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Bioprospecting of endophytic fungi isolated from *Azadirachta indica* (A. Juss) with the potential to produce hydrolytic enzymes and control the phytopathogen *Macrophomina phaseolina*

Bioprospecção de fungos endofíticos isolados de *Azadirachta indica* (A. Juss) com potencial de produzir enzimas hidrolíticas e controlar o fitopatógeno *Macrophomina phaseolina*

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ABSTRACT

Endophytic microorganisms are mostly fungi or bacteria that live inside plants without causing them harm. They establish an intimate mutualistic association, making plants more resistant to stressful environments while receiving nutrients and protection. Studies indicate a significant capacity of these organisms to produce extracellular hydrolytic enzymes such as amylases, lipases, and cellulases from secondary metabolites that inhibit the development of pathogens. This work aimed to bioprospect endophytic fungi isolated from the plant *Azadirachta indica* with the potential to produce hydrolytic enzymes and control the microorganism *Macrophomina phaseolina*. These fungi had been previously isolated from *A. indica*, stored in the mycotheque of the Agricultural Entomology Laboratory B09 of the Don Bosco Catholic University, and molecularly identified. For enzyme production, the isolates were inoculated in specific media for each enzyme: lipase, esterase, pectinase, amylase, and protease. Antagonism tests were conducted in paired cultures, evaluating antagonism indices. It was possible to identify the genera *Colletotrichum, Diaporthe, Phyllosticta, Alternaria, Trichoderma, Phomopsis*, and *Preussia*, besides one identified only at the class level Sordariomycetes. In terms of enzyme production, the isolates *Preussia isomera* (AI17B) and *Alternaria* sp. (AI30B) stood out for their high protease production and the diversity of enzymes produced, respectively. In the presence of *M. phaseolina*, only *Alternaria* sp. (AI30B) and *Phyllosticta capitalensis* (AI25B) demonstrated antagonistic activity. Based on the results obtained, the *A. indica* plant can serve as a host for endophytic fungi with biotechnological and biocontrol potential.

Keywords: antagonism; biocontrol; biotechnology.

RESUMO

Microrganismos endofíticos são, em sua maioria, fungos ou bactérias que vivem no interior das plantas sem lhes causar prejuízos, estabelecendo uma associação mutualística íntima, tornando as plantas mais resistentes a ambientes com estresse, enquanto recebem nutrientes e proteção. Estudos indicam a grande capacidade destes organismos na produção de enzimas extracelulares hidrolíticas como amilases, lipases e celulases de metabólitos secundários que inibem o desenvolvimento de patógenos. O presente trabalho objetivou a bioprospecção de fungos endofíticos isolados da planta *Azadirachta indica,* com o potencial de produção de enzimas hidrolíticas e controle do microrganismo *Macrophomina phaseolina*. Os fungos foram isolados previamente de *A. indica,* armazenados na micoteca do Laboratório de Entomologia Agrícola B09 da Universidade Católica Dom Bosco e identificados molecularmente. Para produção enzimática, os isolados foram inoculados em meios específicos para cada enzima: lipase, esterase, pectinase, amilase e protease. O teste de antagonismo foi conduzido em cultura pareada, avaliando os índices de antagonismo. Foi possível identificar os gêneros *Colletotrichum*, *Diaporthe*, *Phyllosticta*, *Alternaria*, *Trichoderma*, *Phomopsis* e *Preussia*, além de um identificado apenas ao nível da classe Sordariomycetes. Na produção enzimática, os isolados *Preussia isomera* (AI17B) e *Alternaria* sp. (AI30B) se destacaram, respectivamente, pela elevada produção de protease e pela diversidade de enzimas produzidas. Frente à *M. phaseolina*, apenas *Alternaria* sp. (AI30B) e *Phyllosticta capitalensis* (AI25B) demonstraram atividade antagonística. Com base nos resultados obtidos, a planta *A. indica* pode servir como hospedeira de fungos endofíticos com potencial biotecnológico e de biocontrole.

Palavras-chave: antagonismo; biocontrole; biotecnologia.

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Introduction

Fungi or bacteria often inhabit the interior of plants without causing them harm, characterizing them as endophytic microorganisms. They colonize the organs and tissues of plants without causing visible symptoms, establishing a mutualistic association. This association makes plants more resistant to stressful environments while the endophytes are provided with nutrients and protection (Raimi and Adeleke, 2021; Vahobovna et al., 2023).

The term "endophyte," originally described by De Bary (1866), refers to any microorganism that lives within plant tissues, distinct from epiphytes that inhabit the surface (Kandasamy and Kathirvel, 2023). Endophytes have captured the interest of the scientific community due to numerous discoveries of bioactive compounds derived from these organisms. Additionally, they play a crucial role in controlling plant pathogens through various mechanisms, including metabolite antibiosis, competition for resources, and mycoparasitism (Fontana et al., 2021).

Studies indicate the significant capacity of these organisms to produce extracellular hydrolytic enzymes of industrial interest such as amylases, lipases, cellulases, and pectinases. These enzymes are derived from the need to invade and colonize plant tissues (Sopalun and Iamtham, 2020). In the pharmaceutical industry, endophytes can also contribute to the production of secondary metabolites that inhibit pathogen development, demonstrating bactericidal, fungicidal, leishmanicidal, and antitumoral activity (Orlandelli et al., 2012; Almeida et al., 2017; Keshri et al., 2021; Damavandi et al., 2023).

The neem tree, scientifically known as *Azadirachta indica* (A. Juss), is a plant used in the experimental pharmaceutical industry, widely applied as a folk medicine for various therapeutic purposes, as well as a source of agrochemicals for many centuries in agriculture (Kharwar et al., 2020).

Medicinal plants have been used since ancient times to treat diseases and are even widely used in traditional medicine systems of some Asian countries, such as India and China. Due to the presence of diverse metabolites, they are employed against various types of diseases worldwide, and numerous species such as *Momordica charantia, Zingiber officinale,* and *Cinnamomum zeylanicum* are already described in the scientific literature (Sharma et al., 2020). Endophytic fungi can produce the same bioactive compounds as their host plants, making them an alternative to generating compounds derived from plant species of interest (Ye et al., 2021).

In addition, the prospecting of microorganisms with the potential for use in biocontrol of diseases in economically important crops became one of the main targets of biotechnological research, through the use of microorganisms as natural agents, which may replace conventional pesticides (Pirttilä et al., 2021). Some endophytes found within the endophytic microbiome of *A. indica* may exhibit antimicrobial activity (Kadam and Kanase, 2022). Regarding antifungal activity, several endophytic fungi isolated from this plant have already been shown capable of combating phytopathogenic fungi, either by biocontrol or by producing compounds. Examples include the endophytic isolates *Trichoderma* sp., *Verticillium* sp., *Chaetomium* sp., *Fusarium* sp., *Penicillium* sp., and *Phoma* sp. against the phytopathogen *Alternaria* sp. (Ododa et al., 2023) and the isolates *Alternaria tenuissima* and *Alternaria alternata* against the phytopathogen *Fusarium oxysporum* f. sp. *cubense* (Nthuku et al., 2023).

Macrophomina phaseolina is a phytopathogen that causes various diseases affecting soybean production, among other crops, leading to gray stem rot or charcoal rot, which results in significant economic losses (Marquez et al., 2021). Since it is considered a phytopathogen responsible for causing great losses in production, and considering that, to date, no cultivar was identified with resistance to this fungus (Mengistu et al., 2011), it is relevant to evaluate the possibility of enzymatic action on this microorganism.

Therefore, this study aimed to bioprospect endophytic fungi isolated from the plant *A. indica*, with the potential to produce hydrolytic enzymes and control the microorganism *M. phaseolina*.

Material And Methods

Molecular identification of endophytic isolates

Endophytic fungi were previously isolated from the leaves of *A. indica*, collected from Don Bosco Catholic University (UCDB School Farm), located at 20°23'49.6"S 54°36'57.7"W, Campo Grande (MS), Brazil. After isolation, the microorganisms were stored in sterile distilled water and subsequently activated in a potato dextrose agar (PDA) medium.

The fungi were grown on PDA medium for seven days on dialysis membranes in Petri dishes and then macerated in liquid nitrogen. The resulting material was subjected to total deoxyribonucleic acid (DNA) extraction using the PowerSoil® DNA Isolation Kit – QIAGEN.

Amplification of the internal transcribed spacer (ITS) region of ribosomal DNA (White et al., 1990) was performed according to Almeida et al. (2015). The resulting amplicons were purified with ExoSAP-IT™ Product Cleanup Reagent (Applied Biosystems) and sequencing of samples was performed by ACTGene Análises Moleculares Ltda. (Biotechnology Center of the Federal University of Rio Grande do Sul – *Centro de Biotecnologia*, UFRGS, Porto Alegre, RS) using the AB 3500 Genetic Analyzer automated sequencer.

To determine the genetic distance of the isolates, the sequences obtained by sequencing were compared with the sequences available in GenBank (https://www.ncbi.nlm.nih.gov/) using Standard Nucleotide Basic Local Alignment Search Tool (BLASTn) to search for the closest sequences.

The phylogenetic tree was constructed using MEGA 11 software (Tamura et al., 2021). The sequences obtained through sequencing were aligned using ClustalW (Thompson et al., 1994), with the phylogeny performed through the neighbor-joining method (Saitou and Nei, 1987) with the bootstrap test of 10,000 repetitions.

Evaluation of enzyme production by endophytes isolated from Azadirachta indica

The enzyme activity of the microorganisms was evaluated qualitatively and semi-quantitatively, according to Silva et al. (2016), with modifications. The fungal isolates were grown in PDA medium and then inoculated onto Petri dishes with the specific culture media for each enzyme. They remained incubated at 28°C for 2–7 days.

Amylolytic activity

The isolates were grown on plates with minimal M9 medium, replacing glucose with 1% soluble starch (w/v) and containing 0.5% yeast extract, in the proportion of 10 g soluble starch, 5 g yeast extract, 12.8 g $Na_2HPO_4 \cdot 7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 2H_2O$, 15 g agar, and 1000 mL distilled H_2O.

After microbial growth, 10 mL of iodine solution was added and washed immediately with water. The presence of a colorless halo around the colony indicated amylase production (Hankin and Anagnostakis, 1975). For the control, α-Amylase enzyme (SIGMA – code A3176-500KU) was used.

Lipolytic activity

The medium used for lipase detection was the one proposed by Sierra (1957) with modifications, where the PDA medium (Kasvi®) was prepared according to the manufacturer's instructions, and after sterilization, 1% (w/v) of Tween 20 was added, previously sterilized. The presence of halos formed by crystals indicated the secretion of lipase by the isolates.

Esterase activity

The same methodology as for lipase was used to observe esterase production, replacing Tween 20 with Tween 80. Esterase production was indicated by the presence of clear halos around the isolates (Sierra, 1957).

Pectinolytic activity

The microorganisms were grown on minimal M9 medium, replacing glucose with 1% pectin (w/v), containing 0.5% yeast extract, in the proportion of 10 g citric pectin, 5 g yeast extract, 12.8 g $Na_2HPO_4 \cdot 7H_2O$, 3 g KH_2PO_4, 0.5 g NaCl, 1 g NH_4Cl, 5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2 \cdot 2H_2O$, 15 g agar, and 1000 mL distilled H_2O.

After microbial growth, 10 mL of Lugol's iodine solution was added and immediately washed with water. The presence of a colorless halo around the colony indicated pectinase production (Sierra, 1957).

Proteolytic activity

To assess proteolytic activity, the protease detection medium was used, in the proportion of 5 g tryptone, 2.5 g yeast extract, 2.5 g NaCl, 1 g glucose, 15 g agar, 900 mL distilled H_2O, and 100 mL skim milk. After sterilization, for every 900 mL of medium, 100 mL of skim milk was added. Proteolytic activity was indicated by a transparent halo formation around the isolate (Sierra, 1957).

Enzymatic index and statistical analysis

The enzymatic activity of endophytic fungi was qualitatively evaluated. To do this, all isolates were tested in each specific culture media, and only those that yielded positive results underwent a semi-quantitative analysis. The diameter of the halo and colony was measured using a millimeter ruler, and the enzymatic index (IE) was determined according to the Equation 1 below:

$$EI = \frac{halo\ diameter}{colony\ diameter} \quad (1)$$

Higher EI values indicate greater enzymatic productivity. All tests were performed in triplicate. The data obtained were subjected to analysis of variance (ANOVA) using R software. The means were compared by the Scott-Knott test, at a 5% significance level.

In vitro antagonistic activity of isolated endophytes against the phytopathogen *Macrophomina phaseolina*

Endophytic fungi isolated from leaf tissues were evaluated for their antagonistic activity against the phytopathogenic fungus *M. phaseolina* using a modified paired-culture technique (Campanile et al., 2007).

Six-millimeter discs of 7-day-old endophytic and phytopathogenic fungal isolates were inoculated on opposite sides of Petri plates containing PDA culture medium, 4 cm apart. The tests were performed in triplicate, as well as a negative control, with the phytopathogen on one (C1) or both sides of the plates (C2). The plates were incubated in a Biochemical Oxygen Demand (BOD) incubator at 28°C for seven days.

The antagonism index (AI) for each endophyte was calculated according to Campanile et al. (2007). Competitive interactions between endophytes and pathogens were analyzed according to the Badalyan scale (Badalyan et al., 2002). The *in vitro* antagonism test was statistically evaluated using ANOVA, and the means were compared by the Scott-Knott test ($p<0.05$), using the R program package ExpDes.pt.

Results and Discussion

In the present work, the plant *A. indica* exhibited a diverse cultivable endophytic fungal community, including fungi from various genera. These fungi demonstrated the ability to produce enzymes such as lipase, esterase, pectinase, and protease *in vitro*. Additionally, these endophytes displayed antagonistic activity against the phytopathogen *M. phaseolina*.

Isolation and molecular identification of endophytic fungi

The study utilized 13 endophytic fungi previously isolated from the *A. indica* plant. The specific isolates were chosen based on the availability of sample material.

The sequencing of the ITS1-5.8S-ITS2 region of rDNA revealed the presence of seven fungal genera (*Colletotrichum*, *Diaporthe*, *Phyllosticta*, *Alternaria*, *Trichoderma*, *Phomopsis*, and *Preussia*) and one fungal class (Sordariomycetes).

Phylogenetic analysis confirmed the molecular identification of 12 isolates at the genus level. Among them, five of the genus *Colletotrichum* (AI14B, AI15A, AI15B, AI9A, and AI28A), one of the genus *Diaporthe* (AI16B), two of the genus *Phyllosticta* (AI6 and AI25B), one of the genus *Alternaria* (AI30B), one of the genus *Trichoderma* (AI18A), one of the genus *Phomopsis* (AI23A), and one of the genus *Preussia* (AI17B), while the class level was confirmed for the Sordariomycetes isolate (AI32) (Table 1 and Figure 1). Other studies with isolation from *A. indica* corroborate the results obtained. The genera *Alternaria, Colletotrichum*, and *Trichoderma* have already been recorded on the leaves of this plant (Taware et al., 2017; Chatterjee et al., 2019). *Phomopsis* and *Phyllosticta* have also been described in *A. indica* (Wu et al., 2008), the latter being isolated from the inner bark (Tejesvi et al., 2006).

Enzymatic activity

Microorganisms have been studied for their ease of large-scale enzyme production in various industries (Bhadra et al., 2022). In the present work, after the selection and identification of endophytic fungi, favorable results were obtained regarding the efficiency in the production of most of the tested hydrolytic enzymes: lipase, esterase, pectinase, and protease, with the latter being the most prominent.

Out of the 13 endophytic isolates tested, none were positive for the enzyme amylase. For lipase, only isolate AI30B (*Alternaria* sp.) was positive, obtaining an EI of 1.23. For esterase, isolate AI30B showed a positive result, obtaining an EI of 1.17. Isolates AI25B and AI6, both from genus *Phyllosticta*, presented statistically similar positive results for pectinase, with EI of 2.70 and 2.71, respectively.

For protease production, the endophytic isolates that showed positive results were AI9A (*Colletotrichum* sp.), AI15A (*Colletotrichum* sp.), AI17B (*Preussia* sp.), AI30B (*Alternaria* sp.), and AI32 (Sordariomycetes), with EI ranging between 1.33 and 2.60, divided into three statistical groups, as observed in Table 2.

Isolates AI28A (*Colletotrichum* sp.), AI18A (*Trichoderma* sp.), AI16B (*Diaporthe* sp.), and AI14B (*Colletotrichum* sp.) showed negative results for the production of all tested hydrolytic enzymes.

In the present study, the obtained enzymatic indices ranged from 1.33 to 2.71, which represent significant results for the confirmation of enzyme excretion, since, according to Fungaro and Maccheroni Junior (2002), for such an assertion, the results need to be greater than 1.00. Most of the fungi produced protease, with the highlight being the isolate *Preussia isomera* (AI17B), with an enzymatic index of 2.60. The genus *Preussia* is environmentally diverse, being primarily coprophilic, but is also found in various environments such as plant tissues, and is poorly understood phylogenetically (Mapperson et al., 2014). It comprises several species that produce diverse cellular metabolites with useful properties for medicine, and even extracellular enzymes (Seddouk et al., 2022). In the context of hydrolytic enzyme production, some of these enzymes were found to be secreted by *Preussia* spp. such as amylase, cellulase, lipase, phosphatase, and glucosidase (Liang et al., 2015; Khan et al., 2016; Oliveira et al., 2022). However, data on the production of protease by this genus is scarce in the scientific literature.

Alternaria is a genus primarily known for its saprophytic and phytopathogenic species, but it has attracted attention due to its enzyme production capacity. Some species, such as the well-known *Alternaria niger*, synthesize enzymes like cellulase, xylanase, pectinase, and hemicellulase, which are associated with the propagation lifestyle in plant tissues of this group, as well as catalase and L-asparaginase. Therefore, it is an important genus for industrial enzyme production (Robl et al., 2013; Bhadra et al., 2022). The isolate *Alternaria* sp. (AI30B) synthesized three enzymes: protease, esterase, and lipase, among which protease exhibited the highest EI. Rajput et al. (2016) and Zaferanloo et al. (2014) obtained promising results in protease production by *Alternaria alternata*.

Table 1 – Isolated and identified endophytes from Azadirachta indica and the identity percentages found on the National Center for Biotechnology Information website.

Endophytic isolate	Closest lineage in GenBank	% Identity	GenBank accession number
AI14B	*Colletotrichum karstii*	99.82	KC244166
AI15A	*Colletotrichum scovillei*	99.64	ON961750
AI15B	*Colletotrichum boninense*	99.64	KM520013
AI16B	*Diaporthe phaseolorum*	97.58	MT984339
AI32	Sordariomycetes sp.	97.59	JX174135
AI9A	*Colletotrichum boninense*	97.27	KP900268.1
AI6	*Phyllosticta fallopiae*	98.29	MT043804.1
AI30B	*Alternaria* sp.	99.62	KP211537.1
AI18A	*Trichoderma harzianum*	99.48	MK738146.1
AI23A	*Phomopsis* sp.	99.45	GU066693.1
AI25B	*Phyllosticta capitalensis*	97.65	MT085755.1
AI28A	*Colletotrichum gloeosporioides*	99.43	MN873010.1
AI17B	*Preussia isomera*	99.00	KX710221.1

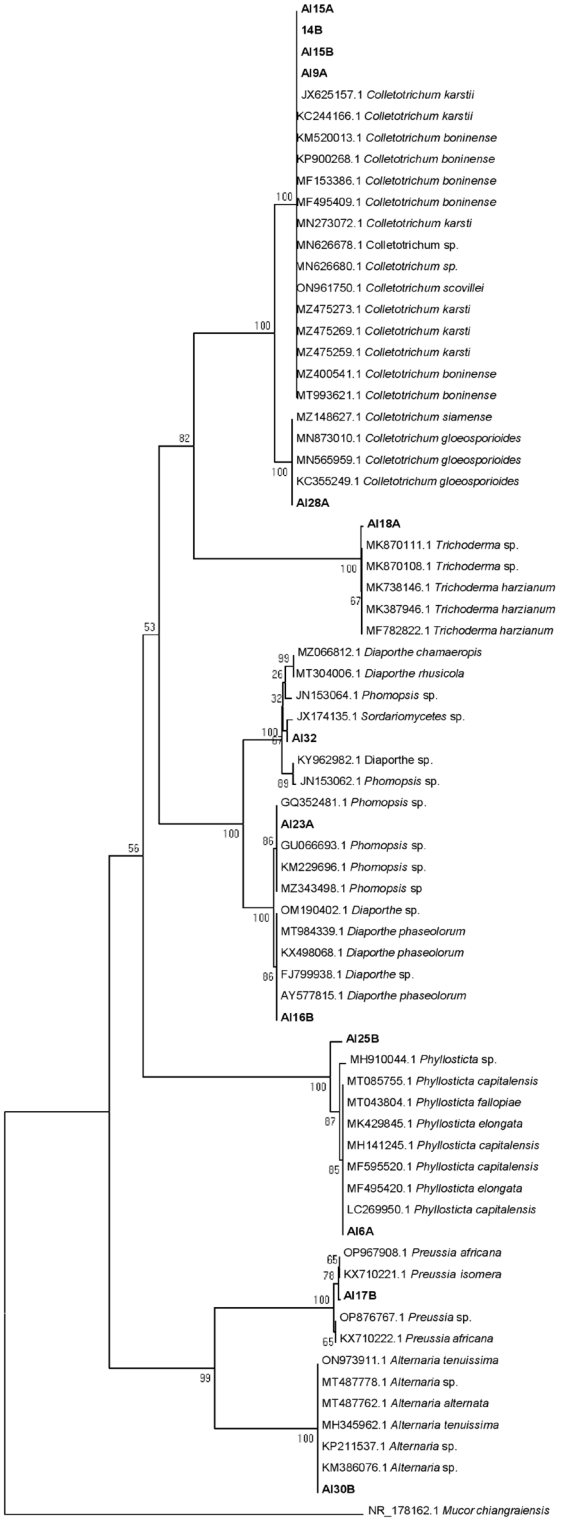

Figure 1 – Phylogenetic tree constructed with sequences of endophytic fungi isolated from *Azadirachta indica* and sequences from GenBank (indicated by the database code), through the neighbor-joining method using p-distance for nucleotides, with the option of pairwise gap deletion. The numbers above and below each node indicate the frequency (in percentage) of each branch in bootstrap analyses of 10,000 replicates.

Phyllosticta spp. are known to produce a wide range of secondary metabolites; however, their ability to produce hydrolytic enzymes, derived from mechanisms of penetration into plant tissues, is still not fully known, especially when compared to other endophytic or pathogenic fungal species (Wikee et al., 2017). The non-phytopathogenic isolate *Phyllosticta capitalensis* (AI25B) produced a remarkable result for pectinase, in agreement with Wikee et al. (2017), who obtained a high production of pectinase from this species in the treatment of lavender plantation residues.

Antagonism index

Microorganisms have long been a promising source of biotechnological resources due to the discovery of fungi with potential applications in agriculture (Santos et al., 2021). These organisms have been used for decades in agriculture for phytopathogens biocontrol, employing various direct or indirect mechanisms such as enzyme production, toxin production, and competition. Therefore, they can be considered biopesticides, which are currently sought after in agriculture as a way to replace conventional chemical pesticides (Boro et al., 2022).

In evaluating the AI against the phytopathogen *M. phaseolina*, the 13 endophytic fungi isolated from *A. indica* were assessed using the paired culture technique. The isolates AI30B (*Alternaria* sp.) and AI25B (*Phyllosticta capitalensis*) showed positive results against the phytopathogen *M. phaseolina*.

Through the analysis of variance (ANOVA) of the AI, statistically significant differences were observed between the tested endophytic strains against the phytopathogen. The obtained AI ranged from 20 to 22%, thus statistically generating only one similar group with 11 isolates showing no inhibition of the phytopathogen, as observed in Table 3.

According to the Badalyan scale (Badalyan et al., 2002), the competitive interactions between endophytes (*A. indica* isolates) against the phytopathogen *M. phaseolina* were of the types: A=inhibition by mycelial contact, observed in isolate AI30B (*Alternaria* sp.); and B=inhibition at a distance, observed in isolate AI25B (*Phyllosticta capitalensis*), as shown in Figure 2 and Table 3.

The isolate *Phyllosticta capitalensis* (AI25B) inhibited the phytopathogen *M. phaseolina* at a distance, demonstrating control potential. According to the scientific literature, although it can be phytopathogenic under conditions of stress to the host plant, this species is described as presenting metabolites with applications in the pharmaceutical and agricultural industries such as phyllotoxin, phyllosticnins, phyllostin, and even taxol (Lateef et al., 2019).

Imbeloni et al. (2015) used isolates of *Alternaria* sp. and observed promising results against phytopathogenic fungi. These results corroborate the present study, where the fungal genera *Alternaria* and *Phyllosticta* demonstrated antagonistic capacity against the phytopathogenic fungus *M. phaseolina*.

Table 2 – Identification and enzymatic activity of endophytic fungi isolated from *Azadirachta indica*.

Endophytic isolate	Enzymatic index*	Esterase	Pectinase	Amylase	Lipase
	Protease				
AI17B (*Preussia isomera)*	2.6^{a}±0	-	-	-	-
AI30B (*Alternaria* sp.)	1.79^{b}±0.21	1.17^{a}±0.18	-	-	1.23^{a}±0.03
AI28A (*Colletotrichum gloeosporioides)*	-	-	-	-	-
AI15A (*Colletotrichum scovillei)*	1.33^{c}±0.08	-	-	-	-
AI9A (*Colletotrichum boninense)*	1.85^{b}±0.14	-	-	-	-
AI25B (*Phyllosticta capitalensis)*	-	-	2.7^{a}±0.17	-	-
AI18A (*Trichoderma harzianum)*	-	-	-	-	-
AI6 (*Phyllosticta fallopiae)*	-	-	2.71^{a}±0.36	-	-
AI16B (*Diaporthe phaseolorum)*	-	-	-	-	-
AI32 (Sordariomycetes sp.)	2^{b}±0.2	-	-	-	-
AI14B *(Colletotrichum karstii)*	-	-	-	-	-
AI15B *(Colletotrichum boninense)*	-	-	-	-	-
AI23A (*Phomopsis* sp.)	-	-	-	-	-

*Means of triplicates. Enzymatic index values followed by the same letter in the columns were not distinguished by the Scott-Knott test ($p<0.05$); ±standard deviation.

Table 3 – *In vitro* antagonistic activity of isolated endophytes with activity against the phytopathogen *Macrophomina phaseolina*.

Endophytic isolate	Antagonism index (%)	Interaction type
AI17B (*Preussia isomera)*	-	-
AI30B (*Alternaria* sp.)	22^{a}	A
AI28A (*Colletotrichum gloeosporioides)*	-	-
AI15A (*Colletotrichum scovillei)*	-	-
AI9A (*Colletotrichum boninense)*	-	-
AI25B (*Phyllosticta capitalensis)*	20^{a}	B
AI18A (*Trichoderma harzianum)*	-	-
AI6 (*Phyllosticta fallopiae)*	-	-
AI16B (*Diaporthe phaseolorum)*	-	-
AAI32 (Sordariomycetes sp.)	-	-
AI14B *(Colletotrichum karstii)*	-	-
AI15B *(Colletotrichum boninense)*	-	-
AI23A (*Phomopsis* sp.)	-	-

*Different letters above the columns indicate that the values are different by the Scott-Knott test ($p<0.05$).

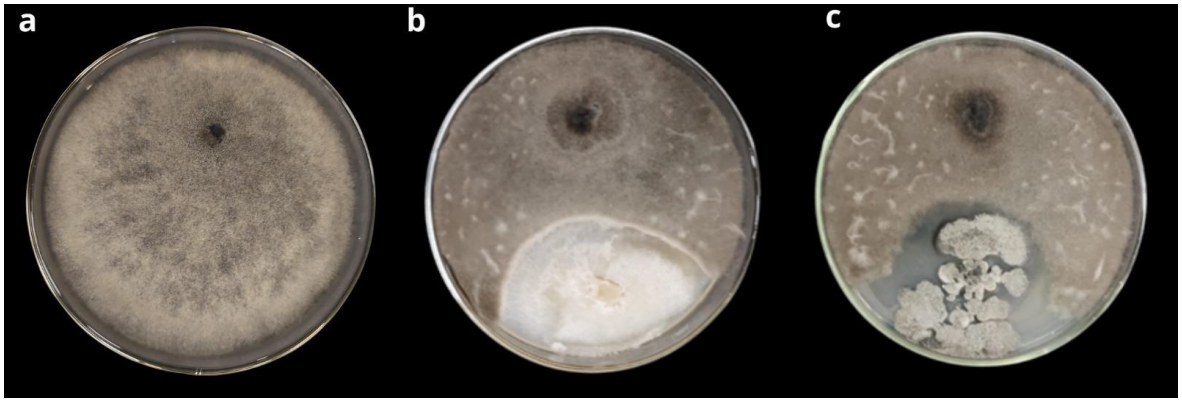

Figure 2 – Antagonistic test of fungal isolates from *Azadirachta indica* against the phytopathogenic fungus *Macrophomina phaseolina*: A) Control M. *phaseolina*; B) Isolate AI30B (*Alternaria sp*); and C) Isolate AI25.

Conclusion

In this study, we observed that *A. indica,* commonly known as neem, effectively hosts endophytic fungi from different genera and classes. The microorganisms isolated from this plant exhibited promising potential for *in vitro* enzyme production of lipase, esterase, pectinase, and protease. Additionally, these endophytes exhibited antagonistic activity against the phytopathogen *M. phaseolina*.

Thus, the biotechnological potential of the evaluated endophytic fungi can be noted, with emphasis on the genera *Alternaria* (for the production of the enzymes protease, esterase, and lipase) and *Phyllosticta* (for the production of the pectinase enzyme). Moreover, both demonstrated inhibition potential against the phytopathogen *M. phaseolina*.

Authors' Contributions

Elias, H.O.: conceptualization; investigation; writing – original draft. Almeida, T.T.: conceptualization; methodology; project administration; formal analysis. Freitas, G.F.: investigation; writing – review & editing. Ferrandin, G.J.: investigation; writing – review & editing. Petucco, D.C.: investigation. Loosli, A.W.M.: writing – review & editing. Carvalho, C.M.E.: funding acquisition; supervision.

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