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Bioprospection and molecular phylogeny of culturable endophytic fungi associated with yellow passion fruit

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ABSTRACT. Endophytic microorganisms live inside the plants without causing any damage to their hosts. In the agricultural field, these endophytes might be a strategy of biological control for phytopathogens. We aimed to isolate endophytic fungi from yellow passion fruit (*Passiflora edulis*) leaves, evaluating its biocontrol capacity by *in vitro* antagonism against phytopathogen *Colletotrichum* sp. CNPU378. We also carried out greenhouse experiments in bean seedlings. A high colonization frequency was obtained (89%), and the molecular identification based on DNA sequencing attested *Colletotrichum* as the most frequent genus and minor occurrence of *Curvularia* endophytes. The endophytes tested showed different types of competitive interactions in *in vitro* antagonism inhibition rate ranging from 28.8 to 48.8%. There were 10 promising antagonists tested for their antagonist activity of crude extracts of secondary metabolites, in which strain PE-36 (20.8%) stood out among the other strains evaluated. In the greenhouse assay, plants inoculated only with endophyte *Colletotrichum* sp. PE-36 was symptomless and suggest that the endophyte strengthened the growth promotion in common bean plants, especially in the root length and number of leaves when compared to control plants and other treatments. Despite many fungi of *Colletotrichum* genus being described as causative agents of anthracnose, in this study, the plant sampled was colonized predominantly by *Colletotrichum* endophytes living in asymptomatic relationship. By the way, we come across a *Colletotrichum* sp. endophyte able to antagonize a *Colletotrichum* sp. pathogen.

Keywords: fungal isolation; *Passiflora edulis*; antagonism; molecular identification; phylogenetic analysis.

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Introduction

Endophytic microorganisms constitute a polyphyletic group of microorganisms that inhabit the interior of healthy plants and do not cause damages to their hosts (Azevedo & Araújo, 2007; Kusari, Hertweck, & Spiteller, 2012; Kusari, Singh, & Jayabaskaran, 2014). The endophytic communities can be employee in different areas (Toghueo, Zabalgoeazcoa, Aldana, & Boyom, 2017; Kyekyeku et al., 2017; López-González et al., 2017; Khan et al., 2017; Pascale et al., 2017; Ribeiro et al., 2018; Sharma, Tiwari, & Jadhav, 2018). In the agricultural field, endophytes might be a strategy of biological control for phytopathogens (Rondot & Reineke, 2016). Many studies have ready been conducted with endophytic fungi to demonstrate its ability as a biocontrol (Romeralo, Santamaría, Pando, & Diez, 2015; Martínez-Álvarez, Fernández-González, Sanz-Ros, Pando, & Diez, 2016; Larran, Simón, Moreno, Siurana, & Perolló, 2016; Preto, Martins, Pereira, & Baptista, 2017).

Passiflora genus (Passifloraceae) has approximately 500 species. Its potential for the identification and application of bioactive pharmaceutical-interest molecules has been exploited extensively in the last decades (Figueira, Freitas, Cruz, Figueira, & Câmara, 2015; Corrêa et al., 2016). Studies with endophytic microbiota of this genus of plant have also been reported successfully (Seetharaman et al., 2017; Rathnayake, Kumar, Jayasinghe, Araya, & Fujimoto, 2017).

Colletotrichum phytopathogens genus affects practically all crops grown worldwide, which are likely to be affected by one or more species of the genus (Dean et al., 2012). The common bean is one of the greatest crops in the world and is a food legume for human consumption (Talaat, 2019). Several classes of pesticides have been used annually to maintain high agricultural production. Nevertheless, the high use of these

agrochemicals causes serious environmental damage (Ying, 2018), making it necessary to develop strategies that prioritize sustainable agriculture to mitigate the adverse effects of these xenobiotic chemical defenses. The formulation and sale of these agrochemicals have had a significant increase per year (Carvalho, 2017). As an alternative control, we can mention the use of microorganisms capable of producing antifungal substances or antagonizing phytopathogens (Talaat, 2019).

In this context, the current study aimed to isolate endophytic fungi from yellow passion fruit (*Passiflora edulis*) and to select strains with biological control potential, capable of antagonizing the phytopathogen *Colletotrichum* sp. CNPUV378 in *in vitro* conditions. We also carried out greenhouse experiments with the best endophyte in bean seedlings.

Material and methods

Isolation of foliar endophytic fungi

Healthy leaves of *P. edulis* were randomly collected in the municipality of Planaltina do Paraná, Paraná State, southern Brazil (latitude: 23°00'24"S, longitude: 52°54'50"W) and transferred to hermetic sealing plastic bags and stored at 4°C for 24 hours. The endophytic fungi isolation was performed as previously described by Bongiorno et al. (2016), with slight modifications. Briefly, leaves were rinsed with 70% ethanol for 1 min., surface-disinfected with sodium hypochlorite solution (1% available Cl⁻) for 2 min., and rinsed once in 70% ethanol (for 30 s) and twice in sterile distilled water. In a laminar airflow chamber, leaves were aseptically cut into 500 fragments (5-mm² area). There were five foliar fragments placed on each PDA dish (Potato Dextrose Agar, pH 6.6) (Himedia, Mumbai, India) supplemented with 50 µg.mL⁻¹ tetracycline (Sigma, St. Louis, MO) and they remained incubated at 28°C for 7 days. The determination of colonization frequency (%) and fungal purification followed the protocols described by Ribeiro et al. (2018).

For the molecular identification, genomic DNA was extracted as described by Pamphile and Azevedo (2002) except that endophytes were previously grown for seven days in PDB dishes (Potato Dextrose Broth, pH 6.8) (Himedia, Mumbai, India). Amplification of the ITS1-5.8S-ITS2 (V9G 5'-TTACGTCCCTGCCCTTTGTA-3' and ITS4 5'-TCCGTAGGTGAACCTGCGG-3') (Invitrogen, Carlsbad, CA, USA) was carried out as described by White, Marrow, and Jones (1990) and Van Den Ende and De Hoog (1999), with initial denaturation at 94°C for 5 min.; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 48°C for 50 s, and extension at 72°C for 2 min.; followed by a final extension at 72°C for 5 min. To amplify the partial gene of β -tubulin (T1 5'-AACATGCGTGAGATTGTAAGT-3' and Bt-2b 5'-ACCTCAGTGTAGTGACCCTTGGC-3') (Invitrogen, Carlsbad, CA, USA) were used the protocol described by Glass and Donaldson (1995) and O' Donnell and Cigelnik (1997) with initial denaturation at 94°C for 4 min.; followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min., and extension at 72°C for 1 min.; followed by a final extension at 72°C for 3 min. Amplification of the glyceraldehyde-3-phosphate dehydrogenase (GPD1F 5'-CAACGGCTTCGGTCGCATTG-3' and GPD2R 5'-GCCAAGCAGTTGGTTGTGC-3') (Invitrogen, Carlsbad, CA, USA) was amplified according to Berbee, Pirseyedi, & Hubbard (1997) with initial denaturation at 96°C for 2 min.; followed by 25 cycles of denaturation at 96°C for 1 min., annealing at 52°C for 45 s, and extension at 72°C for 45 s; followed by a final extension at 72°C for 10 min.

PCR products were sequenced by ACTGene Análises Moleculares Ltd. (Ludwigbiotec). Sequences were compared to those deposited in the GenBank (Altschul, Gish, Miller, Myers, & Lipman, 1990; <http://www.ncbi.nlm.nih.gov/genbank>) using the BLASTn tool with a restricted search to type strains. Based on data available in the TreeBase database (Piel et al., 2009; <http://www.treebase.org>), closely related species were also selected (Studies S17061 and S14881). Sequence alignment and phylogenetic analysis were performed according to Polonio et al. (2016). The sequences obtained were deposited in GenBank corresponding to the ITS1-5.8S-ITS2 (accession numbers: MK804479 to MK804492), β -tubulin (MK848597 to MK848609) and GPDH (MK861119).

In vitro antagonism and competitive interactions between endophytes and *Colletotrichum* sp. CNPUV378

There were 43 endophytic isolates randomly selected and tested for their biocontrol capacity against the pathogen *Colletotrichum* sp. CNPUV378 (provided by EMBRAPA Grape and Wine, Bento Gonçalves, RS, Brazil). For this, the dual culture method of Campanile, Ruscelli, and Luisi (2007) modified by Polonio et al. (2015) was used. All assay was performed in triplicate.

The percentage inhibition rate of mycelial growth (IP%) was calculated following Polonio et al. (2015): $IP\% = (1 - MT/MC) \times 100$, where IP% = inhibition index percentage of mycelial growth, MT = mean of triplicate area measured for treatment in cm^2 , and MC = average of triplicate control area in cm^2 .

Competitive interactions (CI) between endophytes and phytopathogens were determined according to the rating scale proposed by Innocenti, Garibyan, and Badalyan (2002) and slightly modified by Ribeiro et al. (2018), which considers four main types of interactions (A, B, C and D) and their subtypes. The types are: A = inhibition of mycelial growth with contact; B = inhibition from a distance; C = endophytic growth on the pathogen without initial inhibition; and C = partial (CA1) and complete (CA2) endophytic growth on the pathogen after initial inhibition with mycelial contact; C = partial (CB1) and complete (CB2) endophytic growth on the pathogen after initial inhibition from a distance; D = partial (DA1) and complete (DA2) pathogen growth on the endophyte after initial inhibition with mycelial contact.

Screening of antifungal activity of metabolic extracts

Ten top endophytes (named by codes PE-08, PE-14, PE-17, PE-22, PE-27, PE-34, PE-36, PE-39, PE-40 and PE-41) ranked in the dual culture experiment were randomly selected for the obtainment of crude ethyl acetate extracts containing secondary metabolites (CEAEs). There were 3 mycelia plugs (6-mm diameter) of seven-day-old cultures of each endophyte inoculated into 500-mL Erlenmeyer flasks containing 250 mL of PDB (Potato Dextrose Broth; pH 6.8) (Himedia, Mumbai, India) and incubated at 28°C for 21 days under stationary condition. The metabolic extraction from the fermented culture broth, by using ethyl acetate P.A (Synth, Diadema, SP, Brazil) as solvent, followed the protocol described by Polonio et al. (2015).

To evaluate the bioactivity of CEAEs against *Colletotrichum* sp. CNPUV378, triplicates of PDA dishes received 5 mm autoclaved filter paper plugs inoculated with 10 μ L of CEAEs (10 $mg \cdot mL^{-1}$ in methanol) in an opposite position of phytopathogen plugs and remained incubated at 28 °C for 7 days. For controls, the paper plugs received (instead of CEAEs) the fungicide Benlate® (30 $mg \cdot mL^{-1}$) (C1) or methanol P.A (Synth, Diadema, SP, Brazil) (C2); C3 consisted of a phytopathogen plug inoculated in only one point of the PDA dishes. The percentage inhibition rate of mycelial growth (IP%) was calculated according to Polonio et al. (2015).

Greenhouse assay with *Colletotrichum* sp. endophyte and *Colletotrichum* sp. pathogen

For the preparation of fungal inoculum, 6 mm diameter plugs of endophyte PE-36 and pathogen were macerated into microtubes containing 1.5 mL of autoclaved distilled water and then vortexed until completely homogenized. *P. vulgaris* seeds were treated by immersion in 70% ethanol for 1 min., surface-disinfected with sodium hypochlorite solution (2.5% available Cl⁻) for 3 min. and 70% ethanol for 30 s, and finally rinsed three times in sterile distilled water. To evaluate the efficiency of surface-disinfection, aliquots (100 μ L) of water from the last rinsing was spread on Petri dishes containing PDA and TSA (Tryptone Soy Agar, pH 7.3) (Himedia, Mumbai, India). Then, seeds were transferred to dishes containing cotton soaked with 20 mL of water (both of them were previously autoclaved) and incubated at 28°C without photoperiod for 3 days. On the 3rd day, germinated seeds were transferred to 250 mL polypropylene containers with a mixture of Mecplant® substrate + vermiculite (3:1 v v⁻¹) and soil (1:1 v v⁻¹). The soil was collected at *Universidade Estadual de Maringá* (23°24'12.18" S and 51°56'30.54" W), autoclaved at 121°C for 120 min.

There was one germinated seed placed in each container (with prepared soil and previously moistened with water) at approximately 3 cm depth, with the radicle facing down. The treatments were: control with water (C), plants only inoculated with the endophyte (T1), plants only inoculated with the phytopathogen (T2) and plants simultaneously inoculated with the endophyte + pathogen (T3). A total of 800 μ L of each fungal suspension was used with 10 plants for each treatment (one plant per container). The presence or absence of symptoms and measurement of the biometric parameters (height, number of leaves, and root length) was performed 25 days after inoculations.

Statistical analysis

Data from the *in vitro* antagonism and antifungal activity the IP% values were analyzed using analysis of variance and the means were compared by the Scott-Knott test ($p < 0.05$ was taken to indicate a statistically

significant difference) using the statistical program SISVAR 5.6 (Ferreira, 2011). To greenhouse experiment, the means of biometric parameters were compared by the Tukey's test also taking $p < 0.005$ to indicate a statistical significant difference (Ferreira, 2011).

Results and discussion

Isolation and molecular identification of endophytic fungi

From the 500 leaf fragments sampled from *P. edulis*, we obtained a colonization frequency of 89%. The efficiency of the surface-disinfection process was attested by the absence of microbial growth in the control dishes. In order to isolate strains from the endophytic community, the surface disinfection of host plant material is necessary to ensure the elimination of epiphytes. In agreement with this current study, sodium hypochlorite and alcohol solutions have been employed in endophytic isolation protocols (Rani, Sharma, Chaturvedi, & Yadav, 2017; Salazar-Cerezo, Martinez-Montienel, Cruz-Lopez, & Martinez-Contreras, 2018; Lateef et al., 2019). For a look into the properties of endophytic microorganisms, the fragmentation of plant material represents a successful methodology that demonstrates satisfactory results for isolating the endophytic community, as already reported for different host plants (Wang et al., 2015).

The results of the BLASTn analysis are shown in Table 1. Phylogenetic analysis confirmed the molecular identification of endophytes PE-08, PE-14, PE-22, PE-27, PE-34, PE-36, PE-40, and PE-41 at the genus level as *Colletotrichum* sp. The taxonomy of PE-10, PE-17, PE-19, PE-26, and PE-38 was confirmed at the species level as *Colletotrichum brevisporum* [ITS BLASTn 98%; β -tubulin BLASTn 100%; 100% Bayesian probability (BP)], while PE-39 was confirmed as *Curvularia senegalensis* (ITS BLASTn 97%; 100% PB) (Figure 1).

Table 1. Endophytic fungi isolated from passion fruit and sequences with the greatest identity when aligned to the GenBank database (NCBI).

Endophyte	Gene	Fungus with highest similarity	GenBank code	Identity
PE-08	ITS	<i>Colletotrichum gloeosporioides</i>	AJ301979.1	96%
	TUB	<i>Colletotrichum cliviae</i>	KJ955361.1	100%
PE-10	ITS	<i>Colletotrichum gloeosporioides</i>	MF076617.1	98%
	TUB	<i>Colletotrichum brevisporum</i>	MF033887.1	99%
PE-14	ITS	<i>Colletotrichum gloeosporioides</i>	AJ301979.1	99%
	TUB	<i>Colletotrichum cliviae</i>	KJ955361.1	99%
PE-17	ITS	<i>Colletotrichum gloeosporioides</i>	MF076617.1	98%
	TUB	<i>Colletotrichum brevisporum</i>	MF033887.1	99%
PE-19	ITS	<i>Colletotrichum gloeosporioides</i>	MF076617.1	98%
	TUB	<i>Colletotrichum brevisporum</i>	MF033887.1	99%
PE-22	ITS	<i>Colletotrichum gloeosporioides</i>	AJ301979.1	98%
	TUB	<i>Colletotrichum cliviae</i>	KU743289.1	99%
PE-26	ITS	<i>Colletotrichum gloeosporioides</i>	MF076617.1	98%
	TUB	<i>Colletotrichum brevisporum</i>	MF033887.1	99%
PE-27	ITS	<i>Colletotrichum gloeosporioides</i>	AJ301979.1	99%
	TUB	<i>Colletotrichum cliviae</i>	KJ955361.1	100%
PE-34	ITS	<i>Colletotrichum gloeosporioides</i>	AJ301979.1	99%
	TUB	<i>Colletotrichum cliviae</i>	KJ955361.1	100%
PE-36	ITS	<i>Colletotrichum gloeosporioides</i>	AJ301979.1	99%
	TUB	<i>Colletotrichum cliviae</i>	HM585423.1	99%
PE-38	ITS	<i>Colletotrichum gloeosporioides</i>	MF076617.1	98%
	TUB	<i>Colletotrichum brevisporum</i>	MF033887.1	99%
PE-39	ITS	<i>Curvularia senegalensis</i>	HG779001.1	97%
	GPDH	<i>Curvularia affinis</i>	LT715785.1	98%
PE-40	ITS	<i>Colletotrichum gloeosporioides</i>	AJ301979.1	99%
	TUB	<i>Colletotrichum cliviae</i>	KJ955361.1	100%
PE-41	ITS	<i>Colletotrichum brevisporum</i>	9KU498265.1	98%
	TUB	<i>Colletotrichum sp.</i>	GU994315.1	91%

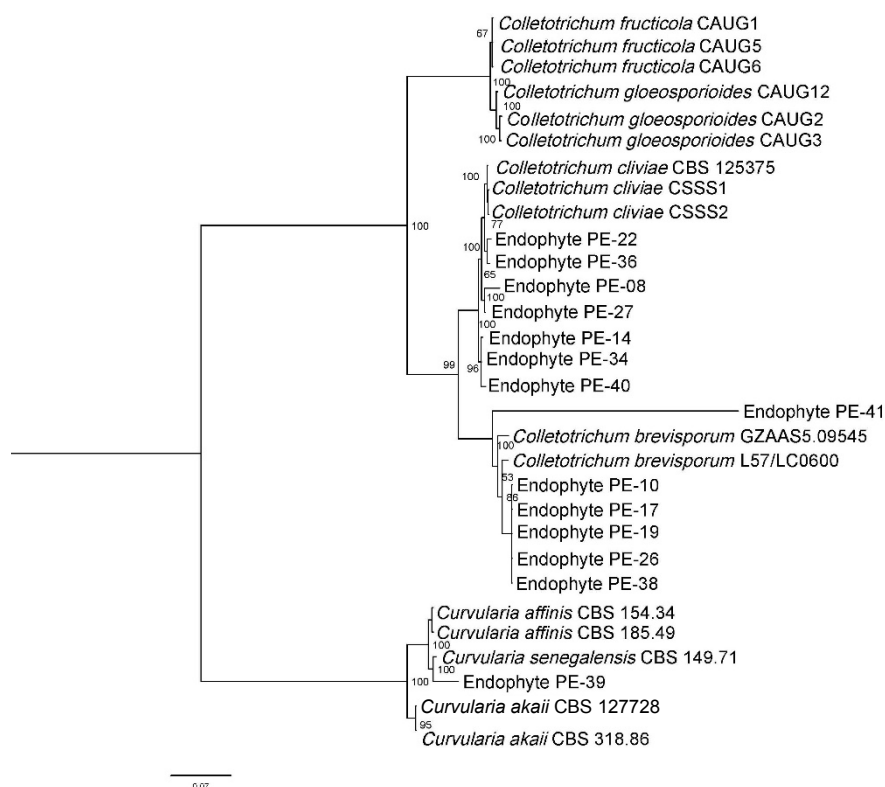


Figure 1. Cladogram results of the Bayesian analysis of endophytic fungi from *Passiflora edulis* with the alignment of three genes combined (ITS, β -Tubulin and GPDH*). The Bayesian probability was demonstrated on the nodes between each individual. *Only for PE-39.

Literature data had already reported the abundant occurrence of *Colletotrichum* in the endophytic community (Vieira, Michereff, Morais Jr, Hyde, & Câmara, 2014; Nascimento et al., 2015; Gonzaga, Costa, Santos, Araújo, & Queiroz, 2015; Correia, Lira, Assis, & Rodrigues, 2018). Herein, *Curvularia* endophytes were less frequent in the *P. edulis* community; this genus was also isolated as an endophytes from other vegetal species (Bezerra et al., 2013; Nascimento et al., 2015; Correia et al., 2018).

***In vitro* antagonistic and antifungal activity of endophytes against *Colletotrichum* sp. CNPU378**

Dual culture results showed differences among the antagonistic actions, ranging from 28.8% to 48.8% of IP% against *Colletotrichum* sp. CNPU378. Based on the rating scale proposed by Innocenti et al. (2002) and modified by Ribeiro et al. (2018), the types of CI observed were: A = deadlock (inhibition of phytopathogen) with mycelial contact (77.2%); DA1 = partial pathogen growth on the endophyte after initial inhibition with mycelial contact (13.6%); CA1 = partial endophyte growth on the pathogen after initial deadlock with mycelial contact (4.5%), and DB1 = partial pathogen growth on the endophyte after initial deadlock at a distance (4.5%). The Scott-Knott test highlighted 15 endophytic strains as promising antagonists in comparison with the control 2 (phytopathogen plug inoculated in only one point of Petri dish). More details regarding the antagonism index and competitive interaction are shown in Table 2 and Figure 2.

Table 2. Inhibition index of pathogen mycelial growth and competitive interaction between *Passiflora edulis* endophytes and *Colletotrichum* sp. CNPU378 in dual culture assay.

Endophytes code	Antagonism index (%) [*]	Competitive interaction ^{**}
PE-39	48.4 ^a	DA1
PE-34	48.0 ^a	A
PE-40	47.7 ^a	A
PE-17	47.5 ^a	A
PE-36	47.1 ^a	A
PE-27	46.5 ^a	CA1
PE-22	45.8 ^a	A
PE-08	45.1 ^a	DB1
PE-14	44.8 ^a	DA1
PE-41	44.5 ^a	A

Endophytes code	Antagonism index (%) [*]	Competitive interaction ^{**}
PE-26	44.3 ^a	A
PE-38	44.1 ^a	A
PE-10	43.3 ^a	DA1
PE-19	43.2 ^a	DA1
PE-21	40.6 ^b	A
PE-12	39.9 ^b	A
PE-24	39.6 ^b	A
PE-13	39.5 ^b	A
PE-06	39.4 ^b	A
PE-18	38.9 ^b	A
PE-30	38.3 ^b	A
PE-11	38.1 ^b	A
PE-44	37.9 ^b	A
PE-04	37.8 ^b	CA1
PE-01	37.5 ^b	DA1
PE-31	37.5 ^b	A
PE-37	37.5 ^b	A
PE-43	37.3 ^b	A
PE-33	35.9 ^b	A
PE-25	35.8 ^b	A
PE-29	35.1 ^c	A
PE-16	35.0 ^c	A
PE-15	34.3 ^c	A
PE-05	34.1 ^c	A
PE-09	33.7 ^c	A
PE-42	33.6 ^c	A
PE-07	33.2 ^c	A
PE-28	32.7 ^c	A
PE-02	32.4 ^c	DB1
PE-23	31.3 ^c	A
PE-32	31.2 ^c	A
PE-35	30.8 ^c	A
PE-03	28.8 ^c	DA1
Control 1	45.5 ^a	---
Control 2	0.0 ^d	---

^{*}Means of triplicates. Means followed by the same letter in column do not differ by the Scott-Knott test ($p < 0.05$). ^{**}Rating scale based on Innocenti et al. (2002) and Ribeiro et al. (2018). The absence of competitive interaction was indicated by (---).

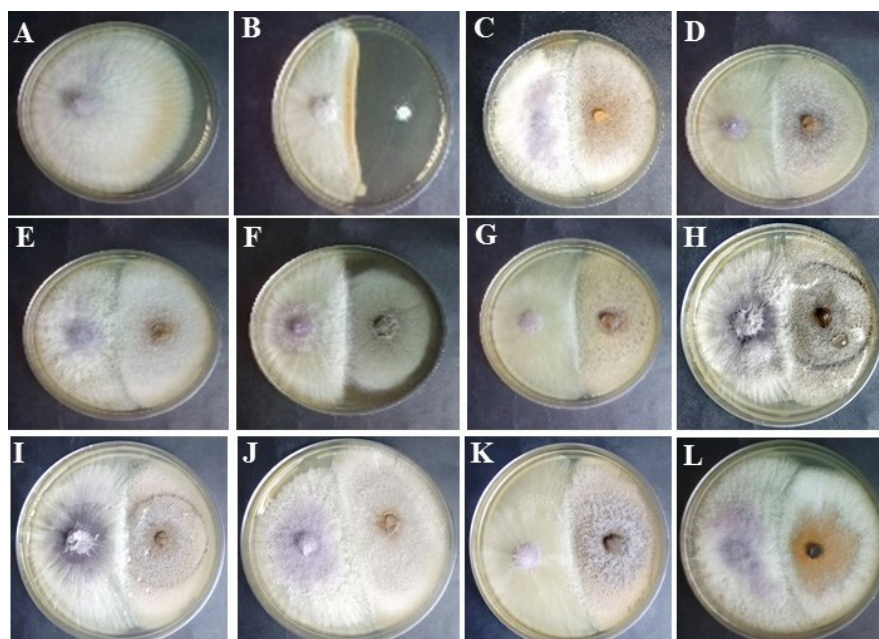


Figure 2. *In vitro* antagonism of endophytic fungi associated with *Passiflora edulis* against pathogen *Colletotrichum* sp. CNPUV38. **A.** Negative control with pathogen; **B.** Positive control with commercial fungicide; **C.** PE-17 (right) against pathogen (left); **D.** PE-34 (right) against pathogen (left); **E.** PE-36 (right) against pathogen (left); **F.** PE-39 (right) against pathogen (left); **G.** PE-40 (right) against pathogen (left); **H.** PE-08 (right) against pathogen (left); **I.** PE-14 (right) against pathogen (left); **J.** PE-22 (right) against pathogen (left); **K.** PE-27 (right) against pathogen (left); **L.** PE-41 (right) against pathogen (left).

Many studies have investigated the *in vitro* activity of endophytes against *Colletotrichum* pathogens. Rabha, Naglot, Sharma, Gogoi, and Veer (2014) showed that the indexes of antagonistic activity of fungal endophytes against *Colletotrichum camelliae* ranged from 18.33 to 36.73. The inhibition indexes (17.52 to 49.71%) obtained by Bongiorno et al. (2016.) were similar to those obtained in this study (28.8 to 48.8%). Supporting our results, literature data has already been reported on the deadlock (mutual inhibition) at mycelial contact (type A) as the most frequent in the pathogen-endophytes competitive interaction (Campanile et al., 2007; Polonio et al., 2015; Wang et al., 2015; Bongiorno et al., 2016), in which mutual fungal inhibition occurs and neither microorganism is able to overgrow the other (Innocenti et al., 2002; Oliveira et al., 2019).

Table 3 describes the results obtained for the *in vitro* antifungal activity of CEAEs of secondary metabolites. The higher IP% (=20.8%) against the *Colletotrichum* sp. CNPU378 was reported for the extract (named as code CEAE_{PE-36}) obtained from the endophyte PE-36.

Table 3. *In vitro* antifungal activity of crude ethyl acetate extracts (CEAEs) from *Passiflora edulis* endophytes, based on the inhibition index of mycelial growth (%) of *Colletotrichum* sp. CNPU378.

CEAEs(fungal code)/ controls	Inhibition index %	Mycelial growth of phytopathogen (cm ²)
CEAE _{PE-36}	20.8 ^b	38.76
CEAE _{PE-39}	14.5 ^c	41.88
CEAE _{PE-08}	13.6 ^c	42.30
CEAE _{PE-17}	13.1 ^c	42.53
CEAE _{PE-14}	12.0 ^c	43.08
CEAE _{PE-41}	10.9 ^d	43.60
CEAE _{PE-22}	6.8 ^d	45.60
CEAE _{PE-27}	9.1 ^d	44.48
CEAE _{PE-34}	8.5 ^d	44.78
CEAE _{PE-40}	6.7 ^d	45.67
Control 1	45.8 ^a	26.54
Control 2	12.5 ^c	42.82
Control 3	0.0 ^e	48.94

* Means of triplicates. Means followed by the same letter in column do not differ by the Scott-Knott test ($p < 0.05$). Control 1 = paper plug with 10 μ L of fungicide Benlate® (30 mg.mL⁻¹); Control 2 = paper plug with 10 μ L of methanol; C3 = phytopathogen plug inoculated in only one point of Petri dish. Concentration of CEAEs = 10 μ L of 10 mg.mL⁻¹ solution of crude extracts.

Landum et al. (2016) reported that extracts with non-volatile compounds, produced by endophytes from olive plants, reached up to 26.8% of inhibition rates in dual culture tests against *C. acutatum*, similar to rates found in this study (up to 20.8% of mycelial inhibition).

Greenhouse assay

Table 4 details the biometric parameters related to the vegetative growth of bean plants. Plants inoculated only with the endophyte *Colletotrichum* sp. PE-36 (T1) was symptomless and showed the difference for number of leaves and root length when compared to control plants (C) and other treatments (T2 and T3). Our findings suggest that the endophyte strengthened the growth promotion of the treated plants (T1). The T2 treatment presented inferior results in all biometric parameters analyzed. In addition, the T2 treatment presented whitish lesions in the root system apparently caused by the mycelial growth of *Colletotrichum* sp. CNPUV378, which were not found in the other treatments, suggesting that plants inoculated with endophyte + pathogen (T3), the endophyte was able to soften the propagules of the pathogen Figure 3.

Table 4. Biometric parameters related to the vegetative growth of bean plants with and without inoculation of the endophyte *Colletotrichum* sp. (PE-36) and phytopathogen *Colletotrichum* sp. CNPU378.

Treatments	Height (cm)	Root length (cm)	Leaves number
Control (C)	34.9 ^a	20.2 ^{ab}	9.1 ^{ab}
Endophyte <i>Colletotrichum</i> sp. PE-36 (T1)	33.0 ^{ab}	21.5 ^a	11 ^a
Pathogen <i>Colletotrichum</i> sp. CNPU378 (T2)	19.2 ^c	17.1 ^b	8.2 ^b
Pathogen x Endophyte PE-36 (T3)	23.50 ^{bc}	19.4 ^{ab}	10 ^{ab}

Means of triplicates followed by lower-case letters indicate that values are not significantly different according to the Tukey test ($p < 0.05$).

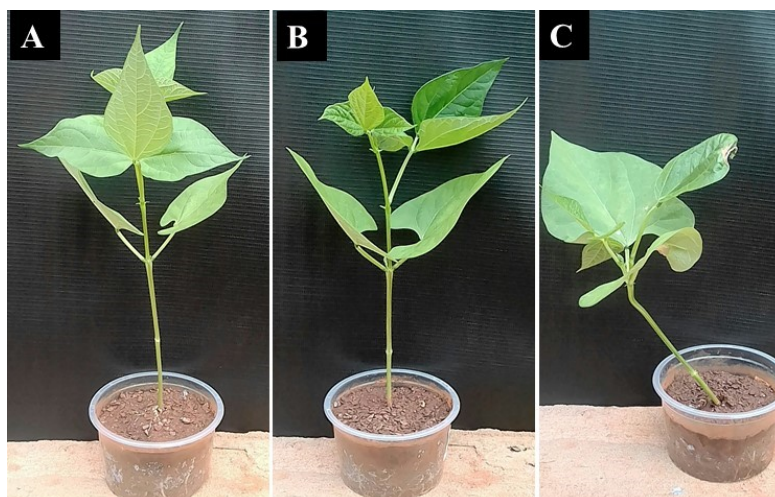


Figure 3. Evaluation of the endophytic strain PE-36 against the pathogen *Colletotrichum* sp. CNPUV38 under greenhouse conditions. A. Control plant (C); B. plant inoculated with pathogen *Colletotrichum* sp. CNPUV38 and endophyte *Colletotrichum* sp. PE-36 (T3); C. Plant only with the pathogen *Colletotrichum* sp. CNPUV38 (T2).

Different mechanisms of *in vivo* biocontrol can be employed alone or together with other strategies to trigger direct or indirect responses to overcome the pathogenicity of the causal agent (Ghorbanpour, Omidvari, Dahaji, Omidavar, & Kariman, 2018). Consistent with our *in vivo* assay, Rodríguez et al. (2016) reporting that the application of a suspension of an endophytic fungus on necrotic leaves reduced the spore production of the pathogen after 21 days. In addition, the authors evaluated the endophytic colonization through plant height, root length, biomass, and the number of leaves. Li, Hwang, Huang and Huang (2018) described an endophyte able to carry out the suppression of *Fusarium* wilt of tomato plants and beefing up their growth, mainly through the absorption of nutrients.

Conclusion

Despite many fungi of the genus *Colletotrichum* being described as causative agents of anthracnose, in this study, the plant sampled was 89% colonized predominantly by *Colletotrichum* endophytes living in asymptomatic relationship with the host *Passiflora edulis*, especially the isolate *Colletotrichum* sp. PE-36 with a highlight in *in vitro* and *in vivo* experiments.

In this work, a *Colletotrichum* endophytic fungus highlighted in antagonistic activity against a pathogen in the agricultural system, reinforcing the idea that endophytic microorganisms are a possible tool for the development of more sustainable, eco-friendly and easy field application as an inoculant. agricultural.

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