Research article

Effect of Culture Medium Composition on the Antagonistic Capacity of Commercial Basidiomycetes against *Fusarium spp.* and *Alternaria alternata*

Efecto de la composición del medio de cultivo sobre la capacidad antagónica de los basidiomicetos comerciales contra *Fusarium spp.* y *Alternaria alternata*

Jeny Paola Pérez Holguín[1], Juan Carlos Quitian[2], Alexander Puentes Santos[3], Astrid Maribel Aguilera Becerra[4], Ingrid Rocio Fonseca-Guerra[5]

Resumen

Objetivo. Evaluar la capacidad antagónica de basidiomicetos frente a fitopatógenos relevantes in vitro, con el fin de identificar cepas con potencial antagónico. **Métodos.** En este estudio se realizaron ensayos de antagonismo en ocho medios de cultivo que diferían en fuentes de carbono y nitrógeno, sales minerales y vitaminas. **Resultados.** Las observaciones cualitativas y cuantitativas demostraron antibiosis por parte de H. erinaceus y T. versicolor, los cuales exhibieron un marcado micoparasitismo contra todas las especies fitopatógenas evaluadas, mientras que Ganoderma mostró un antagonismo in vitro limitado. **Conclusión.** Estos hallazgos destacan la utilidad de los ensayos de antagonismo como herramienta de bioprospección para la selección e identificación de basidiomicetos con potencial de control frente a hongos fitopatógenos.

Palabras clave: composición del medio de cultivo; actividad antifúngica; antagonismo en cultivo dual; control biológico; hongos

1. Bacteriología, Universidad de Boyacá.
ORCID: https://orcid.org/0009-0006-4758-3241

2. Research Group Química de Hongos Macromicetos, Universidad Nacional de Colombia.
ORCID: https://orcid.org/0009-0008-4824-9530

3. Research Group GRIBAC, Universidad de Boyacá.
ORCID: https://orcid.org/0000-0003-2109-343X

4. Research Group Gestión Ambiental, Universidad de Boyacá.
ORCID: https://orcid.org/0000-0003-2892-6916

5. Research Group Gestión Ambiental, Universidad de Boyacá.
ORCID: https://orcid.org/0000-0002-1037-4984

Correspondencia: irfonseca@uniboyaca.edu.co

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Abstract

Objective. To evaluate the antagonistic capacity of basidiomycetes against relevant phytopathogens in vitro, in search of strains with antagonistic capacity. **Methods.** In the present study, antagonism assays were conducted in eight culture media differing in carbon and nitrogen sources, mineral salts, and vitamins. Results. Qualitative and quantitative observations demonstrated antibiosis by *H. erinaceus* and *T. versicolor*, which exhibited marked mycoparasitism against all phytopathogenic species tested, whereas *Ganoderma* showed limited *in vitro* antagonism. **Conclusion.** These findings highlight the utility of antagonism assays as a bioprospecting tool for screening and identifying basidiomycetes with controlled potential against fungal phytopathogens.

Keywords: culture-medium composition; antifungal activity; dual-culture antagonism; biological control; mushrooms

Introduction

Phytopathogenic fungi of the genera *Fusarium* spp. and *Alternaria* spp. pose a major threat to agriculture, infecting a wide range of crops and causing substantial yield and quality losses. In addition, both genera synthesize mycotoxins that compromise the safety of food products for human consumption[1].

Fusarium spp. is notorious for inducing vascular wilt and root rot, particularly in tomato, maize, banana, and rice, where yield reductions of up to 60 % have been documented[2]. These species can survive in soil for extended periods by forming chlamydospores, which facilitate their persistence and dispersal in agricultural environments[3].

Certain *Alternaria* species, agents of late blight and pathogens of citrus, fruit trees, cereals, and several solanaceous crops, diminish photosynthetic capacity by inducing foliar lesions, leading to yield losses of up to 30%[2].

The challenges posed by these two phytopathogens demand effective and sustainable management strategies. Such strategies must integrate agronomic practices, soil-health monitoring, and the deployment of biocontrol agents or biopesticides[1]. In Colombia, biocontrol strains such as *Bacillus subtilis* have demonstrated broad antagonistic capacity against *Fusarium* sp., making biological control alternatives one of the most promising options for the country's agriculture[4].

Basidiomycetes constitute an important group of fungi responsible for organic-matter degradation, thereby recycling carbon and nitrogen and contributing to ecological balance. Interest in these organisms has grown owing to their diverse biological activities, notably antimicrobial properties. For example, *Schizophyllum commune* produces compounds that inhibit the growth of *Rhizoctonia solani, Diaporthe* sp., *Botrytis cinerea*, and *Alternaria solani*[5]. Metabolites from *Stereum ostrea* suppress *Colletotrichum miyabeanus*[6], and ganodermin from *Ganoderma lucidum* inhibits *Botrytis cinerea, Fusarium oxysporum*, and *Physalospora piricola*[7].

The production of secondary metabolites such as antibiotics depends not only on the microorganism but also on growth conditions and medium composition[8]. For instance, Jorcin et al.[9] reported that the antimicrobial activity of the basidiomycete *Ganoderma resinaceum* against *Xanthomonas vesicatoria* is influenced by culture medium, with glucose concentration being the most decisive factor. Carbon source and pH likewise affect metabolite production in endophytic fungi from *Schinus terebinthifolius*[8]. Moreover, secondary metabolite synthesis can be induced through solid state cocultivation of fungi, as demonstrated by Bertrand et al.[10], who showed—via dual confrontations and metabolomic analyses, that such interactions stimulate metabolite production.

Dual culture confrontation assays offer a rapid means of exploring antimicrobial metabolite production by revealing antagonistic capacity, manifested as mycoparasitism, competition, or antibiosis—between organisms. Antibiosis is especially relevant for identifying antimicrobial compounds, as it involves growth inhibition of other microorganisms (in this case, phytopathogenic fungi) via extracellular lytic enzymes, antibiotics, and low molecular weight compounds[11].

Hernández Ochoa et al.[12] used dual-culture screening to detect antagonistic relationships between basidiomycetes and moulds, demonstrating that culture filtrate from a *Macrolepiota* sp. strain could serve as a biocontrol alternative against the early blight agents of tomato.

Against this backdrop and considering both the potential of basidiomycetes and the limited knowledge of their interactions with phytopathogenic fungi, the present study examined the antagonistic relationships of *Hericium erinaceus, Trametes versicolor*, and *Ganoderma multipileum* against *Fusarium oxysporum, F. cerealis, F. equiseti*, and *Alternaria alternata*. Eight culture media of distinct composition were employed, and antagonism was assessed through quantitative and qualitative analyses.

Materials and Methods

Commercial strains of *Ganoderma multipileum* (50602), *Trametes versicolor* (nr), and *Hericium erinaceus* (0526) were obtained from Setas de Siecha® (Machetá, Cundinamarca, Colombia). As reference phytopathogenic fungi, strains previously isolated from quinoa crops and identified as *Fusarium equiseti* (AM0156), *F. oxysporum* (AM0155), *F. cerealis* (AM0231), and *Alternaria alternata* (AM0203) were used[13,14]. These strains belong to the Fungal and Microbial Collection of the Universidad de Boyacá (UBCHM, Tunja, Colombia). All microorganisms were stored at −80 °C and reactivated on potato dextrose agar (PDA). Dual-culture confrontation assays were performed on eight different media, whose compositions are detailed in Table 1.

Table 1. Composition of culture media used in dual culture assays

Concentration in medium g L^{-1}	PDA	YM	Malt	Czapek (CZP)	Sabouraud	YPD	SNA	AGY
Agar	15	15	15	15	15	15	20	15
Glucose	20	10	–	–	40	25	0.2	10
Sucrose	–	–	–	30	–	–	0.2	–
Starch	4	–	–	–	–	–	–	–
Malt extract	–	3	30	–	–	–	–	10
Peptone	–	5	5	–	10	2	–	–
Yeast extract	–	3	–	–	–	3	–	10
Thiamine	–	–	–	–	–	–	–	0.05
KH_2PO_4	–	–	–	1	–	1	1	0.5
$MgSO_4 \cdot 7H_2O$	–	–	–	0.5	–	0.25	0.5	0.5
$FeCl_3$	–	–	–	–	–	–	–	0.05
KCl	–	–	–	0.5	–	–	0.5	–
$NaNO_3$	–	–	–	3	–	–	–	–
$FeSO_4$	–	–	–	0.01	–	–	–	–
pH	5.6	5.6	5.0	–	5.6	7.0	–	6.5

Dual-culture confrontation tests were carried out according to Mejía and Alvarado[15]. In each Petri dish containing the medium under evaluation, a 6 mm agar plug bearing actively growing mycelium (7–14 days old on PDA at 27 °C) of the basidiomycete and the phytopathogen was placed 3 cm apart. The monocultures of each fungus on the same media served as controls. Plates were incubated at 29 ± 2 °C for 7 days, or until the control cultures had completely colonized the dish. After incubation, the percentage of radial growth inhibition (PICR) was calculated with equation[16]:

Where *Rc* is the radial growth of the control and *Rt* is the radial growth in dual culture.

Additionally, observations were made to determine whether an inhibition halo or invasive growth of either fungus occurred. The antagonism index of the basidiomycete against the pathogen was scored using the scale proposed by Agamez et al.[16] This index ranges from 0 to 4, as presented in Table 2; however, in the present study a value of 4 could not be reached because the basidiomycetes were unable to sporulate on Petri dishes within the assay period.

Table 2. Antagonism index scale employed in dual-culture assays between the antagonist (basidiomycete) and the host (pathogen). Scale proposed Agamez et al.[16]

Antagonism index	Interpretation
0	No invasion by the antagonist
1	Invasion and destruction of 25 % of the host colony
2	Invasion and destruction of 50 % of the host colony
3	Invasion and destruction of 100 % of the host colony
4	Invasion and destruction of 100 % of the host colony with sporulation of the antagonist on the host

Statistical analysis

All experiments were conducted in triplicate in separate assays. Differences in the percentage of radial growth inhibition (PICR) among culture media were assessed by analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$. When significant effects were detected, mean separation was performed with Tukey's test using Minitab 19® (Minitab LLC, State College, PA, USA).

Results

In all assay's, marked differences were observed in the interaction between the confronted fungi depending on the culture medium used. In some cases, the phytopathogen's growth was favored, whereas in others the basidiomycete predominated. Figure 1 exemplifies this behavior for the interaction between *Fusarium oxysporum* and *Trametes versicolor*.

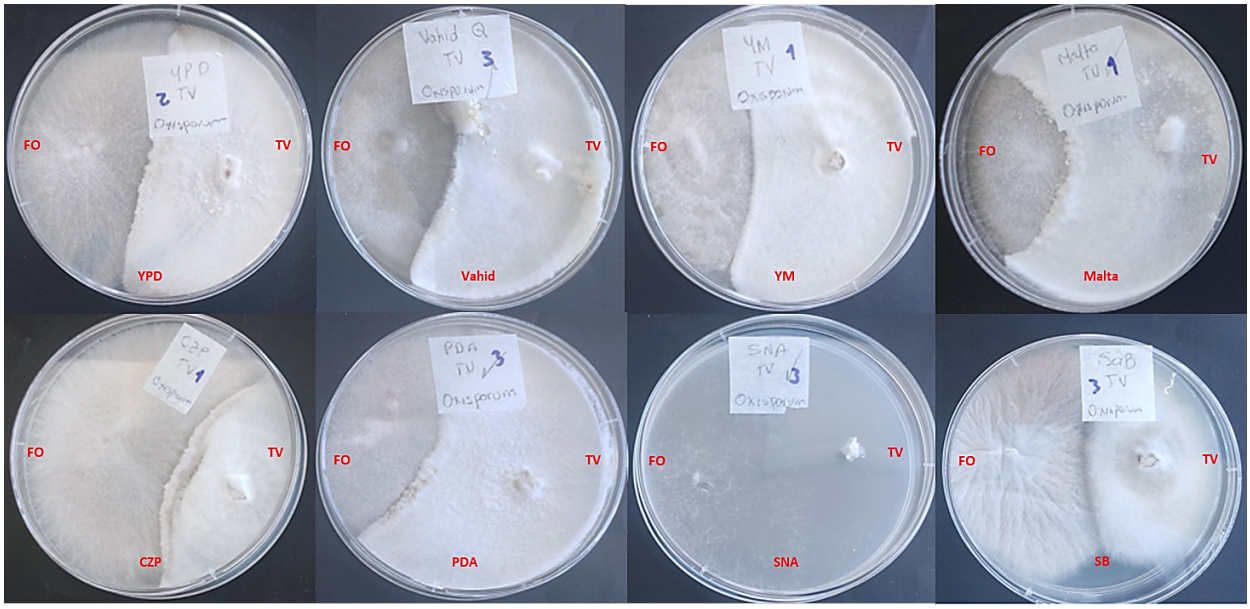

Figure 1. Dual-culture confrontation assays between *Fusarium oxysporum* (FO) and *Trametes versicolor* (TV) on YM, Sabouraud (SB), Malt, PDA, AGY, GPY, SNA, and Czapek (CZP) media, as indicated by the red lettering on the plates.

Additionally, the three anticipated interaction types—competition, mycoparasitism, and antibiosis—were observed, as illustrated by representative examples in Figure 2.

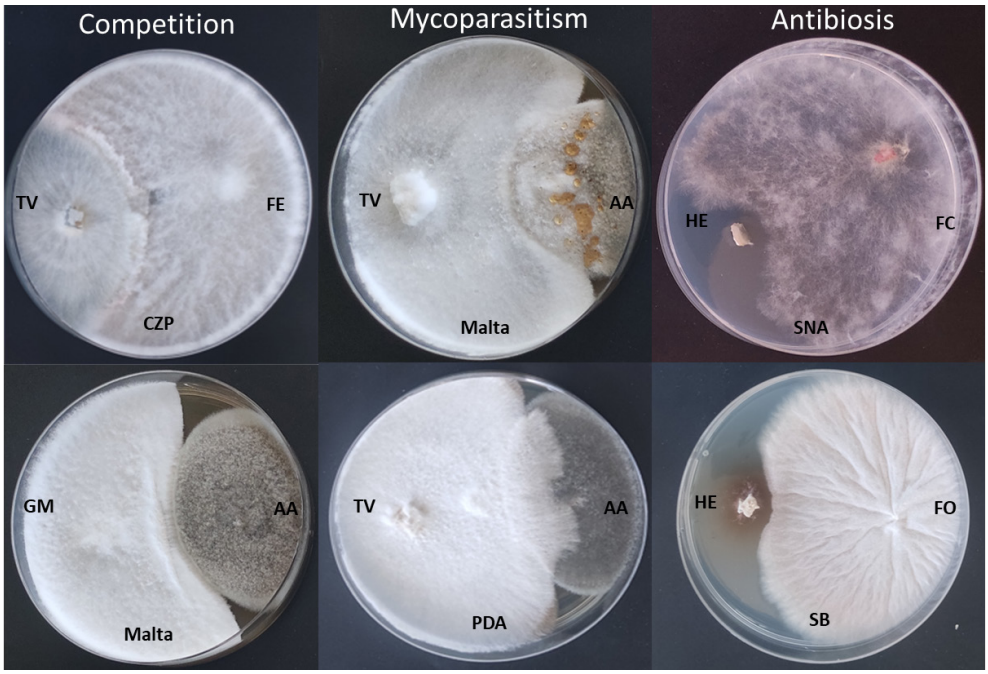

Figure 2. Interactions observed between phytopathogenic fungi and basidiomycetes—competition, mycoparasitism, and antibiosis. TV, *T. versicolor*; FE, *F. equiseti*; AA, *A. alternata*; HE, *H. erinaceus*; FC, *F. cerealis*; GM, *G. multipileum*; FO, *F. oxysporum*. Culture media: Czapek (CZP), malt, SNA, PDA, and Sabouraud (SB).

In general, confrontation with *H. erinaceus* did not result in inhibition greater than 17% in any case; however, it is important to mention that inhibition halos were observed in PDA and Sabouraud media. Regarding the comparative analysis among media, significant differences were observed, with PDA, AGY, and YM media consistently favoring inhibition in all confrontations, while no inhibition was observed in YPD. The other media showed variable effects depending on the strain but did not present significant pathogen inhibition. *H. erinaceus* exhibited moderate and more variable PIRG values, with AGY and YM being the media that least favored the growth of phytopathogens, whereas in YPD, an absolute growth of Fusarium spp. and Alternaria was observed (Figure 3).

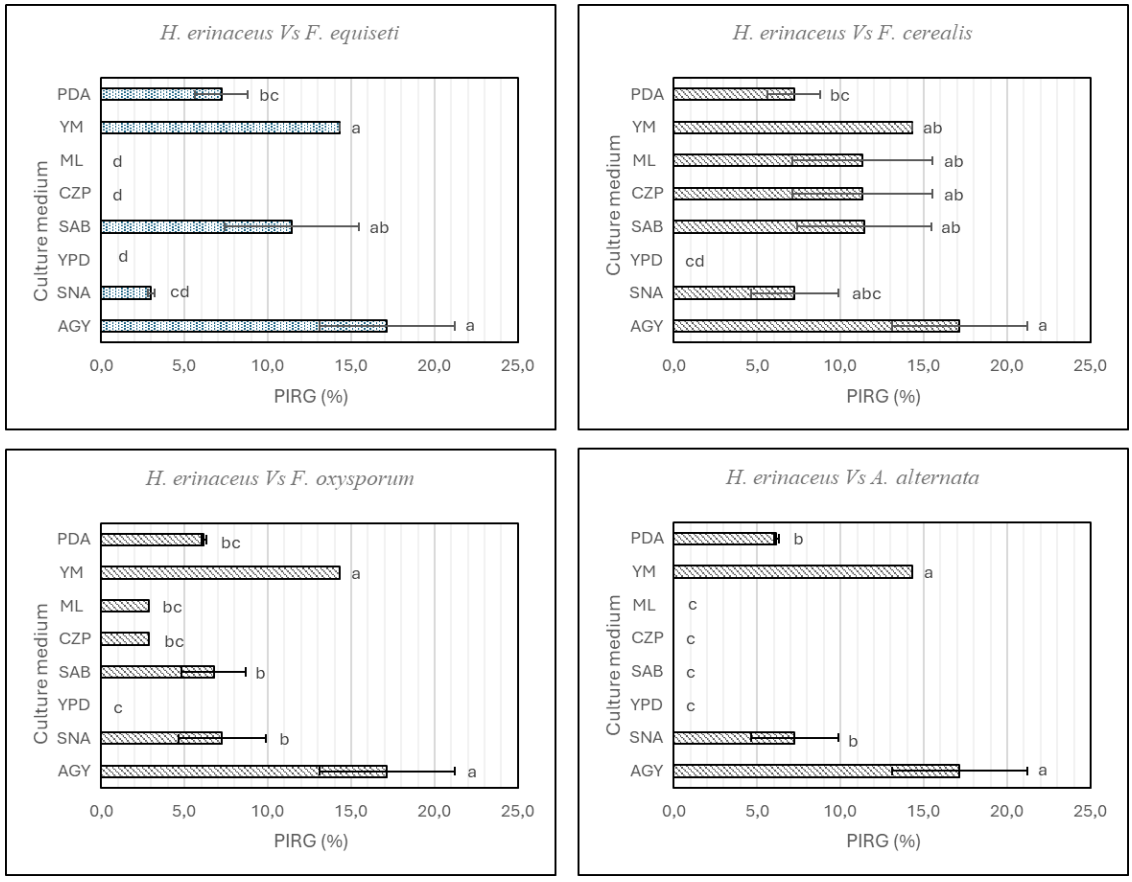

Figure 3. Analysis of variance (ANOVA) results from the antagonism assays performed with *H. erinaceus* on the eight culture media. Different letters above the bars indicate statistically significant differences according to Tukey's test ($\alpha = 0.05$). Against *F. equiseti*, significant differences were observed (ANOVA: $F = 37$, $p < 0.001$), with AGY and YM media showing the highest inhibition (a). SNA and SAB exhibited intermediate inhibition (b), PDA, ML, and CZP showed low to no inhibition (b-c), and YPD showed no inhibition (c). Against *F. cerealis*, the medium significantly influenced inhibition (ANOVA: $F = 7.28$, $p = 0.003$); AGY showed the greatest inhibition, SAB and CZP/ML showed intermediate inhibition, PDA low inhibition, and YPD and SNA showed no inhibition. Against *F. oxysporum*, differences were also significant (ANOVA: $F = 25.32$, $p < 0.001$); the highest inhibition was observed in AGY (17.14%) and YM (14.29%) (a), intermediate inhibition in SNA and SAB (b), low inhibition in PDA, ML, and CZP (b-c), and no inhibition in YPD (c). Against *A. alternata*, a marked effect of the medium was observed (ANOVA: $F = 62.28$, $p < 0.001$); AGY and YM had the highest PIRG values (a), SNA and PDA intermediate (b), and YPD, SAB, ML, and CZP showed no inhibition (group c).

T. versicolor exhibited a significant competitive effect, being the fungus that showed representative inhibition of the growth of the four phytopathogen strains, with inhibition reaching 100% in media such as PDA. In this confrontation, it was observed that the inhibitory effect was greater than 50% in almost all media; however, no antagonistic effect was observed in SNA medium (Figure 4).

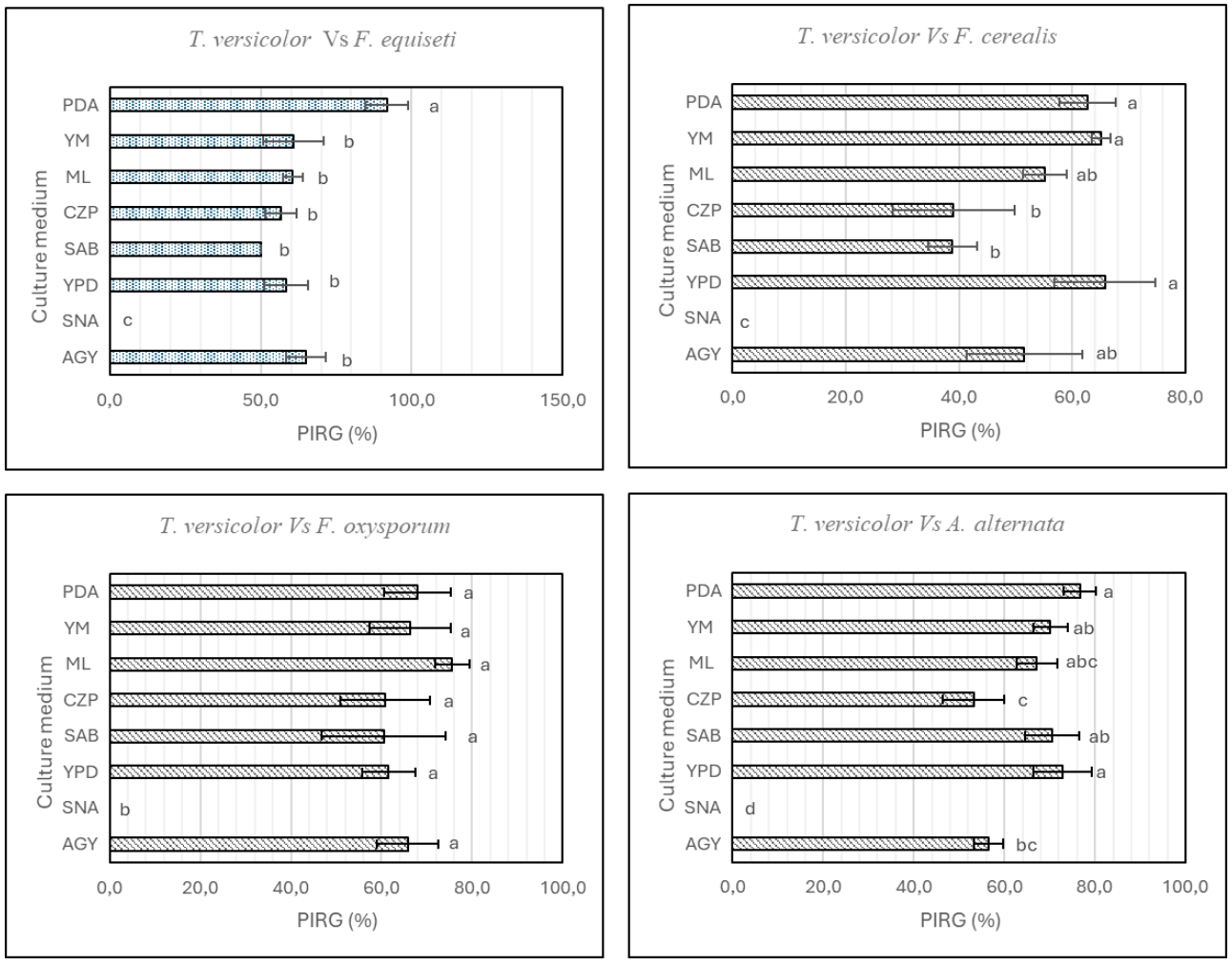

Figure 4. Analysis of variance (ANOVA) results from the antagonism assays performed with *T. versicolor* on the eight-culture media. Different letters above the bars indicate statistically significant differences according to Tukey's test ($\alpha = 0.05$). Significant differences were observed against *F. equiseti* (ANOVA: $F = 55.07$, $p < 0.001$), with PDA being the medium showing the highest inhibition (a); AGY, YM, ML, YPD, CZP, and SAB exhibited intermediate inhibition (b), while SNA showed no inhibition (c). Against *F. cerealis*, the medium significantly influenced inhibition (ANOVA: $F = 20.45$, $p < 0.001$); YPD, YM, and PDA (also ML and AGY) demonstrated the greatest inhibition (a), CZP and SAB showed intermediate inhibition (b), and SNA showed no inhibition (c). Significant differences were also found against *F. oxysporum* (ANOVA: $F = 23.87$, $p < 0.001$); ML, PDA, YM, AGY, YPD, CZP, and SAB exhibited the highest inhibition (a), whereas SNA was the only clearly inferior medium (b). Against *A. alternata*, a marked effect of the medium was observed (ANOVA: $F = 71.95$, $p < 0.001$); PDA, YPD, SAB, YM, and ML showed the highest PIRG values (a), AGY and CZP were intermediate (b), and SNA showed no inhibition (c).

In general, *G. multipileum* showed moderate to no inhibitory activity, depending on the culture medium. Resource competition was highly variable, and overall, this fungus did not exhibit colony growth of the basidiomycete overcoming that of the phytopathogen, which was particularly evident in comparison with *T. versicolor*. Therefore, the antagonistic effect of this basidiomycete was not very promising (Figure 5).

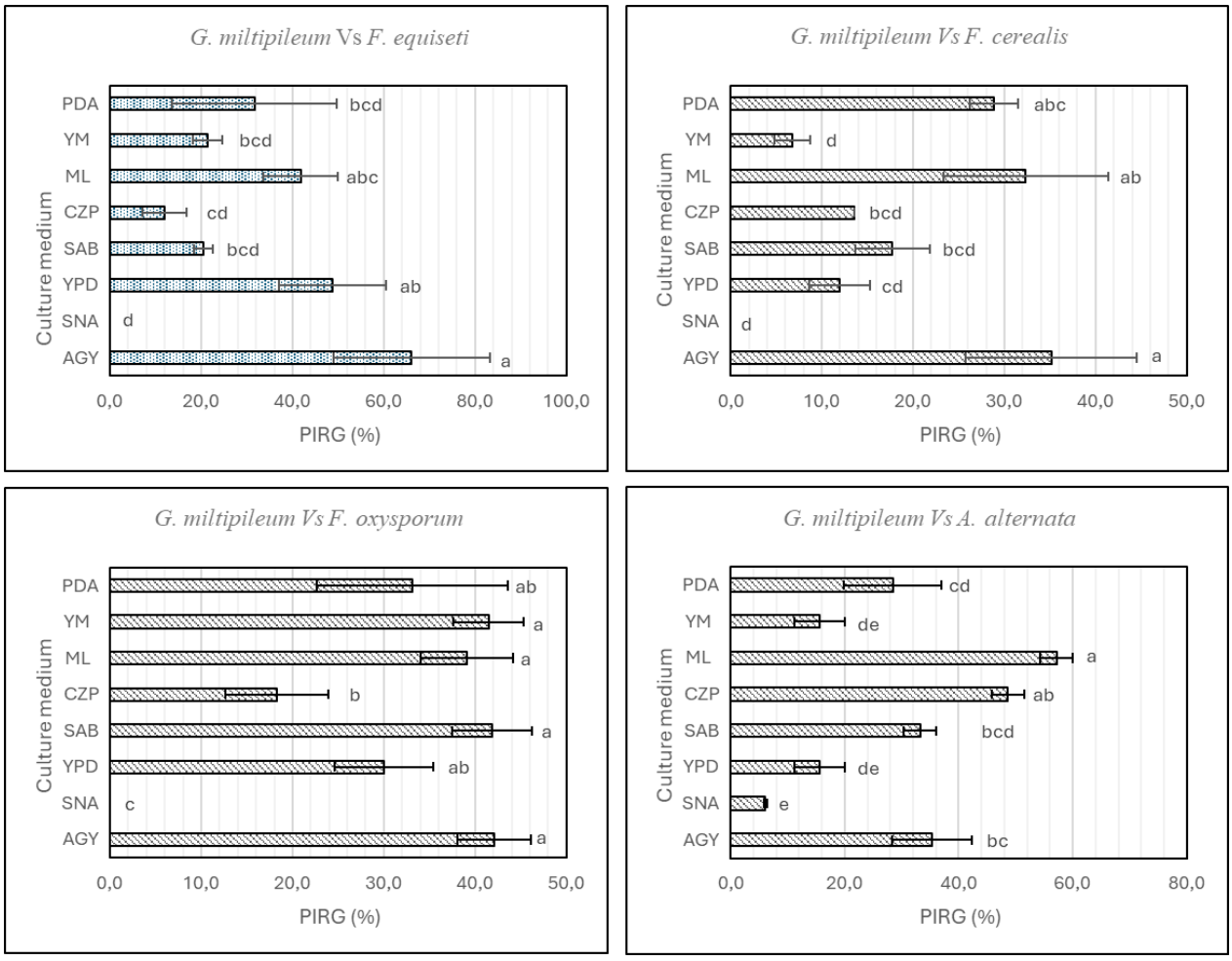

Figure 5. Analysis of variance (ANOVA) results from the antagonism assays performed with *G. multipileum* on the eight-culture media. Different letters above the bars indicate statistically significant differences according to Tukey's test ($\alpha = 0.05$). Significant differences were observed against *F. equiseti* (ANOVA: $F = 12.27$, $p < 0.001$), with AGY being the medium with the highest inhibition (a), YPD showing high to intermediate inhibition (a-b), ML intermediate inhibition (a-b-c), PDA, YM, and SAB showing low inhibition (b-c-d), CZP very low inhibition (c-d), and SNA no inhibition (d). Against F. cerealis, the medium significantly influenced inhibition (ANOVA: $F = 11.61$, $p < 0.001$), with AGY showing the greatest inhibition (a), ML in A–B, PDA in A–B–C, SAB, CZP, YPD, and YM in b-c-d, and SNA in d. Against F. oxysporum, differences were also significant (ANOVA: $F = 25.45$, $p < 0.001$), with AGY, SAB, YM, and ML forming the superior group (a), PDA and YPD intermediate (a-b), CZP low (B), and SNA showing no inhibition (c). Against *A. alternata*, a marked effect of the medium was observed (ANOVA: $F = 33.33$, $p < 0.001$), with ML showing the highest inhibition (a), CZP high to intermediate (a-b), AGY intermediate (b-c), SAB and PDA low (b-c-d), YPD and YM very low (d-e), and SNA showing no inhibition (e).

Regarding the confrontations and competition for resources, it was generally observed that *H. erinaceus* exhibited slow growth, allowing the phytopathogens to grow more extensively. *T. versicolor* induced inhibitions of up to 100%; however, this antagonistic effect was dependent on the culture medium. Overall, *G. multipileum* showed moderate inhibition without a marked antagonistic effect (table 3).

Dual culture assay	Culture medium	Mean	σ	Dual culture assay	Culture medium	Mean	σ	Dual culture assay	Culture medium	Mean	σ
H. erinaceus Vs F. equiseti	PDA	7,21	1,56	T. versicolor Vs F. equiseti	PDA	91,87	7,05	G. multipileum Vs F. equiseti	PDA	31,68	18,05
	YM	14,29	0,00		YM	60,83	10,10		YM	21,31	3,24
	ML	0,00	0,00		ML	60,65	3,22		ML	41,81	8,14
	CZP	0,00	0,00		CZP	56,63	5,25		CZP	11,98	4,82
	SAB	11,43	4,04		SAB	50,00	0,00		SAB	20,40	2,09
	YPD	0,00	0,00		YPD	58,33	7,22		YPD	48,84	11,74
	SNA	2,99	0,19		SNA	0,00	0,00		SNA	0,00	0,00
	AGY	17,14	4,04		AGY	65,00	6,61		AGY	66,11	17,10
H. erinaceus Vs F. cerealis	PDA	7,21	1,56	T. versicolor Vs F. cerealis	PDA	62,70	4,96	G. multipileum Vs F. cerealis	PDA	28,90	2,65
	YM	14,29	0,00		YM	65,07	1,63		YM	6,76	1,91
	ML	11,31	4,21		ML	55,13	3,84		ML	32,33	9,02
	CZP	11,31	4,21		CZP	39,04	10,85		CZP	13,51	0,00
	SAB	11,43	4,04		SAB	38,79	4,35		SAB	17,72	4,07
	YPD	0,00	0,00		YPD	65,75	8,95		YPD	11,97	3,37
	SNA	7,25	2,61		SNA	0,00	0,00		SNA	0,00	0,00
	AGY	17,14	4,04		AGY	51,54	10,28		AGY	35,14	9,36
H. erinaceusVs F. oxysporum	PDA	6,16	0,13	T. versicolor Vs F. oxysporum	PDA	67,95	7,40	G. multipileum Vs F. oxysporum	PDA	33,13	10,48
	YM	14,29	0,00		YM	66,25	8,98		YM	41,48	3,84
	ML	2,86	0,00		ML	75,65	3,71		ML	39,12	5,08
	CZP	2,86	0,00		CZP	60,77	9,93		CZP	18,25	5,61
	SAB	6,76	1,91		SAB	60,49	13,75		SAB	41,90	4,36
	YPD	0,00	0,00		YPD	61,52	5,85		YPD	30,01	5,43
	SNA	7,25	2,61		SNA	0,00	0,00		SNA	0,00	0,00
	AGY	17,14	4,04		AGY	65,81	6,79		AGY	42,13	4,01

Dual culture assay	Culture medium	Mean	σ	Dual culture assay	Culture medium	Mean	σ	Dual culture assay	Culture medium	Mean	σ
H. erinaceus Vs A. alternata	PDA	6,16	0,13	T. versicolor Vs A. alternata	PDA	76,71	3,53	G. multipileum Vs A. alternata	PDA	28,43	8,62
	YM	14,29	0,00		YM	70,15	3,84		YM	15,63	4,42
	ML	0,00	0,00		ML	67,17	4,48		ML	57,14	2,86
	CZP	0,00	0,00		CZP	53,23	6,82		CZP	48,57	2,86
	SAB	0,00	0,00		SAB	70,48	6,02		SAB	33,27	2,86
	YPD	0,00	0,00		YPD	72,76	6,45		YPD	15,63	4,42
	SNA	7,25	2,61		SNA	0,00	0,00		SNA	6,13	0,21
	AGY	17,14	4,04		AGY	56,51	3,30		AGY	35,35	7,00

For comparative purposes, the antagonism scale of Agamez et al.[16], was applied. Although *H. erinaceus* showed smaller mycelial colonies than all phytopathogens on every medium tested, it was the only fungus that produced inhibition halos, evidence of potential antibiosis (Table 3, Figure 2). In *T. versicolor*, a mycoparasitic effect was evident, reaching an antagonism index of 3 in the interaction with *A. alternata* on YPD medium. Overall, T. versicolor displayed antagonistic activity against the phytopathogenic fungi in most cases, and this response was dependent on the culture medium employed.

Table 3. Antagonistic interactions and antagonism indices exhibited by *H. erinaceus*, *T. versicolor* and *G. multipileum* against *Fusarium* spp. and *A. alternata* on eight culture media.

Basidiomycete	Phytopathogen	Medium	Antagonism type	Index
H. erinaceus	*F. equiseti*	PDA; Malt; Sabouraud	Antibiosis	0
H. erinaceus	*F. oxysporum*	PDA; YM; Malt; CZP; Sabouraud; YPD; SNA; AGY	Antibiosis	0
H. erinaceus	*F. cerealis*	Malt; SNA	Antibiosis	0
H. erinaceus	*A. alternata*	PDA; YM; Malt; CZP; YPD; SNA; AGY	Antibiosis	0
T. versicolor	*F. equiseti*	PDA	Mycoparasitism	3
		YM; Malt; CZP; Sabouraud; YPD; AGY	Mycoparasitism	1
T. versicolor	*F. oxysporum*	Malt	Mycoparasitism	2
		PDA; YM; CZP; Sabouraud; YPD; SNA; AGY	Mycoparasitism	1
T. versicolor	*F. cerealis*	PDA; Malt	Mycoparasitism	2
		YM; CZP; Sabouraud; YPD; AGY	Mycoparasitism	1
T. versicolor	*A. alternata*	CZP	Mycoparasitism	3
		PDA; YM; Malt	Mycoparasitism	1
		YPD; SNA; AGY	Mycoparasitism	0
G. multipileum	*F. equiseti*	Sabouraud	Mycoparasitism	2

Basidiomycete	Phytopathogen	Medium	Antagonism type	Index
		PDA; YM; Malt; YPD; AGY	Mycoparasitism	1
G. multipileum	*F. oxysporum*	PDA; YM; Malt; CZP; Sabouraud; YPD; AGY	Competition	0
G. multipileum	*F. cerealis*	PDA; YM; Malt; CZP; Sabouraud; YPD; AGY	Competition	0
G. multipileum	*A. alternata*	YM	Mycoparasitism	0
		Malt; Sabouraud; YPD; AGY	Mycoparasitism	1

Discussion

In this study, it was determined that the interactions between the four phytopathogens and the three basidiomycetes were modulated by the C/N ratio and nitrogen bioavailability, which in turn influenced the PIRG values. It has been documented that this ratio is key for the secretion of antifungal compounds and extracellular enzymes, as their expression responds to signaling from this ratio with strongly conditional regulation[17]. It was thus observed that media containing organic nitrogen (peptone/yeast extract) and multiple carbon sources (glucose ± starch/maltose) favored inhibition, whereas nitrogen-poor media or those with nitrate nitrogen (CZP) attenuated the effect, and the SNA medium—with 0.2 g L^{-1} glucose and sucrose—was broadly unfavorable. Furthermore, the antagonistic effect of *T. versicolor* was very promising, showing inhibitions greater than 50% in PDA (20 g L^{-1} glucose + 4 g L^{-1} starch; pH 5.6), YPD (25 g L^{-1} glucose, 2 g L^{-1} peptone, 3 g L^{-1} yeast extract; pH 7.0) and YM (10 g L^{-1} glucose, 3 g L^{-1} yeast extract, 3 g L^{-1} malt extract, 5 g L^{-1} peptone; pH 5.6). This is attributed to the formulations of these media simultaneously maximizing biomass growth and enzymatic secretion (e.g., ligninolytic system) and/or diffusible metabolites, which explains why PIRG values exceed this inhibition percentage in most comparisons, and why SNA—with its severe carbon and nitrogen limitation—exhibited such low inhibition.

Although all three basidiomycetes exhibited some degree of antagonistic interaction, neither the interaction pattern nor the fungal growth was uniform: significant variations were detected when the same species were confronted under different culture media.

It has previously been documented that microorganisms with biocontrol capabilities, such as bacteria of the genus Bacillus, can produce multiple biologically active metabolites effective against phytopathogenic fungi such as *Fusarium*[18]. Notably, the results indicate that *H. erinaceus* is a substantial source of secondary metabolites, as numerous interactions displayed inhibition halos. Son et al.[19] previously reported the

antagonistic capacity of *H. erinaceus*, describing the pyran alkaloid erinacean lactone and other erinacines, the latter showing strong antifungal activity against *Candida albicans* and *C. neoformans* with MIC values of 31.3 and 62.5 μg mL^{-1}, respectively. Likewise, Meneses documented metabolites from *H. erinaceus* fruiting bodies that effectively suppressed *Salmonella* sp. in vivo by stimulating the immune system, and Pajares and Gisbert[15] reported a direct inhibitory effect of ethanol and ethyl acetate extracts from this basidiomycete against *Helicobacter pylori* in patients with chronic gastritis and gastric ulcers. Several studies thus suggest that biologically active extracts of *H. erinaceus* possess significant antimicrobial effects that could be further enhanced for pharmacological purposes; in addition, their immunomodulatory activity could help overcome bacterial infections by boosting the host's immune response.

Another relevant outcome concerns the antagonistic interactions displayed by *T. versicolor*, which colonised and sporulated on the mycelia of *Fusarium* and *Alternaria* strains in media that favoured its growth, exhibiting high antagonism indices and PICR values against *F. equiseti, F. oxysporum* and *F. cerealis*. Comparable findings were reported by Ezziyyani et al.[20], who demonstrated the mycoparasite capacity of *T. harzianum* against *Phytophthora capsici*, observing intense competition for space and nutrients that promoted greater *T. harzianum* growth. Similarly, Agamez et al.[16] used *Trichoderma* isolates as an ecologically sound alternative to control *Fusarium*, showing mycoparasitism that obstructed the pathogen's physiological functions and halted its growth. Studies conducted in 2021 also confirmed the antagonistic capacity of *H. erinaceus* and *T. versicolor* against bacteria and fungi, evidencing their competitive potential against phytopathogens.

With respect to *G. multipileum*, no significant antagonistic interactions were detected in the present work; however, earlier studies documented bioactive secondary metabolites from this genus, including triterpenes, polysaccharides, nucleotides, sterols, steroids, fatty acids, and proteins/peptides, with antifungal, antibacterial, and antiviral properties[21]. Species such as *G. lucidum* synthesize ganodermin, a protein that inhibits the mycelial growth of *F. oxysporum*, *Penicillium*, *Aspergillus*, and *Mucor*. Metabolite production in *Ganoderma* is strongly influenced by nutritional factors and cultivation conditions—pH, incubation time, and concentration, each significantly affecting the yield of antimicrobial compounds potentially useful against phytopathogens[9].

The contrast between *H. erinaceus* and *T. versicolor* suggests that differential inhibition of phytopathogens may be explained by synergistic routes such as the secretion of lytic enzymes, e.g., chitinases and cellulases, which show high activity

in dual-cultures, and the emission of low molecular weight volatile organic compounds capable of diffusing through agar and disrupting host hyphae[22].

The findings corroborate that medium composition is a key factor in evaluating antagonistic interactions. Moreover, the percentage inhibition of radial growth does not always reflect basidiomycete antifungal capacity because diffusion of secreted metabolites through agar may be limited, masking inhibitory effects. Conditions affecting both basidiomycetes and phytopathogens, such as nutrient availability and biomass production, can significantly influence metabolite synthesis. Although temperature was not examined here, it can modulate production of bioactive compounds: higher temperatures may enhance synthesis of certain metabolites while promoting degradation of others[23,24]. Incubation time likewise affects both biomass accumulation and antimicrobial compound output, making optimization of these parameters critical for basidiomycete cultures[25,26]. Macro and micronutrient balance in the culture medium is also decisive; carbohydrate rich media may favor production of a limited metabolite range, whereas nutritionally balanced media can induce a broader spectrum of bioactive compounds[27,28].

Carbon sources are among the most important nutrients for microbial growth. Glucose at an optimal concentration of 20 g L^{-1} has been identified as the principal carbon source promoting mycelial development, presumably due to its ready metabolization as a respiratory substrate. In contrast, arabinose, galactose, and mannitol are generally less favorable for fungal growth[29].

Regarding nitrogen sources, studies evaluating yeast extract, malt extract, sodium nitrate ($NaNO_3$), and peptones have shown that growth effectiveness varies significantly among fungal species, although yeast extract is generally highly beneficial owing to its rich content of amino acids, carbohydrates, proteins, minerals, and vitamins[27,30,20].

In most interactions tested, the CZP medium, rich in sucrose and poor in nitrogen, favoured phytopathogen growth. Carranza et al.[31] similarly found that modified Czapek Dox media composition significantly influenced *Aspergillus* growth, suggesting that nitrogen-limited media can be more conducive to filamentous fungi. Zhang et al.[32] also reported that nitrogen source markedly affects fungal development, with responses depending on species and taxonomic group.

This research provides an approximation of the antagonistic power of basidiomycete fungi. However, it is important to determine whether enzymes such as chitinase and extracellular cellulases, which have traditionally been associated with antifungal antagonism in rhizosphere fungi[33], are the mechanisms mediating these interactions.

Additionally, it would be essential to evaluate conditions and variables such as changes in pH and temperature, which influence growth kinetics and metabolite production, as this would allow for the optimization of antagonistic interactions when scaling up basidiomycete cultures[34,35,36] Becoming a promising solution, as documented in the country for the control of Phytophthora, to combat the effects of chemical fungicides, which represent a problem due to improper use, making it very difficult to eliminate the disease because of the emergence of new resistant species[37].

Conclusions

The results of this study demonstrate that, by adjusting culture conditions and carbon and nitrogen sources, antagonism assays can be a useful screening tool for basidiomycete fungi with antagonistic potential. Such assays are especially valuable in preliminary exploratory work when the specific growth requirements of native or rarely studied species are unknown, such as colony morphology, mycelial growth, and synthesis of antifungal compounds can vary markedly with these factors. All species examined were able to grow on the different culture media tested and could exploit broad ranges of carbon and nitrogen sources, enabling adaptation to varying temperature, pH, and incubation time conditions.

Future work should focus on isolating and quantifying key metabolites to identify the specific pathways involved in these interactions, as well as conducting in vivo validations in plant models to assess the practical potential of basidiomycete-derived compounds, particularly those from *T. versicolor*, as antagonists.

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Data Availability Statement

The datasets generated may be obtained from the corresponding author upon reasonable request.

AI-assistance statement

ChatGPT (OpenAI GPT4, March 2025 model) and Grammarly® (v 1.2.86) were used solely for translation and grammar checking; all content was reviewed and approved by the authors. No AI tools were used for data analysis, figure preparation, or content generation.

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