunde *et al.* 2016). As a result, they show great potential for use in various industries, agriculture, and medicine. One of the advantages of using *Trichoderma* species is their safety, as they are avirulent and pose no threat to humans or plants (Bai *et al.* 2023; Zhang *et al.* 2021).

Many *Trichoderma* species can create beneficial associations with plants (Tseng *et al.* 2020). Most *Trichoderma* spp. strains remain associated with plants roots, competing soilborne phytopathogens. Other *Trichoderma* spp. strains can also grow as endophytes, colonizing the internal root tissue, intercellular, up to the second or third cells layer (Dutta *et al.* 2023). Such strains can prime the plant response to various detrimental factors (Tseng *et al.* 2020), protecting plants against biotic and abiotic stress (Irshad *et al.* 2023). They produce secondary metabolites that are highly beneficial to the plants (Contreras-Cornejo *et al.* 2016). These metabolites influence *Trichoderma* spp. interactions with other competing microorganisms, and stimulate certain plant physiological responses, by triggering gene expression in their plant hosts (Bailey *et al.* 2006). Endophytic *Trichoderma* spp. also produce phytohormones that enhance the uptake of nitrogen fertilizers, and bioactive secondary metabolites that inhibit a wide range of pathogens, thus increasing plant protection against various diseases (Tyśkiewicz *et al.* 2022). They also help plants to adapt to environmental stressors such as drought, heavy

metal toxicity, low pH, and high salinity, and to produce more photosynthetic products (Cummings *et al.* 2016).

Almost 200 secondary metabolites were discovered in *Trichoderma* spp. These metabolites are classified based on their chemical structures, which include peptaibols, terpenoids, pyrones, and polyketides (Bai *et al.* 2023). There is an increasing evidence highlighting these secondary metabolites for their critical role in promoting the beneficial effects of *Trichoderma* spp. on both crop production and human health (Zhang *et al.* 2021).

Trichoderma spp. terpenoids have diverse structures, with many containing different types of diterpenes and sesquiterpenes. These compounds exhibit various biological activities, including antimicrobial properties (Leylaie & Zafari 2018), inhibition of marine plankton species (Zou *et al.* 2021), and cytotoxicity (Abd El-Rahman *et al.* 2014). Given their numerous bioactive secondary metabolites and beneficial effects on plant growth, *Trichoderma* species are regarded as crucial fungal agents for the development of eco-friendly agrochemicals and drugs (Bai *et al.* 2023).

Synthetic fungicides are commonly used in agriculture and forestry but their unrestricted use has led to water and soil contamination with pesticide residues, prompting the search for natural alternatives that avoid harming the environment and minimizing risks to human and animal health. Many *Trichoderma* species found in the soil, particularly in the rhizosphere, can act as avirulent plant symbionts, with antagonistic and hyperparasitic effects against various phytopathogens. Such symbiotic fungi are able to protect plants from diseases (Tozlu *et al.* 2018). The biocontrol properties of *Trichoderma* spp. fungi rely on both direct and indirect mechanisms. Direct mechanisms include the production of lytic enzymes and active metabolites that suppress pathogens' growth, as well as mycoparasitism, while indirect mechanisms involve microbial competition for nutrients and space, induction of plant defense response, and stimulation of plant growth promotion. This makes *Trichoderma* spp. a promising source for natural alternatives to synthetic pesticides for agriculture and forestry (Baazeem *et al.* 2021).

Trichoderma species are producing various natural secondary metabolites of high value not only for plant protection and growth promotion, but also for human health. With the emergence of antibiotic-resistant pathogens, there is a pressing need to discover new antimicrobial agents. Therefore, *Trichoderma* secondary metabolites have garnered considerable interest in drug development research.

The aim of this study was to evaluate three strains of *Trichoderma* spp., sourced from Turkey and Romania, for their potential use in further biotechnological applications. To reveal the potential use of these strains for agricultural purposes, their antifungal activity was evaluated against important plant pathogens. To highlight the potential use of naturally derived secondary metabolites of these three *Trichoderma* strains for medicinal purposes, the crude extracts of studied *Trichoderma* spp. strains was evaluated for antibacterial and antibiofilm properties.

MATERIAL AND METHODS

Trichoderma spp. strains

Three strains of *Trichoderma* spp. were used in this study. Two of these strains, Td-Exp1 and Td-Exp 2, were isolated from soil samples, in Romania. The soils were collected from agricultural lands cultivated with wheat (in the case of Td-Exp1 isolation), and a mixture of cereals and winter leguminous plants (in the case of Td-Exp2 isolation). For isolation, serial dilutions were made on Rose Bengal Chloramphenicol agar (RBC medium). The Turkish strain *Trichoderma* sp. Ş4 was isolated from bottled water, by membrane filtration method.

Their identification was previously made based on their microscopic and macroscopic traits, as well as molecular characteristics, according to the ITS1-5.8S-ITS2 region (Tunç 2021).

The strains were routinely grown on Potato Dextrose Agar (PDA medium) and stored on slants at 4°C.

Antifungal assay

The antifungal activity of the *Trichoderma* strains was evaluated against eight important fungal pathogens responsible for plant diseases and spoilage: *Alternaria* sp. Nees, *Botrytis cinerea* Pers., *Fusarium culmorum* (Wm.G.Sm.) Sacc., *F. graminearum* Schwabe, *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *Macrophomina phaseolina* (Tassi) Goid. and *Sclerotinia sclerotiorum* (Lib.) de Bary.

The antagonistic activity was performed by dual culture technique on PDA. Each *Trichoderma* strain was co-inoculated, at the same time, with the mentioned fungal pathogens. Control plates for each tested pathogen were also prepared. Plates were incubated at 26°C. Biometrical measurements of the pathogens' growth were made after seven days of incubation. Antagonistic efficacy (E%) was calculated based on the pathogen radius in the test plates (RT) compared with the control (RC), as follows: E (%) = (RC-RT)/RC*100 (Yang *et al.* 2018; Achimón *et al.* 2021; Olowe *et al.* 2022; Yassin *et al.* 2022; Chan *et al.* 2023).

Potential hyperparasitism of the *Trichoderma* spp. was visually evaluated after 10 to 14 days of co-culturing with the pathogenic fungi. *Trichoderma* strains able to colonize and grow over the pathogenic fungi were considered to be hyperparasitic.

Extractions from *Trichoderma* spp.

The crude extracts of *Trichoderma* spp. secondary metabolites were obtained from liquid cultures. For these, the *Trichoderma* spp. strains were first grown on PDA for 7-10 days. Five mycelial discs, of 6 mm diameter, were aseptically removed from these cultures and used to inoculate 100 mL of sporulation medium, containing 40 g/L maltose, 10 g/L yeast extract, and 10 g/L peptone. The flasks were incubated at 25°C in an orbital shaker at 200 rpm. After four days, 5 mL cultures were transferred in 100 mL of wheat peptone broth (ATCC medium 344, without agar). These cultures were incubated for two weeks, as previously mentioned. After incubation, the secondary metabolites produced by each culture

Table 1. — Antifungal activity of *Trichoderma* spp. strains against certain plant pathogens. The data are presented as average values of antifungal efficacy (E%) ± standard deviation (SD). Different letters attributed within the same fungal interaction indicate a significant difference among the experimental variants.

	Trichoderma spp. strains		
Phytopatogenic fungi	**Ş4**	**Td-Exp1**	**Td-Exp2**
Alternaria sp. Nees	68.6 ± 4.1 ab	70.9 ± 1.8 a	62.8 ± 2.5b
Botrytis cinerea Pers.	92.1 ± 2.0 a	91.3 ± 0.9 a	84.2 ± 4.0 a
Fusarium culmorum (Wm.G.Sm.) Sacc.	72.8 ± 3.6 a	72.0 ± 4.2 a	72.0 ± 3.3 a
Fusarium graminearum Schwabe	76.9 ± 2.3 a	70.3 ± 6.0 a	65.7 ± 5.7 a
Fusarium oxysporum Schltdl.	81.1 ± 0.9 a	77.2 ± 1.6 ab	71.4 ± 2.7 b
Fusarium proliferatum (Matsush.) Nirenberg ex Gerlach & Nirenberg	76.3 ± 5.3 a	76.6 ± 2.2 a	65.8 ± 4.7 a
Macrophomina phaseolina (Tassi) Goid.	58.0 ± 1.2 a	60.1 ± 0.2 a	50.7 ± 5.1 a
Sclerotinia sclerotiorum (Lib.) de Bary	65.6 ± 6.4 a	68.8 ± 7.5 a	64.2 ± 7.2 a

Table 2. — Comparison of antimicrobial activities of different extracts obtained from *Trichoderma* spp. strains with agar diffusion method. Negative control (methanol): 0.0 mm.

	Inhibition zone diameter (mm ± SD)				
Organisms	**Td-Exp 1**	**Td-Exp 2**	**Ş4**	**Gen**	**Pen**
Staphylococcus aureus Rosenbach	14.1 ± 0.19	17.1 ± 0.09	17.2 ± 0.29	–	29.0 ± 0.00
Enterococcus faecalis (Andrewes & Horder) Schleifer & Kilpper-Bälz	9.0 ± 0.08	20.0 ± 0.05	10.4 ± 0.14	–	18.0 ± 0.00
Escherichia coli (Migula) Castellani & Chalmers	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	23.0 ± 0.50	–
Bacillus subtilis (Ehrenberg) Cohn	17.5 ± 0.50	21.7 ± 0.58	19.2 ± 0.29	22.0 ± 0.00	–
Pseudomonas aeruginosa (Schroeter) Migula	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	18.0 ± 0.50	–
Listeria monocytogenes (Murray *et al.*) Pirie	12.0 ± 0.00	15.9 ± 0.05	12.2 ± 0.08	26.0 ± 0.00	–
Salmonella typhimurium (Loeffler) Castellani & Chalmers	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	21.0 ± 0.00	–

of *Trichoderma* spp. were extracted in 150 mL ethyl acetate, by incubating at 25°C, for two hours at 120 rpm. The phases were separated, and the ethyl acetate was evaporated at room temperature, for 30 minutes. The crude extracts containing *Trichoderma* spp. secondary metabolites were dried and stored at 4°C, and tested for antifungal measures (Khan *et al.* 2020).

Antibacterial efficacy test

Antibacterial susceptibility tests were performed using Gram-positive *S. aureus* ATCC 6538, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *L. monocytogenes* NCTC 11994, and Gram-negative *P. aeruginosa* ATCC 15442, *E. coli* ATCC 10536, and *S. typhimurium* ATCC 14028 bacteria. Reference bacteria for the antibacterial assay were grown in TSA medium for 18-24 hours. For the experiments, a suspension of 0.5 McFarland density was prepared from bacterial cultures.

Cultures were transferred to Mueller Hinton Agar medium at a final concentration of 10^6 cfu/mL and poured into Petri dishes. After the media was solidified, 25 µL of crude extract dissolved in methanol were transferred to blank discs, placed in the center of the petri dishes. Gentamicin (10 µg/mL) and penicillin (10 µg/mL) discs were used as the positive controls, and pure methanol impregnated discs were used as the negative control. After incubation, the zones of inhibition formed around the discs were measured and recorded (Tong *et al.* 2018).

Antibiofilm effect of *Trichoderma* spp. and metabolic activity of biofilm

The metabolic activity was determined by XTT colorimetric assay kit (Mossman 1983; Mohammadi-Bazargani *et al.* 2017). The cell cultures of *S. aureus* ATCC 6538, *B. subtilis* ATCC 6633, *E. fecalis* ATCC 29212, *L. monocytogenes* NCTC 11994, *P. aeruginosa* ATCC 15442 and *E. coli* ATCC 10536 were adjusted to 0.5 MacFarland. To each well of 96-well microtiter plates, TSB medium and cell culture were transferred and *Trichoderma* spp. extract was added with increasing doses, ranging from 5 to 30 µl, and incubated at 37°C for 24 hours. Then, the cell culture was discarded, and each well was washed three times with 0.01 M phosphate-buffered saline (PBS; pH 7.2) to remove unattached cells. After the microplates were dried, 100 µl PBS and 50 µl XTT reaction solution (Cell Proliferation Kit; Biological Industries) were added to each well. The plates were incubated at 37°C for five hours. Absorbance was measured at 450 nm using an Epoch Microplate Spectrophotometer (BioTek, United States), and the percentages of antibiofilm and metabolic activity were calculated. Triplicates were used for each experiment.

Statistical analysis

The experiments were performed in triplicate and the data were expressed as means ± SD. The statistical differences between the groups were analyzed by Two-way ANOVA, GraphPad v.9. P-values < 0.05 were statistically significant.

RESULTS

Antifungal efficacy results

The antifungal activity of the *Trichoderma* spp. strains against eight important plant pathogens was assayed (Table 1).

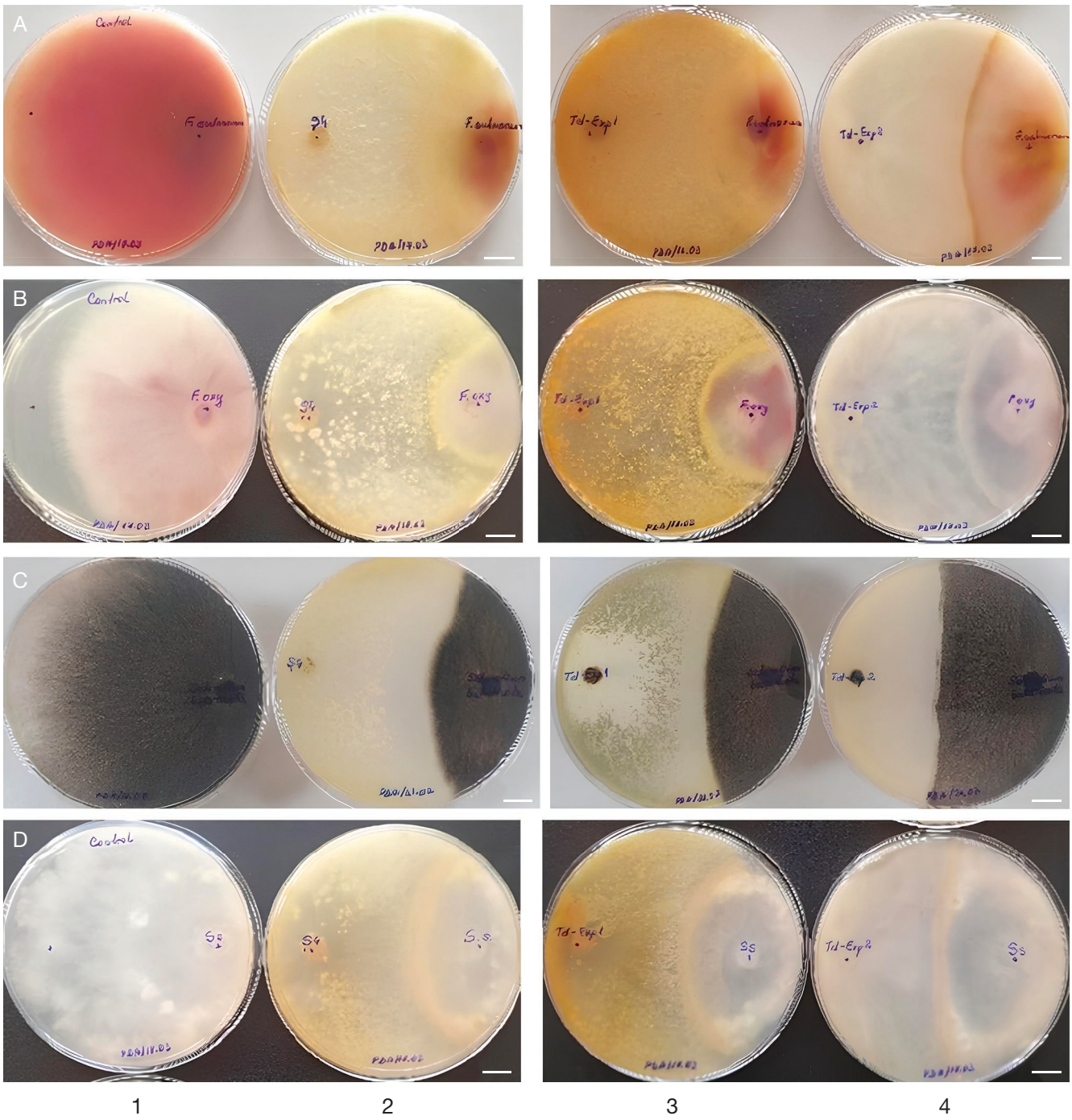


Fig. 1. — *In vitro* antifungal activity of tested *Trichoderma* strains Ş4 (**2**), Td-Exp1 (**3**) and Td-Exp2 (**4**) against various plant pathogens (**1**), such as *Fusarium culmorum* (Wm.G.Sm.) Sacc. (**A**), *F. oxysporum* Schltdl. (**B**), *Macrophomina phaseolina* (Tassi) Goid. (**C**) and *Sclerotinia sclerotiorum* (Lib.) de Bary (**D**). Scale bars: 1 cm.

The tested *Trichoderma* spp. strains revealed an antifungal activity of at least 50% efficacy against all tested plant pathogens. However, the best results were obtained against *Botrytis cinerea*, which was inhibited for at least 84.2% by Td-Exp2 strain, and up to 92.1% by the Turkish *Trichoderma* sp. Ş4 strain.

No significant differences were revealed among the *Trichoderma* spp. strains in most of the antagonistic interactions performed. However, the Td-Exp2 strain revealed less antifungal activity against *Alternaria* sp. and *F. oxysporum*, compared to the other two biocontrol strains tested (Fig. 1).

Mycoparasitic development of tested *Trichoderma* spp. strains varied, depending on the microbial interaction (Fig. 2). However, compared to Ş4 and Td-Exp1 (Fig. 3), the Td-Exp2 strain showed reduced mycoparasitic activity against some of the tested phytopathogens, this strain was able to colonize

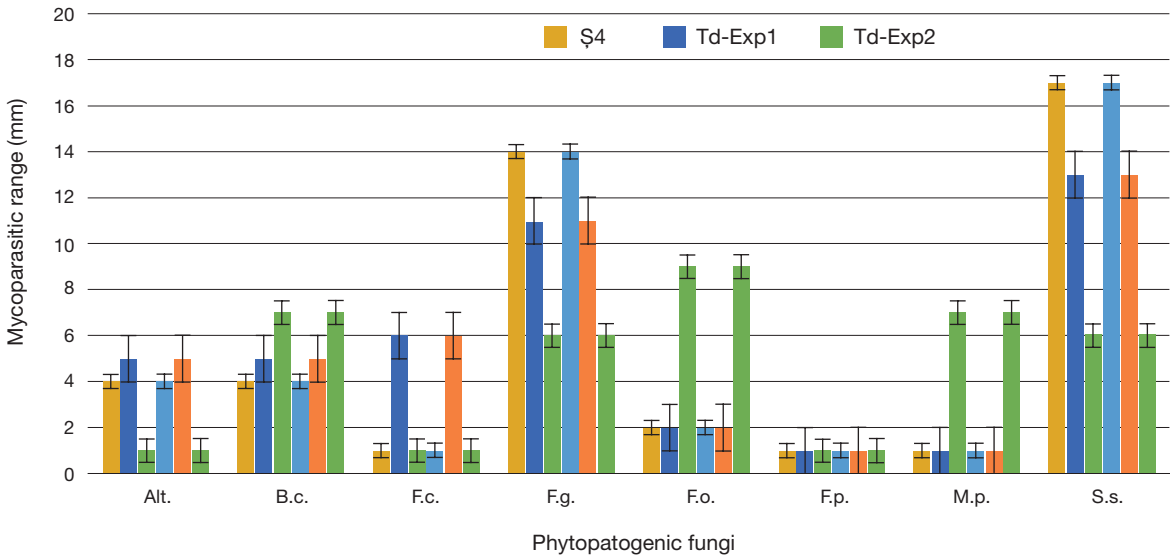


Fig. 2. — *Trichoderma* Pers. hyperparasitic development on various plant pathogens: **Alt.**, *Alternaria* sp. Nees; **B.c.**, *Botrytis cinerea* Pers.; **F.c.**, *Fusarium culmorum* (Wm.G.Sm.) Sacc.; **F.g.**, *F. graminearum* Schwabe; **F.o.**, *F. oxysporum* Schltdl.; **F.p.**, *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg; **M.p.**, *Macrophomina phaseolina* (Tassi) Goid.; **S.s.**, *Sclerotinia sclerotiorum* (Lib.) de Bary.

and hyperparasitize four to six-time larger mycelia areas of *F. oxysporum* and *M. phaseolina* respectively, than the other two biocontrol strains tested.

Antibacterial efficacy results

Diffusion assay was performed to determine the antibacterial activity of crude extract obtained from 3 different *Trichoderma* spp. strains. The data of the inhibition zones formed are given in the Table 2. The Td-Exp2 strain showed highest activity among tested extracts. The inhibition zone ranged of 12.00-17.50 mm in diameter with Td-Exp1, 15.9-21.7 mm with Td-Exp2, and 12.2-19.2 mm with Ş4 strain. Notwithstanding the spore-forming nature of *B. subtilis* ATCC 6633, all *Trichoderma* species demonstrated notable efficacy against this bacterium. Results suggest that isolates Td-Exp 2 and Ş4 strain exhibit notable inhibitory activity against *S. aureus* Rosenbach. The extracts obtained from tested *Trichoderma* spp. strains demonstrated significant inhibitory efficacy against *L. monocytogenes* (Murray *et al.*) Pirie, a food pathogen that can cause serious health problems such as meningitis, abortus, and hydrocephalus (Fig. 4). Despite their high effectiveness against gram-positive strains, all extracts did not exhibit any activity against the gram-negative test strains.

Antibiofilm efficacy results

The antibiofilm activity of *Trichoderma* spp. samples against *S. aureus*, *B. subtilis*, *E. faecalis* (Andrewes & Horder) Schleifer & Kilpper-Bälz and *L. monocytogenes* were determined by XTT Assay kit. After incubation for 24 hours, no decreasing of metabolic activity of cells treated with Td-Exp1 and Td-Exp2 was observed, implying that these samples do not have antibiofilm activity against all selected bacteria. However, *Trichoderma* sp. Ş4 showed significant inhibitory activity against tested pathogens (Fig. 5).

DISCUSSION

Agriculture is an essential part of any country, not only to ensure the food supply for the people but also for economic progress. However, agricultural crops are under constant threat of pests and diseases, and this situation will continue to increase with the climate change. Chemical pesticides are commonly used for plant protection. However, due to consumers' interests for organic or more sustainable food products, as well as for environmentally friendly production systems, more attention is given to the biologic alternative agro-inoculants, such as *Trichoderma*-based products. In order to contribute to these requirements, the antimicrobial effects of some *Trichoderma* spp. strains were evaluated. It has been observed that the studied strains inhibit the development of important plant pathogenic fungi (Boiu-Sicuia & Cornea 2021), opportunistic human pathogens (Toma *et al.* 2023) and food contaminants (Ankita & Jayanthi 2018). Similar aspects are revealed by various studies with different other *Trichoderma* spp. strains (Nuankeaw *et al.* 2019; Tyśkiewicz *et al.* 2022).

Many *Trichoderma*-based bioproducts are currently available on the market (Tyśkiewicz *et al.* 2022), while in the European Union, there are 11 strains approved as active ingredients in plant protection products: *Trichoderma asperellum* ICC012, T25, T34, TV1, *T. atrobrunneum* ITEM 908, *T. atroviride* I-1237, IMI 206040, SC1, T11, *T. gamsii* ICC080, and *T. harzianum* T-22 strains (European Union Pesticides Database 2023). Numerous studies confirm the wide antifungal and mycoparasitic activity of *Trichoderma* species against various plant pathogens (Guzmán-Guzmán *et al.* 2023; Yao *et al.* 2023). In dual culture antagonism studies, various strains of *T. virens* (J.H.Miller, Giddens & A.A.Foster) Arx, *T. pseudokoningii* Rifai and *T. harzianum* Rifai revealed

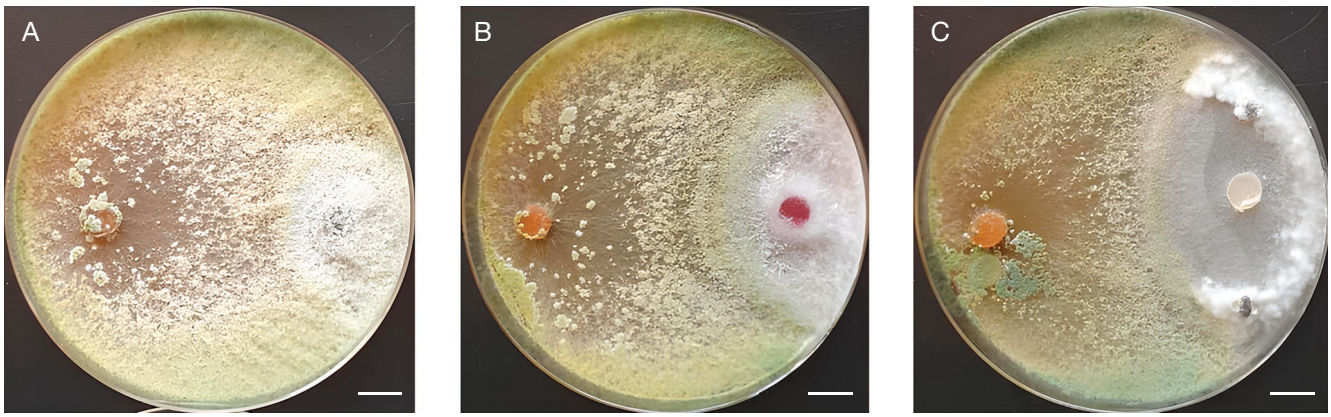
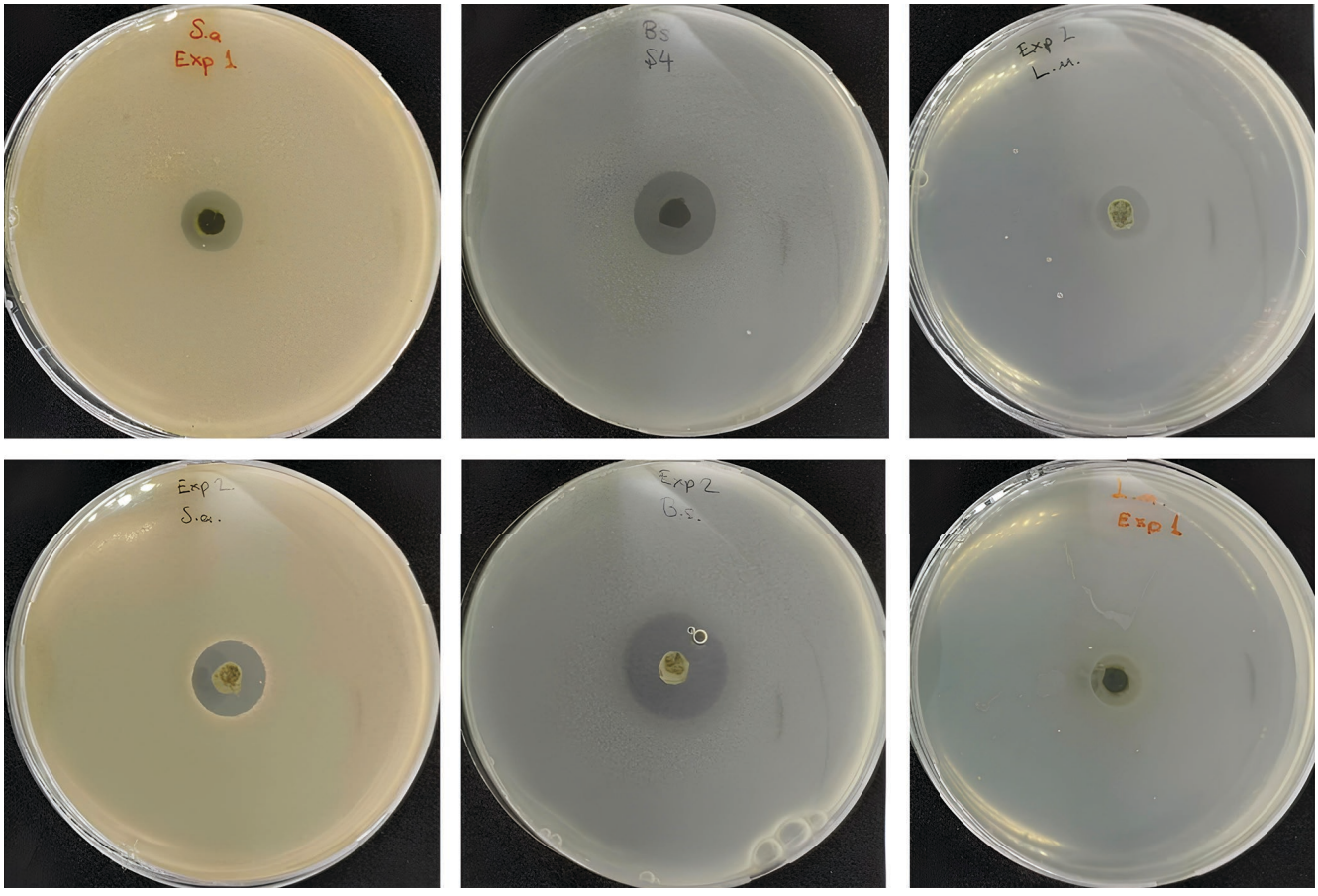


FIG. 3. — Mycoparasitic activity of *Trichoderma* Td-Exp1 strains against plant pathogens: **A**, *Botrytis cinerea* Pers.; **B**, *Fusarium graminearum* Schwabe; **C**, *Sclerotinia sclerotiorum* (Lib.) de Bary. Scale bars: 1 cm.



S. aureaus *B. subtilis* *L. monocytogenes*

FIG. 4. — Clear inhibition zone revealed by the *Trichoderma* spp. crude extract against some human pathogenic bacteria.

Alternaria sp. inhibition percentages of 43.62%, 36.6% and 52.55%, respectively, as well as hyperparasitic mycelial overgrowth (Rahman *et al.* 2020). Similar dual culture antagonism studies against *Botrytis cinerea*, revealed 62.05% pathogen growth inhibition when using a biocontrol *T. harzianum* strain (Geng *et al.* 2022). Against *F. culmorum*, certain *T. viride* Pers., *T. viridescens* (A.S.Horne & H.S.Will.) Jaklitsch & Samuels and *T. atroviride* P.Karst. revealed 30 to 65.6% mycelia inhibition (Modrzewska *et al.* 2022). Compared to all these, the *Trichoderma* spp. strains used in this study revealed superior antifungal activity.

Similar antifungal activity was seen among tested *Trichoderma* spp. strains and various *T. asperellum* Samuels, Lieckf. & Nirenberg and *T. harzianum* strains against *F. graminearum* (Li

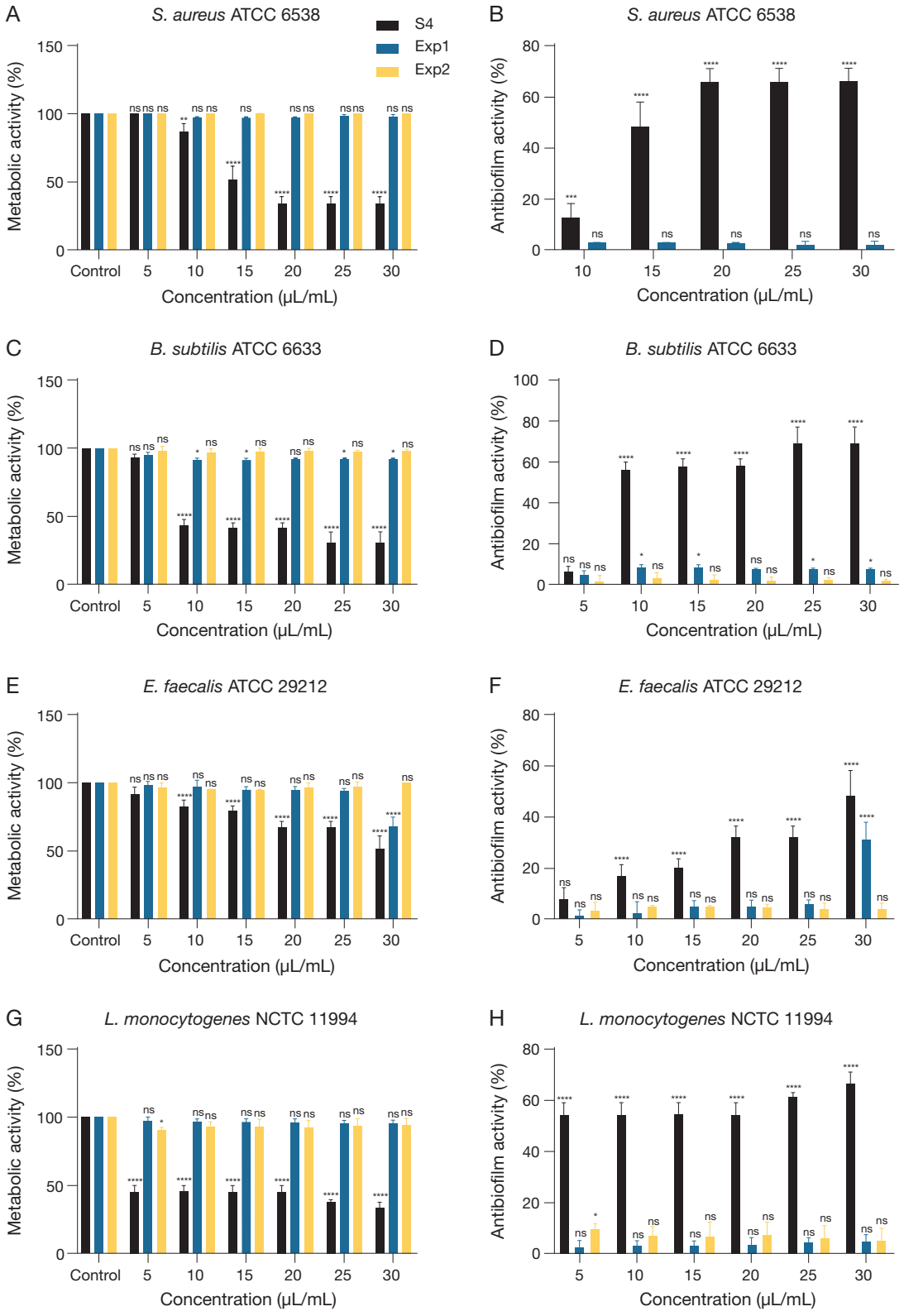


FIG. 5. — Metabolic activity in biofilm and antibiofilm effect of *Trichoderma* spp. against human pathogenic bacterial strains. Symbols: **ns**, no significant; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$.

et al. 2016). Other Romanian native *Trichoderma* spp. strains revealed weaker antifungal activity compared to the currently presented strains, showing only 56.15 to 67.69 % inhibition efficacy against *F. oxysporum* (Petrișor *et al.* 2017). According to Bai *et al.* (2023), terpenoids are the most abundant bioactive compounds in *Trichoderma* species. Moreover, they have stated

that a total of 253 terpenoids, including 202 sesquiterpenes, 48 diterpenes, two monoterpenes and one meroterpenoid, were isolated and identified from *Trichoderma* species between 1948 and 2022 (Bai *et al.* 2023). Thus, we considered that the strong antibiofilm activity of Ş4 may be related to the synergism between similar secondary metabolites.

In vitro studies performed by Khan *et al.* (2021) to suppress *M. phaseolina* growth, revealed that tested *T. viride* has higher antagonistic potential (63%) than the strains of *T. koningii* Oudem., *T. hamatum* (Bonord.) Bainier, *T. longipile* Bissett (46-47%) and *T. harzianum* (28%). Other antagonistic studies performed with *T. atroviride*, *T. gamsii* Samuels & Druzhinina, *T. koningiopsis* Samuels, Carm.Suárez & H.C.Evans and *T. viridescens* against *Sclerotinia sclerotiorum*, revealed 46-60% antifungal potential, with best results obtained with *T. atroviride* (Hidayah *et al.* 2022). Higher antifungal activity, of 63.8%, against *S. sclerotiorum* was seen when using *T. ghanense* T11 strain. However, the biocontrol potential can differ between the strains of the same species. Among different strains of *T. atroviride* the antifungal efficacy against *S. sclerotiorum* varied from 54.4 to 58.2%, with *T. asperellum* the efficacy fluctuated from 52.9 to 58.5%, among *T. citrinoviride* Bissett strains the antifungal efficacy varied from 45.0 to 58.8%, within *T. harzianum* from 54.4 to 60.3%, while within *T. longibrachiatum* Rifai from 54.1 to 63.8% (Hernandez Castíllo *et al.* 2011), all these being less effective compared to the strains presented in the current study.

Studies performed with *T. atroviride* against pathogenic bacteria revealed that it inhibited the growth of gram-positive bacteria, *S. aureus* and *S. epidermidis* (Winslow & Winslow) Evans, rather than gram negative (Víglaš & Olejníková 2019). Similar features were observed also in the present study, with the tested *Trichoderma* strains, which also inhibited bacterial biofilm development (Fig. 5). Although there is no precise estimate for clinically important fungi, it has been calculated that approximately 80% of recurrent and chronic bacterial infections (Davies 2003) and 500 000 deaths annually (Sharifi *et al.* 2018) are attributed to biofilms.

Most diseases caused by *Candida albicans* Berkhout are associated with biofilm formation on abiotic or host surfaces (Nett & Andes 2006; Mathe & Van Dijck 2013). More importantly, *C. albicans* is adept at adhering to catheters and various medical implants. It is currently classified by the Centers for Disease Control and Prevention in the United States as the third most frequently isolated bloodstream pathogen in hospitalized patients (Wisplinghoff *et al.* 2004; Tournu & Van Dijck 2012; Mathe & Van Dijck 2013).

Trichoderma species are well known for their antifungal activities against both human and plant pathogenic fungi, and exhibit a broad spectrum of antifungal activity against various fungi, such as *C. albicans*, *C. krusei* (Castell.) Berkhout, *Saccharomyces cerevisiae* (Desm.) Meyen, *Torulopsis glabrata* (H.W.Anderson) Lodder & N.F.de Vries, *Aspergillus fumigatus* Fresen. and *A. niger* van Tieghem. Watanabe *et al.* (1990) revealed remarkable antifungal activity of *Trichoderma* spp. especially against different strains of *Candida* Berkhout, with MIC values ranging from 0.4 to 12.5 lg/mL.

Further studies should be done to characterize the secondary metabolites synthetized by the studied *Trichoderma* strains, in order to identify the nature of their antimicrobial compounds. Such findings could support the use of valuable biocontrol strains that can reduce the use of chemical pesticides or even replace them.

CONCLUSION

The *Trichoderma* spp. strains Td-Exp 1, Td-Exp 2 and Ş4 used in this study, inhibited various fungal phytopathogens as well as human pathogenic bacteria. Notably, Ş4 strain revealed the highest inhibitory activity, followed by Td-Exp 1 for the antifungal action, and Td-Exp 2 for antibacterial action.

Additionally, the crude extract of *Trichoderma* sp. Ş4 strain was shown antibiofilm activity against important human pathogens such as *S. aureus*, *L. monocytogenes* and *E. faecalis*.

These results highlight the potential use of *Trichoderma* sp. Ş4 strain as biocontrol agent against important plant and human pathogens, which makes it a promising alternative to synthetic pesticides.

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REFERENCES

Abd El-Rahman A. A., El-Shafei S. M., Ivanova E. V., Fattakhova A. N., Pankova A. V., El-Shafei M. A., El-Morsi M. A. & Alimova F. K. 2014. — Cytotoxicity of *Trichoderma* spp. cultural filtrate against human cervical and breast cancer cell lines. *Asian Pacific Journal of Cancer Prevention* 15 (17): 7229-7234. https://doi.org/10.7314/apjcp.2014.15.17.7229

Achimón F., Brito V. D., Pizzolitto R. P., Ramirez Sanchez A., Gómez E. A. & Zygadlo J. A. 2021. — Chemical composition and antifungal properties of commercial essential oils against the maize phytopathogenic fungus *Fusarium verticillioides*. *Revista Argentina de Microbiología* 53 (4): 292-303. https://doi.org/10.1016/j.ram.2020.12.001

Ankita C. & Jayanthi A. 2018. — Microbial Contamination, Prevention, and Early Detection in Food Industry, in Holban A. M. & Grumezescu A. M. (eds), *Handbook of Food Bioengineering, Microbial Contamination and Food Degradation*. Academic Press, Cambridge: 21-47.

Baazeem A., Almanea A., Manikandan P., Alorabi M., Vijayaraghavan P. & Abdel-Hadi A. 2021. — In vitro antibacterial, antifungal, nematocidal and growth promoting activities of *Trichoderma hamatum* FB10 and its secondary metabolites. *Journal of Fungi* 7 (5): 331. https://doi.org/10.3390/jof7050331

Bai B., Liu C., Zhang C., He X., Wang H., Peng W. & Zheng C. 2023. — *Trichoderma* species from plant and soil: an excellent resource for biosynthesis of terpenoids with versatile bioactivities. *Journal of Advanced Research* 49: 81-102. https://doi.org/10.1016/j.jare.2022.09.010

Bailey B. A., Bae H., Strem M. D., Roberts D. P., Thomas S. E., Crozier J., Samuels G. J., Choi I. Y. & Holmes K. A. 2006. — Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. *Planta* 224 (6): 1449-1464. https://doi.org/10.1007/s00425-006-0314-0

Boiu-Sicuia O. A. & Cornea C. P. 2021. — Bacterial strains involved in soilborne phytopathogens inhibition. *Scientific Papers*, Series A, Agronomy 64 (1): 641-646.

Chan M. E., Tan J. Y., Lee Y. Y., Lee D., Fong Y. K., Mutwil M., Wong J. Y. & Hong Y. 2023. — Locally isolated *Trichoderma harzianum* species have broad spectrum biocontrol activities against the wood rot fungal species through both volatile inhibition and mycoparasitism. *Journal of Fungi* 9 (6): 675. https://doi.org/10.3390/jof9060675

Contreras-Cornejo H. A., Macías-Rodríguez L., Del-Val E. & Larsen J. 2016. — Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. *FEMS Microbiology Ecology* 92 (4): fiw036. https://doi.org/10.1093/femsec/fiw036

Cummings N. J., Ambrose A., Braithwaite M., Bissett J., Roslan H. A., Abdullah J., Stewart A., Agbayani F. V., Steyaert J. & Hill R. A. 2016. — Diversity of root-endophytic *Trichoderma* from Malaysian Borneo. *Mycological Progress* 15: 50. https://doi.org/10.1007/s11557-016-1192-x

Davies D. 2003. — Understanding biofilm resistance to antibacterial agents. *Nature Reviews Drug Discovery* 2: 114-122. https://doi.org/10.1038/nrd1008

Dutta P., Mahanta M., Singh S. B., Thakuria D., Deb L., Kumari A., Upamanya G. K., Boruah S., Dey U., Mishra A. K., Vanlaltani L., Vijay Reddy D., Heisnam P. & Pandey A. K. 2023. — Molecular interaction between plants and *Trichoderma* species against soil-borne plant pathogens. *Frontiers in Plant Science* 14: 1145715. https://doi.org/10.3389/fpls.2023.1145715

European Union Pesticides Database 2023. — Available from https://ec.europa.eu/food/plant/pesticides/eupesticides-database/activesubstances/?event=search.as (accessed on 16 April 2023).

Ferreira F. V. & Musumeci M. A. 2021. — *Trichoderma* as biological control agent: scope and prospects to improve efficacy. *World Journal of Microbiology & Biotechnology* 37 (5): 90. https://doi.org/10.1007/s11274-021-03058-7

Geng L., Fu Y., Peng X., Yang Z., Zhang M., Song Z., Guo N., Chen S., Chen J., Bai B., Liu A. & Ahammed G. J. 2022. — Biocontrol potential of *Trichoderma harzianum* against *Botrytis cinerea* in tomato plants. *Biological Control* 174: 105019. https://doi.org/10.1016/j.biocontrol.2022.105019

Guzmán-Guzmán P., Kumar A., De Los Santos-Villalobos S., Parra-Cota F. I., Orozco-Mosqueda M. C., Fadiji A. E., Hyder S., Babalola O. O. & Santoyo G. 2023. — *Trichoderma* species: our best fungal allies in the biocontrol of plant diseases – A Review. *Plants* 12 (3): 432. https://doi.org/10.3390/plants12030432

Hernandez Castíllo F. D., Berlanga Padílla A. M., Gallegos Morales G., Cepeda Síller M., Rodríguez Herrera R., Aguílar Gonzales C. N. & Castíllo R. 2011. — In vitro antagonist action of *Trichoderma* strains against *Sclerotinia sclerotiorum* and *Sclerotium cepivorum*. *American Journal of Agricultural and Biological Sciences* 6 (3): 410-417. https://doi.org/10.3844/ajabssp.2011.410.417

Hidayah B. N., Khangura R. & Dell B. 2022. — Biological control potential of *Trichoderma* species and bacterial antagonists against *Sclerotinia sclerotiorum* on canola in western Australia. *International Journal of Agriculture and Biology* 27: 215-227. https://doi.org/10.17957/IJAB/15.1919

Irshad K., Shaheed Siddiqui Z., Chen J., Rao Y., Hamna Ansari H., Wajid D., Nida K. & Wei X. 2023. — Bio-priming with salt tolerant endophytes improved crop tolerance to salt stress via modulating photosystem II and antioxidant activities in a sub-optimal environment. *Frontiers in Plant Science* 14: 1082480. https://doi.org/10.3389/fpls.2023.1082480

Khan I. H., Javaid A. & Ahmed D. 2021. — *Trichoderma viride* controls *Macrophomina phaseolina* through its DNA disintegration and production of antifungal compounds. *International Journal of Agriculture and Biology* 25: 888-894. https://doi.org/10.17957/IJAB/15.1743

Khan R. A. A., Najeeb S., Mao Z., Ling J., Yang Y., Li Y. & Xie B. 2020. — Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenicbacteria and root-knot nematode. *Microorganisms* 8 (3): 401. https://doi.org/10.3390/microorganisms8030401

Leylaie S. & Zafari D. 2018. — Antiproliferative and antimicrobial activities of secondary metabolites and phylogenetic study of endophytic *Trichoderma* species from Vinca plants. *Frontiers in Microbiology* 9: 1484. https://doi.org/10.3389/fmicb.2018.01484

Li Y., Sun R., Yu J., Saravanakumar K. & Chen J. 2016. — Antagonistic and biocontrol potential of *Trichoderma asperellum* ZJSX5003 against the maize stalk rot pathogen *Fusarium graminearum*. *Indian Journal of Microbiology* 56 (3): 318-327. https://doi.org/10.1007/s12088-016-0581-9

Mathe L. & Van Dijck P. 2013. — Recent insights into *Candida albicans* biofilm resistance. *Current Genetics* 59: 251-264. https://doi.org/10.1007/s00294-013-0400-3

Modrzewska M., Błaszczyk L., Stępień Ł., Urbaniak M., Waśkiewicz A., Yoshinari T. & Bryła M. 2022. — *Trichoderma* versus *Fusarium* inhibition of pathogen growth and mycotoxin biosynthesis. *Molecules* 27: 8146. https://doi.org/10.3390/molecules27238146

Mohammadi-Bazargani M., Rohloff J. & Atapour M. 2017. — Comparative analysis of essential oil and different extracts of *Ajugachamaecistus* ssp. *scoparia* on antibacterial activity. *Journal of Essential Oil Bearing Plants* 20 (4): 1117-1124. https://doi.org/10.1080/0972060X.2017.1365017

Mossman T. 1983. — Rapid colorimetric assay for cellular growth and survival; application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65 (1-2): 55-63. https://doi.org/10.1016/0022-1759(83)90303-4

Nett J. E. & Andes D. R. 2006. — *Candida albicans* biofilm development, modeling a host-pathogen interaction. *Current Opinion in Microbiology* 9 (4): 340-345. https://doi.org/10.1016/j.mib.2006.06.007

Nuankeaw K., Chaiyosang B., Suebrasri T., Kanokmedhakul S., Lumyong S. & Boonlue S. 2019 — First report of secondary metabolites, Violaceol I and Violaceol II produced by endophytic fungus, *Trichoderma polyalthiae* and their antimicrobial activity. *Mycoscience* 61 (1): 16-21. https://doi.org/10.1016/j.myc.2019.10.001

Olowe O. M., Nicola L., Asemoloye M. D., Akanmu A. O., Sobowale A. A. & Babalola O. O. 2022. — Characterization and antagonistic potentials of selected rhizosphere *Trichoderma* species against some *Fusarium* species. *Frontiers in Microbiology* 13: 985874. https://doi.org/10.3389/fmicb.2022.985874

Petrișor C., Paica A. & Constantinescu F. 2017. — Effect of secondary metabolites produced by different *Trichoderma* spp. isolates against *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Fusarium solani*. *Scientific Papers*, Series B, Horticulture LXI: 407-412.

Rahman M. A., Rahman M. A., Rahman Moni Z. & Rahman M. A. 2020. — Evaluation of bio-control efficacy of *Trichoderma* strains against *Alternaria alternata* causing leaf blight of ashwagandha [*Withania somnifera* (L.) Dunal]. *Journal of Forest and Environmental Science* 36 (3): 207-218. https://doi.org/10.7747/JFES.2020.36.3.207

Sharifi R., Lee S.-M. & Ryu C.-M. 2018. — Microbe-induced plant volatiles. *New Phytologist* 220 (3): 684-691. https://doi.org/10.1111/nph.14955

Toma R. C., Boiu-Sicuia O. A., Diguță F. C., Ciucă M., Matei F. & Cornea C. P. 2023. — Selected plant protection *Bacillus* strains increase food safeness by inhibiting human pathogenic bacteria. *Romanian Agricultural Research* 40: 36. https://doi.org/10.59665/rar4057

Tong X., Shen X. Y. & Hou C. L. 2018. — Antimicrobial activity of fungal endophytes from *Vaccinium dunalianum* var. *urophyllum*. *Sains Malaysiana* 47 (8): 1685-1692. https://doi.org/10.17576/jsm-2018-4708-07

Tournu H. & Van Dijck P. 2012. — *Candida* biofilms and the host: models and new concepts for eradication. *International Journal of Microbiology* 2012: 845352. https://doi.org/10.1155/2012/845352

Tozlu E., Tekiner N. & Örtücü S. 2018. — Investigation on the biological control of *Alternaria alternata*. *Indian Journal of Agricultural Sciences* 88 (8): 1241-1248. https://doi.org/10.56093/ijas.v88i8.82561

Tseng Y. H., Rouina H., Groten K., Rajani P., Furch A. C. U., Reichelt M., Baldwin I. T., Nataraja K. N., Uma Shaanker R. & Oelmüller R. 2020. — An endophytic *Trichoderma* strain promotes growth of its hosts and defends against pathogen attack. *Frontiers in Plant Science* 11: 573670. https://doi.org/10.3389/fpls.2020.573670

Tunç G. 2021. — *Isolation, identification and toxin production potentials of fungi in bottled waters sold in Izmir and its vicinity*. PhD thesis. Ege University, Turkey, 184 p.

Tyśkiewicz R., Nowak A., Ozimek E. & Jaroszuk-Ściseł J. 2022. — *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *International Journal of Molecular Sciences* 23 (4): 2329. https://doi.org/10.3390/ijms23042329

Víglaš J. & Olejníková P. 2019. — *Trichoderma atroviride*: an isolate from forest environment with secondary metabolites with high antimicrobial potential. *Acta Chimica Slovaca* 12 (1): 46-55. https://doi.org/10.2478/acs-2019-0008

Waghunde R. R., Shelake R. M. & Sabalpara A. N. 2016. — *Trichoderma*: a significant fungus for agriculture and environment. *African Journal of Agricultural Research* 11: 1952-1965.

Watanabe N., Yamagishi M., Mizutani T., Kondoh H., Omura S., Hanada K. & Kushida K. 1990. — CAF-603: a new antifungal carotane sesquiterpene. Isolation and structure elucidation. *Journal of Natural Products* 53 (5): 1176-1181. https://doi.org/10.1021/np50071a006

Wisplinghoff H., Bischoff T., Tallent S. M., Seifert H., Wenzel R. P. & Edmond M. B. 2004. — Nosocomial bloodstream infections in US hospitals: analysis of 24 179 cases from a prospective nationwide surveillance study. *Clinical Infectious Diseases* 39 (3): 309-317. https://doi.org/10.1086/421946

Yang M., Huang C., Xue Y., Li S., Lu L. & Wang C. 2018. — Biofumigation with volatile organic compounds from *Streptomyces alboflavus* TD-1 and pure chemicals to control *Aspergillus ochraceus*. *Annals of Applied Biology* 173 (3): 313-322. https://doi.org/10.1111/aab.12465

Yao X., Guo H., Zhang K., Zhao M., Ruan J. & Chen J. 2023. — *Trichoderma* and its role in biological control of plant fungal and nematode disease. *Frontiers in Microbiology* 14: 1160551. https://doi.org/10.3389/fmicb.2023.1160551

Yassin M. T., Mostafa A. A.-F. & Al-Askar A. A. 2022. — In vitro antagonistic activity of *Trichoderma* spp. against fungal pathogens causing black point disease of wheat. *Journal of Taibah University for Science* 16 (1): 57-65. https://doi.org/10.1080/16583655.2022.2029327

Zhang J. L., Tang W. L., Huang Q. R., Li Y. Z., Wei M. L., Jiang L. L., Liu C., Yu X., Zhu H. W., Chen G. Z. & Zhang X. X. 2021. — *Trichoderma*: A treasure house of structurally diverse secondary metabolites with medicinal importance. *Frontiers in Microbiology* 12: 723828. https://doi.org/10.3389/fmicb.2021.723828

Zou J. X., Song Y. P. & Ji N. Y. 2021. — Deoxytrichodermaerin, a harziane lactone from the marine algicolous fungus *Trichoderma longibrachiatum* A-WH-20-2. *Natural Product Research* 35 (2): 216-221. https://doi.org/10.1080/14786419.2019.1622110

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