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Toxicity of contact fungicides to four *Trichoderma* species: an *in vitro* compatibility approach

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ABSTRACT

Objective/Background. The transition towards responsible agricultural practices is essential to promote the health of agroecosystems and ensure food security. Promoting comprehensive research that combines chemical and biological methods represents a significant advance in the management of phytopathogens. This novel approach is based on the premise that the joint action between fungicides and an antagonistic agent such as *Trichoderma* spp. can offer robust protection compared to individual approaches. The objective of the study is to investigate the *in vitro* resistance and compatibility of four *Trichoderma* species against three fungicides widely used in Mexico.

Materials and Methods. The controlled poisoning technique was used in PDA medium under controlled conditions with three concentrations (450, 900 and 1350 mg L^{-1}) for the active ingredients Captan and Chlorothalonil, while for Mancozeb 600, 1200 and 1800 mg L^{-1} were used. Compatibility was determined in relation to the control group using the statistical software SPSS Statistics version 26 for the Windows operating environment.

Results. The study revealed that the strains of *T. harzianum*, *T. hamatum*, *T. koningiopsis* and *T. asperellum* exhibited an overall compatibility of 60.04% for the active ingredients evaluated, with the fungicide Captan 50® showing the highest percentage of compatibility (79.87%) at concentrations of 450, 900 and 1350 mg L^{-1}. *T. harzianum* showed greater tolerance to the active ingredient Chlorothalonil

at a concentration of 450 mg L^{-1}, however, at higher concentrations it showed greater toxicity, with *T. koningiopsis* exhibiting the lowest resistance at its three tested concentrations.

Conclusion. Treatments with different concentrations of the fungicides Captan, Mancozeb and Chlorothalonil showed a marked variability in terms of prevalence and toxicity towards the tested *Trichoderma* species *in vitro*. This approach allows the design of integrated management strategies minimizing the dependence on chemical products and promoting compatibility between biological agents and fungicides.

Keywords: Fungi, phytopathogens, toxicity, resistance.

Introduction

In modern agriculture, controlling pathogens associated with crop diseases faces significant challenges due to the frequent and widespread use of industrial inputs, commonly known as agrotoxins (Hensh *et al*., 2022). The use of these inputs has risen sharply in recent years to boost the productivity of agricultural systems (Zhang *et al*., 2018; Balaska *et al*., 2023). However, overuse has resulted in the development of resistance, diminishing their effectiveness and raising serious environmental concerns (Lamichhane *et al*., 2018; Sharma *et al*., 2019; Andrade-Hoyos *et al*., 2023).

In Mexico, the introduction of Green Revolution technologies during the second half of the 20th century brought conventional farming practices along with the extensive use of fertilizers and pesticides for crop production (Rivera *et al*., 2022). It is estimated that Mexican farmers use around 900 pesticides across various crops.

Some of these pesticides are classified as highly hazardous according to the list published by Pesticide Action Network International (PAN, 2016), causing significant ecological harm and adverse health effects on local populations and consumers (Muhie *et al*., 2022; Rosas-Sánchez *et al*., 2023). Available data indicate that regions in Mexico with the highest pesticide usage include Sinaloa, Chiapas, Veracruz, Jalisco, Nayarit, Colima, Sonora, Baja California, Tamaulipas, Michoacán, Tabasco, Estado de México, Oaxaca, and Puebla, where 80% of the country's total pesticides are applied. Additionally, contamination by 28 pesticides has been reported in 15 states of the Mexican Republic, according to data from the Ministry of Environment and Natural Resources (Ortiz-Hernández *et al*., 2017; SEMARNAT, 2020).

Year after year, the quantities of fungicides used fluctuate significantly, ranging from 395 to 13.16 tons, highlighting the country's heavy dependence on these

products for crop protection (García-Gutiérrez *et al*., 2012). Furthermore, no public information is available regarding the names, active ingredients, or quantities of the fungicides authorized for nationwide use (Cruz *et al*., 2022). According to García *et al*. (2018), active ingredients such as Captan, Mancozeb, and Chlorothalonil are among the most commonly applied in the country.

The search for agricultural strategies to improve the effectiveness of pathogen control in food production has led to exploring beneficial synergies between fungicides and biological antagonists like *Trichoderma* spp. (Abd *et al*., 2019). This innovative approach is based on the premise that combining fungicides with *Trichoderma* spp. can provide stronger protection against pathogens compared to using either method alone (Yao *et al*., 2023; Parraguirre *et al*., 2023). Research in this area aims to understand and optimize these interactions to develop effective and environmentally sustainable agricultural practices. This represents a significant step toward integrating chemical and biological methods in managing crop pathogens (Terrero-Yépez *et al*., 2028; Ruano-Rosa *et al*., 2018; Zin *et al*., 2020; Rodríguez *et al*., 2023).

The integration of chemical and biological methods, such as the use of the genus *Trichoderma*, represents a shift in agricultural management, emphasizing the need to balance treatment effectiveness with biodiversity preservation and agricultural sustainability. Adopting this approach allows farmers to efficiently combat various pathogens while promoting the long-term health of agroecosystems (Tyśkiewicz *et al*., 2022).

This transition toward practices that reduce the negative impacts of fungicides marks a significant step forward and establishes a foundation for a resilient and sustainable future in producing healthy food (Mishenin *et al*., 2021). In this context, exploring innovative solutions becomes essential. Accordingly, this study aimed to evaluate the resistance and *in vitro* compatibility of four *Trichoderma* species with three broad-spectrum active ingredients commonly used in Mexico.

Materials and Methods

The study was conducted in Laboratory 204 of Phytopathology at the Center for Agroecology, VAL 1 Building, Institute of Sciences, Benemérita Universidad Autónoma de Puebla, Puebla.

Biological material. The strains used were previously characterized and identified both taxonomically and molecularly (Andrade-Hoyos *et al*., 2020). For this study, *Trichoderma harzianum* (T-H4) was collected in Tetela de Ocampo, Puebla; *Trichoderma koningiopsis* (T-K11) in Guadalajara, Jalisco; and *Trichoderma*

asperellum (T-AS1) in Hidalgo, all isolated from the rhizosphere of avocado (*Persea americana*). Additionally, the strain *Trichoderma hamatum* (T-A12) was collected in Tlaltipa, Veracruz, isolated from the rhizosphere of cinnamon (*Cinnamomum verum*). The sequences are deposited in the National Center for Biotechnology Information (NCBI) database with accession numbers MK779064, MK791648, MK778890, and MK791650, respectively. They are also preserved in Laboratory 204 of Phytopathology at the Center for Agroecology, Institute of Sciences, BUAP.

Characterization of growth under standard conditions. Ten-day-old fragments, 5 mm in diameter, with active growth of *Trichoderma* strains cultured on PDA medium (Bioxon, Becton Dickinson, Mexico City, Mexico) were used. Each fragment was placed in a Petri dish (9 cm in diameter) containing 20 mL of PDA (Bioxon, Becton Dickinson, Mexico City, Mexico) and incubated at 28 ± 2 °C in darkness for 10 days. Mycelial growth was measured every 12 hours using a digital Vernier caliper (CD-6 Mitutoyo, Naucalpan de Juárez, Mexico), with the average taken from four measurements per experimental unit (4 Petri dishes). The diameter (d) was calculated using the formula: $d = 2 \times r$ (Mannerucci *et al*., 2023). The experiment was performed in duplicate using a completely randomized design, with four replicates for each treatment.

***In vitro* assay of resistance and tolerance to fungicides.** The controlled poisoning technique described by Azza *et al*. (2021) was applied, using 5 mm diameter discs with 10-day-old active mycelium from the strains T-H4 of *T. harzianum*, T-K11 of *T. koningiopsis*, T-AS1 of *T. asperellum*, and T-A12 of *T. hamatum*. PDA medium (Bioxon, Becton Dickinson, Mexico City, Mexico) was prepared according to the manufacturer's instructions and supplemented with the active ingredient from one of three protective fungicide brands (Captán 50®, Mancosol 80®, Talonil 75®) at three different concentrations, measured in mg L^{-1}: (a) low (half the recommended commercial dose), (b) recommended (the commercial dose authorized by the manufacturer), and (c) high (double the recommended commercial dose) (Table 1).

Table 1. Fungicides used at different evaluated concentrations.

Fungicidal (Commercial name)	Active ingredient	Molecular formula	Concentration (mg L^{-1})		
			Low	Recommended	High
Captán 50®	Captan	$C_9H_8Cl_3NO_2S$	450	900	1350
Mancosol 80®	Mancozeb	$C_4H_6MnN_2S_4$	600	1200	1800
Talonil 75®	Clorotalonil	$C_8Cl_4N_2$	450	900	1350

Finally, 5 mm diameter discs from 10-day-old *Trichoderma* cultures grown on PDA medium (Bioxon, Becton Dickinson, Mexico City, Mexico) were placed on 9 cm diameter Petri dishes containing 20 mL of PDA supplemented with the different fungicide treatments, in triplicate. The plates were incubated at 28 ± 2 °C with a 12-hour photoperiod for 10 days to induce sporulation (Escudero-Leyva *et al*., 2022). PDA medium prepared according to the manufacturer's instructions, without fungicide addition, served as the control group.

The evaluation ended when the mycelium in the control group for each strain completely covered the Petri dish. The percentage of mycelial growth inhibition was calculated using the formula PI = [(X − Y)/X] × 100, as proposed by Sundar *et al*. (1995), where PI = Percentage of inhibition, X = Colony diameter on the control plate, and Y = Colony diameter on the treated plate.

To assess the effect of the fungicide active ingredients at different concentrations on conidial formation capacity (CFC), the method described by Castellanos *et al*. (2015), with minor modifications, was employed. Ten-day-old plates of *Trichoderma* strains grown on media containing the different fungicides and the control group were used. For each plate, 9 mL of sterile water mixed with 1 mL of 0.01% Tween 80 was added. Tween 80 was included to reduce water surface tension and ensure a uniform distribution of conidia for precise microscopic counting. The conidial suspension was then transferred to 160 × 20 mm test tubes and adjusted to a final volume of 20 mL using sterile water in a volumetric flask. Each tube was vortexed for 30 seconds, and conidial counts (conidia mL^{-1}) were performed using a Neubauer counting chamber. Additionally, the sporulation percentage (SP) for each fungicide treatment was calculated relative to the control group.

***In vitro* compatibility assay with fungicides**. The two previously evaluated indicators were used: the percentage of mycelial growth inhibition and the percentage of sporulation at 10 days. These were calculated using the formula C = [20(CV) + 80(SP)]/100, as established by Alves *et al*. (1998), where C = Corrected value for product classification, CV = Percentage of mycelial growth inhibition of the treatment compared to the control group, and SP = Percentage of sporulation of the treatment compared to the control. The compatibility of each active ingredient was then determined based on the C values, using the scale proposed by Alves *et al*. (1998): A) Very toxic, 0–30%; B) Toxic, 31–45%; C) Moderately toxic, 46–60%; and D) Compatible, greater than 60%. This approach provides a quantitative assessment of each pesticide's compatibility with *Trichoderma* strains, enabling an objective evaluation of the observed effects.

Statistical analysis. The collected data were analyzed using a multivariate linear model with a full factorial design (MANOVA) in SPSS Statistics version 26 for

Windows. The response variables included mycelial growth diameter, sporulation rate, and percentage of mycelial growth inhibition. The fixed factors were the active ingredient, the evaluated concentrations, and the four *Trichoderma* species studied. Bartlett's test of sphericity was performed to assess the homogeneity of covariances, followed by Tukey-Kramer multiple comparison tests at a significance level of $p \leq 0.05$.

The variables percentage of mycelial growth inhibition (PI) and sporulation percentage (SP) were transformed using the angular arccosine function ($\sqrt{x} + 1$). Compatibility (C) was then analyzed using principal component analysis (PCA) and meta-analysis, conducted with Jamovi Statistics version 2.0 for Windows. Meta-analysis results were presented as mean differences with their 95% confidence intervals (CI). Heterogeneity values (I^2) and statistical significance were also reported.

Results

The results from the Type III Multivariate Analysis of Variance (MANOVA) show that the independent variables—active ingredients, *Trichoderma* spp. species, and the different concentrations of each active ingredient—have a highly significant effect ($p = 0.0001$) on the percentage growth index (PI%), colony diameter, and conidial concentration per milliliter. Furthermore, the interactions between active ingredients and species, as well as between active ingredients and concentrations, were also statistically significant. This demonstrates that the efficacy of active ingredients varies depending on the *Trichoderma* spp. species and the concentration applied. These findings highlight that the efficacy of active ingredients is not uniform but differs significantly based on the *Trichoderma* species and concentration, suggesting that certain species may exhibit resistance or sensitivity to specific chemical compounds. This is crucial for developing integrated and targeted management strategies.

The statistical model used is robust, explaining between 92.2 and 94% of the variability in the data, as reflected in the adjusted R^2 values. This indicates that the evaluated variables are key drivers of the observed response in *Trichoderma* spp. and reinforces the importance of accounting for multiple factors and their interactions in studies on the efficacy of biocontrol agents (Table 2). These results provide a strong basis for future research and practical applications in agricultural disease management. This is particularly important in the context of integrated disease management, where the careful selection of active ingredients, combined with appropriate species and concentrations, can optimize outcomes while minimizing adverse effects on the agroecosystem.

Table 2. Partial multifactorial analysis of variance (MANOVA) for the effects of active ingredients, species (*Trichoderma* spp.), and concentration on the percentage of mycelial growth inhibition (PI), diameter (mm), and conidial formation capacity (CFC).

Origin	Dependent variable	Type III sum of squares	dF	Root mean square	F	Sig.
Corrected model[x]	X_1= PI (%)	123455.289[a]	39	3165.52	64.664	<0.001
	X_2= Diameter (mm)	95923.155[b]	39	2459.56	48.886	<0.001
	X_3= CFC (Conidia mL^{-1})	245 E+05[c]	39	6.30E+23	61.986	<0.001
Active ingredients * Species * Concentration n[y]	X_1	4048.447	12	337.371	6.892	<0.001
	X_2	4.1764E+23	12	3.4803E+22	2.702	<0.001
	X_3	2270.496	12	189.208	4.768	<0.003

xDesign: Intercept + Active ingredients + Species (*Trichoderma* spp.) + Concentration n; yActive ingredients * Species (*Trichoderma* spp.) * Concentration n; Concentration n: $R^2 = 0.955$ (Adjusted $R^2 = 0.94$)a; Diameter: $R^2 = 0.95$ (Adjusted $R^2 = 0.94$)b; CFC: $R^2 = 0.941$ (Adjusted $R^2 = 0.92$)c; Significance alpha = 0.05.

A clear reduction in fungal colony diameter was observed as the concentration of active ingredients increased, particularly at high concentrations of Mancozeb (1800 mg L^{-1}) and Chlorothalonil (1350 mg L^{-1}). In terms of sporulation, the results showed that the control group allowed for a high concentration of conidia in all *Trichoderma* species, with values exceeding 1.4E+12 conidia mL^{-1} (Table 3).

The DTH0 treatment, used as the control, exhibited a larger colony diameter in *T. hamatum* compared to other treatments, while its sporulation capacity was significantly higher than that of other *Trichoderma* species under the same conditions. *T. harzianum* demonstrated a colony diameter of 22.31 mm at 1800 mg L^{-1} of Mancozeb and 10.50 mm at 1350 mg L^{-1} of Chlorothalonil.

The active ingredient Captan caused a moderate reduction in colony diameter and was less effective at low concentrations (450 mg L^{-1}) compared to Mancozeb and Chlorothalonil. However, Chlorothalonil had a pronounced negative impact on conidial formation capacity, especially in *T. hamatum* and *T. asperellum*, which reached only 1.13E+11 and 1.25E+11 conidia mL^{-1}, respectively, at a concentration of 1350 mg L^{-1}. Mancozeb at a concentration of 600 mg L^{-1} maintained higher sporulation levels compared to Chlorothalonil, though it still showed a noticeable reduction relative to the control (Table 3).

Figure 1 shows the percentage of mycelial growth inhibition (PI) in different *Trichoderma* species exposed to the active ingredients Captan, Mancozeb, and Chlorothalonil. Treatments with inhibition percentages ranging from 40% to 60% and higher mycelial growth for the different fungicide active ingredients include DTH1-3, DTK1, and DTHR1-2 for Captan at all three concentrations tested, followed by DTA4-6 and DTHR1 for Mancozeb at a concentration of 600 mg L^{-1}.

Table 3. Evaluation of four *Trichoderma* species at different fungicide concentrations under controlled conditions.

Code	Active ingredient	Species	Concentration (mg L^{-1})	Diameter±SD (mm) [x]	CFC±SD (conidial mL^{-1}) [x]
DTHR0	Water (Control)	*T. harzianum*	-	90.00±0.01^{a}	1.66E+12±3.59E+07^{a}
DTK0		*T. konigiopsis*		89.99±0.01a	1.47E+12±2.46E+07^{a}
DTH0		*T. hamatum*		88.95±0.01^{a}	1.41E+12±1.50E+07^{a}
DTA0		*T. asperellum*		89.17±0.01^{a}	1.48E+12±1.13E+07^{a}
DTHR1	Captan	*T. harzianum*	450	42.38±1.30^{cdefg}	4.76E+11±2.14E+11^{bcdef}
DTHR2			900	37.88±1.30c^{defghi}	3.49E+11±1.56E+11^{bcdefg}
DTHR3			1350	35.50±0.58^{cdefghi}	2.26E+11±3.38E+10^{cdefg}
DTK1		*T. konigiopsis*	450	43.88±9.99^{cdef}	5.48E+11±5.70E+10^{bcd}
DTK2			900	31.13±14.75^{defghij}	3.96E+11±1.34E+11 ^{bcdefg}
DTK3			1350	25.19±12.60^{hijkl}	1.85E+11±5.45E+10^{defg}
DTH01		*T. hamatum*	450	39.88±1.65^{cdefgh}	3.54E+11±3.90E+10^{bcdefg}
DTH02			900	34.75±5.04^{cdefghi}	3.24E+11±2.40E+11^{cdefg}
DTH03			1350	27.50±5.26f^{ghijk}	2.08E+11±1.04E+10^{defg}
DTA1		*T. asperellum*	450	49.50±15.22^{c}	4.15E+11±9.46E+10^{bcdefg}
DTA2			900	40.75±15.50^{cdefgh}	3.98E+11±3.07E+10^{bcdefg}
DTA3			1350	35.75±14.19^{cdefghi}	2.74E+11±1.09E+10^{cdefg}
DTHR4	Mancozeb	*T. harzianum*	600	29.75±4.31^{efghij}	1.28E+11±3.18E+10^{efg}
DTHR5			1200	26.56±4.97^{ghijkl}	9.00E+10±1.06E+10^{fg}
DTHR6			1800	22.31±1.95^{ijklm}	5.63E+10±1.30E+10^{g}
DTK4		*T. konigiopsis*	600	47.13±9.55^{cd}	3.47E+11±1.16E+11^{bcdefg}
DTK5			1200	45.81±2.12^{cde}	1.13E+11±6.29E+10^{efg}
DTK6			1800	41.44±2.88^{cdefgh}	1.13E+11±6.29E+10^{efg}
DTH4		*T. hamatum*	600	36.00±2.71^{cdefghi}	3.21E+11±7.92E+10^{defg}
DTH5			1200	34.81±5.32^{cdefghi}	1.58E+11±1.01E+11^{defg}
DTH6			1800	10.75±0.96l^{mn}	1.13E+11±6.29E+10^{efg}
DTA4		*T. asperellum*	600	66.50±12.12^{b}	2.94E+11±1.86E+11^{cdefg}
DTA5			1200	30.50±1.34^{efghij}	5.25E+10±6.12E+09^{g}
DTA6			1800	11.00±0.82^{klmn}	2.06E+10±1.55E+10^{g}
DTHR7	Chlorothalonil	*T. harzianum*	450	12.50±3.42^{klmn}	3.84E+11±8.23E+10^{bcdefg}
DTHR8			900	11.75±1.71^{klmn}	2.84E+11±1.01E+10^{cdefg}
DTHR9			1350	10.50±1.29^{lmn}	2.59E+11±9.93E+10^{cdefg}
DTK7		*T. konigiopsis*	450	17.00±2.58^{jklmn}	5.48E+11±6.06E+10 ^{bcd}
DTK8			900	14.75±2.50^{jklmn}	5.03E+11 ± 6.93E+10^{bcde}
DTK9			1350	11.50±1.91^{klmn}	4.26E+11±4.98E+10^{bcdefg}
DTH7		*T. hamatum*	450	1.50±0.58^{n}	2.00E+11±2.58E+04^{defg}
DTH8			900	1.25±0.50^{n}	2.00E+11±2.58E+04^{defg}
DTH09			1350	1.50 ± 0.58^{n}	1.13E+11 ± 6.29E+10^{efg}
DTA7		*T. asperellum*	450	12.50±1.29^{klmn}	7.39E+11 ± 1.05E+11^{b}
DTA8			900	6.50±2.38^{mn}	6.24E+11 ± 3.75E+10^{bc}
DTA9			1350	1.25±0.50^{n}	1.25E+11 ± 6.45E+10^{efg}

[x]Identical letters indicate no statistically significant differences ($p < 0.05$) between treatments. SD: standard deviation.

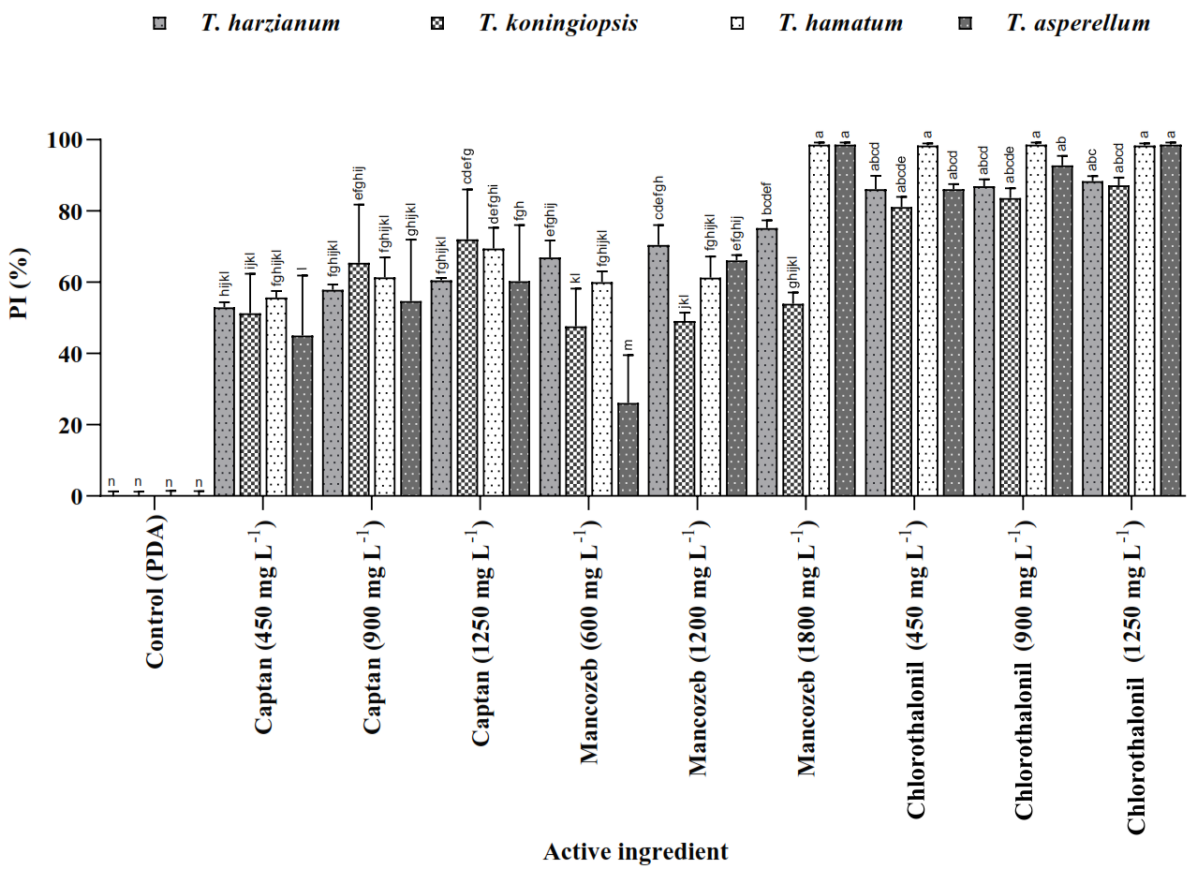

Figure 1. Percentage of mycelial growth inhibition (PI) in four *Trichoderma* species at different fungicide concentrations under controlled conditions. *Identical letters indicate no statistically significant differences ($p < 0.05$) between treatments.

These results indicate a moderate capacity for the studied phenomenon compared to the active ingredient Chlorothalonil, which demonstrated higher efficacy in inhibiting the mycelial growth of the evaluated *Trichoderma* spp. species. Chlorothalonil was effective at all three tested concentrations.

These findings underscore the critical role of fungicide concentration in regulating the percentage of inhibition (PI) in the *Trichoderma* species studied. They also highlight differences in each species' response to the various chemical ingredients of the fungicides, emphasizing the need to account for intraspecific variability in *Trichoderma* species when developing strategies for control, resistance, and compatibility as part of the transition to sustainable agricultural practices targeting phytopathogens.

In this study, the compatibility of different fungicide concentrations with native antagonists isolated from the avocado rhizosphere showed highly significant variations ($p = 0.0001$). Compatibility decreased as fungicide concentration

increased, suggesting potential differences in tolerance among the evaluated *Trichoderma* species. Principal component analysis revealed a clustering of treatments based on the compatibility and toxicity scale established by Alves *et al*. (1998) (Figure 2).

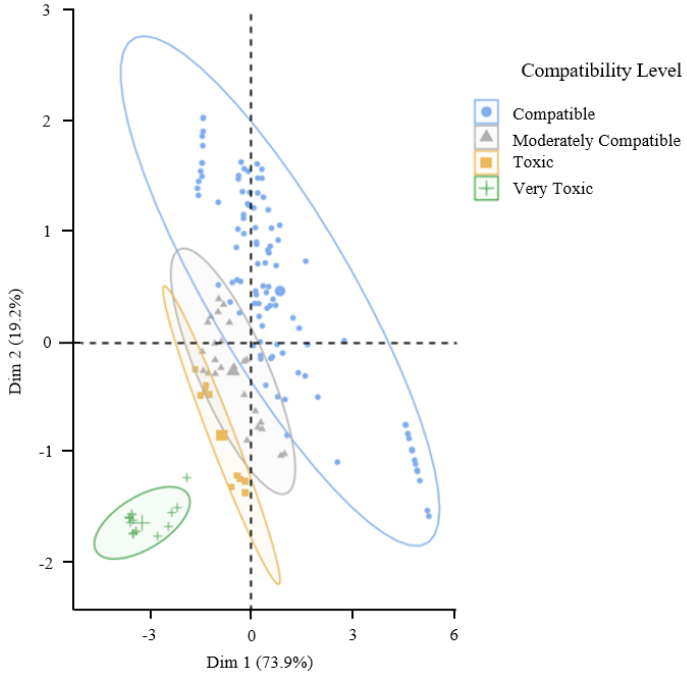

Figure 2. Principal component analysis explained 93.1% of the variance in two components regarding the compatibility of four native *Trichoderma* strains at different fungicide concentrations, according to the scale established by Alves *et al.* (1998).

This analysis revealed that 93.1% of the variability was explained by two principal components, labeled as "Dim 1 (73.9%)" and "Dim 2 (19.2%)," representing the dimensions of the principal component analysis. Approximately 65% of the treatments were classified as compatible, followed by those classified as moderately compatible (15.6%) and very toxic (13.1%). The toxic classification category had the lowest percentage distribution, accounting for only 6.3% of the analyzed cases.

A meta-analysis was also conducted, using the mean difference ($\bar{x}$) and standard deviation (SD) of the treatments (K = 40) as the outcome measure. Mean differences (T) ranged from 1.38 to 99.98, with nearly all estimates being positive (100%). The estimated average mean difference, based on the random-effects model, was $\mu\hat{} = 60.04$ (95% CI: 49.00 to 71.08). This value was significantly different from zero ($t(39) = 10.99$, $p < 0.0001$), indicating a clear trend in the results. Additionally, the

heterogeneity test revealed significant variability (Q(39) = 411015.47, $p < 0.0001$, $\tau^2 = 1184.30$, $i^2 = 100\%$), suggesting substantial differences across the studies included in the analysis. This provided a detailed understanding of the observed effects, with a 95% prediction interval (Figure 3).

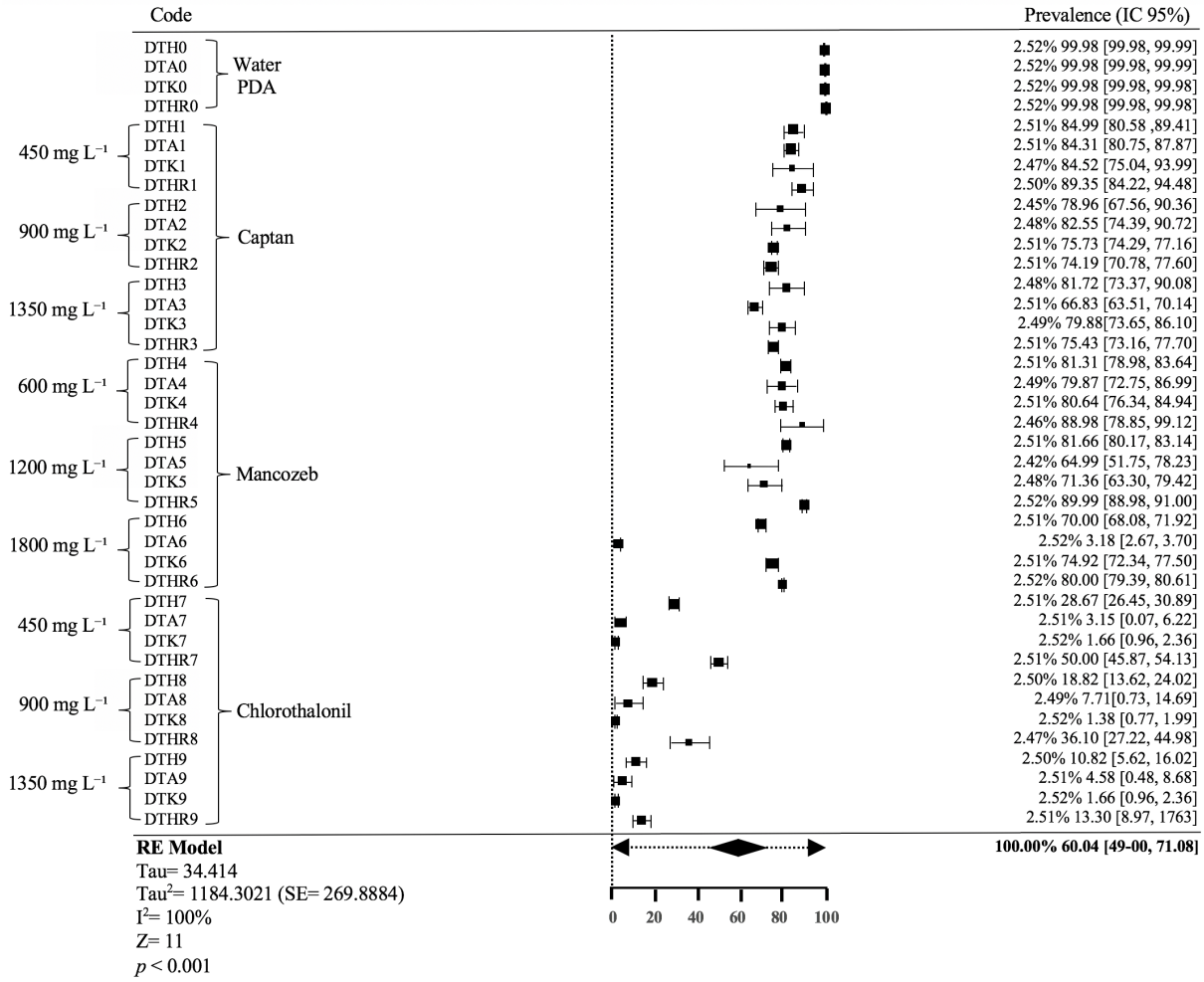

Figure 3. Forest plot representing compatibility (C%), indicated by the means and their 95% confidence intervals, associated with each combination of strain and fungicide concentration, showing relative resistance and toxicity.

The meta-analysis revealed that the strains T-H4 of *T. harzianum*, T-K11 of *T. koningiopsis*, T-AS1 of *T. asperellum*, and T-A12 of *T. hamatum* exhibited an overall compatibility of 60.04%.

Among the active ingredients evaluated, Captan (Captan 50®) at concentrations of 450, 900, and 1350 mg L^{-1} showed the highest compatibility percentage (79.87%),

compared to Mancozeb (Mancosol 80®) and Chlorothalonil (Talonil 75®), which exhibited compatibilities of 72.24% and 14.82%, respectively.

The strain T-A12 of *T. hamatum* showed the highest resistance to Captan at a concentration of 1350 mg L^{-1}, while the strain T-AS1 of *T. asperellum* displayed the lowest resistance at the same concentration. For Mancozeb, the strain T-H4 of *T. harzianum* exhibited high compatibility at concentrations of 600, 1200, and 1800 mg L^{-1}, whereas the strain T-AS1 of *T. asperellum* showed high toxicity at 1800 mg L^{-1}.

For Chlorothalonil, the strain T-H4 of *T. harzianum* exhibited moderate toxicity at 450 mg L^{-1}. However, the strains T-AS1 of *T. asperellum*, T-K11 of *T. koningiopsis*, and T-A12 of *T. hamatum* showed toxicity at all three concentrations tested. Among these, T-K11 of *T. koningiopsis* displayed the lowest resistance to this fungicide, as shown in Figure 3.

Discussion

The biological control of plant pathogens has emerged as a promising approach in plant health management. This strategy not only reduces dependence on synthetic pesticides but is also cost-effective and practical (Kumar *et al*., 2023). In agroecology, enhancing agricultural production through combined technologies, such as plant protection strategies, plays a vital role in increasing crop yield and productivity (Deguine *et al*., 2023). *Trichoderma* spp. is widely used for managing plant pathogens and is a key component of integrated phytopathogen management (Maheshwary *et al*., 2020).

Species of the genus *Trichoderma* are of significant interest due to their benefits in agriculture and natural ecosystems. They exhibit antagonistic activity against a wide range of plant pathogens, primarily fungi such as *Fusarium oxysporum*, *F. solani*, *B. cinerea*, *S. sclerotiorum*, *S. minor*, *Rhizoctonia solani*, *Phytophthora capsici*, *Phytophthora parasitica*, *Chondrostereum purpureum*, *Macrophomina phaseolina*, *Podosphaera xanthii*, *Alternaria alternata*, *Pythium aphanidermatum*, and *Pythium ultimum*, among others (Aceves *et al*., 2001; Guigón-López *et al*., 2010; Ruiz-Cisneros *et al*., 2017; Correa-Pacheco *et al*., 2018; Miguel-Ferrer *et al*., 2021; Sánchez-Montesinos *et al*., 2021).

The findings provide essential information for selecting and applying fungicides, facilitating efficient and targeted disease management. Additionally, principal component analysis and meta-analysis serve as robust tools to understand variability in treatment responses, offering valuable insights for future integrated disease management strategies in agricultural systems.

Peláez-Álvarez *et al*. (2016) reported the combined use of *T. asperellum* (T8a) and a low dose of Captan (100 mg/L^{-1}), which resulted in significant inhibition

of *C. gloeosporioides* (ATCC MYA 454) growth under *in vitro* conditions. This pathogen strain is responsible for causing anthracnose in mango crops. However, Captan is subject to specific usage restrictions in Europe due to its association with carcinogenic effects in humans, and Mancozeb is also restricted, highlighting the importance of studies like this one (Gensch *et al*., 2024).

In this study, the *T. koningiopsis* strain T-K11 demonstrated moderate compatibility with the active ingredient Mancozeb. These findings are consistent with those of González *et al*. (2020), who showed under *in vitro* conditions that adding Mancozeb to the culture medium at concentrations below 5 mg/mL does not significantly inhibit the mycelial growth of *Trichoderma*. *Trichoderma* is known to tolerate relatively high concentrations of various synthetic and natural toxic compounds, relying on a complex system of membrane pumps that facilitate efficient cellular detoxification mechanisms (Ruocco *et al*., 2009; Asad, 2022).

The compatibility of *T. harzianum* with fungicides like Mancozeb has been reported in *in vitro* evaluations on basal media, where no inhibition of radial growth was observed at 25 and 50 mg/L^{-1} after 192 and 240 hours of incubation, respectively (Ajay *et al*., 2018). Huilgol *et al*. (2022) also reported a 71.80% compatibility of *T. harzianum* with Mancozeb, which aligns with the findings of this study. Several factors contribute to the tolerance of *Trichoderma* strains to pesticides, including changes in the function of oxidoreductase genes and ABC transporter genes, which enable *Trichoderma* spp. to tolerate pesticides such as diclorvos, mancozeb, thiram, tebuconazole, and carbendazim (Hu *et al*., 2016; Hirpara *et al*., 2018; Sun *et al*., 2019).

These results, in line with previous research, highlight the potential of *Trichoderma* strains combined with the active ingredients Captan and Mancozeb for integrated disease management, contributing to the shift toward agroecosystem-friendly agricultural practices. However, as these studies were conducted *in vitro*, further research is necessary in diverse production environments under specific phytopathogen pressures. It is also essential to understand the ecology of antagonistic fungi and conduct experiments in fungicide-free environments. In this context, evaluating the tolerance or resistance of *Trichoderma* spp. to chemical fungicides is a critical step in including these fungi in biological control programs (Garman *et al*., 2006; Adnan *et al*., 2019; Alfiky *et al*., 2021; Parraguirre *et al*., 2023; Zapata-Narváez *et al*., 2023).

The incompatibility between *Trichoderma* spp. and the active ingredient Chlorothalonil poses significant challenges to implementing sustainable agricultural practices for producing healthy food (Elshahawy *et al*., 2016). This study found that the four *Trichoderma* spp. strains evaluated were incompatible with Chlorothalonil at concentrations of 450, 900, and 1350 mg/L^{-1}. These findings are consistent with the work of Gangopadhyay *et al*. (2009), which reported high toxicity of

Chlorothalonil to *T. viride*, further corroborating the present study's results by demonstrating Chlorothalonil's incompatibility with the *T. hamatum* strain T-A12, even though it is a different species.

Notably, the active ingredient Chlorothalonil is a widely used foliar fungicide worldwide. It functions as a polychlorinated aromatic compound with broad-spectrum activity, disrupting cellular respiration and ATP synthesis in fungi, which can result in cell death (Cruz *et al*., 2022). These findings emphasize the importance of understanding fungicide toxicity in biological control agents like *Trichoderma* spp., as demonstrated in the case of Chlorothalonil. Such incompatibility can hinder the development of integrated management strategies and limit farmers' ability to effectively control crop diseases. The integration of plant protection technologies continues to play a vital role in enhancing agricultural productivity (Shang *et al*., 2019; Bokade *et al*., 2021).

According to the results, it is essential to examine a broader range of fungicide concentrations (Captan, Mancozeb, and Chlorothalonil) to determine optimal doses that balance toxicity and compatibility with additional *Trichoderma* species from various geographical regions. Further investigation into mechanisms such as gene expression, enzymatic activity, and cellular physiology related to the tolerance or susceptibility of *Trichoderma* strains to fungicides could uncover specific adaptations and aid in predicting the evolution of resistance. Additionally, validating these findings under field conditions is crucial to assess factors like soil composition, native microbiota, and climatic conditions that influence the compatibility between fungicides and *Trichoderma* in complex agricultural ecosystems.

This research on the toxicity and compatibility of fungicides with *Trichoderma* spp. native to the rhizospheres of avocado (*Persea americana*) and cinnamon (*Cinnamomum verum*) is highly significant in the context of agriculture. It provides a comprehensive assessment of the interaction between biological and chemical control agents, aiming to develop agricultural practices that reduce environmental impacts while enhancing the effectiveness and selection of microbial agents for biological control.

Conclusions

Treatments with varying concentrations of the fungicides Captan, Mancozeb, and Chlorothalonil revealed marked variability in terms of prevalence and toxicity toward the evaluated *Trichoderma* species. Higher concentrations proved significantly more toxic, while lower doses allowed for greater prevalence of the evaluated organisms.

In the case of Mancozeb, a dose of 1800 mg L^{-1} was highly toxic to the *T. asperellum* strain. This contact fungicide is generally effective against a broad spectrum of fungi that infect aerial plant organs.

Captan, when applied at low concentrations, showed a variable reduction in the prevalence of the four *Trichoderma* strains, suggesting that compatibility is directly influenced by the dose used. Among the evaluated strains, *T. harzianum* exhibited the highest compatibility (89.35%), followed by *T. koningiopsis* and *T. asperellum*, particularly at a dose of 450 mg L^{-1}, where lower toxicity was observed. These findings highlight the importance of adjusting Captan doses to maximize fungicide efficacy without compromising the biological activity of *Trichoderma*, which is crucial for its integration into integrated management programs.

Regarding Chlorothalonil, the *T. harzianum* strain T-H4 exhibited greater tolerance at a concentration of 450 mg L^{-1}; however, higher concentrations (900 and 1350 mg L^{-1}) were highly toxic, classified as levels 2 and 1, respectively. In contrast, the *T. koningiopsis* strain T-K11 was identified as the most susceptible to this active ingredient.

The variability observed in the responses of the evaluated species underscores that not all have developed effective resistance or tolerance mechanisms to certain active ingredients, possibly due to specific selective pressures in their local environments. This finding reinforces the importance of comprehensively assessing the interaction between chemical fungicides and biological agents like *Trichoderma*. A deeper understanding of these interactions will enable the design of integrated management strategies that minimize reliance on chemical products and promote compatibility between biological agents and fungicides.

Conflict of interest

The authors declare no conflict of interest.

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draft preparation, A.C.C., C.A.C.P., and A.M.G.; writing—review and editing, C.P.L. and O.R.-A.; visualization, O.R.-A., C.A.C.P., and A.R.T.; supervision, O.R.-A.; project administration, O.R.-A. and C.P.L.; funding acquisition, O.R.-A. and A.R.T. All authors have read and agreed to the published version of the manuscript.

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