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In vitro compatibility of *Trichoderma asperellum* with isotianil and pesticides of chemical and organic origin

Compatibilidad *in vitro* de *Trichoderma asperellum* con isotianil y pesticidas de origen químico y orgánico

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Optical microscopy at 40x magnification of a germinated conidia of *Trichoderma asperellum*.
Photo: C.A. Dodino-Gutiérrez

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ABSTRACT

Synthetic pesticides are used to reduce the adverse effect of pests on the crops, although their indiscriminate use causes environmental pollution and harmful effects on soil microorganisms. The use of *Trichoderma* sp. is established as an alternative for the control of plant diseases and reduction of negative effects of the employment of pesticides through its combination with chemical agents. This study evaluated the inhibition percentage *in vitro* and germination conidia of *Trichoderma asperellum* after exposure to isotianil and chemical and organic pesticides by means of the technique poisoned food and inoculation in water agar. The assay was carried out in a completely randomized experimental design, data were subjected to analysis of variance and means were compared using the LSD Fisher $P<0.01$ test. *Cinnamomun verum* extract (1,050 µL L^{-1}) and the defense inducer isotianil (2,200 µL L^{-1}) were found to be harmless to *T. asperellum* presenting a mycelial growth inhibition percentage (PI) of 0.33±0.73 and 1.92±1.09, respectively; chili bell pepper-garlic extract (400 µL L^{-1}) and azoxystrobin (750 µL L^{-1}) were slightly toxic with a PI of 37.5±11.6 and 45.9±1.56; while glyphosate (6,480 µL L^{-1}), mancozeb (26,666 mg L^{-1}), difenoconazole (1,562 µL L^{-1}) and fenpropimorph (24,200 µL L^{-1}) were toxic with a PI of 100%. The results on conidia germination showed that chili bell pepper and garlic extract, *C. verum* extract and isotianil allowed more than 83% of their germination, unlike glyphosate and azoxystrobin that only allowed the germination of 48.8 and 33.9% of the conidia. The chemical fungicides mancozeb, difenoconazole and fenpropimorph showed negative effects causing less than 2% of germination. These results suggest the development of future studies for the joint application of native strains of *Trichoderma* sp. with pesticides of chemical and organic origin with the objective of evaluating their compatibility and using them jointly in the integrated pest management of crops in the region.

Additional key words: antagonist; biological control; compatibility; plaguicides.

RESUMEN

Los plaguicidas sintéticos se utilizan para reducir el efecto adverso de las plagas en los cultivos; aunque su uso indiscriminado causa contaminación ambiental y efectos perjudiciales en los microorganismos del suelo. El uso de *Trichoderma* sp. se establece como una alternativa para el

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control de enfermedades de las plantas y la reducción de los efectos adversos del empleo de pesticidas mediante su combinación con agentes químicos. Este estudio evaluó el porcentaje de inhibición *in vitro* y germinación de conidios de *Trichoderma asperellum* tras exposición a isotianil y pesticidas químicos y orgánicos por medio de la técnica de alimentos envenenados e inoculación en agar agua. El ensayo se llevó a cabo en un diseño experimental completamente aleatorizado, los datos se sometieron a análisis de varianza y las medias se compararon mediante la prueba LSD Fisher P<0,01. Se encontró que el extracto de *Cinnamomun verum* (1.050 µL L^{-1}) y el inductor de defensas isotianil (2.200 µL L^{-1}) fueron inocuos para *T. asperellum* presentando un porcentaje de inhibición de crecimiento micelial (PI) de 0,33±0,73 y 1,92±1,09 respectivamente; el extracto de ají-ajo (400 µL L^{-1}) y azoxistrobina (750 µL L^{-1}) fueron ligeramente tóxicos con un PI de 37,5± 11,6 y 45,9±1,56; mientras que el glifosato (6.480 µL L^{-1}), mancozeb (26.666 mg L^{-1}), difenoconazole (1.562 µL L^{-1}) y fenpropimorf (24.200 µL L^{-1}), fueron tóxicos con un PI de 100%. Los resultados sobre la germinación de conidios mostraron que el extracto de ají y ajo, extracto de canela e isotianil permitieron más del 83% de su germinación, a diferencia del glifosato y azoxistrobina que solo permitieron la germinación del 48.8 y 33.9% de los conidios. Los fungicidas químicos mancozeb, difenoconazol y fenpropimorf demostraron efectos negativos ocasionando menos del 2% de germinación. Estos resultados sugieren desarrollar futuros estudios para la aplicación conjunta de cepas nativas de *Trichoderma* sp. con pesticidas de origen químico y orgánico con el objetivo de evaluar su compatibilidad y emplearlos de manera conjunta en el manejo integrado de plagas de los cultivos de la región.

Palabras clave adicionales: antagonista; compatibilidad; control biológico; plaguicida.

INTRODUCTION

Substances used in agriculture such as fungicides, herbicides, rodenticides, insecticides, nematicides and molluscicides are part of a group or mixture of substances known as pesticides (Agarwal and Pandey, 2017; Tudi *et al*., 2021). These products are applied to crops to reduce the impact caused by plants pests and diseases (Sangiorgio *et al*., 2022; Ahmad *et al*., 2024), which is currently a worrying problem with negative effects on economic income and world food security (Cerda *et al*., 2017). From the 1960s onwards, the green revolution increased fertilizer use fourfold and led to the application of multiple agrochemicals (Poudel *et al*., 2024). Thus, nearly 3 billion kilograms of pesticides are used worldwide each year at an approximate cost of

~$40 billion (Sharma *et al*., 2020; Kumar *et al*., 2023). Although the employment of synthetic fungicides is generally associated with improved crop quality, their indiscriminate use has caused environmental pollution (Poudel *et al*., 2024), intensified harmful consequences on soil and crop biodiversity, impacting native microorganisms and their natural antagonistic activity (Meena *et al*., 2020; Dodino-Gutiérrez *et al*., 2023). Also, the constant application of fungicides and organophosphate pesticides leads to eutrophication processes in surface waters and freshwater ecotoxicity (Lu *et al*., 2019; Liu *et al*., 2021; Tsalidis, 2022).

One of the challenges of using fungicides with the same action properties is that it leads to the emergence of resistance in plant pathogenic fungi, which poses a serious threat to the safeguarding of crops (McLaughlin *et al*., 2023; Yin *et al*., 2023). Despite the high effectiveness of fungicides, there are limitations in their application; one of these shortcomings lies in the lack of specificity with respect to non-target species (Marinho *et al*., 2020; Andreolli *et al*., 2023). For example, in the field, bees assimilate a variety of combinations of insecticides and fungicides that can influence larval and physiological growth and increase mortality of these pollinators, according to the type of pesticide used (Schuhmann *et al*., 2022; McLaughlin *et al*., 2023). In humans, frequent and direct exposure to chemical pesticides and fungicides causes both acute and chronic neurotoxicity, can be carcinogenic, cytotoxic and mutagenic (Weisenburger, 1993; Fang *et al*., 2020; Ahmad *et al*., 2024).

Currently, there are several biological control agents (BCAs) available for the control of plant diseases (Singh *et al*., 2021), and their use has increased significantly in the agricultural sector due to consumer and producer interest in reducing the application of synthetic pesticides (Sánchez-Montesinos *et al*., 2021). Therefore, based with the problem of the detrimental effect of fungicides on human health and their severe impact on the environment (Zhang *et al*., 2021; Slaboch *et al*., 2022), it is crucial to investigate environmentally friendly microbial alternatives to manage plant disease pathogens (Poudel *et al*., 2024).

As a reaction to this situation, a significant opportunity for biocontrol through *Trichoderma* spp. strains has been generated in the context of integrated pest management (IPM), substantiated on the combination of physical, chemical and biological control agents (Celar and Kos, 2021). These measures involve the use of chemical fungicides in combination with the inoculation of BCAs, such as non-pathogenic fungi (*Trichoderma* spp., *Xylaria* sp. and *Clonostachys* sp.) and bacteria (*Bacillus* spp., *Solibacillus* sp. and *Lysinibacillus* sp.) (Kannan and Sureendar, 2009;

Tirado-Gallego *et al*., 2016; Gonzalez *et al*., 2020; Ons *et al*., 2020). This combination has the ability to reduce the fungicide dose below the maximum residue limits (MRLs) (Ons *et al*., 2020), decrease the development of resistance pathogen resistance to fungicides (Maheshwary *et al*., 2020; Dinkwar *et al*., 2023), minimizes the frequent application of pesticides and reduces costs through a single atomization of the active component (Singh *et al*., 2021; Bharadwaz *et al*., 2023).

Of the multiple microorganisms that have been used as BCAs, *Trichoderma* is considered the gold standard of biological control (Sánchez-Montesinos *et al*., 2021); many species genus *Trichoderma* include *T. asperellum* Samuels, Lieckf and Nirenberg; *T. viride* Pers; *T. harzianum* Rifai; *T. atroviride* P. Karst; *T. citrinoviride* Bissett; *T. aggressivum* Samuels and W. Gams; *T. saturnisporum* Hamill, as efficient biopesticides against a wide variety of pathogens (Jiang *et al*., 2016; Martínez *et al*., 2016; Fraceto *et al*., 2018; Bunbury-Blanchette and Walker, 2019; Gezgin *et al*., 2020; Aydoğdu, 2022; Bharadwaz *et al*., 2023). It has been demonstrated that the species of *Trichoderma* manifest mechanisms of action to control phytopathogens, such as competition for space and nutrients (Sharma *et al*., 2019); is also attributed to mycoparasitism, an ability to attack other fungal species through a process of ramming, penetration and death of the host (Adnan *et al*., 2019). In addition, they secrete secondary metabolites, antibiotics (Asad, 2022), and induce systemic resistance in plants to several fungal diseases (Brotman *et al*., 2010; Haque *et al*., 2023). Although *Trichoderma* spp. are attributed with the ability to resist many agrochemicals and to degrade synthetic pesticides due to their specific enzymatic activity to break down these compounds (Mendarte-Alquisira *et al*., 2024), *Trichoderma* spp. strains are differentiated by their sensitivity to different pesticides (Thomas, 2010).

Due to the above reasons, species of the genus *Trichoderma* are widely studied and also marketed as biofertilizers, biofungicides and soil amendments (Kredics *et al*., 2014). *Trichoderma asperellum*, has the ability to acidify the growth environment through the generation of organic acids, such as gluconic, citric and fumaric acids, while producing phytohormones; thereby, acids derived from fungal metabolism have the ability to solubilize phosphates, micronutrients and elements such as magnesium, manganese and iron (Harman *et al*., 2004; Chagas Junior *et al*., 2022). *T. asperellum* has been reported as a phytosanitary agent that has the ability to counteract phytopathogenic fungi such as *Fusarium graminearum*, *Phytophthora megakarya*, *Alternaria tenuissima* and *Colletotrichum gloeosporioides* (Li *et al*., 2016; Tchameni *et al*., 2017; Kaissoumi *et al*., 2023; Madrid-Molina *et al*., 2023). *Trichoderma*-based agricultural bioinputs; in which *T. asperellum* is included, are offered to the market in various formulations (Calle-Cheje *et al*., 2023), such as oil dispersion, concentrated solution, soluble powder, granular products, microparticulates and microcapsules (Vindas-Reyes *et al*., 2024). Kolombet *et al.* (2007), developed a liquid paste formulation from *T. asperellum* biomass that shows the ability to remain active *in vitro* for at minimum 6 months. Formulations based on *Trichoderma asperellum* are incorporated into crops for disease biocontrol through applications as foliar sprays (Chien and Huang, 2020). Additions of live spores of the fungi can occur before prior sowing of seeds, incorporations directly into the soil, irrigation and by root dipping (Woo *et al*., 2014). Therefore, the aim of this study was to evaluate the *in vitro* compatibility of *Trichoderma asperellum* and the percentage of conidia germination against exposure to isotianil and pesticides of chemical and organic origin.

MATERIALS AND METHODS

Location

The study was carried out in the microbiology laboratory of the agricultural bioinputs laboratory unit - Agrotec belonging to C.I. Técnicas Baltime de Colombia S.A. located in the banana-growing area of the department of Magdalena, Colombia; during the months of September and October 2024.

Trichoderma strain

The strain used was *Trichoderma asperellum* (Th2), supplied from the collection of the agricultural bioinputs laboratory -Agrotec. This strain was isolated from rhizospheric soil of banana crops in the department of Magdalena, Colombia. This isolate was selected for its higher inhibitory activity against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (Foc TR4), based on antagonism by the dual-culture *in vitro* confrontation technique. The Th2 strain was molecularly identified using primers ITS1-F 5'-CTTGGTCATTTAGAGGAAGTAA -3' and ITS4-R 5'-TCCTCCGCTTATTGATATATGC-3' of the ITS region. The sequence obtained was verified in the NCBI fungal database using the blastn search algorithm (https://www.ncbi.nlm.nih.gov/) obtaining 98.97% sequence homology of the Th2 strain with

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Trichoderma asperellum accession MN516469. For the study, the strain was maintained in Papa dextrose agar (PDA, Merck KGaA, Germany) with 0.05% lactic acid at 26±2°C for 10 d of growth. The characteristics of the conidiophores of the genus allow the confirmation of *Trichoderma* by optical microscopy (Sánchez-Montesinos *et al*., 2021).

Active ingredients

A total of eight active ingredients were used. The general data of the isotianil and chemical and organic pesticides used in this study are in table 1.

Table 1. General data of active ingredients used in the test.

Active ingredient	Commercial product	Dose *	Concentration (mg L^{-1} / µL L^{-1})
Chili bell pepper and garlic extract	Alisín	2.0 mL L^{-1}	400
Glyphosate	Panzer 648 SL	1.2 L ha^{-1}	6,480
Cinnamomun verum extract	Provius 70%	1.5 mL L^{-1}	1,050
Azoxystrobin	Azobin 250 SC	0.6 L ha^{-1}	750
Isotianil	Routine 200 SC	0.25 L ha^{-1}	2,200
Mancozeb	Invezeb 80 WP	2.0 kg ha^{-1}	26,666
Difenoconazole	Kurdo 250 EC	0.4 L ha^{-1}	1,562
Fenpropimorph	Volley 88 OL	0.5-0.7 L ha^{-1}	24,200

* The concentration of the chemical and organic fungicides, herbicide and insecticide corresponds to the conversion of the product into a mixture used for ground or aerial application in the field according to the manufacturer's indications. Dose of chemical fungicides (mancozeb, difenoconazole and fenpropimorph), corresponds for the control of black sigatoka caused by *Pseudocercospora fijiensis* in banana crops. Glyphosate herbicide dose is for weed control in banana crops. In the organic fungicide, cinnamon extract corresponds to control of *Botrytis* sp. in roses and the dose of the organic insecticide chili bell pepper and garlic extract, corresponds to that recommended for the control of the insect pest *Colaspis submetallica* in banana crops. For azoxystrobin, the dose used for the control of the grain spot complex in rice crops was taken into account.

Mycelial growth inhibition of *Trichoderma asperellum*

The evaluation of the compatibility of *T. asperellum* with isotianil and pesticides was carried out to calculate the percentage inhibition of mycelial growth, using the poisoned food technique (Sinclair and Dhingra, 2017). Sterile PDA (Potato dextrose agar), was poured into 90 mm diameter Petri dishes mixed with the pesticides at the field doses presented in (Tab. 1). The mixture was then homogenized by vigorously shaking. The medium was then allowed to solidify and 10 µL of a suspension of 10^5 conidia/mL of *T. asperellum* quantified by Neubauer chamber counting (Boeco, Germany) was sown in the center of the culture medium (Terrero-Yépez *et al*., 2018). Twenty replications were used for each type of treatment, including the control. For the control, the fungus was sown in Petri dishes with PDA culture medium without active ingredient. The boxes were then sealed with Parafilm® and incubated at 26±2°C until the control grew throughout the Petri dish. The fungal growth diameter (mm) measurements were made in all boxes using a digital caliper and the mean value were recorded. The percentage of inhibition of fungal mycelial growth was calculated according to the formula described by Vincent (1947):

$$PI= [C-T / C]* 100$$

PI = Percent inhibition, C = *Trichoderma* growth on control plate (mm), y T = *Trichoderma* growth in the treatments (mm).

In another aspect, toxicity was classified, that is, the compatibility between the active ingredients evaluated and *T. asperellum*, using the International Organization for Biological Control (IOBC) criteria. This categorization between microorganisms and compatibility is based on the percentage of inhibition as opposed to control (<30%: harmless; 30-75%: slightly toxic; 75-90%: moderately toxic; >90%: toxic) (Vinuela and Jacas, 1993).

In vitro test of conidial germination

The effect of isotianil and synthetic and organic pesticides used on the germination of *T. asperellum* conidia was evaluated *in vitro* on sterile 1% water agar (AW) (Mohammadi and Amini, 2015). Thus, a suspension of 100 mL *T. asperellum* mixed with each of the products was prepared in sterile demineralized water; for which the dose of *T. asperellum* cultivated in sterile rice substrate ten days old in relation to 300 g ha^{-1} and each of the concentrations of the products established in table 1 were mixed, leaving it in contact for 30 min simulating field conditions. Serial 10^{-1} and 10^{-2} dilutions were then made in test tubes containing 9 mL of sterile demineralized water and 5 µL of each dilution was inoculated in duplicate at five sites equidistant from the surface of the AW medium. The AW medium inoculated without mixing it with any active ingredient was considered as control. Plates were incubated at 26±2°C. Conidial germination was assessed by light microscopy at 18±2 h of incubation. Conidia were stained with lactophenol blue in each inoculation zone (Lopes *et al*., 2013). Conidia were considered viable if they had germinative tubes larger than their diameters (Oliveira *et al*., 2015; Ramos-González *et* *al*., 2022). Germinated and non-germinated conidia (100 conidia in total) were counted for each treatment including the control and two readings and five replicates were performed.

Statistical analysis

Quantitative data were obtained from a totally randomized experimental design. *In vitro* mycelial growth inhibition and conidial germination data were subjected to a one-factor analysis of variance and Fisher's least significant difference (LSD) test was applied for comparison of means at a significance level of $P<0.01$, using Minitab® Statistical Software-Desktop version 22.1 (Minitab, LLC., USA).

RESULTS AND DISCUSSION

Morphological and microscopic identification of *Trichoderma asperellum*

Three days of incubation, *T. asperellum* strain grown on PDA agar (Fig. 1), at 26±2°C. *T. asperellum* exhibited macroscopic characteristics, starting with an intense green coloration in the center of the PDA Petri dish, cream-colored colony backing, absence of color or pigment diffusion in the culture medium, and scattering of green conidia on the surface of the medium. Microscopically, the hyphae were hyaline and septate, the conidiophores had features of a pyramidal system, and the phialides were generally in whorls of two or three (Siddiquee, 2017; Asis *et al*., 2021). The presence of ovoid conidia with globose or subglobose morphology and chlamydospores was observed.

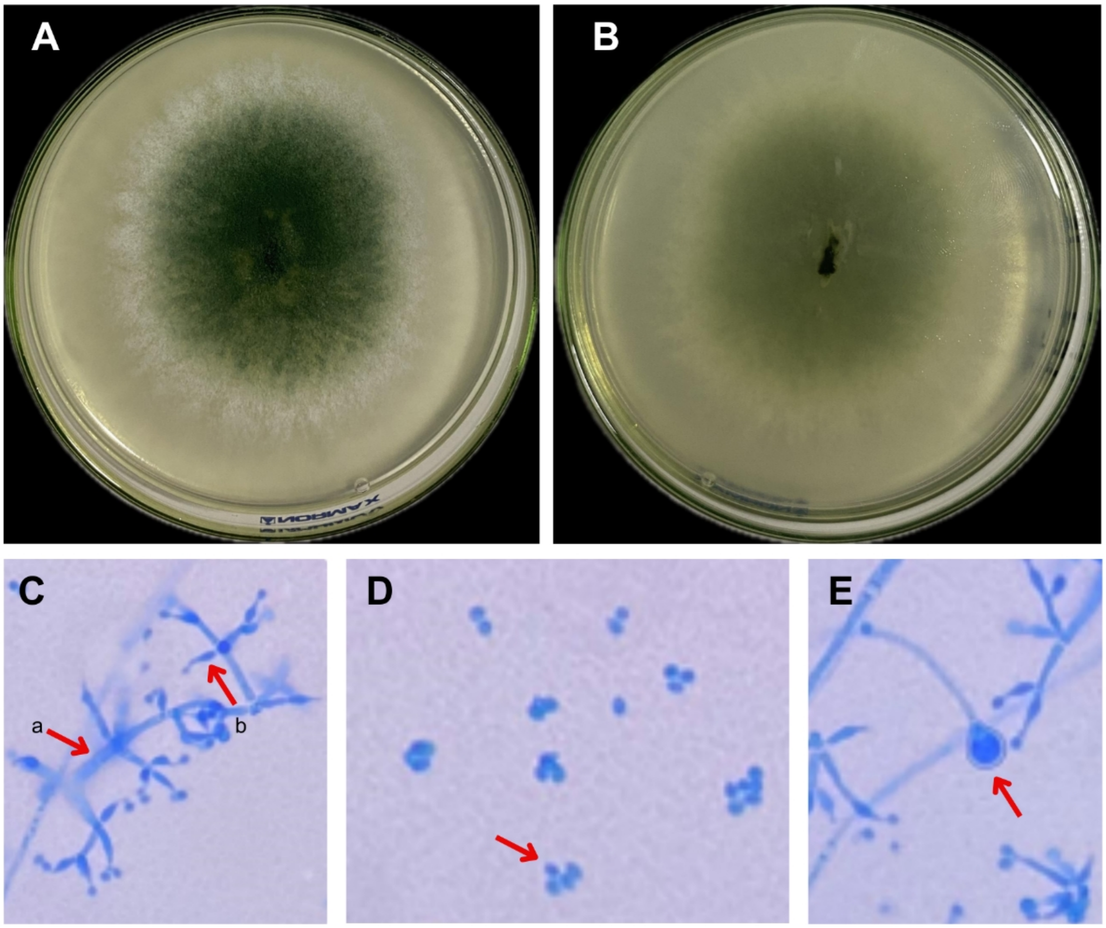

Figure 1. Macroscopic and microscopic characteristics of *T. asperellum.* A. Colony front on PDA after three days of incubation. B. Back of the colony. C. a) conidiophores and b) phialides. D. Conidia and E. Chlamydospore, observed under light microscopy at 40x magnification.

Mycelial growth inhibition of *Trichoderma asperellum*

Fungicides that adhere in a thin layer to the plant surface are adsorbed and those that can be incorporated into the plant and the active ingredient is diffused are absorbed; therefore, since fungicides can be adsorbed or absorbed, they have two mobility modes: contact (adsorbed to the plant surface) and systemic or penetrating (absorbed by the plant) (Oliver and Beckerman, 2022). Systemic fungicides are those that, when sprayed on the upper surface of plants, move into their tissues and transfer their toxicity to the target fungus (Moharram *et al*., 2004) and the contact type remain on the surface of the plant after application without entering the plant tissues and are normally used to control foliar diseases (Tsalidis, 2022), therefore, it is possible that it may be removed from the surface of the plant due to rainfall or irrigation (Carmona *et al*., 2020). Of the chemical fungicides used in this study, three active ingredients are of systemic mobility (difenoconazole, fenpropimorph and azoxystrobin) and one of contact type (mancozeb). In general, systemic fungicides may be more detrimental to *Trichoderma* species. In a study, Sarkar *et al.* (2010), showed that, among the fungicides evaluated, systemic fungicides derived from benzimidazoles were more toxic than contact fungicides in relation to the percentage of mycelial inhibition on *Trichoderma harzianum*.

Regarding mycelial growth, figure 2 shows the effect of chemical and organic active ingredients on the development of *T. asperellum* after 96 h of incubation. Isotianil and azoxystrobin showed percentage inhibition values of 1.92 and 45.9%, respectively (Tab. 2). Isotianil was harmless for *T. asperellum*, which may be due to the fact that it is a phytosanitary agent that does not directly exert antimicrobial activity, because it acts such as a defense inducer (Toquin *et al*., 2011; Bektas and Eulgem, 2015; Portz *et al*., 2020), that promotes systemic acquired resistance in plants (Zhou *et al*., 2023). To our knowledge, this is the first *in vitro* compatibility study of *T. asperellum* with isotianil. Azoxystrobin was shown to be slightly toxic to *T. asperellum*. Sunkad *et al*., (2023), found that *T. asperellum* was compatible with azoxystrobin at 500; 1,000 y 1,500 mg L^{-1}, while Maheshwary *et al*. (2020) obtained a 38.14% inhibition of mycelium at 100 mg L^{-1}. However, at a lower dose than in this study (150 mg L^{-1}) and mixed with difenoconazole, 28.8 and 0% inhibition of *T. asperellum* and 34.81% inhibition of *T. harzianum* mycelium were observed (Dinkwar *et al*., 2023). Therefore, different levels of tolerance and sensitivity in *Trichoderma* strains to azoxystrobin have been evidenced. The tolerance and resistance of fungi to fungicides is an evolutionary process, where exposure to an active component applies selectivity pressure to a population, eliminating the initial wild-type population but not the mutationally modified population (FRAC, 2019).

Table 2. Effect of chemical and organic active ingredients on *Trichoderma asperellum* mycelial growth diameter and percentage inhibition.

Treatment	**Mean mycelial growth diameter (mm)**	**PI**	**Toxicity**
Chili-garlic extract	56.2	37.5 (±11.6) c	slightly toxic
Glyphosate	0	100 a	toxic
Cinnamomun verum extract	89.7	0.33 (±0.73) d	harmless
Azoxystrobin	48.6	45.9 (±1.56) b	slightly toxic
Isotianil	88.3	1.92 (±1.09) d	harmless

Mancozeb	0	100 a	toxic
Difenoconazole	0	100 a	toxic
Fenpropimorph	0	100 a	toxic
Control	90	-	-

Data correspond to the mean of twenty replicates. Means (± standard deviation), with different letters between rows, indicate significant statistical differences ($P<0.01$) by Fisher's least significant (LSD) test. Toxicity was classified according to the scale of the International Organization for Biological Control (IOBC).

Four active ingredients, glyphosate, difenoconazole, mancozeb and fenpropimorph, had a completely inhibitory effect on *T. asperellum* at the concentrations evaluated. Da Silva *et al.* (2024), showed that *T. asperelllum* under continuous exposure in glyphosate-poisoned medium at 446 mg L^{-1}, the mycelial growth rate was 0 mm d^{-1}. While, in *T. harzianum*, the mean inhibitory concentration (DI50) was 69.38 mg L^{-1}, values well below those recommended by the manufacturer at a concentration of 8,000 mg L^{-1} (Blanco *et al*., 2024). Glyphosate is a phosphonomethyl herbicide derived from the glycine, whose active ingredient is N-(phosphonomethyl) glycine (Davoren and Schiestl, 2018). Glyphosate is usually applied to the foliar zones of weeds; after application, glyphosate has the ability to infiltrate plants through leaves, roots, green tissues, trunk and shoots emerging from both roots and trunk (Sharma and Singh, 2001), after its entry, it quickly moves to the active growth regions of the plant (Kanissery *et al*., 2019), exercising its mechanism of action that interferes in the enzyme 5-enolpyruvylsikimate-3-phosphate synthase (Duke, 2018), in the metabolic pathway of shikimate in plants and also in the main groups of fungi, archaea, bacteria and protozoa, which prevents the aromatic amino acids synthesis (Van Bruggen *et al*., 2021).

The systemic fungicide difenoconazole has been reported to be poorly compatible on *T. asperellum*, causing 77.77% mycelial growth inhibition at a dose of 100 mg L^{-1}, lower than that of this study (Baby *et al*., 2022). Maheshwary *et al.* (2020), demonstrated that the combination of difenoconazole + azoxystrobin at 1,000; 1,500; 2,000 and 3,000 mg L^{-1} completely inhibits *in vitro* growth of *T. asperellum*. Furthermore, the lethal dose 90 (LD90) of difenoconazole on *T. asperellum* was only 39.7 mg L^{-1} (Arain *et al*., 2022).

Fenpropimorph was found to be toxic to *T. asperellum*. Carver *et al.* (1996), they found that this chemical at 2 mg L^{-1} prevented radial growth of *T. aureoviride*. The effect of mancozeb on the growth of *Trichoderma* spp. has been extensively studied *in vitro* (Bhale and Rajkonda, 2015; Kumar *et al*., 2017; Marcellin *et al*., 2018; Ramanagouda and Naik, 2021; Ajith *et al*., 2024). Mancozeb applied at field doses of 2 kg ha^{-1} showed a critical inhibition on the growth of *T.*

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asperellum; similar results have been described by Coca and Gakegne (2020), who evidenced between 94.93 and 98.08% of the percentage of inhibition *in vitro* at 72 h of inoculation using the same dose. However, at lower doses than those evaluated in this study, namely 2,000 mg L^{-1}, the harmless effect of mancozeb on *T. asperellum* has been demonstrated, obtaining only 12.59% mycelial growth inhibition (Maheshwary *et al*., 2020) and 26.11% mycelial growth inhibition on *T. asperelloides* (Saha *et al*., 2023). This difference in the percentage of inhibition has been attributed to the progressive increase in the concentration to which the fungus is exposed (Wedajo, 2015; Baby *et al*., 2022) and ability to degrade chemical molecules and their inherent resistance to a variety of fungicides (Papavizas, 1985). The sensitivity or resistance of *Trichoderma* sp. strains differs according to the ecological conditions from which they are isolated growth restriction, sporulation and conidial germination fluctuates depending on strain, type of active ingredient and the dose (Celar and Kos, 2021).

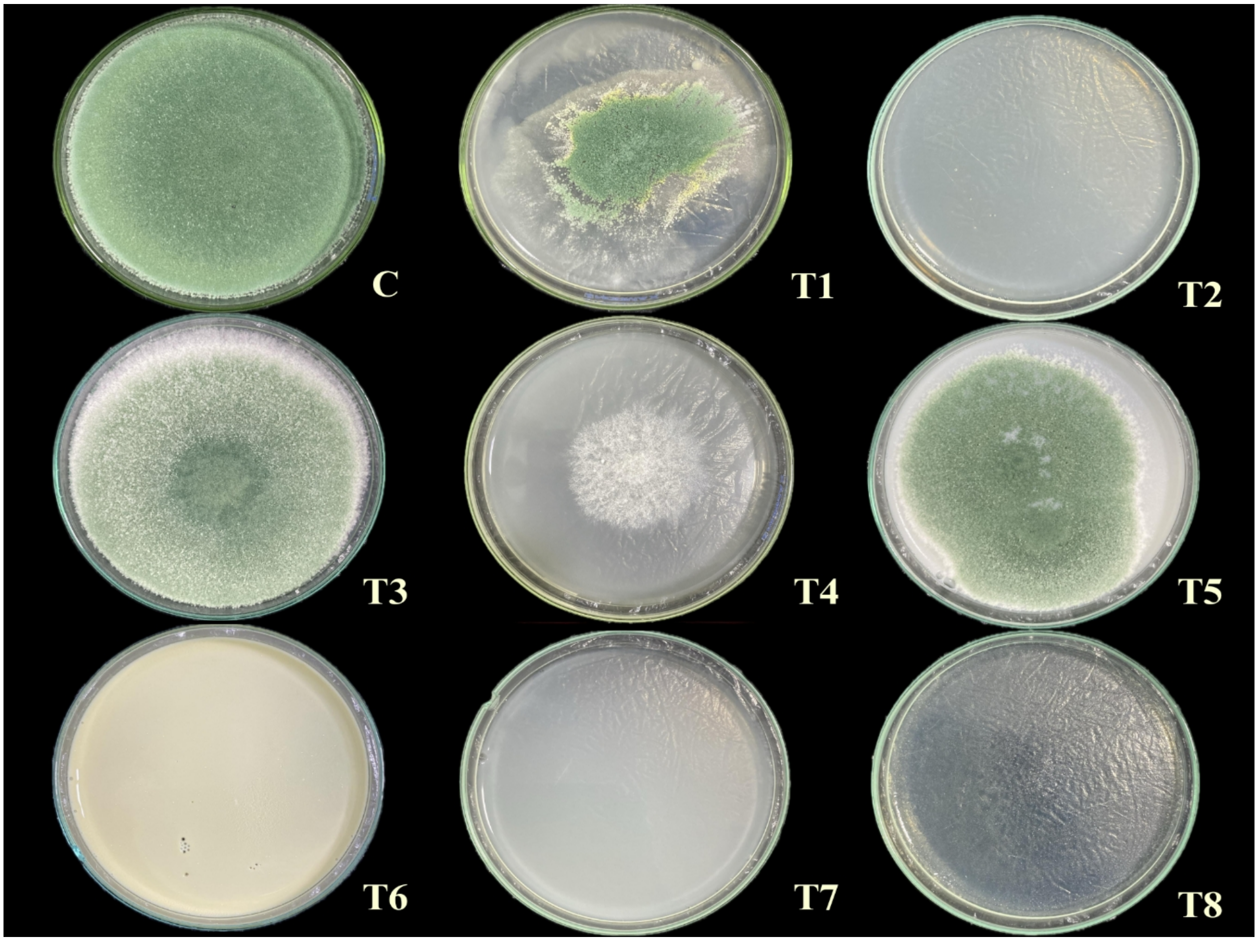

Figure 2. Mycelial growth of *Trichoderma asperellum* after 96 h of inoculation in PDA culture medium poisoned with isotianil and different chemical and organic pesticides. C:

Control, T1: Chili bell pepper and garlic extract, T2: Glyphosate, T3: *Cinnamomun verum* extract, T4: Azoxystrobin, T5: Isotianil, T6: Mancozeb, T7: Difenoconazole and T8: Fenpropimorph.

The organic pesticides, *Cinnamomun verum* extract and chili bell pepper and garlic extract showed an inhibition of 37.5 and 0.33%, respectively. Compatibility studies of *Trichoderma* species have been documented in botanical extracts. The botanical products tested, 10% extract of karanj leaves (*Pongamea pinnata*), presented 46.7 and 54.4% inhibition compared to the control in *T. harzianum and T. viride*; while, in extracts of cumin leaves (*Cuminum cyminum*), 25.2 and 34.1% mycelial inhibition were evidenced in *T. harzianum and T. viride* respectively (Bagwan, 2010). In neem leaves extracts (*Azadirachta indica*) the PI was found to be 0% at concentrations of 5, 10 and 15% (Thomas *et al*., 2022). Maheshwari (2014), found that at a concentration of 5% garlic extract (*Allium sativum*), the percentage of inhibition of *Trichoderma* spp. varied between 9.51 and 17.11%. *T. harzianum* showed greater sensitivity to garlic botanical extract at a lower dose (15 mg L^{-1}) than the *T. asperellum* strain used in this study, obtaining a higher percentage of inhibition, i.e. 51.02% (Poudel *et al*., 2023). On the other hand, *Cinnamomun verum* extract was highly compatible with *T. asperellum* at the dose evaluated. However, the findings of this investigation vary from those obtained by Yan *et al.* (2021), who found that out of seven essential oils, *cinnamon* oil presented the lowest minimum inhibitory concentration results (31.25 µL L^{-1}), on *Trichoderma viride*.

In vitro test of conidial germination

The *in vitro* germination test showed that the fungicides difenoconazole, mancozeb and fenpropimorph severely affected the viability of *T. asperellum* conidia (Fig. 3).These results are in agreement with those published by Zaine (2011), who evaluated the germination of conidia upon exposure to difenoconazole and mancozeb, obtaining that 100 mg L^{-1} of the active principles completely inhibited spore germination in *Trichoderma* spp. strains. Mancozeb belongs to the M3 group, which has a multisite contact mode of action; while difenoconazole belongs to group 3 of the FRAC (Fungicide Resistance Action Committee) and acts by causing the inhibition of ergosterol synthesis of the fungal membrane (Fishel and Dewdney, 2012; FRAC, 2024). Fenpropimorph is a systemic fungicide of the FRAC group 5 morpholine chemical class and its mode of action is also related to specific inhibition processes in the biosynthesis of ergosterols (Bjørnlund *et al*., 2000; FRAC, 2024), which impedes the germination process of the fungus and affects cell permeability.

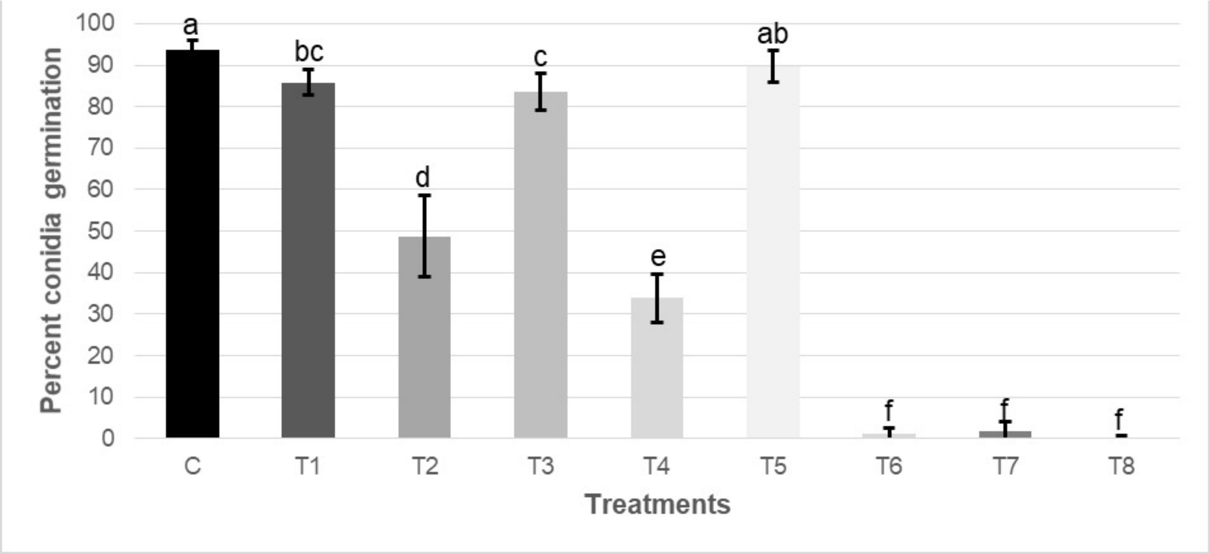

Figure 3. Percent conidia germination at 18±2 h of inoculation on AW. Data correspond to the mean of 10 replicates. Means (error bars: standard deviation) with different letters between treatments indicate significant statistical differences (*p*< 0.01), by Fisher's least significant (LSD) test. C: Control, T1: Chili bell pepper and garlic extract, T2: Glyphosate, T3: *Cinnamomun verum* extract, T4: Azoxystrobin, T5: Isotianil, T6: Mancozeb, T7: Difenoconazole and T8: Fenpropimorph.

Isotianil showed no significant statistical differences compared to the control containing no active ingredient ($P<0.01$). In addition, the organic fungicide based on *Cinnamomun verum* extract and chili bell pepper and garlic extract allowed germination of *T. asperellum* conidia of 83.7 and 85.8%, respectively. Poudel *et al.* (2023) observed that as time elapsed the percentage of inhibition of *Trichoderma harzianum* decreased and this may be related to the efficacy of the BCAs in neutralizing the active compounds present in the botanical extracts. In addition, *Trichoderma* sp. has been attributed the ability to assimilate a wide variety of substrates (Lyubenova *et al*., 2023), including pesticides and herbicides (Nykiel-Szymańska *et al*., 2020; Szpyrka *et al*., 2020).

Azoxystrobin and the herbicide glyphosate, at the doses used, significantly decreased the germination of *T. asperellum*, allowing its viability by 33.9 and 48.8%, respectively. These results are consistently with the published by Da Silva *et al.* (2018), who demonstrated that azoxystrobin reduced the conidia viability in *T. asperellum* and *T. asperelloides* at a concentration of 10 µL L^{-1}. Azoxystrobin had no commensurate effect on mycelial growth, but greatly reduced conidia germination in *T. asperellum*. Whereas azoxystrobin is a strobirulin that belongs to group 11 (external quinone inhibitor) of FRAC (Wedge *et al*., 2007, FRAC, 2024). This group of fungicides prevents fungal cellular respiration and therefore, the germination of conidia, which is an energy-demanding process (Bartlett *et al*., 2002). As for glyphosate, it has been shown that even *Trichoderma* members of the same species show varying degrees of tolerance to exposure to this herbicide. Two *T. asperellum* strains (GRB-HA1 and GRB-HA2), exposed for 30 min to a dose of 12.5 mL L^{-1} of glyphosate + 0.2 g L^{-1} metsulfuron methyl, had an effect on the percentage of conidia germination of 84 and 15% (Ramírez-Olier *et al*., 2021). Interestingly, it was observed that the mycelial growth of *T. asperellum* was highly affected after exposure to glyphosate, but allowed a lower percentage of germination; this has been linked to the modification of the electrostatic charge on the surface of the fungi and a possible suppression of the mucous layer covering the conidia (Zapata-Narváez and Botina-Azain, 2023).

In Integrated Pest Management (IPM), it is necessary to deepen the knowledge of the combination of *Trichoderma* sp. strains and different pesticides of chemical and organic origin. For this reason, the inclusion of BCA species showing resistance to agrochemicals is crucial (Dinkwar *et al*., 2023). However, it is suggested to continue evaluating the effect of *Trichoderma asperellum* against fungicides, herbicides and insecticides with the aim of contribute to sustainable agriculture by reducing pesticide application cycles and using them in combination with *Trichoderma* sp. to ensure safe management against crop pathogens and minimize the unwanted negative effects of over-application of synthetic pesticides.

CONCLUSION

Under the test conditions of this study, *T. asperellum* strain evaluated was compatible with chili bell pepper and garlic extracts, *Cinnamomun verum* extract and the isotianil compound in both mycelial growth and conidial germination. They could therefore be used with this fungus in integrated pest management in the region. Azoxystrobin showed a notable reduction in conidial germination; however, it was slightly toxic to *T. asperellum* in relation to the percentage of mycelial inhibition. In addition, the herbicide glyphosate and the fungicides mancozeb, difenoconazole and fenpropimorph at recommended field rates was toxic to *T. asperellum*.

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Author's contributions: C.A. Dodino-Gutiérrez: Methodology, research, formal analysis, writing original paper, review and editing paper. J. Rodríguez-Escobar: Methodology, formal analysis, editing paper, funding adquisition.

Open data not available.

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