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Scientific Article

Evaluation of fungicides and *Trichoderma* spp. for controlling soil-borne fungal pathogens in melon crops

Avaliação de fungicidas e *Trichoderma* spp. no controle de fungos patogênicos habitantes do solo em meloeiro

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ABSTRACT - Soil-borne fungal pathogens pose an increasing challenge to melon cultivation globally. The demand for reduced agrochemical use in melon farming, driven by limitations on chemical residues in the fruit, underscores the need for alternative control strategies. This study assesses the effectiveness of various fungicides-difenoconazole, fluazinam, fludioxonil, and procymidone -and *Trichoderma* spp. strains (*T. asperellum*, *T. harzianum*, and two strains of *T. longibrachiatum*) in combatting *Ceratobasidium* sp., *Fusarium falciforme*, *Macrophomina phaseolina*, and *Monosporascus cannonballus*. Fluazinam (EC_{50} from 0.01 to 0.88 mg/L) and fludioxonil (EC_{50} from 0.01 to 0.07 mg/L) emerged as the most effective fungicides in suppressing the mycelial growth of the pathogens in vitro, whereas procymidone (EC_{50} from 2.31 to 9.77 mg/L) was the least effective. Fludioxonil demonstrated significant efficacy against *Ceratobasidium* sp., *F. falciforme*, *M. phaseolina*, and *M. cannonballus*. *In vitro* assays revealed that all tested *Trichoderma* spp. strains significantly inhibited mycelial growth, with over 70% reduction for all pathogens examined. Field trials indicated that *Trichoderma* treatments could decrease disease incidence (28.00 to 69.33%) and severity (0.95 to 2.25) in melon crops. These findings illuminate the potential of various fungicides and *Trichoderma* spp. in managing soil-borne pathogens in melon cultivation. Such control methods might be employed independently or synergistically with other strategies like grafting onto resistant rootstocks or breeding for resistance to mitigate the threats these pathogens pose to global melon production.

Keywords: Cucurbits. Biological control. Soil-born fungi. Hypocreaceae.

RESUMO - Fungos patogênicos habitantes do solo estão se tornando um dos maiores desafios para o cultivo de melão globalmente. Existe também uma necessidade para uma redução no uso de agroquímicos devido às restrições de resíduos químicos em melões. Assim, o objetivo deste estudo foi avaliar a eficiência de diferentes fungicidas (difenoconazol, fluazinam, fludioxonil e procimidona) e *Trichoderma* spp. (*T. asperellum*, *T. harzianum*, e dois isolados de *T. longibrachiatum*), no controle de *Ceratobasidium* sp., *Fusarium falciforme*, *Macrophomina phaseolina* e *Monosporascus cannonballus*. Fluazinam (EC_{50} variando de 0,01 a 0,88 mg/L) e fludioxonil (EC_{50} variando de 0,01 a 0,07 mg/L) foram os fungicidas mais eficientes, com redução do crescimento micelial, e procimidona (EC_{50} variando de 2,31 a 9,77 mg/L) foi o menos eficiente nos ensaios *in vitro*. *Ceratobasidium* sp., *F. falciforme*, *M. phaseolina* e *M. cannonballus* demonstraram alta sensibilidade a fludioxonil. Os isolados de *Trichoderma* spp. apresentaram alta eficiência no controle dos patógenos nos ensaios *in vitro*, com inibição do crescimento micelial acima de 70%. Nos experimentos em campo, os tratamentos com *Trichoderma* demonstraram um bom potencial para reduzir incidência (28,00 a 69,33%) e severidade (0,95 a 2,25) de doenças. Os resultados encontrados demonstram potencial do uso de diferentes fungicidas e *Trichoderma* spp. para controle de patógenos, *in vitro* e em campo. Estas estratégias de controle podem ser usadas isoladamente ou em combinação com outras técnicas de manejo, como enxertia e/ou melhoramento genético para resistência, no campo para manejo desses patógenos que ameaçam a produção de melão em todo o mundo.

Palavras-chave: Cucurbitáceas. Controle biológico. Fungos habitantes do solo. Hypocreaceae.

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INTRODUCTION

Melon (*Cucumis melo* L.), indigenous to tropical and subtropical regions, ranks among the globally most consumed fruit crops. Brazil stands as the 12th leading melon producer globally, with the fruit being the nation's second most exported (FAO, 2023; SALVIANO et al., 2017; SILVA JÚNIOR et al., 2020). In 2022, Brazil exported 222,355 tons of melons, generating over US$ 156 million in revenue. Notably, the northeastern region of Brazil accounts for approximately 97% of the country's melon production, predominantly in the states of Rio Grande do Norte (RN), Ceará (CE), and Bahia (BA) (IBGE, 2022).

Cucurbit species, including melons, are vulnerable to soil-borne fungi and oomycetes, leading to significant root and crown diseases. Symptoms include stem girdling, stunted growth, reduced root density, root necrosis, root rot, damping-off, vine decline, wilting, and plant collapse (AYALA-DOÑAS et al., 2020). In northeastern Brazil, the primary soil-borne pathogens in melon cultivation include *Fusarium* spp. (causing root rot and wilt) (KESH; KAUSHIK,

2021), *Macrophomina* spp. (causing black stem and root rot) (NEGREIROS et al., 2019), *Rhizoctonia solani* Kühn (causing seedling damping-off and root rot) (SALES JÚNIOR et al., 2015), and *Monosporascus cannonballus* Pollack & Uecker (causing root rot and vine decline) (CAVALCANTE et al., 2020).

Traditionally, the management of these pathogens has relied on soil disinfestation and fungicide applications. Yet, there is a growing trend towards integrated control strategies and ecological practices to reduce agrochemical reliance, driven by international market demands for minimal residue levels on fruits (AYALA-DOÑAS et al., 2020; GONZÁLEZ; ARMIJOS; GARCÉS-CLAVER, 2020). These alternatives include cultural management, biosolarization, biopesticides, and biocontrol agents, such as *Bacillus*, *Gliocladium*, *Pseudomonas*, *Purpureocillium*, and *Trichoderma*, which have shown promise in promoting sustainable growth and yield in cucurbits (AYALA-DOÑAS et al., 2020; GONZÁLEZ; ARMIJOS; GARCÉS-CLAVER, 2020).

In the melon farms of northeastern Brazil, despite the prevalent use of fungicides, there is an increasing adoption of integrated approaches, combining fungicides with crop rotation, and physical and biological controls (BELLE; FONTANA, 2018; CAVALCANTE et al., 2020; MEDEIROS; SALES JÚNIOR; MICHEREFF, 2006). Particularly, *Trichoderma* species are being explored for their disease management potential, attributed to their mycoparasitism and the production of extracellular lytic enzymes like chitinases, cellulases, and proteases (FONSÊCA NETO et al., 2016; ZHANG et al., 2018).

Given the rising prevalence of soil-borne fungal pathogens in melon cultivation in northeastern Brazil, this study aims to identify new fungicides for pathogen control and evaluate *Trichoderma* spp. for their potential to either supplement or replace chemical treatments, aligning with the move towards reduced agrochemical use in melon farming.

MATERIALS AND METHODS

Fungal strains

In the experiments, eight fungal strains were utilized, comprising four soil pathogen strains and four *Trichoderma* product strains. The soil-borne fungi were isolated from the roots of symptomatic melon plants cultivated in commercial fields in northeastern Brazil (Table 1). The pathogens underwent identification through morphological examination and Sanger sequencing, complemented by Blastn searches and phylogenetic inference analyses. The identification of *Ceratobasidium* sp. and *Monosporascus cannonballus* involved the ITS genomic region's partial sequences, utilizing ITS1 and ITS4 primers (GARDES; BRUNS, 1993). *Fusarium falciforme* (Carrion) Summerb. & Schroers, within clade 3 of the *F. solani* complex, was identified by sequencing the partial elongation factor (EF) genes using the EF1/EF2 primers (O'DONNELL et al., 2022). *M. phaseolina* (Tassi) Goid identification was based on elongation factor-1alpha (tef -1α) gene sequences (NEGREIROS et al., 2019). All isolates were prepared by hyphal tipping, except for *F. falciforme*, which was isolated as a single spore. For preservation, two methods were employed: the 'Castellani' technique and organic-sandy substrate (GONÇALVES; ALFENAS; MAFIA, 2016).

Table 1. Soil-borne fungi and *Trichoderma* species and strains used in this study.

Soil-borne fungi	Strain number	Geographic Coordinates [1]
Ceratobasidium sp.	UFERSA 001[3]	4°58'46.5"S 37°25'23.2"W
Fusarium falciforme	UFERSA 002[3]	4°59'08.9"S 37°25'14.8"W
Macrophomina phaseolina	CMM 1556[2]	4°55'14.1"S 37°19'56.0"W
Monosporascus cannonballus	UFERSA 003[3]	8°51'31.8"S 37°48'19.6"W
Trichoderma species	Strain number	Products
Trichoderma asperellum	URM 5911	Quality
Trichoderma harzianum	ESALQ 1306	Trichodermil
*Trichoderma longibrachiatum**	CMIAT 236	Supress-L
*Trichoderma longibrachiatum***	NA[4]	TrichonemateMax

[1]Melon growing areas where strains were collected from. [2]CMM = Collection of Phytopathogenic Fungi Cultures "Prof. Maria Menezes" from the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil). [3] Collection of Phytopathogenic Fungi Cultures from the Laboratory of Phytopathology II of the Universidade Federal Rural do Semi-Árido (Mossoró, RN, Brazil). [4]Information not released by the manufacturer.

The four *Trichoderma* product strains included *T. asperellum* Samuels, Lieckf & Nirenberg; *T. harzianum* Rifai; and two strains of *T. longibrachiatum* (Rehm) Chaverri, Samuels & Rocha. These strains are included in commercial products for biological control (Table 1). Before the experiments, all fungal strains were cultivated on Petri dishes containing potato dextrose agar (PDA) culture medium (Merck KGaA, Darmstadt, Germany). These cultures were then incubated at 25 °C in a dark environment for seven days.

In vitro assay of soil-borne pathogen sensitivity to fungicides

Sensitivity testing of fungal mycelia to four fungicides-difenoconazole (Score®), fluazinam (Frowncide 500 SC®), fludioxonil (Maxim®), and procymidone (Sumilex 500 WP®)

was conducted following a modified methodology outlined by Tonin et al. (2013). The assay utilized five concentrations of the active ingredients (ai) (0.01, 0.1, 1, 10, and 100 mg/L) and included a control group without fungicide. Each pathogen-treatment combination was replicated five times, and the entire experiment was conducted twice for robustness.

For the assay, 8 mm diameter discs from each fungal strain were placed at the center of five Petri dishes containing PDA medium infused with varying concentrations of the fungicides. These plates were then incubated at 28°C for seven days under a controlled 12-hour dark/light cycle, or until the growth in the control plates (without fungicide) reached full coverage. To assess fungal growth, colony diameters were measured in two perpendicular directions using a millimeter-graduated ruler. These measurements were taken at the bottom of each plate where two perpendicular lines had been drawn.

An initial ANOVA test was conducted to evaluate the consistency between the two experimental repeats and to decide if their data could be pooled. The calculation of the half-maximum effective concentration (EC_{50}) for each fungicide-pathogen pairing was based on the Percentage of Growth Inhibition (PGI). This analysis was facilitated by TableCurve 2D v.5.01 software (Systat Software, Inc., San Jose, CA, USA). In this test, equations with three $\left\{f(x,\beta) = \frac{\beta_1}{1+\beta_3 e^{\beta_2 x}}\right\}$ and four $\left\{f(x,\beta) = \beta_1 + \frac{\beta_2 - \beta_1}{1+e^{\beta_4(\log x - \beta_3)}}\right\}$ parameters, such as a logistic model based on plotting transformed probit values of fungicide concentrations and PGI (%), respectively.

In vitro assay of *Trichoderma* strain antagonism to soil-borne pathogens

The experimental procedure followed the modified Skidmore and Dickinson (1976) methodology, incorporating double confrontations between the four *Trichoderma* strains and each of the fungal pathogens. In this setup, 8 mm diameter discs from the *Trichoderma* spp. and pathogenic fungal isolates were positioned on opposite edges of Petri dishes containing PDA medium. These dishes were then incubated at 28 °C for 7 days, subjected to an automatic 12-hour dark/light cycle. For control, pathogen-only cultures were grown on PDA without *Trichoderma*. These measurements were taken at the bottom of each plate where two perpendicular lines had been drawn.

The experimental design was completely randomized, with five replicates for each treatment and the entire trial repeated thrice. Colony diameters were measured along two perpendicular lines drawn at the base of each plate. The Percentage of Growth Inhibition (PGI) was calculated using the formula: PGI = (R1 - R2 / R1) x 100, where: R1 = mycelium radius of the fungal pathogens in the control plates, R2 = mycelium radius of the fungal pathogens with the presence of the antagonist.

To assess the antagonistic activity (AA) of *Trichoderma* against the pathogens, the Korsten and Jager (1995) rating scale was employed, which categorizes inhibition as: 0 for no inhibition, 1 for 1 to 25% inhibition, 2 for 25 to 50% inhibition, 3 for 50 to 75% inhibition, and 4 for 75 to 100% inhibition. This test is based in the PGI and assesses the control level of *Trichoderma* species on each pathogen. According to Louzada et al. (2009), an AA score of 3 or less signifies effective biological control by the antagonist.

A preliminary ANOVA tested for significant differences between the three trial repetitions to determine if data could be pooled. The PGI values were statistically analyzed using Tukey's Test ($p < 0.05$) via Sisvar software v. 5.6 (FERREIRA, 2019), and the AA was categorized based on the established rating scale, providing a quantitative measure of Trichoderma's biocontrol efficacy against each pathogen.

Field evaluation of fungicides and biological control agents

The field experiments were conducted in a naturally infested area with a variety of soil-borne pathogens impacting melon crops, located at the farm *Agrícola Famosa* (04° 51'59.40"S 37°19'40.40"W), in Tibau, Rio Grande do Norte. The chosen cultivar for this study was the yellow melon 'Natal'. The experiment was set up in three repeated trials, with planting in October 2020 and harvesting in December 2020.

Soil preparation involved plowing, harrowing, and then making furrows 1.8 meters apart. Compost was incorporated at a rate of 15 tons per hectare using a rotary hoe for fertilization. Additional topdressing fertilization was applied through fertigation, tailored to the nutritional requirements of the 'Natal' cultivar. Seedlings were transplanted 12 days after sowing at a spacing of 0.35 meters, resulting in a planting density of 14,284 plants per hectare. Each treatment was separated by two border plants.

For phytosanitary control, aerial applications of insecticides were conducted weekly to manage whiteflies and leaf miners. The fruits were manually harvested 65 days post-sowing, and their number and weight were recorded to calculate field productivity in tons per hectare.

The experimental design comprised five blocks per repetition, with six treatments and 10 plant replicates per treatment, arranged in a randomized block design. *Trichoderma*-based treatments included applications of Quality® (100 g/ha) and TrichonemateMax® (2 L/ha) starting from transplanting and repeated weekly, and preventive applications of Supress-L® (5 kg/ha) and Trichodermil® (1 L/ha) one week before transplanting. Chemical control involved applications of Score® (30 mL/100L water) starting 28 days after transplanting, with additional applications at 42 and 55 days. Doses corresponded to each manufacturer's recommendation, and control treatment did not receive any fungicide or biocontrol agent.

Upon completion of the field trials, 65 days post-transplantation, the melon plants were extracted from the soil and individually bagged for transport to the laboratory, where fungal isolation procedures were undertaken. Each plant's roots were meticulously rinsed under flowing water to remove soil and debris. Disease incidence (INC) was quantified by the ratio of diseased plants to the total number of plants per treatment. Severity (SEV) of root lesions was gauged using a scale developed by Ambrósio et al. (2015), which categorizes the extent of infection: 0 for asymptomatic tissues, 1 for less than 3% tissue infection, 2 for 3-10% infection, 3 for 11-25% infection, 4 for 26-50% infection, and 5 for over 50% infection.

To isolate the fungal pathogens, seven segments of necrotic root tissue from each plant were cultured on PDA plates supplemented with streptomycin sulfate (500 mg/L),

referred to as PDAS. This process aimed to promote the growth of the fungal organisms while inhibiting bacterial contamination. The isolated fungi were then identified to the genus level. The percentage of plants infected with each fungal genus was recorded, providing a measure of the prevalence of different fungal pathogens within the treated populations.

A preliminary ANOVA test was performed to ascertain significant differences between experiments and the feasibility of combining them. INC and SEV data were analyzed using the Kruskal-Wallis's test ($p < 0.05$) (FERREIRA, 2019). Data from the replicates of all experiments were combined due to the absence of significant differences between replicates from the ANOVA test ($p > 0.05$).

RESULTS AND DISCUSSION

The results showed significant variability in the effectiveness of the studied fungicides and biological control agents.

In vitro assay of soil-borne fungal pathogen sensitivity to fungicides

Figure 1 shows the impact of varying fungicide concentrations on inhibiting the mycelial growth of soil-borne fungal pathogens. The EC_{50} values for difenoconazole ranged from 0.21 to 31 mg/L, from 0.01 to 0.88 mg/L for fluazinam, from 0.01 to 0.07 mg/L for fludioxonil, and from 2.31 to 9.77 mg/L for procymidone. Notably, fluazinam and fludioxonil emerged as the most effective fungicides, while procymidone showed lesser efficacy in suppressing mycelial growth across all tested fungal isolates. Specifically, *Ceratobasidium* sp. (0.07 mg/L), *M. phaseolina* (0.03 mg/L), *M. cannonballus* (0.01 mg/L) and *Fusarium falciforme* (0.01 mg/L) showed high sensitivity to fludioxonil, indicated by their low EC_{50} values.

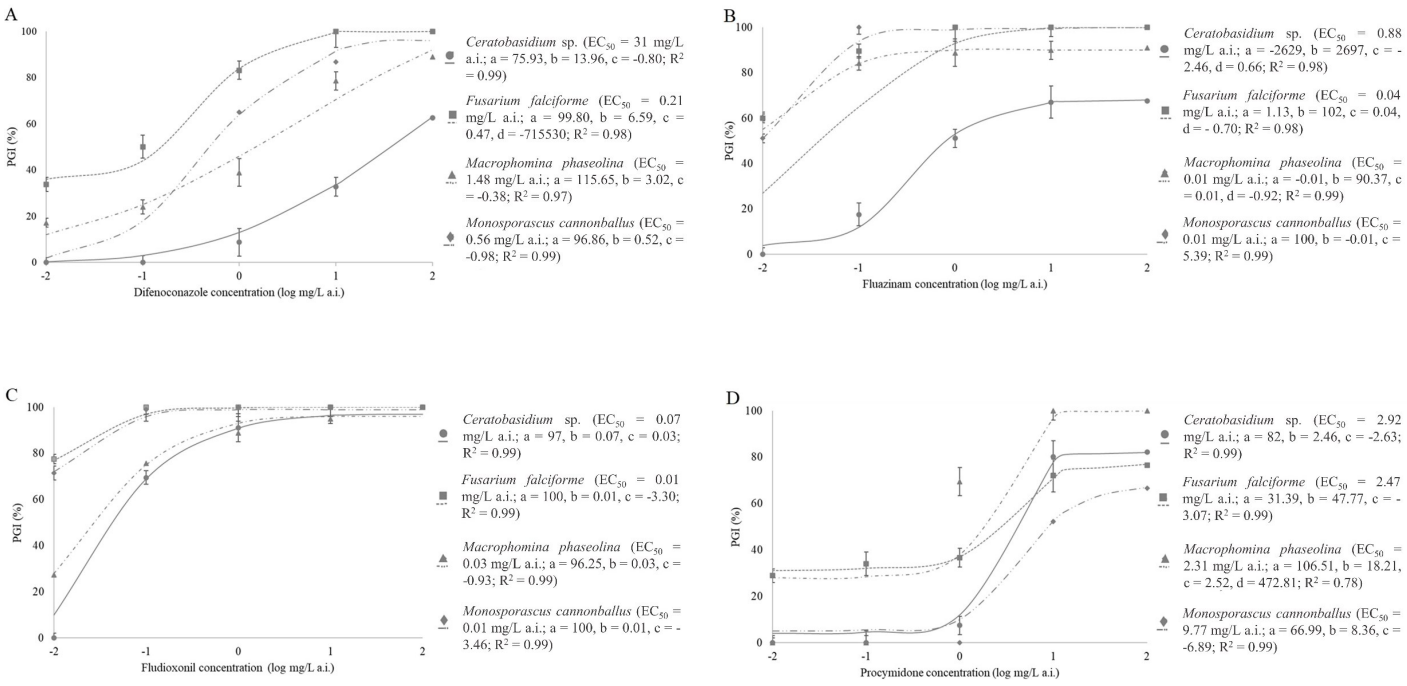

Figure 1. Regression equation, coefficient of determination (R^2), and mean effect concentration (EC_{50}) of the fungal species for (A) difenoconazole, (B) fluazinam, (C) fludioxonil, and (D) procymidone. y= adjusted with percentage mycelial growth inhibition (PGI) values at concentrations of 0.01, 0.1, 1, 10, and 100 mg/L of active ingredient by fungicide. EC_{50} = concentration calculated for 50% inhibition of mycelial growth based on the regression equation (mg/L).

Fluazinam inhibited the mycelial growth of all tested fungal isolates, with *M. phaseolina* and *M. cannonballus* being particularly sensitive to this fungicide. The EC_{50} value, a metric for efficacy, signifies the isolate's susceptibility when below 1 mg/L, indicating a specific and constant response to the active ingredient (TONIN et al., 2013). This observation aligns with Cavalcante et al. (2020), who found that only fludioxonil and fluazinam, among various tested fungicides, effectively controlled *Monosporascus* species on melon with EC_{50} values under 1 mg/L. Negreiros et al. (2020) reported that 62 *M. pseudophaseolina* isolates from northeastern Brazilian melon crops were extremely sensitive to carbendazim, with EC_{50} values ranging from 0.013 to 0.089 mg/L. The same study indicated difenoconazole's high efficacy in two of four fungal isolates, although *Ceratobasidium* sp. showed low sensitivity. Procymidone did not exhibit effectiveness against the evaluated soil-borne pathogens. Despite fluazinam and fludioxonil being unauthorized for melon cultivation in Brazil (AGROFIT, 2022), difenoconazole is approved for managing *Dydimella bryoniae* (Auersw.) Rehm in melon (AGROFIT, 2022), justifying its inclusion in field trials.

***In vitro* assay of *Trichoderma* species antagonism against soil-borne fungal pathogens**

For *T. asperellum*, a significant difference was observed, with *F. falciforme* exhibiting the highest mean PGI at 88.16 ± 1.27%, compared to *Ceratobasidium* sp. (80.42 ± 2.30%), *M. phaseolina* (81.66 ± 1.58%), and *M. cannonballus* (80.88 ± 1.37%) (Figure 2A). For *T. harzianum*, *M. phaseolina* was statistically different from the other isolates, with a higher mean PGI (87.51 ± 1.54%) compared *to F. falciforme* and *M. cannonballus* (85.93 ± 1.52% and 83.15 ± 1.81%, respectively), and *Ceratobasidium* sp. (72.09 ± 5.01%), with the lowest mean PGI. For *T. longibrachiatum**, *M. cannonballus* showed the highest mean PGI (84.09 ± 1.48%), which was statistically different from *F. falciforme* (76.66 ± 0.98%), with the lowest mean PGI. *Trichoderma longibrachiatum*** had *F. falciforme* as the isolate with the highest mean PGI (88.16 ± 1.51%), which was statistically different from *Ceratobasidium* sp. (81.14 ± 2.04%), with the lowest mean PGI.

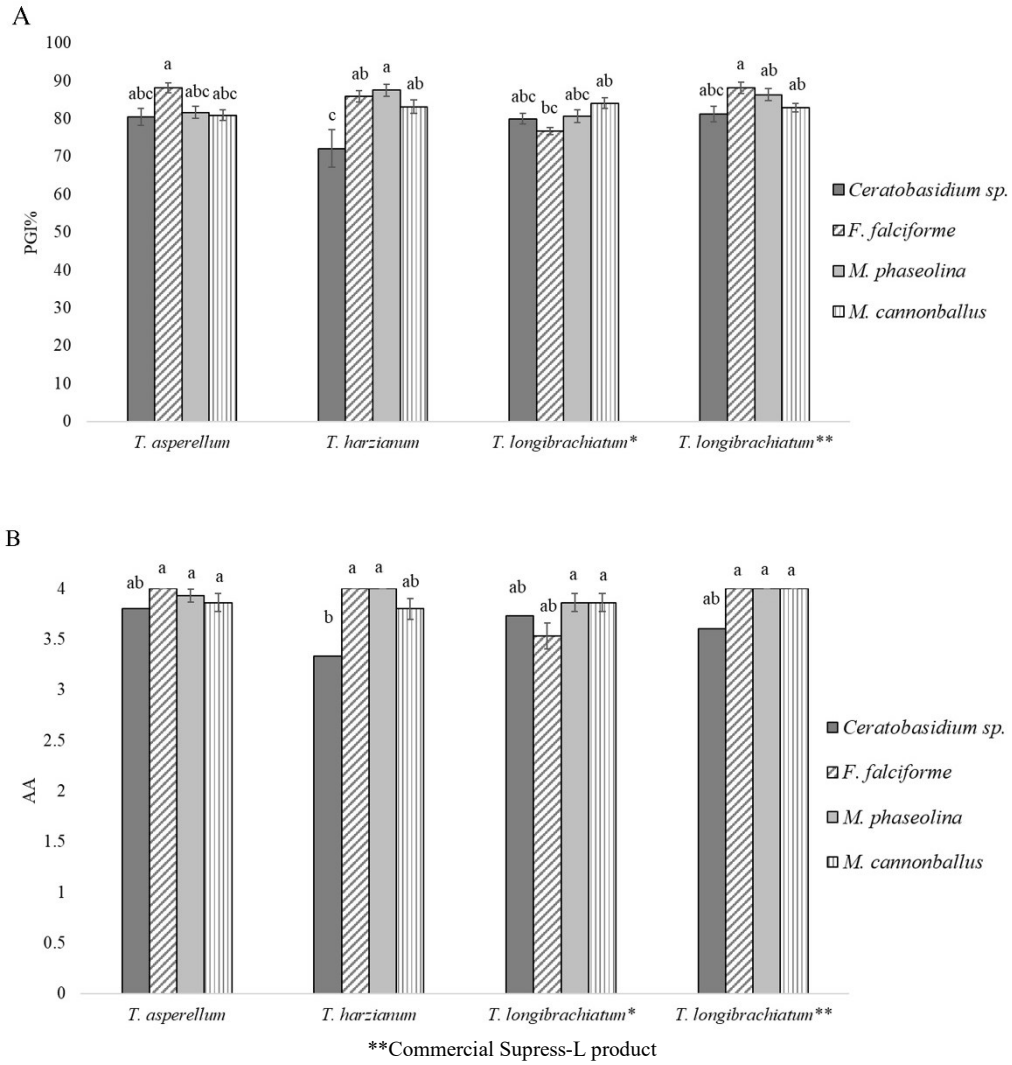

Figure 2. Percentage growth inhibition - PGI% (A) and antagonistic activity - AA (B) of *Trichoderma* spp. on soil-borne pathogens in dual confrontation experiments. Means of three experiments ± standard error, values with the same letter within column are not significantly different by the Tukey's test at 5% probability. *TrichonemateMax commercial product.

Regarding antagonist activity (Figure 2B), *T. asperellum* showed a significant difference compared to *Ceratobasium* sp. (scoring 3.80), which differed from other phytopathogenic isolates that showed no statistical differences among themselves (*F. falciforme*, 4.00; *M. phaseolina*, 3.93; and *M. cannonballus*, 3.86). For *T. harzianum*, a significant difference was noted between *F. falciforme* (4.00) and *M. phaseolina* (4.00) isolates when compared to *Ceratobasium* sp. (3.33) and *M. cannonballus* (3.80). For *T. longibrachiatum**, *M. phaseolina* (3.86) and *M. cannonballus* (3.86) were statistically different from *Ceratobasidium* sp. (3.73) and from *F. falciforme* (3.53). As for *T. longibrachiatum***, a significant difference was noted between *Ceratobasium* sp. (3.60) and other isolates (*F. falciforme*, 4.00; *M. phaseolina*, 4.00; and *M. cannonballus*, 4.00).

All *Trichoderma* species showed high efficacy in the *in vitro* assays, with above 70% inhibition of mycelial growth

for all soil-borne pathogens tested. This demonstrates the competitive capacity of antagonists in the presence of fungal pathogens (SÁNCHEZ et al., 2015). Similar *in vitro* assay results were found by Mokhtari et al. (2017). They obtained PGI values ranging from 91.5 to 99.1% and from 67.2 to 93.3%, for *R. solani* and *F. oxysporum*, respectively, when these pathogens were confronted with a *T. longibrachiatum* strain isolated from soil in Morocco. Likewise, González, Armijos and Garcés-Claver (2020) evaluated the antagonistic effect of different endophytic isolates against soil-borne fungal pathogens of melon and watermelon crops in Spain. These authors conducted *in vitro* experiments and found that *T. harzianum* and *T. lentiforme* inhibited the mycelial growth of *M. cannonballus* (93.4 and 93.3%, respectively), *M. phaseolina* (72.2 and 76.1%, respectively), *F. solani* f. sp. *cucurbitae* race1 (=*Neocosmospora cucurbitae*; 74.1 and 73.4%, respectively), *F. solani* f. sp. *cucurbitae* race 2 (=*N. petroliphila*; 70.4 and 65.1%, respectively), *F. oxysporum* f. sp. *melonis* (race 0) (67.6 and 65.6, respectively), and *F. solani* (=*N. keratoplastica*; 66.1 and 68.2%, respectively). In the same study, González, Armijos and Garcés-Claver (2020) conducted *in vivo* assays using melon seedlings (cultivar "Charentais T") to test the ability of *T. harzianum* and *T. lentiforme* to reduce symptoms and incidence of pathogens in an artificially infested soil. Both species showed good efficacy to control *F. oxysporum* f. sp. *melonis* (race 2), with symptomatology degrees of 2.00 ± 0.00 and 1.00 ± 0.00 (*T. harzianum* and *T. lentiforme*, respectively) compared to the control inoculated with only *F. oxysporum* f. sp. *melonis* (race 2) (3.33 ± 1.03).

In Brazil, Fonsêca Neto et al. (2016) demonstrated that associating *T. harzianum* with green manure (*Crotalaria juncea* L.) could reduce *F. solani* population in soils cultivated with melon. The study revealed a statistically significant difference between treatments, showing 1.34 x 10^4 CFU in asymptomatic plants, compared to 3.31 x 10^4 CFU in the control group with *F. solani*, which had symptomatic plants. In Tunisia, Rhouma et al. (2018) assessed the effectiveness of endophytic fungi isolated from the rhizosphere of cucurbits in controlling various strains of *M. cannonballus* in watermelon crops. They reported *in vitro* mycelial inhibition rates with *T. harzianum* ranging from 89.23% to 95.16% and with *T. viride* Pers. from 89.23% to 94.62%. Furthermore, their *in vivo* tests indicated that preventive treatments with *T. harzianum* and *T. viride* did not show an incidence of *M. cannonballus* strains MT3 and MT4. However, there was a 25% incidence for the MT41 strain in seedlings treated with these fungi. The incidence of damage caused by this pathogen varied from 12.5% to 25% when using *T. harzianum* and from 0% to 25% with *T. viride*.

Field evaluation of fungicides and biological control agents against soil-borne fungal pathogens

All treatments exhibited significant differences in INC ($\chi2$ = 91.45) and SEV ($\chi2$ = 128.14) compared with the control group, which had 97.33% INC and 3.88% SEV, respectively. Although there were no statistical differences in INC among the treatments - Quality (40.00%), Supress-L (48.00%), Trichodermil (50.67%), and TrichonemateMax (28%) - TrichonemateMax emerged as the most effective treatment, showing the lowest incidence rate. Notably, the highest INC value was observed in the Quality treatment (69.33%). In terms of SEV, similar patterns were observed, with TrichonemateMax demonstrating the lowest mean SEV value (0.95) (Table 2).

Table 2. Effect of biological control agents and fungicides on incidence and severity of root rot diseases caused by soil-borne fungi on melon plants (cv. 'Natal') grown in naturally infested soil.

Treatment	Incidence (%)		Severity	
	Rank[1,2]	Mean (%)[3]	Rank[1,2]	Mean[3]
Control	319.50 c	97.33 ± 16.22	358.19 c	3.88 ± 1.24
Quality	190.50 a	40.00 ± 49.32	173.98 a	1.03 ± 1.42
Score	256.50 b	69.33 ± 46.42	254.65 b	2.25 ± 1.66
Supress-L	208.50 ab	48.00 ± 50.29	206.85 ab	1.56 ± 1.85
Trichodermil	214.50 ab	50.67 ± 50.33	194.09 a	1.32 ± 1.57
TrichonemateMax	163.50 a	28.00 ± 45.20	165.25 a	0.95 ± 1.70
χ^2	91.45		128.14	

χ^2 = significant chi-square values by the nonparametric Kruskal-Wallis's test ($p < 0.05$); [1]Values followed by the same letter within the columns show no statistical difference between them. [2]Data comprise mean values of three experiments, each experiment with five replicates per treatment and one plant per replicate. [3]Mean ± standard error. *CV (%) - coefficient of variation from the Tukey's test at 5% probability.

Four genera of soil-borne fungal pathogens were isolated from the roots of all melon plants collected from the field trials: *Macrophomina* with isolation rates ranging from 6% to 36%, Rhizoctonia from 2% to 12%, Monosporascus from 8% to 14%, and Fusarium from 14% to 20%. Trichoderma was only isolated from the treated plants (Quality, Supress-L, Trichodermil, and TrichonemateMax), with isolation rates ranging from 2% to 11% (Figure 3). Additionally, several genera considered saprophytes *Aspergillus*, *Curvularia*, *Alternaria*, *Penicillium*, and *Rhizopus*-were also isolated. These are included in Figure 3 under the designation 'Others'.

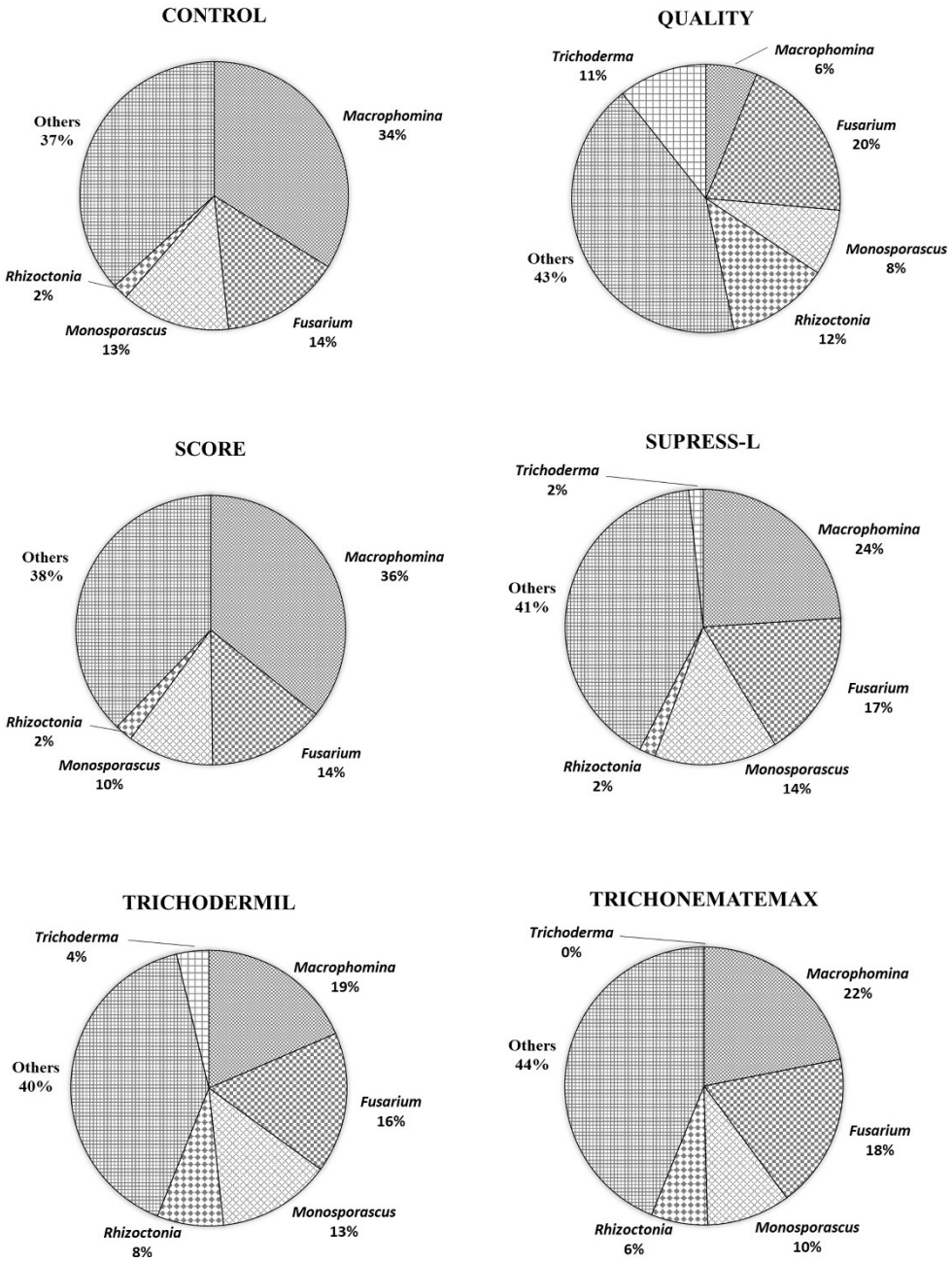

Figure 3. Isolation frequency of soil-borne fungal genera in melon roots under different field trial treatments.

In field evaluations, all genera of soil-borne pathogens were detected across all treatments, with incidence values ranging from 28% to 97% (Table 2). The high incidence observed may be attributed to the field experiments being conducted on highly naturally infested soil, where the use of plastic mulching creates ideal conditions for the proliferation of soil-borne fungi (CAVALCANTE et al., 2020). Notably, *Macrophomina* sp. and *Fusarium* sp. emerged as the predominant genera in these evaluations, possibly due to their ability to produce resistant structures that enable survival under elevated temperatures and environmental challenges (SALVIANO et al., 2017). Additionally, Sales Júnior et al. (2012) suggested that various *Macrophomina* species might persist in the environment by using weeds as alternative hosts in melon-producing regions.

Regarding disease severity, the scores varied among treatments, ranging from 0.95 to 3.88. This variability could be linked to the distinct mechanisms of action of the treatments being tested. For example, difenoconazole disrupts fungal metabolic pathways (KARTASHOV et al., 2019), while *T. asperellum* and *T. harzianum* are known for producing volatile antifungal metabolites and exhibiting mycoparasitism. Similarly, *T. longibrachiatum* is recognized for its antibiotic production in the melon rhizosphere

(ZHANG et al., 2018). Overall, the *Trichoderma*-based treatments demonstrated promising potential in reducing both incidence and severity of diseases caused by soil-borne pathogens in melon crops (Table 2).

CONCLUSIONS

This study clarifies the *in vitro* and field activity of various fungicides and *Trichoderma* spp. against soil-borne pathogens infecting melon. These control strategies can be implemented alone or combined with other management approaches in the field, such as grafting on resistant rootstocks and/or breeding programs. This integrated approach has the potential to effectively manage these important pathogens that threaten melon production. However, further research is necessary to optimize the integration of these different management strategies for improved disease control in intensive melon cropping systems, particularly those employed in northeastern Brazil.

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