

Antagonism potential mediated by volatile organic compounds produced by *Trichoderma* spp. on *Agroathelia rolfsii*

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ABSTRACT. A diversity of polyvalent strains of *Trichoderma* spp. has been widely employed in the biological control of plant pests and diseases, being recognized for its effectiveness by contributing sustainable alternatives to agriculture. In this study, 21 *Trichoderma* spp. isolates (wild and transformed) were analyzed with the aim of identifying those capable of producing volatile organic compounds (VOCs) with antagonistic activity against *Agroathelia rolfsii*, a phytopathogen originating from soybean. The assay was conducted in vitro using the double-plate technique, which allows microorganism interaction through a shared atmosphere without direct contact. The experimental design was completely randomized, consisting of 21 *Trichoderma* spp. isolates with three replicates (paired with one isolate of *A. rolfsii*), totaling 63 experimental units (excluding controls for the phytopathogen and *Trichoderma* spp. isolates). The plates were incubated at 27 °C with a 12-hour photoperiod. After complete colonization of the controls, colony diameters (mm) were measured using a digital caliper, and the mean percentages of mycelial growth inhibition (%) were calculated. Data were subjected to analysis of variance, and means were compared using the Scott-Knott test (1974) at a 5% significance level, with the aid of R software. Regarding the area under the curve of mycelial growth progress of *Trichoderma* spp. isolates (AUMGPC-Tri), isolates IF 114, IF 123, IF 132, and IF 135 showed the highest values, whereas the lowest values were observed for IF 113, IF 124, IF 127, IF 130, and IF 137. Concerning the apparent mycelial growth rate of *Trichoderma* spp. isolates (AUMGPC-Tri), the highest values were recorded for IF 113, IF 125, IF 126, and IF 127, while the lowest values were observed for IF 114, IF 124, IF 130, IF 131, and IF 135. With respect to the antagonistic effect itself, the lowest values of the area under the curve of mycelial growth progress of the pathogen *A. rolfsii* (AUCMG-Pat), indicative of greater pathogen inhibition, were observed for isolates IF 114, IF 119, IF 132, IF 133, IF 134, and IF 135, whereas the highest values were recorded for isolates IF 124, IF 126, IF 130, and IF 137, which were therefore considered less effective in inhibiting the growth of *A. rolfsii*. Isolates IF 113, IF 119, IF 121, IF 126, IF 127, IF 130, IF 131, IF 132, IF 133, and IF 135 more markedly reduced the mycelial expansion rate of *A. rolfsii*, whereas IF 114, IF 120, IF 122, IF 123, IF 124, IF 125, IF 128, IF 129, IF 134, IF 136, and IF 137 showed a lower effect on this variable. *Trichoderma* spp. isolates exhibited considerable variation in their ability to inhibit mycelial growth and expansion rate of *A. rolfsii*. Isolates such as IF 114, IF 119, IF 132, IF 133, IF 134, and IF 135 demonstrated greater antagonistic potential, indicating their relevance as candidates for the biocontrol of this pathogen. Thus, the results highlight that the selection of promising isolates for the biocontrol of *A. rolfsii* should jointly consider growth vigor and, most importantly, efficiency in pathogen suppression.

Key words: Biological control, mycelial growth, interaction; agricultural sustainability.

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill, Fabaceae), native to Ancient China, is one of the most important agricultural crops worldwide, both because of its wide

range of uses and its significant economic contribution. In Brazil, the world's leading soybean producer in the 2020/21 growing season, 38.5 million ha were cultivated, with a production of 135.9 million t and an average yield of 3,527 kg ha⁻¹ (Conab, 2024). Soybean grains are widely used in human and animal nutrition, as well as in the production of oil, meal, biodiesel, lubricants, and fertilizers, which ensures high market liquidity for the crop (Gaonkar & Rosentrater, 2019).

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Root and collar diseases have proven to be particularly damaging because they impair soybean development from the earliest growth stages, potentially causing significant and severe yield losses. Three soilborne pathogens are especially noteworthy in this context, as they cause damage either individually or as part of disease complexes: *Agroathelia rolfsii* (southern blight), *Macrophomina phaseolina* (charcoal rot), and *Sclerotinia sclerotiorum* (white mold) (Belkar & Gade, 2013).

The fungus *Agroathelia rolfsii* (Curzi) C.C. Tu & Kimbr. is a necrotrophic basidiomycete belonging to the order Atheliales and the family Atheliaceae (Kirk, 2021). It causes southern blight, a disease characterized by collar rot followed by complete plant desiccation. The pathogen shows optimal development between 27 and 30 °C and has a broad host range, infecting more than 500 plant species (Bulluck & Ristaino, 2002). Symptoms include sudden plant wilting, abundant white mycelial growth, and the formation of spherical sclerotia that develop on the collar and roots. These structures, approximately 1 mm in diameter, act as primary inoculum sources and can persist in the soil for more than five years (Granados & Wang, 2008). Disease development is favored by high humidity, accumulation of organic residues, and canopy closure between rows.

The genus *Trichoderma* exhibits wide diversity, with more than 468 described species, and is recognized as the most widely used group in biofungicide formulations worldwide, accounting for approximately 60% of registered products (Raymaekers et al., 2020). In Brazil, strains such as *T. afroharzianum*, *T. asperellum*, *T. harzianum*, *T. koningiopsis*, and *T. stromaticum* are commercially available, totaling 42 registered products—41 as microbiological fungicides and one as a microbiological nematicide (Agrofit, 2025). The ability of this genus to adapt to different ecological niches makes it particularly attractive for the development of new bioproducts.

In addition, *Trichoderma* spp. are recognized for promoting plant growth in rhizosphere soils, playing a crucial role in protecting plants against soilborne diseases and enhancing overall plant development (Asgar et al., 2024). These fungi are capable of inducing local and systemic defenses in associated plants through the recognition of microbe-associated molecular patterns (MAMPs) or via the action of volatile metabolites (Lee et al., 2016). Through these strategies, *Trichoderma* spp. can indirectly antagonize phytopathogens (Poveda et al., 2024).

Fungi of this genus produce a wide variety of secondary metabolites that facilitate their interactions with plants and other microorganisms. Compounds such as harzianolides, peptaibols, and various volatile organic compounds (VOCs) have been reported to possess antifungal potential, in addition to promoting

plant growth, resulting in increased plant resistance to pathogen attack (Khan et al., 2020).

In agriculture, *Trichoderma* spp. are widely used as biocontrol agents due to their effectiveness in managing several difficult-to-control plant diseases, such as root and collar diseases, which are particularly harmful because they compromise plant development from the early growth stages and may cause severe losses. Among these soilborne pathogens, *Agroathelia rolfsii* deserves special attention (Meyer et al., 2019). These biocontrol agents can induce a combination of antagonistic mechanisms, such as antibiosis involving the production of secondary metabolites with antifungal activity; mycoparasitism through the production of cell wall-degrading enzymes targeting plant pathogens; competition for nutrients or space; and the induction of plant resistance via the production and secretion of elicitor molecules that stimulate plant defenses against pathogens (Gomes et al., 2015).

Among the alternatives to conventional chemical control, the use of biological agents has shown great promise, particularly with the application of fungi of the genus *Trichoderma*. These microorganisms exhibit multiple mechanisms of action against phytopathogens, including competition for space and nutrients, mycoparasitism, induction of systemic resistance in plants, and, more recently, the production of volatile organic compounds (VOCs). Such compounds exhibit antifungal activity at a distance, acting without the need for direct contact with the pathogen (Harman, 2011; Kredics et al., 2014; Silva et al., 2022).

MATERIAL AND METHODS

The experiment was conducted at the Bioinputs Laboratory of the Instituto Federal Goiano – Campus Urutaí, using 21 matrices of axenic *Trichoderma* spp. isolates obtained from different hosts (Table 1) and belonging to the reference mycological collection of IF Goiano, Campus Urutaí, GO, Brazil.

Under a completely randomized design, 21 isolates were evaluated with three replications per combination, totaling 63 experimental units. For the assay, Petri dishes containing a universal solid culture medium for fungi, potato dextrose agar (PDA) [200 g potato/1.2 L water, 20 g dextrose, 20 g agar; recipe for 1 L of medium] (Atlas, 2010), were used. In one plate, a 5-mm-diameter mycelial disc of *Trichoderma* spp. isolates (Table 1) was placed; in another, a disc of *Agroathelia rolfsii* (originating from soybean, Urutaí, GO). The plates were overlapped so that they shared the same internal atmosphere (without direct contact), with the antagonists positioned on opposite plates (bottoms of the plates containing PDA medium). The set was sealed with plastic film to ensure effective sealing and accumulation of volatile organic

compounds (VOCs) released by the *Trichoderma* spp. isolates.

Positioning *Trichoderma* sp. on the lower plate served two purposes: (1) to facilitate the upward diffusion and release of VOCs, which tend to rise due to their low molecular weight, and (2) to avoid contamination of the *A. rolfsii* colony (pathogen).

Evaluations were performed by measuring colony diameter (mm) using a digital caliper over a seven-day period. Data were collected on days 1, 2, 3, 4, 5, 6, and 7 after inoculation (DAI).

From the integration of colony diameter measurements over the DAI, the independent variable X was represented by DAI and Y by colony diameter (subtracted from the mycelial disc diameter). The area under the mycelial growth progress curve (AUMGPC) was calculated by integrating the progress curve for each isolate (colony diameter × DAI) (Shanner & Finney, 1977). Thus, AUMGPC-Tri represented the area under the growth progress curve of *Trichoderma* spp. isolates, and AUMGPC-Pat represented the *A. rolfsii* isolate.

The apparent mycelial growth rate (AMGR) was calculated by linear regression, with DAI as X and colony diameter as Y. The slope coefficient was obtained in Excel® (procedure = slope (y;x)) and corresponded to the growth rate expressed as mycelial growth (mm day⁻¹). Thus, the apparent mycelial growth rate of *Trichoderma* spp. isolates was represented by the acronym AMGR-Tri, and that of the pathogen *Agroathelia rolfsii* by AMGR-Pat.

The plates were incubated at 27 °C with a 12-h photoperiod. The control treatment consisted of plates containing only *A. rolfsii*, without exposure to VOCs produced by *Trichoderma* spp. isolates.

Mycelial growth of the pathogen was measured using a digital caliper at the end of complete colonization in the control plates. Inhibition was calculated using the formula:

$$I = (C - T) \times 100 \quad (1)$$

where *I* represents the percentage of inhibition, *C* is the mean colony diameter in the control, and *T* is the mean diameter in plates treated with VOCs produced by *Trichoderma* spp. isolates.

Data was subjected to verification of the assumptions of homogeneity and normality for the application of ANOVA. Residual normality was assessed using the Shapiro-Wilk test, and homogeneity of variances using Bartlett's test. When assumptions were violated, the nonparametric Friedman test was applied, followed by the ranked Scott-Knott mean comparison test using the ScottKnott package (Jelihovschi et al., 2014). All statistical analyses were performed using R software, version 4.4.2 (R Core Team, 2025).

Table 1. Codes of wild and transformed *Trichoderma* spp. isolates, source substrates, and city/state of isolate origin.

Isolates	Source Substrates	Origin
IF 114	Collected from forest area (IF)	Urutaí-GO
IF 132	Common bean	Urutaí-GO
IF 136	Common bean	Urutaí-GO
IF 128	Cotton	Urutaí-GO
IF 121	Johnson grass (<i>Sorghum halepense</i>)	Urutaí-GO
IF 134	Morning glory (<i>Ipomoea</i> spp.)	Urutaí-GO
IF 129	Purple trumpet tree (<i>Handroanthus impetiginosus</i>)	Urutaí-GO
IF 133	Purple trumpet tree (<i>Handroanthus impetiginosus</i>)	Urutaí-GO
IF 120	Soybean	Urutaí-GO
IF 120	Soybean	Urutaí-GO
IF 137	Soybean	Urutaí-GO
IF 135	Soybean	Urutaí-GO
IF 122	Soybean	Urutaí-GO
IF 113	Maize	Urutaí-GO
IF 131	<i>Ficus</i> sp.	Urutaí-GO
IF 127	Native cotton (Cerrado)	Urutaí-GO
IF 130	Maize	Urutaí-GO
IF 123	Soybean seeds	Argentina
IF 125	Commercial product (Ecotrich)	Urutaí-GO
IF 126	Transformed isolates	Enzymology Laboratory, UFG/ICB
IF 124	Transformed isolates	Enzymology Laboratory, UFG/ICB

RESULTS AND DISCUSSION

Overall, all evaluated variables showed statistically significant differences among the isolates ($p < 0.05$), indicating wide functional variability among the *Trichoderma* spp. isolates evaluated (Table 2). Such variability is desirable in biocontrol agent selection programs, as it reflects different growth and antagonistic strategies.

Initially, when analyzing the intrinsic growth of *Trichoderma* spp. isolates (AUMGPC-Tri), isolates IF 114, IF 123, IF 132, and IF 135 exhibited the highest values, indicating high mycelial vigor (Table 2). These isolates formed the most efficient group in terms of medium colonization, a trait frequently associated with greater competitiveness for space and nutrients, as described for *Trichoderma* spp. isolates in different pathosystems (Rahman et al., 2009; Medrado et al., 2022). An intermediate group included isolates IF 119, IF 120, IF 121, IF 122, IF 125, IF 126, IF 128, IF 129, IF 131, IF 133, IF 134, and IF 136, whereas the lowest AUMGPC-Tri values were observed for IF 113, IF 124, IF 127, IF 130, and IF 137, characterizing these isolates as less vigorous under the assay conditions (Table 2; Figure 1).

The apparent mycelial growth rate of *Trichoderma* spp. isolates (AMGR-Tri) complements this analysis,

as it expresses the speed of mycelial expansion. The highest values were observed for IF 113, IF 125, IF 126, and IF 127, indicating fast-growing isolates, although these were not accompanied by high AUMGPC-Tri values (Table 2; Figure 1). Isolates such as IF 114, IF 124, IF 130, IF 131, and IF 135 showed the lowest AMGR-Tri values, indicating slower growth, although not always associated with low AUMGPC-Tri (Table 2; Figure 1). This behavior indicates distinct physiological strategies, in which some isolates prioritize rapid initial growth, whereas others show greater biomass accumulation over time, a pattern previously reported for *Trichoderma* spp. in comparative studies of growth and antagonism (Filizola et al., 2019; Lalhruaitluangi et al., 2022).

Regarding the antagonistic effect itself, the most relevant variable was AUMGPC-Pat, which reflects the degree of suppression of *Agroathelia rolf sii* growth by volatile organic compounds produced by *Trichoderma* spp. isolates. The lowest AUMGPC-Pat values, indicative of greater pathogen inhibition, were observed for isolates IF 114, IF 119, IF 132, IF 133, IF 134, and IF 135 (Table 2; Figure 1), corroborating literature reports indicating high antagonistic

efficiency of *Trichoderma* spp. against *Agroathelia rolf sii* in in vitro assays (Medrado et al., 2022; Montalvão et al., 2023). These isolates were classified as highly effective, as they combined vigorous intrinsic growth (high AUMGPC-Tri values) with a strong capacity to suppress the phytopathogen.

An intermediate efficacy group included isolates IF 120, IF 121, IF 122, IF 125, IF 128, IF 129, and IF 136, whereas isolates IF 124, IF 126, IF 130, and IF 137 showed the highest AUMGPC-Pat values and were therefore considered less effective in inhibiting the mycelial growth of *A. rolf sii* (Table 2; Figure 1).

Analysis of the pathogen growth rate (AMGR-Pat) reinforced this classification. Isolates IF 113, IF 119, IF 121, IF 126, IF 127, IF 130, IF 131, IF 132, IF 133, and IF 135 more strongly reduced the mycelial expansion rate of *A. rolf sii* (Table 2), a pattern similar to that reported by Kumari et al. (2024) and Montalvão et al. (2023), who observed a significant reduction in pathogen growth rate in the presence of *Trichoderma* spp. In contrast, isolates IF 114, IF 120, IF 122, IF 123, IF 124, IF 125, IF 128, IF 129, IF 134, IF 136, and IF 137 showed a lower effect on this variable (Table 2; Figure 1).

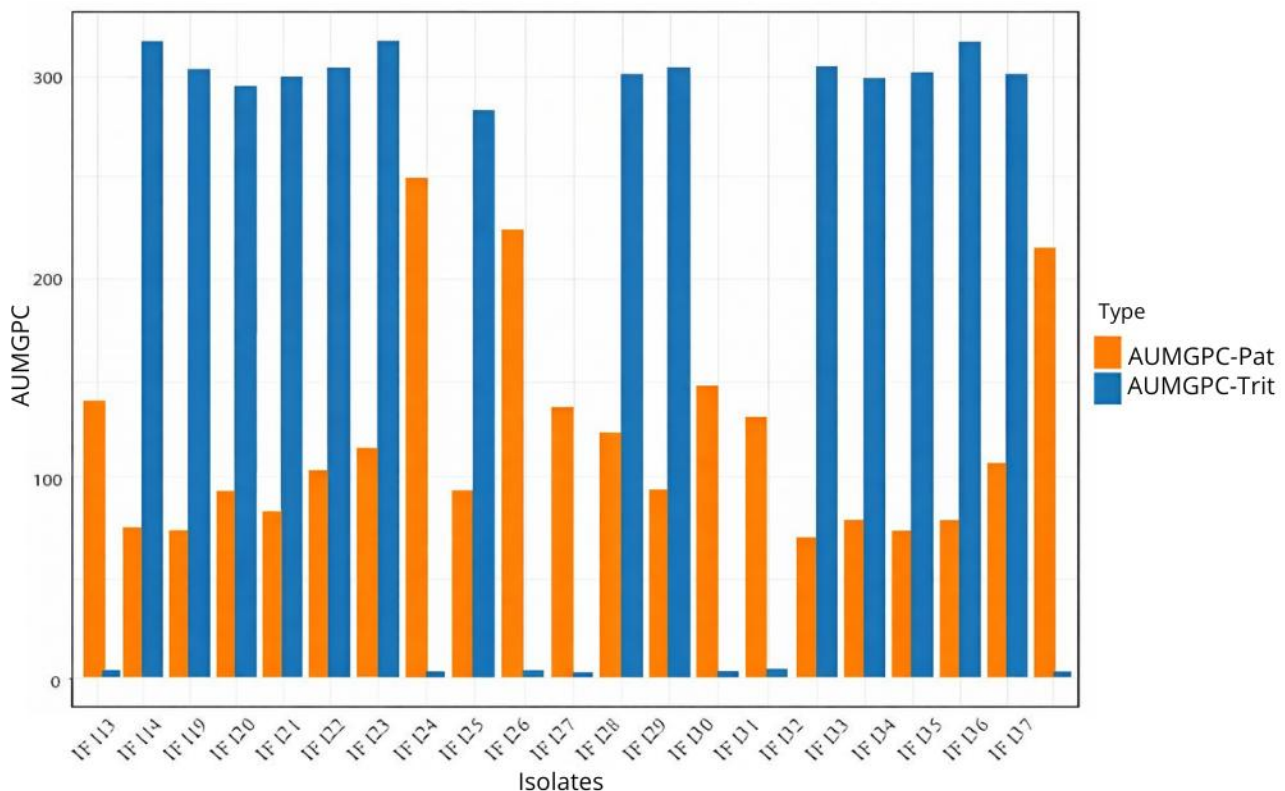


Figure 1. Mean area under the mycelial growth progress curve of *Trichoderma* spp. isolates (AUMGPC-Tri) when confronted with *Agroathelia rolf sii* (AUMGPC-Pat).

Table 2. Means \pm standard deviation of the area under the mycelial growth progress curve of *Trichoderma* spp. (AUMGPC-Tri) when confronted with *Agroathelia rolfsii* (AUMGPC-Pat), and the apparent mycelial growth rate of *Trichoderma* spp. isolates (AMGR-Tri) when confronted with *A. rolfsii* (AMGR-Pat).

<i>Trichoderma</i> spp. isolates	Average <i>Trichoderma</i> spp.		Average <i>Agroathelia rolfsii</i>	
	AUMGPC -Tri	AMGR-Tri	AUMGPC -Pat	AMGR -Pat
IF 113	3,96 \pm 0,07 d	10,42 \pm 1,39 a	154,00 \pm 16,36 b	1,00 \pm 0,59 b
IF 114	315,50 \pm 1,69 a	7,54 \pm 0,14 d	66,50 \pm 4,17 d	2,82 \pm 0,29 a
IF 119	304,00 \pm 1,26 b	8,57 \pm 0,08 c	72,00 \pm 4,50 d	2,18 \pm 0,21 b
IF 120	297,50 \pm 1,86 c	9,11 \pm 0,13 b	110,00 \pm 12,33 c	4,50 \pm 0,14 a
IF 121	301,00 \pm 2,77 b	8,79 \pm 0,31 c	105,00 \pm 21,83 c	2,25 \pm 0,71 b
IF 122	303,50 \pm 3,61 b	8,68 \pm 0,36 c	111,00 \pm 13,51 c	3,64 \pm 0,52 a
IF 123	322,00 \pm 8,13 a	6,71 \pm 0,89 c	119,00 \pm 9,58 b	2,93 \pm 0,42 a
IF 124	3,39 \pm 0,98 d	-13,33 \pm 9,69 d	201,50 \pm 66,73 a	3,90 \pm 3,06 a
IF 125	281,50 \pm 2,19 c	10,18 \pm 0,17 a	92,00 \pm 5,49 c	3,46 \pm 0,44 a
IF 126	5,89 \pm 0,87 c	73,08 \pm 1,28 a	203,00 \pm 29,74 a	1,60 \pm 0,32 b
IF 127	2,14 \pm 0,18 d	40,00 \pm 3,33 a	160,00 \pm 22,09 b	3,30 \pm 0,75 b
IF 128	303,00 \pm 1,42 b	8,79 \pm 0,08 c	126,00 \pm 2,17 b	4,93 \pm 0,35 a
IF 129	306,50 \pm 2,17 b	8,61 \pm 0,15 c	87,00 \pm 6,42 c	2,86 \pm 0,57 a
IF 130	3,14 \pm 0,07 d	1,59 \pm 1,83 d	140,50 \pm 12,65 a	0,80 \pm 0,15 b
IF 131	4,29 \pm 0,48 c	0,00 \pm 2,40 d	131,00 \pm 1,09 b	2,10 \pm 0,39 b
IF 132	306,00 \pm 0,67 a	8,29 \pm 0,05 c	67,50 \pm 3,00 d	1,75 \pm 0,33 b
IF 133	301,00 \pm 4,48 b	9,07 \pm 0,34 b	71,00 \pm 8,33 d	1,71 \pm 0,83 b
IF 134	301,50 \pm 0,58 b	9,18 \pm 0,06 b	80,50 \pm 8,33 d	3,71 \pm 1,01 a
IF 135	316,50 \pm 1,04 a	7,32 \pm 0,05 d	64,00 \pm 17,30 d	0,86 \pm 0,65 b
IF 136	300,50 \pm 1,74 b	8,89 \pm 0,17 b	122,00 \pm 7,84 b	5,82 \pm 1,11 a
IF 137	2,68 \pm 0,47 d	25,00 \pm 8,48 b	185,00 \pm 35,67 a	5,40 \pm 3,57 a
P valour	<0,05	<0,05	<0,05	<0,05
C.V. (%)	2,14	43,9	30,06	64,95

* Means followed by the same lowercase letter within a column do not differ from each other according to the ranked Scott-Knott test at $P \leq 0.05$.

The superior performance of the most effective isolates can be explained by mechanisms well described in the literature. *Trichoderma* spp. isolates with greater antagonistic capacity often produce higher amounts and greater diversity of volatile organic compounds, such as alcohols, ketones, and terpenes, which are capable of inhibiting mycelial growth and the formation of pathogen survival structures, as demonstrated by Ruangwong et al. (2021) and Silva et al. (2022). In addition, secondary metabolites such as gliotoxin, described for *Trichoderma virens*, have been associated with strong suppression of *Agroathelia rolfsii*, acting directly on mycelial growth and pathogen viability (Hua et al., 2021).

The combination of vigorous mycelial growth and high production of antifungal metabolites explains the superior performance of isolates IF 114, IF 132, IF 133, IF 134, and IF 135 compared with the others (Table 2; Figure 1). In contrast, less effective isolates may exhibit limitations in the production or qualitative profile of volatile compounds, or may prioritize intrinsic growth at the expense of antagonist metabolite synthesis. Overall, the results indicate that the selection of

promising isolates for the biocontrol of *A. rolfsii* should jointly consider growth vigor and, most importantly, efficiency in pathogen suppression.

CONCLUSION

Trichoderma spp. isolates exhibited marked variation in their ability to inhibit mycelial growth and reduce the expansion rate of *A. rolfsii*. Isolates such as IF 114, IF 119, IF 132, IF 133, IF 134, and IF 135 showed greater antagonistic potential, indicating their relevance as promising candidates for the biocontrol of this pathogen.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to conducting this study. The research was conducted independently, without the influence of commercial, financial, or institutional interests that impeded the design of the study, the conduct of the experiments, the analysis and interpretation of the data, or the writing of the manuscript. All authors participated in the preparation of the work and

approved the final version of the article for publication.

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