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Biotechnological potential of the endophytic fungi isolated from *Clitoria guianensis*

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INFO

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ABSTRACT

The biodiversity of endophytic fungi in the Cerrado region in the north of Brazil remains largely unexplored, however it is important to explore the biotechnological potential of these endophytes. In this study, we isolated seventeen endophytic fungi strains from the medicinal plant *Clitoria guianensis* and tested the ability of these strain to inhibit the phytopathogenic fungi: *Fusarium oxysporum*, *Bipolaris oryzae*, and *Curvularia lunata*. The *in vitro* antagonistic assay was evaluated on different time scales (six and twelve days), we observed that all strains of endophytic fungi, isolated from *C. guianenses*, inhibited the growth of *F. oxysporum* and *B. oryzae* after six days, for *C. lunata* just ten strains inhibited its growth in the first six days. In twelve days, only the strains CGF1, CGF3, and CGF4 inhibited the growth of *F. oxysporum*, while for the other phytopathogenic fungi the inhibitions were not observed. The endophytic fungi were cultivated in a controlled environment using potato dextrose broth (PDB) medium and the crude extracts were obtained by extracting the fungi culture with ethyl acetate (EtOAc). All the crude extracts exhibited antioxidant activity, as assessed by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) method. Additionally, four different concentrations (C100, C500, C1000 and C2000) of the crude extracts were evaluated in the *in vitro* allelopathic assay using *Lactuca sativa* (lettuce) seeds. Some fungal extracts demonstrated allelopathic effects by inhibiting the growth of *Lactuca sativa* seedlings, for example, the crude extract obtained from the strain CGF7 at a concentration of C2000 inhibited radicle development by 75.2%. While others acted as growth promoters, as is the case with the crude extract of the CGF10 strain, which contributed to lettuce radicle growth by 15.9% in C2000. Overall, these findings suggest that endophytic fungi associated with *C. guianensis* have significant biotechnological potential and can be used as biocontrol agents against phytopathogens and specific plants, in addition to growth promoters, making them valuable tools for sustainable agriculture.

Palavras-chaves
fungos endossimbiontes
vergateza
atividade antioxidante
antagonismo
atividade alelopática

RESUMO

Potencial biotecnológico de fungos endofiticos isolados de Clitoria gianensis

A biodiversidade de fungos endofíticos na região do cerrado no Norte do Brasil permanece pouco explorada, portanto, é importante explorar o potencial biotecnológico desses endófitos. Neste estudo, foram isoladas dezessete cepas de fungos endofíticos da planta medicinal *Clitoria guianensis* e foi testado sua capacidade de inibir três fungos fitopatogênicos: *Fusarium oxysporum*, *Bipolaris oryzae* e *Curvularia lunata*. O ensaio antagônico in vitro foi avaliado em diferentes escalas de tempo (seis e doze dias), observamos que todas as cepas de fungos endofíticos, isoladas de *C. guianenses*, inibiram o crescimento de *F. oxysporum* e *B. oryzae* após seis dias, para *C. lunata* apenas dez cepas inibiram seu crescimento nos primeiros seis dias. Em doze dias de ensaio apenas as cepas CGF1, CGF3 e CGF4 inibiram o crescimento de *F. oxysporum*, enquanto para os demais fungos fitopatogênicos a inibição não foi observada. Os fungos endofíticos foram cultivados em ambiente controlado utilizando meio de batata dextrose (PDB) e os extratos brutos foram obtidos pela extração da cultura do fungo com acetato de etila (AcOEt). Todos os extratos brutos apresentaram atividade antioxidante, avaliada pelo método de 2,2 difenil-1-picrilhidrazil (DPPH). Adicionalmente, quatro concentrações diferentes (C100, C500, C1000 e C2000) dos extratos brutos foram avaliados no ensaio alelopático in vitro utilizando sementes de *Lactuca sativa*. Alguns extratos fúngicos demonstraram efeitos alelopáticos inibindo o crescimento de plântulas de *Lactuca sativa*, como por exemplo o extrato bruto obtido da cepa CGF7 na concentração de C2000 inibiu o desenvolvimento das radículas em 75,2%. Enquanto outros atuaram como promotores de crescimento, como é o caso do extrato bruto da cepa CGF10 que contribuiu para o crescimento da radícula de alface em 15,9% em C2000. De modo geral, estes achados sugerem que os fungos endofíticos associados a *C. guianensis* possuem significativo potencial biotecnológico, podendo ser utilizados como agentes de biocontrole contra fitopatógenos e plantas daninhas, além de promotores de crescimento, tornando-os ferramentas valiosas para a agricultura sustentável.

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INTRODUCTION

Endophytes are microorganisms that inhabit the internal tissues of plants without causing apparent harm to their hosts throughout their life cycle (Manganyi and Ateba 2020). These endophytes play vital physiological and ecological roles in plants, contributing to their protection and overall health (Khare et al. 2018; Khare et al. 2018). They promote plant growth, enhance yields, and provide resilience against various abiotic and biotic stresses, including drought, salinity, extreme temperatures, heavy metal toxicity, oxidative stress, as well as protection against insects and herbivores (Khare et al. 2018; Rai et al. 2014; Yan et al. 2019).

Endophytic fungi occupy the same ecological niche as phytopathogens, allowing them to control these pathogens through nutrient competition or the production of toxic substances (Hamzah et al. 2018; Yan et al. 2019). They have also garnered significant interest due to their ability to produce bioactive compounds, which hold potential applications in pharmaceutical and agricultural industries, particularly in crop protection. These fungi are capable of synthesizing antimicrobial compounds, plant growth hormones, herbicides, and various other agrochemical bioactive metabolites (Rai et al. 2014; Yan et al. 2019). Utilizing agrochemicals derived from endophytes could enhance crop sustainability by reducing the reliance on environmentally harmful chemical pesticides, minimizing the use of fertilizers, and boosting plant resistance (Behie e Bidochka 2013).

The biodiversity and biotechnological potential of endophytic fungi within the Cerrado biome have been relatively unexplored (Dantas, Alves, and Chapla 2021; Ribeiro et al. 2021). For example, the medicinal plant *Clitoria guianensis* lacks scientific studies investigating its chemical composition, biological activities, and fungal biodiversity, highlighting a gap between cultural usage in traditional medicine and scientific research. *Clitoria guianensis* Benth (Fabaceae), is a medicinal plant native to the Cerrado biome in Brazil (Vila Verde et al., 2003). This plant is related in some ethnobotanical studies in which they report its use by part of the population in the form of decoction or bottled, such as sexual stimulant, tonic for the nervous system, purification baths, among others (Cunha et al., 2020; Souza and Felfili, 2006; Vila Verde et al., 2003).

The aim of this study was to isolate the endophytic fungi associated with the medicinal plant *C. guianensis* and evaluate the antagonistic activity of these endophytes against agriculturally significant phytopathogens. The crude extracts of the endophytes were obtained and their antioxidant and allelopathic properties was obtained. This work also aims to contribute to the understanding of fungal biodiversity and its biotechnological potential for sustainable agricultural applications.

MATERIAL AND METHODS

Collection and isolation of endophytic fungi

Healthy leaves and stems of *C. guianensis* were collected in the experimental farm of the Federal University of Tocantins (UFT), Gurupi city (at 11.46154" S and 49.02424" W, at an altitude of 300 m). The collection was performed in July 2015, during the dry season. A voucher specimen of the plant was deposited in the Herbarium of Tocantins (HTO) of UFT/Porto Nacional with the number 10.637.

The collected material was first washed with soap and water, immersed in alcohol 70% for 1 minute and then transferred to a hypochlorite solution 2.5% for 1 min as described of Chapla, Biasetto and Araujo (2013). Subsequently, they were transferred to two Beakers (250 mL) containing 100 mL of the sterile distilled water, for 2 min. in each. For endophytic fungi isolation the leaf and stem were cut with the aid of scalpel and inserted into three Petri dishes (90 x 15 mm) containing 20 mL of the Potato Dextrose Agar (PDA) medium with addition of antibiotic penicillin (50 mg/L).

In addition, the plant material underwent two other isolation procedures: dilution and crushing. Specifically, 5 g of stem and leaves were added to separate mortar with PBS buffer. After maceration, a portion of the material was divided into four sterile Falcon tubes (15 mL) and subject to shaking for 1.5 hours. Subsequently, dilutions 10^{-1} and 10^{-2} were inoculated onto petri dishes containing PDA with addition of antibiotic penicillin (50 mg/L). The remaining macerated material was directly inoculated onto six Petri dishes containing 20 mL of PDA with addition of antibiotic penicillin (50 mg/L).

After the growth of the endophytes, serial transfers were performed until pure cultures were obtained. These pure cultures were then preserved in Eppendorf tubes following the Castellani method, and labeled with the designation of CGFX, where X corresponds to specific fungus number (e.g., CGF1 to CGF17).

Antagonist activity assay

The *in vitro* antagonism experiment was conducted using the plate-paired cultures method (Lopes et al. 2017), which involves direct confrontation between endophytes isolated from *C. guianensis* and the phytopathogenic fungi *Fusarium oxysporum*, *Curvularia lunata* and *Bipolaris oryzae*, made available by Laboratory AgroMicrobiology

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at the UFT. Each Petri dish containing 20 mL of PDA medium, was inoculated with two paired 6 mm discs placed equidistantly. One disc was taken from the edge of the pathogenic fungus colony, while the other disc was obtained from the edge of the endophytic fungus colony. After pairing, the plates were incubated in BOD at 25°C for 14 days. For the control, the phytopathogenic fungus was inoculated without the presence of endophytes. Evaluation was performed when the fungal colony (control) reached the edges of the plate, as described by Bell (1982). All tests were performed in triplicate. Once the pairing of all endophytes was completed, the measured data of the size of the phytopathogen colonies were used to generate an inhibition table using statistical program Assistat (Francisco and Carlos 2016). The Duncan test with 5% probability was applied to compare means and determine significant differences, indicated by alphabetical letters. The percentage of inhibition was calculated according to the following formula: PI% = (Dc – Dt /Dc) x 100, where Dc is the average diameter of the pathogen colony on the control plates (without antagonist) and Dt is the average diameter of the pathogen colony compared to the antagonist (endophytic isolate).

Fermentation in a liquid medium and obtaining crude extracts

The isolated endophytes were grown in petri dishes containing 20 mL PDA medium for seven days. At the end of this period, each fungus was inoculated in two Erlenmeyer flasks (500 mL) with 300 mL of Potato Dextrose Broth (PDB) medium and incubated at 25°C for 15 days. After incubation, conventional filtration using filter paper and Buchner funnel was performed to obtain clarified broth. The filtrate was submitted to liquid/liquid extraction with ethyl acetate (EtOAc) (3 x 300 mL), this being evaporated into a rotary evaporator under reduced pressure, providing the EtOAc crude extract of each strain of the endophytic fungus isolated.

The crude extracts obtained were analyzed by TLC silica gel 60 (F254-Filter-Bio) and mobile phase chloroform:methanol (9:1 v/v). The plates were revealed in iodine vapors and ultraviolet light (λ= 254 nm). And by HPLC-DAD in Shimadzu equipment: Shimadzu LC-10AD pump; auto gun Shimadzu SIL-10A; Ultraviolet detector, in diode arrangement, Shimadzu SPD-M10A; a Phenomenex Luna analytical column (C-18, 250 x 4.60 mm and 5 µm) and H_2O:CH_3OH gradient elution (95:05 v/v to 0:100% in 45 minutes remaining in this condition for another 10 min.) with a flow rate of 1.0 mL/min and 254 nm.

Antioxidant activity assay

The crude extracts were submitted to evaluation regarding reactivity with 2,2 diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) by the chromatoplate method (Simões-Pires et al. 2005). The extracts were applied in silica plate gel 60 (F254-Filter-Bio) and eluted with $CHCl_3$:CH_3OH (9:1 and 8:2 v/v). The methanolic solution of 0.2% DPPH was dispersed under chromatoplates, which were then left under artificial light for 15 min. The antioxidant potential was evidenced by the presence of yellowish spots resulting from the reduction of DPPH against the purple pigmentation of the background.

Allelopathic activity assay

Crude extracts were evaluated for their allelopathic activity using lettuce seeds (*Lactuca sativa*, Feltrin seeds brand). Different concentrations of the crude extract were used: C100, C500, C1000 and C2000 µg/mL. The control group contained only distilled water. The application of the concentrations was performed in triplicate in petri dishes. Following the addition of 1 mL of each concentration, a 24-hours waiting period was observed to allow for solvent evaporation. Subsequently, 15 lettuce seeds were placed on each plate, along 4 mL of distilled water. The plates were then incubated at approximately 25°C for a duration of 4 days. At the end of the period, the germinated seeds were counted and their measurement, including the radicle and stem size, were taken using a caliper. The germination rate of the seeds was calculated using the following formula: G= (N/A) x 100, where: G represents germinability; N denotes the number of seeds that germinated, and A corresponds to the total number of seeds placed for germination (Pereira et al. 2019). For statistical analysis, the mean sizes of the radicle and stem, measured in millimeters, were analyzed using the Sisvar program (Ferreira 2014). The Tukey test was applied at a significance level of 5%. Alphabetical indices were used to indicate differences between the means. The percentage of inhibition was calculated according to the following formula: PI% = (Lc – Lt /Lc) x 100, where Lc is the average length of the control seedling and Lt is the average length of the seedling with the crude extract.

RESULTS AND DISCUSSION

Isolation of the endophytes

A total of seventeen strains of the endophytic fungi were isolated from *C. guianensis* (Figure 1), using three different techniques. In the fragmentation method, only two endophytic fungi strain were

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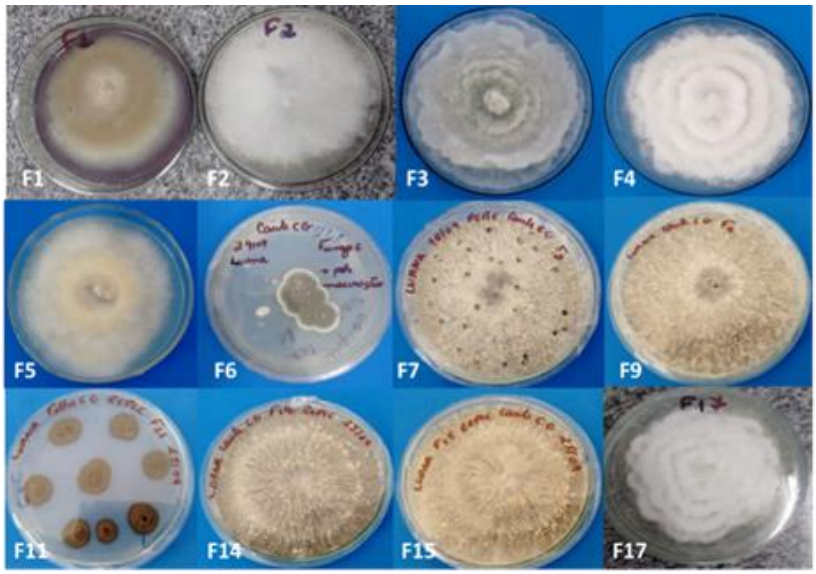
isolated: one from the leaf (code CGF1) and the other from the stem (code CGF2). Due to the limited number of isolated fungi, the plant material collection was repeated, and this time the endophyte isolation was carried out using the crushing and dilution method. However, no growth of endophytic fungi was observed during the dilution isolation. On the other hand, when the material was subjected to crushing isolation, fifteen endophytes strain were found to grow, nine were isolated from leaves and six from stem.



Endophytic fungi isolated	
Strain obtained from leaf	Strain obtained from stem
CGF1	CGF2
CGF3	CGF4
CGF7	CGF5
CGF8	CGF6
CGF10	CGF9
CGF11	CGF14
CGF12	CGF15
CGF13	
CGF16	
CGF17	

Figure 1 - Endophytic fungi isolated from *Clitoria guianensis*

In this study, endophytes were isolated using different methods, which may have influenced the number of endophytes isolated, herein the crushing method demonstrated the most efficient in isolating fungi according to the number of the strain isolated. This method involves breaking the plant tissue, facilitating the release of microorganisms from the interior of the plant (Araujo et al. 2010).

Diversity and frequency of endophytic fungi are also influenced by the season; studies found that the level of endophytic colonization increased during rainy seasons (Mishra et al. 2012; De Oliveira et al. 2020; Sadeghi et al. 2019; Singh et al. 2017). Rain splashes help in release of inoculum materials, and high humidity and low temperature help fungal spore germination and reproduction, causing high infection rate and fungal establishment in rain seasons (Sadeghi et al. 2019). In our work the isolation of the endophytes was performed in the dry season, which may have influenced the number of isolates. Sadeghi and colleagues believe that the low richness in the fungal endophyte community of *Citrus reticulata* in Roudam can be attributed to the extremely difficult environmental conditions such as drought (Sadeghi et al. 2019).

Antagonistic activity

The *in vitro* assay conducted through direct pairing demonstrated the antagonistic potential of certain strain of the endophytic fungi. The results from the *in vitro* test, as supported by statistical analysis, revealed the inhibition of *Fusarium oxysporum* by all endophytic fungi, as presented in Table 1. Notably, the fungi strains CGF12, CGF13, CGF6, and CGF14 displayed the highest inhibition percentages during the 6-day trial, with rates of 71.92%, 67.30%, 66.55%, and 62.12%, respectively.

All endophytes inhibited the phytopathogen *Bipolaris oryzae* in the first six days of cultivation (Table 1), however no inhibition of *B. oryzae* was observed for 12 days. The most effective inhibition percentages during the 6-day trial were exhibited by endophytes CGF10, CGF12, CGF13, and CGF6, with growth inhibition rates of 62.43%, 57.65%, 55.65%, and 55.49%, respectively. The inhibition of *Curvularia lunata* (Table 1) during the 6-day trial was demonstrated by the fungus CGF12, inhibiting the phytopathogen growth by 51.35%, the strain CGF14 followed closely with an inhibition rate of 40.84%. Additionally, CGF9, CGF10, and CGF13 all exhibited 40.55% inhibition of the phytopathogen. As for *B. oryzae* no inhibition of *C. lunata* was observed for 12 days. The type of interaction between endophytes and phytopathogens was the inhibition of mycelial growth with contact or an inhibition halo was formed, suggesting that the competition for space or the production of antifungal compounds by the fungi featured the inhibition mechanism (Dai, Gao, e Liu 2010; Santos et al. 2019).

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The inhibition of *B. oryzae* and *C. lunata* by the endophytes during the initial 6-day period suggests their potential effectiveness in controlling this phytopathogen. However, it is worth noting that the absence of inhibition beyond 6 days raises questions about the long-term efficacy of these endophytes against *B. oryzae* and *C. lunata*. Further studies should investigate the underlying mechanisms and potential limitations of the endophytic fungi in maintaining sustained inhibition over an extended period.

Table 1 - Mycelial growth means (in mm) values from phytopathogenic fungi in the direct confrontation assay against isolated endophytic fungi

Fungi code	**Phytopathogenic fungi[1]**					
	Fusarium oxysporum		*Bipolaris oryzae*		*Curvularia lunata*	
	6 days	12 days	6 days	12 days	6 days	12 days
Control	60.11^{a}	75.36^{a}	68.88^{a}	84.00^{a}	48.63^{b}	80.33^{a}
CGF1	41.12^{b}	43.61^{c}	54.65^{b}	70.00^{b}	46.68^{b}	70.00^{b}
CGF2	32.18^{bc}	70.00^{a}	51.33^{bc}	70.00^{b}	48.53^{b}	70.00^{b}
CGF3	29.34^{c}	57.08^{b}	46.83^{bc}	70.00^{b}	46.27^{b}	70.00^{b}
CGF4	26.37^{c}	31.55^{d}	52.55^{bc}	70.00^{b}	47.15^{b}	70.00^{b}
CGF5	44.16^{b}	70.00 ^{a}	42.31^{de}	70.00^{b}	29.66^{e}	70.00^{b}
CGF6	20.11^{c}	70.00 ^{a}	30.66^{fg}	70.00^{b}	37.70^{c}	70.00^{b}
CGF7	44.13^{b}	70.00 ^{a}	41.30^{de}	70.00^{b}	44.55^{b}	70.00^{b}
CGF8	25.77^{c}	70.00 ^{a}	42.77^{de}	70.00^{b}	35.22^{cd}	70.00^{b}
CGF9	26.67^{c}	70.00 ^{a}	45.66^{cd}	70.00^{b}	28.88^{ef}	70.00^{b}
CGF10	28.11^{c}	70.00 ^{a}	25.88^{g}	70.00^{b}	28.88^{ef}	70.00^{b}
CGF12	16.88^{c}	70.00 ^{a}	29.11^{fg}	70.00^{b}	23.66^{f}	70.00^{b}
CGF13	19.66^{c}	70.00 ^{a}	30.55^{fg}	70.00^{b}	28.88^{ef}	70.00^{b}
CGF14	22.77^{c}	70.00 ^{a}	35.00^{ef}	70.00^{b}	28.77^{e}	70.00^{b}
CGF15	26.77^{c}	70.00 ^{a}	33.88^{ef}	70.00^{b}	31.22^{de}	70.00^{b}
CGF16	25.66^{c}	70.00^{a}	36.44^{ef}	70.00^{b}	30.33^{de}	70.00^{b}
CGF17	29.55c	70.00a	35.88ef	70.00b	58.27a	70.00b

[1]Mean values followed by the same letter do not differ from each other by Duncan's test 5% probability.

The endophytic fungi strains CGF6, CGF10, CGF12, CGF13, and CGF14 show great promise as potential biocontrol agents, as they inhibited all three phytopathogenic fungi within a period of 6 days. These isolates represent 32% of the total isolates obtained. These findings underscore the importance of exploring endophytic fungi as a valuable resource for developing sustainable agricultural practices.

Studies have identified the antagonistic activity of endophytic fungi against different phytopathogenic fungi of agricultural interest (Huang et al. 2020; Grabka et al. 2022; Santos and Varavallo 2011; Silva-Valderrama et al. 2021). For example, Lopes et al. (2017) verified biocontrol with endophytes isolated from *Cymbopogon nardus* L. Rendle against *C. lunata*, *B. oryzae* and *F. oxysporum*; the *Trichoderma* spp. isolates obtained the higher percentages of pathogens inhibition. Santos et al. (2019) evaluated the antagonist activity of endophytic fungi from *Sapindus saponária* agaist *F. solani*, *Moniliophtora perniciosa* and *Glomeralla* sp., the best performance was observed against *F. solani* with an inhibition rate between 41.8 % and 67.5 %. The parasitic activity and other mechanisms of action such as production of volatile antibiotics and non-volatile substances, competition for space and nutrients and enzymatic activity hydrolytic properties make endophytic fungi powerful in control of phytopathogens and promotion of plant growth.

Agriculture has undergone severe production losses due to fungal pathogens. Although decreasing the attack of phytopathogenic insects and microorganisms, synthetic chemical fungicides repre-

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sent high risks for humans, with deleterious environmental impacts. The natural and biological control of diseases that affect crop plants has been focused to reduce the use of pesticides in agriculture. Biocontrol endophytes, such as *Ampelomyces*, one of the first biocontrol fungi used against pathogenic fungi, are environmentally friendly alternatives to chemical fungicides (Dai, Gao, and Liu 2010; Grabka et al. 2022; Santos et al. 2019; Souza and Santos 2017; Strobel 2018). The use of endophytes with biocontrol and/or growth promotion actions have been identified as viable alternative for sustainable agricultural production systems, and the biological control through antagonists has provided a viable solution for several diseases considered difficult to control (Chen et al. 2016; Grabka et al. 2022; Huang et al. 2020).

Crude extracts and bioactivities

After obtaining crude extract from all endophytes strains isolates, it was observed that each fungus exhibited different optimal cultivation conditions, as evidenced by the variation in the mass of crude extracts obtained for each fungus (Table 2).

Table 2 - Total mass of crude extracts obtained from the endophytic fungi in grams

Crude extract	Mass (g)	Crude extract	Mass (g)
CGF1	0.2287	CGF10	0.0928
CGF2	0.1871	CGF12	0.2558
CGF3	0.1132	CGF13	0.1715
CGF4	0.1747	CGF14	0.1711
CGF5	0.0865	CGF15	0.2338
CGF7	0.1679	CGF16	0.2505
CGF8	0.7582	CGF17	0.1343
CGF9	0.1401		

The chromatograms obtained from the crude extracts displayed distinct peaks with varying retention times (Figure 2), indicating a broad range of secondary metabolites production, varying in polarity from high to low, which can represent different classes of chemical compounds. This chemical diversity is advantageous as it increases the likelihood of discovering novel bioactive compounds with potential applications in various fields, including pharmaceuticals and agriculture.

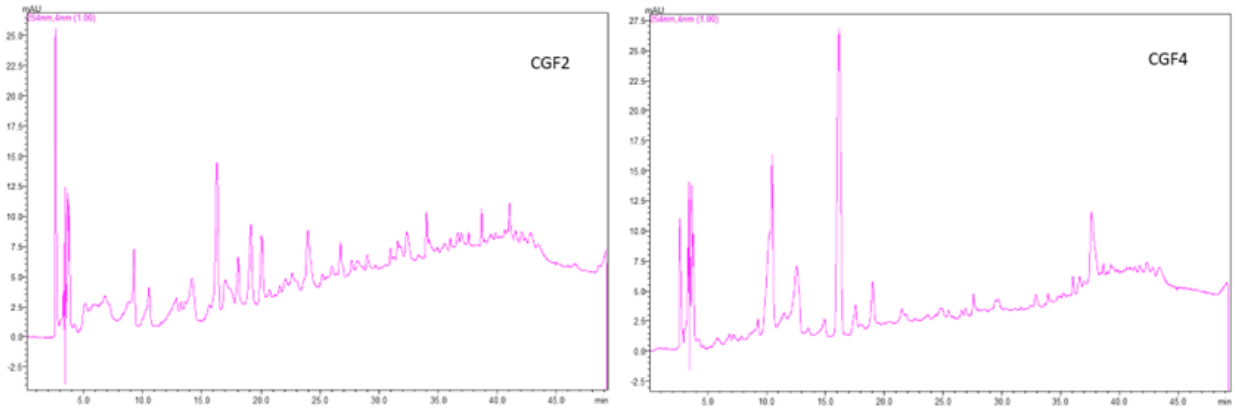

Figure 2 - Selected chromatograms obtained from the crude extracts of the endophytic fungi strains. Chromatograms obtained using the C18 column in λ=254 nm

The antioxidant activity from the crude extracts obtained was revealed by the presence of yellow spots on the chromatoplates, all the tested crude extracts exhibited positive antioxidant activity, demonstrating the biotechnological potential of these endophytic fungi. Antioxidants compounds prevent oxidative damage in plants, secondary metabolites, such as polyphenols, flavonoids and terpenoids, participate in the detoxification of reactive oxygen species (ROS) under different environmental stresses (Llauradó Maury et al. 2020). The ability of the endophytic fungi to

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scavenge free radicals and exhibit antioxidant activity suggests their potential as a source of natural antioxidants for medicinal and functional food purposes (Huang et al. 2007; Llauradó Maury et al. 2020).

Soares (2016) performed the antioxidant assay with DPPH in crude extracts in ethanolic, hexane and ethyl acetate fractions, obtained from leaves of *C. guianensis*. All extracts and fractions have been shown to be active. Crude ethyl acetate extract presented the strongest antioxidant activity. Thus, antioxidant properties are present both in the plant and in endophytic fungi in their association.

Lettuce seeds were incubated at 25°C for a period of four days. The germination percentage indicates that the crude extracts did not exhibit allelopathic activity by inhibiting the germination of lettuce seeds. Another factor that can indicate positive allelopathic activity is the effect of the extract on seedling growth, including the radicle and stem. Therefore, the measurements of seedlings were taken, and the means for each concentration of the extract are presented in Table 3.

The crude extract, CGF7 showed the most significant result in reducing the radicle size at the C2000 concentration, with a 75.2% inhibition. The crude extract of CGF4 strain demonstrated the second-best result, inhibiting 68.25% of radicle size compared to the control, followed by the fungus CGF3, which inhibited growth by 50.1% at C2000. Regarding stem size, once again, CGF3 and CGF7 fungi inhibited growth by 51.39% and 20.05% at C2000, respectively, providing evidence of the allelopathic activity of these fungal extracts.

On the other hand, it was observed that some crude extracts stimulated seedling growth. For example, CGF1 and CGF10 at C2000 concentration enhanced radicle growth by 13.04% and 15.95%, respectively. Additionally, the extracts CGF15 and CGF17 contributed to stem growth by 18.66% and 26.77%, respectively, demonstrating the allelopathic activity of these endophytic fungi.

Table 3 - Radicle growth and stem of *Lactuca sativa* (lettuce) against the crude extracts obtained from endophytes. Measure in millimeters

	Radicle length[1]					**Stem length[1]**				
Crude extracts	**Control**	**C100**	**C500**	**C1000**	**C2000**	**Control**	**C100**	**C500**	**C1000**	**C2000**
CGF1	17.94^{b}	18.10^{c}	17.29^{a}	18.99^{d}	20.28^{e}	31.44^{b}	32.65^{e}	30.53^{a}	32.15^{c}	32.55^{d}
CGF2	17.15^{c}	23.01^{e}	22.55^{d}	16.02^{b}	13.39^{a}	26.27^{c}	30.68^{e}	29.93^{d}	21.41^{b}	18.06^{a}
CGF3	24.66^{d}	24.74^{e}	21.68^{c}	12.63^{b}	12.32^{a}	33.31^{e}	31.71^{d}	27.31^{c}	16.58^{a}	17.12^{b}
CGF4	21.61^{e}	19.46^{d}	18.47^{c}	15.74^{b}	14.75^{a}	28.58^{d}	29.79^{e}	27.14^{c}	23.79^{a}	24.98^{b}
CGF5	22.50^{c}	21.38^{a}	23.73^{e}	22.49^{b}	23.10^{d}	29.61^{c}	28.22^{a}	30.79^{e}	28.57^{b}	30.05^{d}
CGF7	22.91^{e}	18.85^{d}	16.13^{c}	7.55^{b}	5.68^{a}	28.33^{e}	24.18^{d}	21.98^{c}	11.54^{b}	8.67^{a}
CGF8	19.93^{c}	19.76^{b}	21.49^{e}	19.96^{d}	17.69^{a}	25.38^{c}	25.34^{b}	28.07^{e}	26.07^{d}	25.22^{a}
CGF9	16.33^{c}	15.03^{b}	16.85^{e}	16.72^{d}	14.40^{a}	20.66^{d}	19.20^{c}	20.70^{e}	18.79^{b}	16.35^{a}
CGF10	13.67^{a}	14.87^{c}	16.5^{e}	14.58^{b}	15.85^{d}	18.92^{a}	20.01^{c}	21.55^{e}	19.78^{b}	21.02^{d}
CGG12	7.17^{a}	14.80^{e}	8.46^{c}	13.32^{d}	7.48^{b}	10.45^{a}	18.58^{e}	11.16^{c}	16.35^{d}	10.60^{b}
CGF13	9.40^{a}	14.23^{c}	15.23^{e}	14.28^{d}	13.06^{b}	12.60^{a}	19.36^{e}	19.34^{d}	18.69^{c}	16.95^{b}
CGF14	12.32^{c}	15.15^{e}	12.99^{d}	10.05^{b}	7.02^{a}	17.64^{c}	21.02^{e}	18.64^{d}	14.82^{b}	10.50^{a}
CGF15	14.74^{c}	13.52^{b}	17.02^{d}	12.81^{a}	17.81^{e}	18.17^{b}	18.06^{a}	21.31^{d}	20.91^{c}	21.43^{e}
CGF16	13.44^{c}	11.43^{b}	15.60^{e}	14.44^{d}	9.66^{a}	16.40^{c}	14.71^{b}	19.38^{e}	17.91^{d}	12.79^{a}
CGF17	11.07^{a}	11.83^{b}	17.86^{d}	19.56^{e}	17.82^{c}	14.47^{a}	16.51^{c}	14.65^{b}	23.30^{e}	20.93^{d}
CV	(%)[2]		2.40					1.93		

[1]Mean value followed by the same letter do not differ from each other by the Tukey test at 5% significance. [2]CV = Coefficient of variation

Allelopathy refers to the positive and negative effects that one plant or microorganism can have on

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another plant through the release of substances known as allelochemicals. These allelochemicals, which can be found in all plant tissues and are often part of the secondary metabolism, play a role in modifying plant growth and development. The precise mechanisms by which allelochemicals affect plants are not fully understood, but it is known that they interfere with vital plant activities. One of the ways allelochemicals exert their effects is by interacting with essential plant hormones (Khamare et al. 2022; Latif, Chiapusio, and Weston 2017; Pereira et al. 2019).

The significant inhibition of radicle and stem growth by CGF7 and CGF3 extracts, respectively, at the C2000 concentration supports the hypothesis that these fungal extracts possess allelopathic activity. The observed inhibitory effects indicate the presence of bioactive compounds that interfere in the growth and development of lettuce seedlings. Interestingly, some fungal extracts, such as CGF1, CGF10, CGF15, and CGF17, showed a stimulatory effect on seedling growth. This suggests the presence of compounds that promote radicle and stem growth in lettuce seedlings. Exploring the chemical composition of these extracts and identifying the specific compounds responsible for the growth-promoting effects could have practical implications for agricultural practices, such as enhancing seedling establishment or crop productivity. Further analysis and identification of these bioactive compounds could contribute to the development of natural herbicides or plant growth regulators.

It is important to note that allelopathy is a complex phenomenon influenced by various factors, including the concentration of bioactive compounds, interactions between different compounds, and the specific plant species involved. Therefore, further studies are warranted to elucidate the mechanisms underlying the allelopathic activity of these endophytic fungi and to assess their effects on other plant species.

Fungal derived chemicals offer great potential for natural alternatives to chemical weed control because they are biodegradable and comparatively safer for the environment. The use of allelopathy for weed control had considerable importance in the last few decades, due to issues regarding the sustainability of chemical weed control methods (Khamare et al. 2022).

CONCLUSION

The results presented in this study highlight the significant biotechnological potential of the endophytic fungi associated with *C. guianensis*. They demonstrate their ability to inhibit different phytopathogenic fungi and suggest their potential application as biocontrol agents in the management of fungal diseases. Furthermore, the analysis of crude extracts obtained from the endophytic fungi showed antioxidant activity, providing evidence of their ability to protect the host plant against free radicals. Additionally, these endophytic fungi have shown the capacity to produce allelochemical compounds, as demonstrated by their ability to either inhibit or stimulate the growth of *L. sativa* seedlings. In conclusion, this study has contributed to expanding our understanding the biological characteristics of endophytic fungi associated with *C. guianensis* Benth. The results suggest that these endophytic fungi hold great biotechnological potential, particularly in the field of agriculture.

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REFERENCES

Araujo WL, Lacava PT, Marcon J, Lima AOS, Sobral JK, Pizzirani-Kleiner AA, Azevedo JL. Guia prático: isolamento e caracterização de microrganismos endofíticos. Piracicaba: CALO, p.167, 2010.

Behie SW, Bidochka JM. Potential agricultural benefits through biotechnological manipulation of plant fungal associations. BioEssays, v. 35, n. 4, p. 328–331, 2013. https://doi.org/10.1002/bies.201200147

Bell DK, Wells HD, Markham CR. In vitro antagonism of Trichoderma species against six fungal plant pathogens. Phytopathology, v. 72, p. 379–82, 1982.

Chapla VM, Biasetto CR, Araujo AR. Endophytic fungi: An unexplored and sustainable source of new and bioactive natural products. Revista Virtual de Química, v. 5, n. 3, p. 421–437, 2013. https://doi.org/10.5935/1984-6835.20130036.

Chen JL, Shi ZS, Cui PM, Kai W, Chen YW et al. Endophytic Trichoderma gamsii YIM PH30019: A promising biocontrol agent with hyperosmolar, mycoparasitism, and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of Panax notoginseng. Journal of Ginseng Research, v. 40, n. 4, p. 315–324, 2016. https://doi.org/10.1016/j.jgr.2015.09.006.

Cunha CL, Siebeneichler SC, Nascimento IR, Holzbach JC. A New Isoflavone and Other Constituents from Roots of Clitoria guianensis. Journal of the Brazilian Chemical Society, v. 31, n. 8, p. 1753–1757, 2020. https://doi.org/10.21577/0103-5053.20200061.

Dai CC, Gao FK, Liu, XZ. Mechanisms of fungal endophytes in plant protection against pathogens. African Journal of Microbiology Research, v. 4, n.13, p. 1346–51, 2010.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n2.17691

Dantas SBS, Alves FAM, Chapla VM. Chemical, diversity, and biotechnological potential of endophytic fungi isolated from Brazilian Cerrado plants. Biota Neotropica, v. 21, n. 2, 2021. https://doi.org/10.1590/1676-0611-bn-2020-1069

Ferreira DF Sisvar: a guide for its bootstrap procedure in multiple comparisons. Ciência e Agrotecnologia, v. 38, p. 109-112, 2014.

Francisco ASS, Carlos, AVZ. The Assistat Software Version 7.7 and its use in the analysis of experimental data. African Journal of Agricultural Research, v. 11, n. 39, p. 3733–40, 2016. doi: 10.5897/ajar2016.11522.

Grabka R, D'entremont TW, Adam, SJ, Walker AK, Tanney JB, Abbasi PA, Ali S. Fungal Endophytes and Their Role in Agricultural Plant Protection against Pests and Pathogens. Plants, v. 11, n. 3, p. 384, 2022. https://doi.org/10.3390/plants11030384

Hamzah TNT, Lee SY, Hidayat A, Terhem R, Faridah-Hanum I, Mohamed R. Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, Rhizophora mucronata, and identification of potential antagonists against the soil-borne fungus, Fusarium solani. Frontiers in Microbiology, v. 15, n. 9, p. 1705, 2018. https://doi.org/10.3389/fmicb.2018.01707.

Huang LQ, Ni, Y, Su L, Deng H, Lyu H. The potential of endophytic fungi isolated from cucurbit plants for biocontrol of soilborne fungal diseases of cucumber. Microbiological Research, v. 231, 2020. https://doi.org/10.1016/j.micres.2019.126369

Huang WY, Cai YZ, Hyde KD, Corke H, Sun M. Endophytic fungi from Nerium oleander L (Apocynaceae): Main constituents and antioxidant activity. World Journal of Microbiology and Biotechnology, v. 23, n. 9, p. 1253–63, 2007. doi: 10.1007/s11274-007-9357-z.

Khamare Y, Chen J, Marble SC. Allelopathy and its application as a weed management tool: A review. Frontiers in Plant Science, v. 28, n. 13, p. 1034649, 2022. https://doi.org/10.3389/fpls.2022.1034649.

Khare E, Mishra J, Arora NK. Multifaceted interactions between endophytes and plant: Developments and Prospects. Frontiers in Microbiology, v. 15, n. 9, p. 2732, 2018. https://doi.org/10.3389/fmicb.2018.02732.

Latif S, Chiapusio G, Weston LA. Allelopathy and the Role of Allelochemicals in Plant Defence. Advances in Botanical Research, v. 82, p. 19–54, 2017. https://doi.org/10.1016/bs.abr.2016.12.001.

Llauradó MG, Rodríguez DM, Hendrix S, Arranz JCE, Boix YF, Pacheco AO, Díaz JG, Quevedo HJM, Dubois AF, Aleman EI, Beenaerts N, Santos IEM, Ratón TO, Cos P, Cuypers A. Antioxidants in plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. Antioxidants, v. 9, n. 11, p. 1–39, 2020. doi: 10.3390/antiox9111048.

Lopes JC, Chagas Junior AF, Neves ACC, Chapla VM, Batistella AR. Fungos endofíticos isolados do capim citronela e seleção de antagonistas a fitopatógenos. Biota Amazônica, v. 7, n. 3, p. 84–88, 2017. https://doi.org/10.18561/2179-5746/biotaamazonia.v7n3p84-88

Manganyi MC, Ateba CN. Untapped potentials of endophytic fungi: A review of novel bioactive compounds with biological applications. Microorganisms, v. 8, n. 12, p.1934, 2020. https://doi.org/10.3390/microorganisms8121934.

Mishra A, Surendra KG, Kumar A, Sharma VK, Verma SK, Kharwar RN, Sieber TN. Season and Tissue Type Affect Fungal Endophyte Communities of the Indian Medicinal Plant Tinospora cordifolia More Strongly than Geographic Location. Microbial Ecology, v. 64, n. 2, p. 388–98, 2012. doi: 10.1007/s00248-012-0029-7.

Oliveira RJV, Sousa NMF, Pinto Neto WPP, Bezerra JL, Silva GA, Cavalcanti, MAQ. Seasonality affects the community of endophytic fungi in coconut (Cocos nucifera) crop leaves. Acta Botanica Brasilica, v. 34, n. 4, p. 704–11, 2020. doi: 10.1590/0102-33062020abb0106.

Pereira JC, Paulino CLA, Endres L, Santana AEG, Pereira FRS, Souza RC. Allelopathic potential of ethanolic extract and phytochemical analysis of Paspalum maritimum trind. Planta Daninha, v. 37, 2019. https://doi.org/10.1590/S0100-83582019370100053

Rai M, Rathod D, Agarkar G, Dar M, Brestic M, Pastore GM, Marostica Junior MR. Fungal growth promotor endophytes: A pragmatic approach towards sustainable food and agriculture. Symbiosis, v. 62, n. 2, p. 63–79, 2014. https://doi.org/10.1007/s13199-014-0273-3.

Ribeiro BA, Mata TB, Canuto GAB, Silva EO. Chemical Diversity of Secondary Metabolites Produced by Brazilian Endophytic Fungi. Current Microbiology, v. 78, n. 1, p. 33–54, 2021. https://doi.org/10.1007/s00284-020-02264-0

Sadeghi F, Samsampour D, Seyahooei MA, Bagheri A, Soltani J. Diversity and Spatiotemporal Distribution of Fungal Endophytes Associated with Citrus reticulata cv. Siyahoo. Current Microbiology, v. 76, n. 3, p. 279–89, 2019. doi: 10.1007/s00284-019-01632-9.

Santos CM, Ribeiro AS, Garcia A, Polli AD, Polonio JC, Azevedo JL, Pamphile JÁ. Enzymatic and antagonist activity of endophytic fungi from Sapindus saponaria L. (Sapindaceae). Acta Biológica Colombiana, v. 24, n. 2, p. 322–30, 2019. doi: 10.15446/abc.

Santos TT, Varavallo MA. Aplicação de microrganismos endofíticos na agricultura e na produção de substâncias de interesse econômico. Semina: Ciências Biológicas e da Saúde, v. 32, n. 2, p. 199–212, 2011. doi: 10.5433/1679-0367.2011v32n2p199.

Silva-Valderrama I, Toapanta D, Miccono MA, Lolas M, Díaz GA, Cantu D, Castro A. Biocontrol potential of Grapevine endophytic and Rhizospheric Fungi against trunk pathogens. Frontiers in Microbiology, v. 11, p. 1–13, 2021. doi: 10.3389/fmicb.2020.614620.

Simões-Pires CA., Queiroz EF, Henriques AT, Hostettmann K. Isolation and on-line identification of antioxidant compounds from three Baccharis species by HPLC-UV-MS/MS with post-column derivatization. Phytochemical Analysis, v. 16, p. 307–14, 2005. https://doi.org/10.1002.pca.826.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n2.17691

Singh DK, Sharma VK, Kumar J, Mishra A, Verma SK, Sieber TN, Kharwar RN. Diversity of endophytic mycobiota of tropical tree Tectona grandis Linn.f.: Spatiotemporal and tissue type effects. Scientific Reports, v. 7, n. 1, 2017. doi: 10.1038/s41598-017-03933-0.

Soares DF. Análise química e biológica dos extratos brutos, hexânico e acetato de etila obtidos das folhas da espécie Clitoria guianensis Benth. 2016. Monografia (Química Ambiental). Universidade Federal do Tocantins, Gurupi.

Souza BS, Santos TT. Endophytic fungi in economically important plants: ecological aspects, diversity and potential biotechnological applications. Journal of bioenergy and food Science, v. 4, n. 2, p. 113–26, 2017. doi: 10.18067/jbfs.v4i2.121.

Souza CD, Felfili JM. Uso de plantas medicinais na região de Alto Paraíso de Goiás, GO, Brasil. Acta Botânica Brasilica, v. 20, p. 135–142, 2006.

Strobel G. The emergence of endophytic microbes and their biological promise. Journal of Fungi, v. 4, n. 2, 2018. doi: 10.3390/jof4020057.

Vila Verde GM, Paula JR, Carneiro DM. Levantamento etnobotânico das plantas medicinais do cerrado utilizadas pela população de Mossâmedes (GO). Revista Brasileira de Farmacognosia, v. 13, p. 64–66, 2003.

Yan L, Zhu J, Zhao X, Shi J, Jiang C, Shao G. Beneficial effects of endophytic fungi colonization on plants. Applied Microbiology and Biotechnology, v. 103, n. 8, p. 3327–40, 2019. https://doi.org/0.1007/s00253-019-09713-2.

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v12n2.17691