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Control of endophytic fungi in the micropropagation of caçari (*Myrciaria dubia* (Kunth) McVaugh)

Greguy Looban Cavalcante de Lima[1], Edvan Alves Chagas[2]*, Kedma da Silva Matos[3], Daniel Augusto Schurt[2], Maria da Conceição da Rocha Araújo[4], Maria Isabel Garcia Ribeiro[1], Elias Ariel de Moura[3], Deila Cristina Vieira da Silva[1], Caroline Marques Silva[1] and Pollyana Cardoso Chagas[3]

[1]Post-Graduate in Agronomy Program (CCA/UFRR). Campus Cauamé, BR 174, Km 12, Monte Cristo, 69300-000, Boa Vista-RR, Brazil.
[2]Brazilian Corporation of Agricultural Research (Embrapa), Rod. BR 174, Km 08, Distrito Industrial, 69301-970, Boa Vista-RR, Brazil.
[3]Federal University of Roraima, Campus Cauamé, BR 174, Km 12, Monte Cristo, 69300-000, Boa Vista-RR, Brazil.
[4]Biotech Mudas. Rua Nivaldo da Conceição Gutierrez, nº 1105, Sala 1, Pintolândia, 69316-740, Boa Vista- RR.

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The micropropagation of camu-camu (*Myrciaria dubia*) is frequently compromised by endogenous fungal contamination, requiring effective fungicides for explant disinfection. This study evaluated the efficiency of systemic fungicides (strobilurins, triazoles, benzimidazoles, phenylpyridinamines, and aromatic hydrocarbons) in controlling 20 endophytic fungal isolates associated with camu-camu, through two stages: (1) *in vitro* sensitivity tests and (2) application in micropropagation protocols. The experimental design followed a factorial scheme (3×4×20 for *in vitro* tests and 3×4×3 for micropropagation), analyzing mycelial growth inhibition (%MGI) and mycelial growth rate index (MGRI). The *in vitro* results demonstrated that the strobilurin + triazole + aromatic hydrocarbon combination inhibited 100% of the isolates at all tested concentrations (50 to 150 µg/mL). However, in the micropropagation phase, this fungicide was not effective in controlling the fungi, indicating that *in vitro* resistance does not guarantee success under actual cultivation conditions. The study highlights the need to optimize application strategies, such as dosage adjustments or combinations with other agents, to overcome the observed limitations.

Key words: Systemic fungicides, endophytic manifestation, endogenous microorganisms and Strobilurin.

INTRODUCTION

Camu-camu (*Myrciaria dubia*), locally known as caçari, is an economically valuable Amazonian fruit tree species. Recognized as an exceptional source of vitamin C, β-carotene, phenolic compounds, and antioxidants, it possesses remarkable nutraceutical properties that enhance its commercial potential for Brazil and particularly for Roraima State (Chagas et al., 2014; Kaneshima et al., 2017; Chang et al., 2019; Grigio et al.,

*Corresponding author. E-mail: Greguy.lima@ufrr.br.

2021). In Roraima's Northern Amazon region, camu-camu has become an important alternative crop, with its economic value amplified by widespread natural distribution across the territory (Chirinos et al., 2010; Chagas et al., 2014).

Extensive genetic improvement programs have been developed to enable commercial-scale cultivation. However, efficient clonal propagation of selected genotypes remains challenging. While seed propagation occurs naturally, the resulting genetic variability limits its commercial viability. Consequently, micropropagation has emerged as the preferred method for large-scale production of genetically uniform plants (Rodriguez et al., 2014; Nascimento et al., 2018; Grigio et al., 2021).

As an advanced vegetative propagation technique, micropropagation offers distinct advantages over conventional methods, allowing rapid mass production of pathogen-free plantlets while supporting germplasm conservation (Maheshwari, 2017; Delgado-Paredes et al., 2021). Despite these benefits, persistent contamination by endophytic microorganisms remains a major constraint, often causing significant losses. These endogenous contaminants are particularly difficult to manage in woody species like camu-camu, typically compromising cultures during the critical establishment phase by disrupting regeneration processes and preventing progression to subsequent stages (Cançado et al., 2013; Chagas et al., 2014; Esposito-Polesi et al., 2020).

Effective microbial control during establishment is crucial. While incorporating antimicrobial agents into culture media shows promise against endophytes in woody species, systemic fungicides have demonstrated superior efficacy due to their, combined protective and curative action, high fungitoxicity, rapid tissue penetration, efficient vascular translocation (Sarmast, 2018; Dutra, 2019; Chechi et al., 2019; Cheong et al., 2020).

However, limited research has comprehensively assessed fungicide effectiveness against camu-camu endophytes or their practical integration into micropropagation protocols. This study bridges this knowledge gap through a two-stage approach: (1) *in vitro* evaluation of fungicidal compounds against characterized endophytic isolates and (2) implementation of optimal formulations in functional micropropagation systems. By translating laboratory results into practical solutions, this research contributes to developing reliable clonal propagation methods that support sustainable commercialization of this valuable Amazonian species while ensuring genetic integrity and plant health standards.

MATERIALS AND METHODS

Collection of plant material

Sixty stem segment explants (approximately 1.5 cm in length, each containing a pair of apical buds) were collected from five elite caçari genotypes (UAT/1796-7, UAT/1596-7, UAT/1896-7, UAT/1096-5, and UAT/0796-3) maintained in the germplasm collection at Embrapa-RR's Serra da Prata Experimental Field (CESP) in Mucajaí (2°23'49"N, 60°58'40"W). Only explants exhibiting optimal phytosanitary conditions were selected for the study.

Immediately after collection using sterilized pruning shears, the plant material was immersed in a citric acid solution (200 mg L^{-1}) for transport to the laboratory, where it underwent a rigorous surface sterilization protocol consisting of sequential washes in 70% (v/v) ethanol for 1 min followed by 2% sodium hypochlorite for 3 min to effectively eliminate epiphytic microorganisms while preserving explant viability.

All experimental procedures were conducted in Embrapa-RR's specialized facilities in Boa Vista, including the Soil Microbiology Laboratory and the Postharvest, Agroindustry, and Tissue Culture Laboratory, ensuring controlled conditions throughout the study.

In vitro sensitivity of endophytic microorganisms of caçari to different systemic fungicides

The prepared fungicide solutions were aseptically incorporated into BDA medium heated to 45°C to achieve the target concentrations, while control plates contained fungicide-free BDA médium (Figure 1). Following the protocol adapted from Dutra et al. (2019), four different fungal isolates were randomly distributed on each Petri dish, with five replicate plates prepared per isolate (Table 1).

All plates were then incubated in a BOD chamber at 26ºC under a 12-h photoperiod for seven days. Daily measurements of mycelial growth were performed at 24-h intervals by taking two perpendicular diameter measurements of each colony using digital calipers, from which the average colony diameter (DC) was calculated. Fungicide efficacy was evaluated through two key parameters: the Percentage of Mycelial Growth Inhibition (%MGI) and the Mycelial Growth Rate Index (MGRI), analyzed according to the culture medium supplementation method described by Amiri et al. (2008). This dual-metric approach allowed comprehensive assessment of fungicidal activity against the endophytic microorganisms throughout the experimental period. As the tested fungicides can act on spore germination and mycelial growth, this classification was adapted for mycelial growth, employing distinct doses. The study by Weber and Hahn (2011) served as a reference for this adaptation.

Action of systemic fungicides in the *In vitro* control protocol of endophytic microorganisms in the initial establishment of Caçari

A total of 576 explants were distributed equally among 16 Erlenmeyer flasks (12 explants per flask), with eight flasks containing fungicide F2 and eight containing fungicide F3. The flasks were placed on a Tecnal© - 14I orbital shaker at 100 RPM for 24 h to ensure proper exposure to the fungicidal solutions. Following this treatment, the explants were transferred to two variants of culture medium: the basic WPM formulation (Lloyd and McCown, 1981) and a modified WPM medium incorporating the test fungicides. Each explant was then individually inoculated into 25×100 mm test tubes containing 10 mL of the respective WPM medium (Figure 2) (Ribeiro, 2021).

The basic WPM culture medium, as originally described by Lloyd and McCown (1981), served as the control, while the modified WPM culture medium included pre-established fungicides. The experiment consisted of two fungicide products: F1 (Azoxystrobin + Difenoconazole) and F2 (Pyraclostrobin + Epoxiconazole + Heavy Aromatic Solvent [Naphtha, Petroleum]), with their complete compositions detailed in Table 1. Each fungicide was tested at four

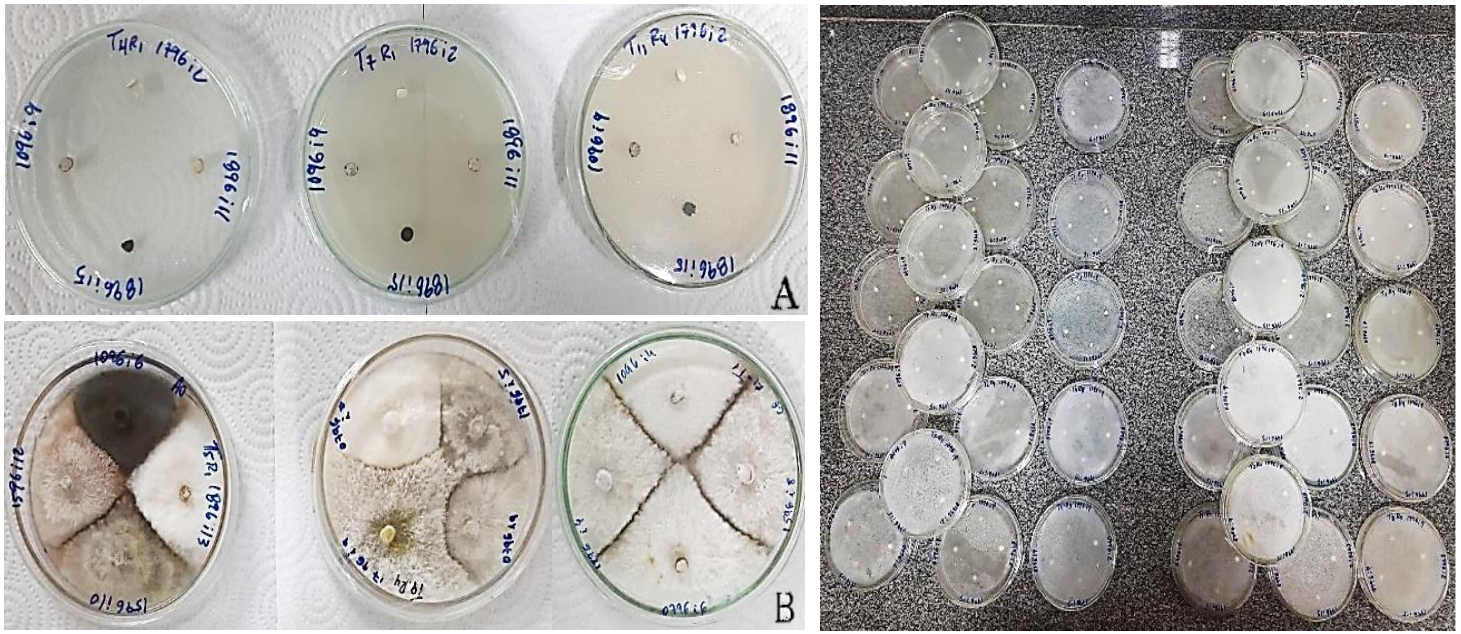

Figure 1. (A) 5 mm mycelial disc collected from the colonies of the 20 selected isolates, all with 0 days of growth, cultivated on BDA medium. (B) 5 mm mycelial plug collected from the colonies of the 20 selected isolates, all with 7 days of growth, cultivated on BDA medium. (C) Experimental setup.

Table 1. Fungicides used in the *in vitro* sensitivity test of endophytic fungal isolates obtained from caçari explants.

Active ingredient	Commercial name	Chemical group	*CIA	Action mechanisms	References
**(F1) Tiofanate-Methyl + Fluazinam	CERTEZA N®	Benzimidazole and Phenylpyridinylamine	350.00 g/L (35% w/v) + 52.50 g/L (5.25% w/v)	- Complex III of cytochrome BC1 (ubiquinol oxidase) at Qo site (cyt b gene). - Uncouplers of Oxidative Phosphorylation	Gwon et al. (2019) Dutra et al. (2019)
**(F2) Azoxystrobin + Difenoconazole	AMISTAR TOP®	Strobilurin and Triazole	200 g/L (20% w/v) + 125 g/L (12.5% w/v)	- Complex III of cytochrome BC1 (ubiquinol oxidase) at Qo site (cyt b gene). - Acts on the formation of ergosterol	Gwon et al. (2019)
**(F3) Pyraclostrobin + Epoxiconazole + Heavy Aromatic Solvent (Naphtha, Petroleum)	OPERA®	Strobilurin, Triazole, and Aromatic Hydrocarbo	133 g/L (13.3% w/v), 50 g/L (5.0% w/v), and 200 g/L (20% w/v)	- Complex III of cytochrome BC1 (ubiquinol oxidase) at Qo site (cyt b gene).	Gwon et al. (2019)

*Concentrations are given in grams per liter (g/L) and percentages (w/v). Replace "Nome do Fungicida" with the actual names of the fungicides and "Concentração" with the corresponding concentrations used in the study.

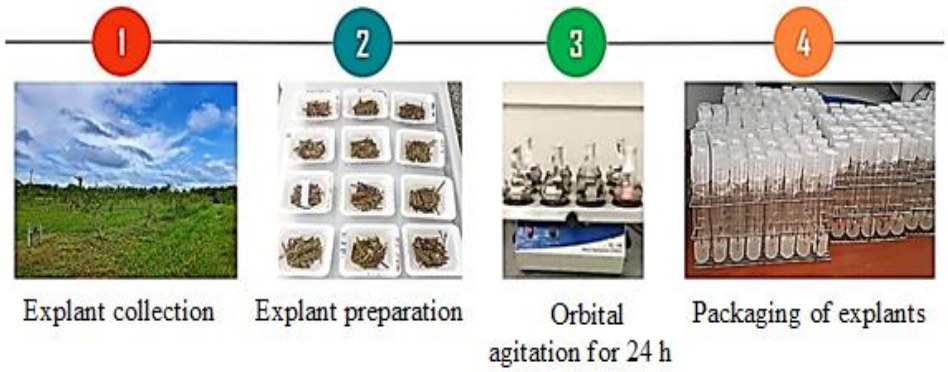

Figure 2. Flowchart of the steps for conducting the experiment with the test for inhibition of the growth of endophytic fungi in the micropropagation of camu-camu.

Table 2. Differential efficacy of systemic fungicides in the control of endophytic fungi in *Myrciaria dubia* genotypes.

Genotype	Effective fungicide	Optimal dose	% Contamination reduction***
UAT 0796	F3* (Opera®)	75 ml.L^{-1}	95.8 → 4.2
UAT 1596	F2** (Amistar Top®)	50 ml.L^{-1}	76.4 → 23.6
UAT 1096	Nenhum	-	100 → 0

*F2: AmistarTop Fungicide (Active Ingredients: Azoxystrobin + Difenoconazole); **F3: Opera Fungicide (Active Ingredients: Pyraclostrobin + Epoxiconazole + Heavy Aromatic Solvent (Naphtha, Petroleum). ***Values represent percentage reduction in contamination compared to the control (n=3). Different letters indicate significant differences (p<0.05).

concentrations (0, 25, 50, and 75 mLL⁻¹) with three replicates per treatment, resulting in a total of 24 treatment combinations. The selection of concentrations was based on preliminary in vitro tests, aiming to balance effective control of endophytic fungi against potential phytotoxicity concerns. The specific treatments were: T1 (F2 at 0 mL·L⁻¹), T2 (F2 at 25 mL·L⁻¹), T3 (F2 at 50 mL·L⁻¹), T4 (F2 at 75 mL·L⁻¹), T5 (F3 at 0 mL·L⁻¹), T6 (F3 at 25 mL·L⁻¹), T7 (F3 at 50 mL·L⁻¹), and T8 (F3 at 75 mL·L⁻¹), all replicated three times to ensure statistical reliability.

Data analysis

For the sensitivity analyses, colony growth was monitored by measuring mean diameter using digital calipers, calculated from two perpendicular measurements of each colony. Evaluations commenced 24 h post-inoculation and continued until control colonies reached the plate edges, ensuring comparable growth conditions across all treatments. The experiment followed a Completely Randomized Design (CRD) with a 3×4×20 factorial arrangement, testing three chemical fungicides at four concentrations (0 [control], 50, 100, and 150 µg/mL) against twenty fungal isolates, with five replicates per treatment combination. Micropropagation assessments were conducted over a 30-day period, with weekly evaluations of key parameters: fungal contamination frequency (%CF), bacterial contamination frequency (%CB), oxidation incidence (%OX), and shoot proliferation rate (NB).

All statistical analyses were performed using SISVAR software (version 5.6; Ferreira, 2011). For significant treatment effects (p<0.05), post-hoc comparisons were made with Tukey's test for fungicide and isolate effects, while dose-response relationships were analyzed through regression models to determine optimal concentrations.

RESULTS

In vitro sensitivity of endophytic microorganisms of caçari to different systemic fungicides

The analysis of variance revealed highly significant triple interactions ($p < 0.001$) between fungal isolates, fungicides, and doses for both mycelial growth rate (IVCM) and inhibition percentage (%ICM), as detailed in Tables 3 and 4. When examining the specific effects of fungicides across different doses for each isolate, the active ingredient F3 consistently demonstrated superior performance, completely inhibiting mycelial growth at just 50 µg/mL while significantly reducing growth rates across all tested isolates, showing statistically significant differences compared to F1 and F2 formulations.

All fungal isolates exhibited a clear negative linear response to increasing fungicide concentrations, though with notable resistance patterns emerging - particularly in *Diaporthe* species. nov. (isolate UAT1896i4) and *Trichoderma asperelloides* (UAT1096i1) which showed complete resistance to F1, while isolates UAT1896i15

Table 3. Summary of analysis of variance of data on the percentage of contamination as a function of culture media, fungicides and concentrations and their interactions with explants.

Fungicide	UAT 0796	UAT 1596
	Contamination (%)	
F3*	95.83[a]	94.79[a]
F2*	89.58[b]	76.04[b]
CV (%)	11.01	23.14

*F2: AmistarTop Fungicide (Active Ingredients: Azoxystrobin + Difenoconazole); *F3: Opera Fungicide (Active Ingredients: Pyraclostrobin + Epoxiconazole + Heavy Aromatic Solvent (Naphtha, Petroleum)).

(unidentified), *Grammothele* species (UAT1896i1), UAT1096i1, and *Curvularia* species (UAT1096i6) proved resistant to F2 across all tested doses (Tables 4 and 5).

The spectrum analysis clearly established F3 as the most comprehensive fungicide, achieving 100% inhibition against all fungal isolates, while F2 showed the narrowest effective range with particularly poor performance against UAT1896i15, *Grammothele* spp., *T. asperelloides*, and *Curvularia* spp.

The *T. asperelloides* isolate presented a particularly interesting case, demonstrating complete resistance to both F1 and F2 at all concentrations while showing exceptional sensitivity to F3, with complete inhibition achieved at the lowest 50 µg/mL dose - a finding that powerfully underscores F3's unmatched efficacy against these challenging endophytic fungi.

Action of systemic fungicides in the *in vitro* control protocol of endophytic microorganisms in the initial establishment of Caçari

The results of the analysis of variance for the data in the experiment with the genotype UAT 0796 showed significant double interaction ($p < 0.005$) for the factors media and concentrations (Tables 2 and 3). In this genotype, the fungicide F3 (Pyraclostrobin + Epoxiconazole) at 75 $ml.L^{-1}$ in the modified medium (MM) promoted a contamination reduction of 4,2%, that is, of 100% of the samples, 95,8% presented contamination.

Regarding the results of the analysis of variance for the experiment with the UAT 1596 genotype (Table 3), the fungicide F2 (Azoxystrobin + Difenoconazole) proved to be more efficient, achieving 23.6% control at a concentration of 50 mL/L (Table 2). However, even at the most effective dose, it was observed that there was a high contamination of explants.

Contrarily, the experiment conducted with the UAT 1096 genotype showed that none of the tested culture media, fungicides, and concentrations demonstrated a significant effect on inhibiting fungal manifestations. This genotype exhibited the highest rates of endophytic manifestations. When there was no treatment with fungicides, there was 100% fungal manifestation, highlighting the need to associate chemical components in disinfection (Tables 2 and 3). From the first week, the incidence of fungi in the controls was observed, early in the explant incubation period.

When there was no treatment with the use of fungicides, there was 100% fungal manifestation, demonstrating the need to associate chemical components in the disinfestation. From the first week onwards, it was possible to observe the incidence of fungi in the controls, right in the initial period of incubation of the explants. Results represent means of three independent replicates, with statistically significant differences ($p < 0.05$) determined by analysis of variance (ANOVA) followed by Tukey's test. In this experiment, oxidation rates were nil, with no oxidized explants observed during the 21-day evaluation period. The tested fungicides did not cause oxidation in the explants, demonstrating that there was no phytotoxic effect.

DISCUSSION

Numerous studies have demonstrated the efficacy of systemic fungicides from the Triazole, Benzimidazole, Strobilurin, and Phenylpyridinylamine families in controlling endophytic microorganisms (Fillinger and Walker, 2016; Dutra et al., 2019; Gwon et al., 2019; Chechi et al., 2019), findings that align with the results showing their effectiveness against *M. dubia* endophytes.

These active ingredients are among the most widely used currently for the control of various phytopathogens and in controlling endophytic manifestations in micropropagation environments. Their efficiency has been confirmed in microorganisms of the genera *Colletotrichum*, *Alternaria*, and *Neopestalotiopsis*, all common in micropropagation (Zhang et al., 2012; Chechi et al., 2019; Gwon et al., 2019; Batzer and Mueller, 2020).

In this study, products combining these fungicide families were used to provide a synergistic activity, explaining the good results obtained in the *in vitro* sensitivity stage. Despite the statistical differences between the tested products, all fungicides showed excellent inhibition results. Particularly noteworthy is the F3 product, for which no isolates resistant to its active ingredients were found at different tested doses, not even the *T. asperelloides* isolate, which expressed resistance to the other tested fungicides.

There are currently no published studies explaining this resistance; however, it may be related to a mutation that encodes an enzyme involved in detoxifying this fungus. This mutation results in a change in the enzyme's conformation, making this fungus less sensitive to the fungicide (Silva et al., 2018).

Table 4. Means of the effect of different concentrations of fungicides F1, F2, and F3 on the Mycelial Growth Rate Index (IVCM) of each isolate.

Insulated	Fungicide	DOSES (μg.mL)				Equation	R^2
		0	50	100	150		
Diaporthe spp. nova	AMIST	24.78^{a}	1.91^{b}	3.05^{a}	2.29^{a}	ŷ = 17.95** - 0.13x**	0.60
	CERT	24.53^{ab}	2.57^{a}	0^{b}	0^{b}	ŷ = 18.20** - 0.15x**	0.68
	OPERA	24.24^{b}	0^{c}	0^{b}	0^{b}	ŷ = 16.97** - 0.15x**	0.60
Diaporthe spp. nova	AMIST	16.36^{a}	2.90^{a}	0^{a}	0^{a}	ŷ = 11.45**- 0.09x**	0.60
	CERT	16.15^{a}	0^{b}	0^{a}	0^{a}	ŷ = 12.47** - 0.10x**	0.73
	OPERA	16.30^{a}	0^{b}	0^{a}	0^{a}	ŷ = 11.41** - 0.09x**	0.60
Diaporthe cerradensis	AMIST	16.34^{a}	2.70^{a}	0^{a}	0^{a}	ŷ = 11.44** - 0.09x**	0.60
	CERT	15.92^{a}	0^{b}	0^{a}	0^{a}	ŷ = 12.22** - 0.10x**	0.73
	OPERA	16.26^{a}	0^{b}	0^{a}	0^{a}	ŷ = 11.38** - 0.09x**	0.60
Diaporthe spp. nova	AMIST	24.53^{b}	0^{b}	0^{a}	0^{a}	ŷ = 17.17** - 0.14x**	0.60
	CERT	25.41^{a}	2.95^{a}	0^{a}	0^{a}	ŷ = 18.97** - 0.15x**	0.69
	OPERA	24.36^{b}	0^{b}	0^{a}	0^{a}	ŷ = 17.05** - 0.14x**	0.60
1896 i15	AMIST	12.14^{ab}	3.83^{b}	3.87^{b}	3.93^{b}	ŷ = 9.63** - 0.04x**	0.58
	CERT	12.29^{a}	7.91^{a}	7.14^{a}	6.19^{a}	ŷ = 11.24** - 0.03x**	0.83
	OPERA	11.78^{b}	0^{c}	0^{c}	0^{c}	ŷ = 8.24** - 0.07x**	0.60
Diaporthe spp. nova	AMIST	16.50^{a}	5.13^{a}	0^{a}	0^{a}	ŷ = 11.55** - 0.09x**	0.60
	CERT	16.20^{ab}	0^{b}	0^{a}	0^{a}	ŷ = 13.12 ** - 0.10x**	0.82
	OPERA	15.81^{b}	0^{b}	0^{a}	0^{a}	ŷ = 11.34** - 0.09x**	0.60
Diaporthe spp. nova	AMIST	16.67^{a}	0^{b}	0^{a}	0^{a}	ŷ = 11.67** - 0.10x**	0.60
	CERT	16.86^{a}	3.09^{a}	0^{a}	0^{a}	ŷ = 13.04** - 0.10x**	0.74
	OPERA	16.52^{a}	0^{b}	0^{a}	0^{a}	ŷ = 11.56** - 0.09x**	0.60
Diaporthe spp. nova	AMIST	15.34^{b}	0^{b}	0^{a}	0^{a}	ŷ = 10.74** - 0.09x**	0.60
	CERT	16.01^{a}	4.25^{a}	0^{a}	0^{a}	ŷ = 12.91** - 0.10x**	0.79
	OPERA	15.29^{b}	0^{b}	0^{a}	0^{a}	ŷ = 10.70** - 0.09x**	0.60
Diaporthe pseudoinconspicua	AMIST	12.85^{b}	3.54^{a}	0^{a}	0^{a}	ŷ = 10.41** - 0.08x**	0.80
	CERT	13.45^{a}	3.71^{a}	0^{a}	0^{a}	ŷ = 10.90** - 0.08x**	0.80
	OPERA	12.64^{b}	0^{b}	0^{a}	0^{a}	ŷ = 8.85** - 0.07x**	0.60
Neopestalotiopsis spp.	AMIST	12.62^{b}	0^{a}	0^{a}	0^{a}	ŷ = 8.83** - 0.07x**	0.60
	CERT	13.38^{a}	0^{a}	0^{a}	0^{a}	ŷ = 9.36** - 0.08x**	0.60
	OPERA	13.10^{a}	0^{a}	0^{a}	0^{a}	ŷ = 9.17** - 0.07x**	0.60
Diaporthe spp. nova	AMIST	13.30^{a}	0^{a}	0^{a}	0^{a}	ŷ = 9.31** - 0.07x**	0.60
	CERT	13.40^{a}	0^{a}	0^{a}	0^{a}	ŷ = 9.38** - 0.08x**	0.60
	OPERA	13.49^{a}	0^{a}	0^{a}	0^{a}	ŷ = 9.44** - 0.08x**	0.60
Diaporthe spp. nova	AMIST	18.36^{a}	0^{b}	0^{a}	0^{a}	ŷ = 12.85** - 0.11x**	0.60
	CERT	18.43^{a}	3.26^{a}	0^{a}	0^{a}	ŷ = 14.21** - 0.11x**	0.73
	OPERA	18.77^{a}	0^{b}	0^{a}	0^{a}	ŷ = 13.13** - 0.11x**	0.60
Diaporthe spp. nova	AMIST	15.72^{a}	0^{b}	0^{b}	0^{a}	ŷ = 11.00** - 0.09x**	0.60
	CERT	15.64^{a}	5.56^{a}	2.61^{a}	0^{a}	ŷ = 13.43** - 0.09x**	0.88
	OPERA	15.74^{a}	0^{b}	0^{b}	0^{a}	ŷ = 11.01** - 0.09x**	0.60
Diaporthe spp. nova	AMIST	16.03^{a}	0^{b}	0^{a}	0^{a}	ŷ = 11.22**-0.09x**	0.60
	CERT	15.87^{a}	3.75^{a}	0^{a}	0^{a}	ŷ = 12.61** - 0.10x**	0.77
	OPERA	15.25^{b}	0^{b}	0^{a}	0^{a}	ŷ = 10.68** - 0.09x**	0.60

Table 4. Cont'd

	AMIST	11.83[b]	0[b]	0[b]	0[b]	ŷ = 8.28** - 0.07x**	0.60
Grammothele spp.	CERT	12.44[a]	11.96[a]	8.13[a]	7.93[a]	ŷ = 12.72** - 0.03x**	0.85
	OPERA	12.38[a]	0[b]	0[b]	0[b]	ŷ = 8.67** - 0 07x**	0.60
	AMIST	24.77[a]	3.27[b]	4.44[b]	5.67[a]	ŷ = 17.96** - 0.11x**	0.50
T. asperelloides	CERT	24.91[a]	16.46[a]	9.84[a]	5.62[a]	ŷ = 23.88** - 0 12x**	0.97
	OPERA	24.57[a]	0[c]	0[c]	0[b]	ŷ = 17.20** - 0.14x**	0.60
	AMIST	12.37[a]	0[b]	0[a]	0[a]	ŷ = 8.65** - 0.07x**	0.60
Neopestalotiopsis species	CERT	12.18[a]	3.05[a]	0[a]	0[a]	ŷ = 9.74** - 0.07x**	0.78
	OPERA	12.30[a]	0[b]	0[a]	0[a]	ŷ = 8.61** - 0.07x**	0.60
	AMIST	12.57[ab]	4.14[a]	0[a]	0[a]	ŷ = 10.45** - 0.08x**	0.83
Diaporthe spp. nova	CERT	12.35[b]	3.32[b]	0[a]	0[a]	ŷ = 9.97** - 0.08x**	0.79
	OPERA	12.89[a]	0[c]	0[a]	0[a]	ŷ = 9.02** - 0.07x**	0.60
	AMIST	12.67[a]	0[b]	0[a]	0[a]	ŷ = 8.87** - 0.07x**	0.60
Diaporthe spp.	CERT	12.41[a]	3.21[a]	0[a]	0[a]	ŷ = 9.97** - 0.08x**	0.79
	OPERA	12.65[a]	0[b]	0[a]	0[a]	ŷ = 8.85** - 0.07x**	0.60
	AMIST	9.61[a]	0[b]	0[b]	0[b]	ŷ = 6.72** - 0.05x**	0.60
Curvularia spp.	CERT	9.79[a]	8.07[a]	6.91[a]	6.87[a]	ŷ = 9.40** - 0.01x**	0.87
	OPERA	9.49[a]	0[b]	0[b]	0[b]	ŷ = 6.64** - 0.05x**	0.60

Fungicides = Certeza N®.amistar Top®and Opera®. In the rows. for the variable Mycelial Growth Speed Index (mm/day).are theaverage concentrations (µg.mL) in the Fungi levels within each fungicide leveland their respective regressions.averages followedby the same letter in the column do not differ from each otherby the t-test ($p \leq 0.01$). ** significantat 0.01 probabilityby the F-test.

Table 5. Resistance pattern of endophytes associated with caçari to fungicides F1, F2, and F3, and classification of phenotypes according to the mean Percentage of Inhibition of Mycelial Growth (%ICM) of each isolate.

Insulated	Fungicide	DOSES (*µ*g.mL)				Equation	R^2	Phenotype
		0	50	100	150			
	AMIST	0[a]	60.79[b]	69.26[b]	66.84[b]	ŷ = 17.87** - 0.41x**	0.66	R
Diaporthe spp. nova	CERT	0[a]	63.31[b]	100[a]	100[a]	ŷ = 15.32** - 0.67x**	0.84	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	CERT	0[a]	76.02[b]	100[a]	100[a]	ŷ = 20.41** - 0.64x**	0.77	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
D. cerradensis	CERT	0[a]	83.01[b]	100[a]	100[a]	ŷ = 23.20** - 0.63x**	0.73	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	CERT	0[a]	59.27[b]	100[a]	100[a]	ŷ = 13.70** - 0.68x**	0.86	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	AMIST	0[a]	84.20[b]	83.97[b]	83.79[b]	ŷ = 25.32** - 0.50x**	0.59	S
1896 i15	CERT	0[a]	3.43c	1.7[c]	24.36[c]	ŷ = -3.66** - 0.13x**	0.52	R
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	CERT	0[a]	35.12[b]	100[a]	100[a]	ŷ = 4.04** - 0.72x**	0.89	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS

Table 5. Cont'd

Diaporthe spp. nova	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	81.56[b]	100[a]	100[a]	ŷ = 22.62** - 0.63x**	0.74	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	73.40[b]	100[a]	100[a]	ŷ = 19.36** - 0.65x**	0.79	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
D. pseudoinconspicua	AMIST	0[a]	93.11[a]	100[a]	100[a]	ŷ = 27.24** - 0.61x**	0.65	SS
	CERT	0[a]	93.08[b]	100[a]	100[a]	ŷ = 27.23** - 0.61x**	0.65	SS
	OPERA	0[a]	100[b]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Neopestalotiopsis spp.	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	82.27[b]	100[a]	100[a]	ŷ = 22.90** - 0.63x**	0.73	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	52.60[b]	77.72[b]	100[a]	ŷ = 8.81** - 0.65x**	0.95	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	81.79[b]	100[a]	100[a]	ŷ = 22.71** - 0.63x**	0.74	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Grammothele spp.	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	4.28[b]	2.04[b]	4.39[b]	ŷ = 3.57 μg.ml	_1	R
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
T. asperelloides	AMIST	0[a]	68.14[b]	64.07[b]	54.24[b]	ŷ = 22.81** - 0.31x**	0.41	R
	CERT	0[a]	0.85[c]	1.23[c]	20.91[c]	ŷ = -3.71** - 0.12x**	0.64	R
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Neopestalotiopsis spp.	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	68.62[b]	100[a]	100[a]	ŷ = 17.44** - 0.66x**	0.82	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp. nova	AMIST	0[a]	91.77[b]	100[a]	100[a]	ŷ = 26.70** - 0.61x**	0.66	SS
	CERT	0[a]	77.81[c]	100[a]	100[a]	ŷ = 21.12** - 0.64x**	0.76	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Diaporthe spp.	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	79.18[b]	100[a]	100[a]	ŷ = 21.67** - 0.64x**	0.75	SS
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
Curvularia spp.	AMIST	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS
	CERT	0[a]	1.17[b]	1.15[b]	1.71[b]	ŷ = 1.34 μg.ml	_1	R
	OPERA	0[a]	100[a]	100[a]	100[a]	ŷ = 30** - 0.60x**	0.6	SS

Fungicides = Certeza N®, Amistar Top® and Opera®. In the rows, for the variable Percentage of Mycelial Growth Inhibition, are the average concentrations (µg.mL) in the Fungi levels within each fungicide level, their respective regressions and classification of sensitivity levels. Averages followed by the same letter in the column do not differ from each other by the t-test ($p \leq 0.01$). _1 = the averages of this factor were considered statistically equal according to the F test and the data did not fit any regression model. ** significant at 0.01 probability by the F test..

The success of the F3 product can be explained by Ebrahimzadeh and Abrinbana (2019), as they report that the synergistic use of a mix of active ingredients in a product provides a broader control spectrum, acting on multiple action sites and enhancing microbial control efficiency. In the case of the F3 product, it contains one more active ingredient in its composition than the F1 and F2 products.

In addition to this benefit, this mix also promotes the important prevention of the development of resistance in microorganisms. When microorganisms have frequent contact with the same active ingredient, they acquire mechanisms that make them resistant a factor widely discussed in microbial control (Ebrahimzadeh and Abrinbana, 2019).

Regarding the fungicides' effects, when the *in vitro* sensitivity experiments were conducted, the active ingredients Pyraclostrobin + Epoxiconazole + Heavy Aromatic Solvent (Naphtha, Petroleum) (Product F3) provided the best growth inhibition results (100%) in all tested isolates. However, the results in the micropropagation of *M. dubia* were different, and none of the fungicides were effective in controlling fungal manifestations.

The most accepted hypothesis for this negative effect is related to the colonization site of the fungi. In the sensitivity tests, the fungi were directly exposed to the medium containing fungicides, while in the micropropagation experiment, the fungi were in better-protected locations, such as the structures forming the explants of woody species.

This is a hypothesis discussed by Esposito-Polesi (2020), who argues that the difficulty fungicides have in accessing fungi that have colonized intra- and intercellular spaces and apical buds is the main reason for the failure of most microbial control protocols.

This hypothesis is also supported by Pasqualini et al. (2019), who observed fungal hyphae in axillary buds of bamboo (*Bambusa oldhamii*) nodal segments, both extra- and intracellularly. This colonization site hindered decontamination efforts, reducing the effectiveness of the decontamination protocol. The *in vitro* environment also poses challenges, as its conditions tend to select specific microorganisms, favoring the growth of more adapted endophytic species, including fungi and bacteria (Fang and Hsu, 2012; Polesi, 2015). This further complicates in vitro control. Regarding the influence of the culture medium, all treatments with Basic Medium (MB) resulted in 100% fungal manifestations, indicating that 24 h of orbital agitation alone was insufficient. However, when used in the MM, orbital agitation demonstrated greater efficiency, suggesting its potential as a complementary method to optimize and enhance the control protocol.

The success of a decontamination protocol for caçari micropropagation may involve combining aseptic methods with chemical treatment. For example, Machado et al. (2020) established a micropropagation method for Gabiroba (*Campomanesia xanthocarpa*) using a controlled and aseptic environment from the germinative phase to acclimatization, relying solely on Plant Preservative Mixture (PPM) as chemical control. Xue et al. (2023) supports the idea that treating the mother plant and explant material is a crucial first step for a successful decontamination protocol.

Factors such as explant type, collection time, and plant health are also essential considerations for establishing a successful micropropagation program for woody species.

Conclusion

Under the experimental conditions of this study, the products F2 (Azoxystrobin + Difenoconazole) and F3 (Pyraclostrobin + Epoxiconazole + Heavy Aromatic Naphtha Solvent) were ineffective in controlling the endophytic fungi that manifested during caçari micropropagation. However, F2 and F3 did not exhibit phytotoxic characteristics for the caçari explants. A 30-day period may be insufficient to fully assess the efficacy of systemic fungicides, as endophytic fungi can persist within internal tissues and re-emerge in subsequent subcultures. This study focused on the critical in vitro establishment phase, where initial contamination is the primary cause of failure. Future research should evaluate protocols involving multiple subcultures in fungicide-supplemented medium to allow for a more comprehensive assessment of the gradual eradication of these microorganisms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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