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ENDOPHYTIC MICROORGANISMS OF AROEIRA TREE AND THEIR POTENTIAL AS PRE-EMERGENT BIOHERBICIDE FOR WEEDS

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Resumo

Microrganismos endofíticos de Aroeira e seu potencial como bioherbicida pré-emergente de plantas daninhas. A microbiota de endofíticos de plantas nativas ainda é pouco conhecida, mas o seu potencial em produzir compostos bioativos de interesse para agricultura tem registrado um número crescente de artigos. Ambientes como as Florestas de Mata Seca reúnem características que podem abrigar uma microbiota endofítica diversa e com particularidades metabólicas com potencial a ser explorado. Os objetivos do trabalho foram isolar fungos e bactérias de folhas de aroeira (*Myracrodruon urundeuva* Freire Allemão) de plantas do Parque Nacional da Mata Seca, município de Manga/MG, identificá-los através do sequenciamento da região ITS e gene 16S rRNA. Três microrganismos foram empregados em experimentos para avaliação do potencial efeito bioherbicida dos seus metabólitos na germinação e vigor de sementes de *Urochloa brizantha* Stapf cv. Piatã, *Bidens pilosa* L. e *Stylosanthes* sp. O gênero *Phomopsis* foi o mais abundante em aroeira preta encontrado em 12 dos 19 fungos isolados. Os metabóltios de *Phomopsis* sp., *Bacillus* sp. e *Brevilacillus* sp. interferiram no vigor das sementes das plantas daninhas apresentando potencial efeito bioherbicida.
Palavras-chave: Phomopsis, Mata Seca, *Bacillus*, *Brevibacillus*, semente.

Abstract

The endophytic microbiota of native plants is still poorly understood, but their potential to produce bioactive compounds of interest for agriculture has registered an increasing number of articles. Environments such as the Dry Forest bring together characteristics that can harbor a diverse endophytic microbiota with metabolic particularities with potential to be explored. The objectives of this study were to isolate fungi and bacteria from aroeira leaves (Myracrodruon urundeuva Freire Alemão) situated at the Mata Seca National Park, Manga/MG; identify them through the sequencing of the ITS region and 16S rRNA gene, and evaluate the potential bioherbicidal effect of their metabolites on germination and vigor of Urochloa brizanthaCV. Piatã, Bidens pilosa L. and Stylosanthes sp. seeds. The genus Phomopsis was the most abundant in the capoeira tree, being found in 12 of the 19 isolated fungi. Phomopsis sp., Bacillus sp., and Brevilacillus sp. metabolites interfered with the vigor of the seeds, presenting a potential bioherbicidal effect.
Keywords: Phomopsis sp, Dry Forest, *Bacillus*, *Brevibacillus*, seed.

INTRODUCTION

The Dry Forest is a phytophysiognomy present in the Amazon, Caatinga, Cerrado, and Atlantic Forest biomes and is characterized by leaf deciduity in the dry season and tree growth in the rainy season (SCARIOT; SEVILHA, 2005). In the North of Minas Gerais, the Dry Forest is identified as Caatinga phytophysiognomy due to the presence of xerophytic plant species. In the region of the municipality of Manga, where the Dry Forest State Park is located, there is a Seasonal Deciduous Forest of Limestone Outcrops due to the karst relief (BELÉM; OLIVEIRA; VELOSO, 2021). This unique environment is situated in the cerrado-caatinga transition, exhibiting specific edaphoclimatic conditions and plant diversity.

Among the plant species of the Dry Forest the Aroeira tree (*Myracrodruon urundeuva* Freire Allemão), Family Anacardiaceae, occurs in greater abundance in the North of Minas Gerais State (ARRUDA *et al.*, 2011). It is widely used as a medicinal plant, and its wood is of excellent quality, which is why its illegal extraction has reduced the number of native trees.

Both common sense and the scientific community are aware of the allelopathic effect of the Aroeira tree. Allelopathy is an ecological phenomenon understood as the harmful or beneficial effect of secondary metabolites from one plant on another plant through root exudates, compounds volatilized or made available by the degradation of plant material (FERGUSON *et al.*, 2016). The allelopathic compound acts as a natural herbicide that prevents the germination of seeds from other plant species.

The allelopathic compound acts as a natural herbicide that prevents the germination of seeds from other plant species. The endophytic microbiota associated with a plant can interfere with its allelochemical profile since

these symbiotic or mutualistic microorganisms provide the plant with defense mechanisms and environmental adaptation (ASCHEHOUG *et al.*, 2014). Endophytic microorganisms can produce or stimulate the production of allelochemicals from their host (CIPOLLINI *et al.*, 2012) through their secondary metabolites. The secondary metabolism of endophytes is a fertile field of investigation to identify bioactive molecules applied to agriculture with herbicide and antimicrobial action, especially.

The increasing demand for alternatives to pesticides that are less harmful to the environment stimulates studies on bioactive compounds. Among pesticides, herbicides have the fewest registered biological products. Thus, the research proposed investigating the cultivable endophytic microbiota associated with the Aroeira tree and the herbicidal potential of bioactive compounds from microorganisms in the pre-emergence of *Urochloa brizantha* Stapf cv. Piatã, *Bidens pilosa* L. and *Stylosanthes* sp.

MATERIAL AND METHODS

Collection of plant material and isolation of fungal and bacterial endophytes

The Aroeira tree leaves were collected at the Dry Forest State Park (-14.87082921824656, -44.00377895901981) in the municipality of Manga/MG - Brazil in May 2017. Five plants were sampled, with five leaves per plant. Leaves without symptoms of disease or insect damage were collected. The plant exsiccate is deposited in the herbarium (collection, reference year 2017) of the Phytopathology Laboratory of the Federal Institute of the North of Minas Gerais, Campus Januária.

In order to isolate microorganisms, the leaves were washed with neutral soap and subjected to 70% alcohol for 1 min, 2% sodium hypochlorite for 3 min, and triple washing in sterilized distilled water (SDW). The set of leaves from each plant constituted a sample. Three fragments were removed from each leaf and placed in Petri dishes with a Potato-Dextrose-Agar (PDA) medium for fungal isolation. The plates were maintained at 25°C with a 12 hours photoperiod until colonies developed.

In the bacterial isolation, five 1 cm^2 leaf fragments, one fragment from each leaf in the sample, were macerated with 50 ml of $MgSO_4$ solution (0.2M) in a porcelain crucible. One milliliter of the macerate suspension was taken, and a serial dilution was performed up to 10^4 times. One hundred microliters were taken from each dilution and seeded with a Drigalski loop on a KADO culture medium in Petri dishes. The plates were kept at 28°C with a 12 hours photoperiod for bacterial growth. Axenic colonies were obtained by successive subcultures.

DNA extraction and sequencing

For molecular characterization of the fungal isolates, the Internal transcribed spacer (ITS) region was amplified with the primers F: ITS1 (TCCGTAGGTGAACCTGCGG) and R: ITS4 (TCCTCCGCTTATTGATATGC). The 16S rRNA gene was amplified with the primers F: 27F (AGAGTTTGATCMTGGCTC-AG) and R: 1492R (GGTTACCTTGTTACGACTT) for the bacteria characterization. Fungal mycelium with seven days of growth in PDA medium or bacterial cells with 48 hours of growth in liquid KADO medium, under shaking, were used. DNA extraction was performed using the Wizard® Genomic DNA Purification kit (Promega Corporation). After extraction, the pellet was resuspended with 50 µl TE buffer (Tris-HCl; 10 mM, pH: 8.0; 0.1 M EDTA) and 2 µl RNase. The tubes were kept at 37ºC for 12 hours, and stored at -20ºC. DNA was quantified and adjusted to ~50 ng/µl using the NanoDrop 2000® Spectrophotometer (Thermo Fisher Scientific).

The PCR conditions were as follows: initial denaturation at 95°C for 5 min; then 35 cycles of denaturation at 95°C for 30 s; annealing at 55°C for 45 s; extension at 72°C for 1 min; final extension at 72°C for 5 min.

The sequencing was performed by the company Macrogen Inc. (South Korea). The sequences were manually edited using the DNA Baser program (DNA Sequence Assembler v4, Heracle BioSoft, www.DnaBaser.com). The edited sequences were compared to sequences deposited in the National Center for Biotechnology Information (NCBI) - Nucleotide Sequence Database (EMBL) using the Basic Local Alignment Search Tool (BLAST).

Obtaining non-volatile metabolites from endophytic microorganisms

In order to produce the metabolite of isolate IM36 (*Phomopsis* sp.), the microorganism was cultivated in PDA at 25ºC with a 12 hours photoperiod for 7 days. Two 10 mm mycelium discs were transferred to Erlenmeyer flasks containing 100 ml of liquid Potato and Sucrose medium (PS). The flasks were manually shaken for 1 minute, twice a day for 7 days, and kept at 25ºC in the dark.

To produce metabolites from bacterial isolates, IF385 (*Bacillus* sp.) and IF386 (*Brevibacillus* sp.) were cultivated in a KADO culture medium and incubated at 28°C in the dark. After two days, a 10 mm disc was taken from the bacterial colony and added to Erlenmeyer flasks with 100 ml of liquid KADO medium, which were then kept in the absence of light at 28°C. Flask agitation was performed as described earlier.

The metabolites were filtered through a triple layer of gauze and diluted in SDW to obtain the desired

concentrations. The diluted suspensions were autoclaved at 120°C for 20 minutes.

Germination of *Urochloa brizantha* Stapf cv. Piatã, *Bidens pilosa* L. and *Stylosanthes* sp. seeds treated with microbial metabolites

Seeds of *U. brizantha* Stapf cv. Piatã and *Stylosanthes* sp. were obtained from seed suppliers. Seeds of *B. pilosa* were collected from plants at the Federal Institute of the North of Minas Gerais – Campus Januária. Five treatments were used, consisting of concentrations of metabolites: T1 – 100% SDW (control); T2 – 25% metabolite - M + 75% SDW; T3 – 50% M + 50% SDW; T4 – 75% M + 25% SDW and T5 – 100% M.

The seeds were disinfected in 70% alcohol for 1 min and 2% sodium hypochlorite for 1 min, followed by rinsing with SDW. *U. brizantha* Stapf cv. Piatã seeds were treated with sulfuric acid for 10 min to overcome dormancy (BRASIL, 2009).

The experimental unit was a Petri dish with two filter papers at the base, moistened with the defined metabolite doses for each treatment at 2.5 times the paper mass (mass:volume) (BRASIL, 2009). Twenty-five seeds were placed on the filter papers in five replicates. The plates were then placed in a B.O.D. with a 12 hours photoperiod at ±25°C (BRASIL, 2009) for seven days.

Germination percentage (GP) was evaluated by counting germinated seeds relative to the total number of seeds. Only seeds with a root protrusion of more than two mm were considered germinated (BRASIL, 2009). The germination speed index (GSI), mean germination time (MGT) (AL-ANSARI; KSIKSI, 2016), root length (RL), and shoot length (SL) were also assessed.

Analysis of microbial metabolite experiment data

GP, GSI, MGT, RL, and SL data were analyzed for normality using the Shapiro-Wilk test and homogeneity using the O'Neils-Mathews test. Once the assumptions were met, the data were subjected to statistical analysis using the F test, and the means were compared using the Tukey test, at 5% probability, using the statistical program Sisvar version 5.6. Germination percentage data were transformed using the arcsine square root transformation $\sqrt{x}/100$.

RESULTS

The different endophytic fungal and bacterial genera associated with Aroeira tree leaves are listed in Table 1, where it is observed that among the 19 fungi, 12 belong to the genus *Diaporthe* (anamorph *Phomopsis*). The remaining observed genera are represented by only one individual each. Except for the fungi *Xylaria* and *Eutypella*, which belong to the Basidiomycota phylum, the other fungi belong to the Ascomycota phylum.

Table 1. Endophytic fungi and bacteria from *Myracrodruon urundeuva* identified through sequencing the ITS region and the 16S rRNA gene, respectively, using the BLAST tool (NCBI).
Tabela 1. Fungos e bactérias endofíticas de *Myracrodruon urundeuva* identificados pelo sequenciamento da região ITS e o gene 16S rRNA, respectivamente, com o uso da ferramenta BLAST (NCBI).

Isolado	Gênero/espécie - BLAST/NCBI
IM36	*Phomopsis* sp.
IM37	*Phomopsis* sp.
IM48	*Diaphorte schini*
IM94	*Diaporthe* sp.
IM96	*Diaporthe* sp.
IM97	*Diaporthe* sp.
IM99	*Phomopsis* sp.
IM102	*Diaporthe endophytica*
IM104	*Dothiorella* sp.
IM105	*Cytospora* sp.
IM106	*Diaporthe phaseolorum*
IM110	*Alternaria* sp.
IM114	*Diaporthe* sp.
IM115	*Phomopsis* sp.
IM116	*Phoma medicaginis*
IM120	*Diaporthe* sp.
IF361	*Paraconiothyrium brasiliense*
IF362	*Xylaria laevis*
IF364	*Eutypella* sp.
IF385	*Bacillus* sp.
IF386	*Brevibacillus* sp.

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The fungi belong to 8 distinct families, which are: Family: Diaporthaceae, Genus: *Diaporthe/Phomopsis*; Family: Botryosphaeriaceae, Genus: *Dothiorella*; Family: Valsaceae, Genus: *Cytospora*; Family: Pleosporaceae, Genus: *Alternaria*; Family: Didymellaceae, Genus: *Phoma*, Species: *Phoma medicaginis*; Family: Didymosphaeriaceae, Genus: *Paraconiothyrium*, Species: *Paraconiothyrium brasiliense*; Family: Xylariaceae, Genus: *Xylaria*; Family: Diatrypaceae, Genus: *Eutypella*. The endophytic bacteria were identified as genera *Bacillus* and *Brevibacillus*.

The evaluation of the herbicidal potential of metabolites from endophytic fungi and bacteria from the Aroeira tree indicated varying responses in weed plants (Table 2).

Table 2. Germination percentage (GP), germination speed index (GSI), and mean germination time (MGT) of *Urochloa brizantha* cv. Piatã, *Bidens pilosa* and *Stylosanthes* sp. seeds under doses of metabolites from *Phomopsis* sp. (IM36), *Bacillus* sp. (IF385) and *Brevibacillus* sp. (IF386).

Tabela 2. Porcentagem de geminação (PG), índice de velocidade de germinação (IVG) e tempo médio de germinação (TMG) de sementes *Urochloa brizantha* cv. Piatã, *Bidens pilosa* e *Stylosanthes* sp. sob doses de metabólitos de *Phomopsis* sp. (IM36), *Bacillus* sp. (IF385) e *Brevibacillus* sp. (IF386).

Urochloa brizantha cv. Piatã									
Dose (%)	GP (%)			GSI (dimensionless)			MGT (days)		
	IM36	IF385	IF386	IM36	IF385	IF386	IM36	IF385	IF386
0	72,0 ns	64,0 a	79,7 a	16,7 ns	13,8 a	14,6 a	5,2 ab	5,3 ns	5,4 ns
25	73,6	51,2 ab	65,0 ab	18,1	9,6 ab	11,5 ab	5,1 ab	5,5	5,8
50	77,6	37,6 abc	50,4 abc	17,7	5,4 abc	7,5 bc	5,2 ab	5,7	5,8
75	68,0	27,2 bc	35,8 bc	19,0	5,0 bc	5,9 bc	4,9 b	4,4	4,6
100	76,0	13,6 c	21,1 c	14,6	1,4 c	1,9 c	5,5 a	3,6	4,8
CV(%)	12,6	23,7	31,2	27,7	34,2	9,9	3,9	33,7	29,3
Bidens pilosa									
Dose (%)	GP (%)			GSI (dimensionless)			MGT (days)		
	IM36	IF385	IF386	IM36	IF385	IF386	IM36	IF385	IF386
0	68,8 ns	52,8 ns	56,8 ns	11,5 ns	20,2 ns	11,4 ns	5,9 b	4,6 b	4,6 b
25	70,4	52,0	49,6	11,9	19,7	10,1	6,1 ab	4,6 b	4,6 b
50	68,8	54,4	65,6	14,2	18,4	12,0	6,6 ab	4,7 ab	4,9 ab
75	64,0	57,6	42,2	14,2	18,6	8,4	6,9 a	4,8 b	4,7 b
100	68,8	55,2	54,4	15,4	13,0	9,4	6,3 ab	5,1 a	5,1 a
CV (%)	19,3	23,9	30,9	27,7	28,0	31,7	7,0	4,1	3,9
Stylosanthes sp.									
Dose (%)	GP (%)			GSI (dimensionless)			MGT (days)		
	IM36	IF385	IF386	IM36	IF385	IF386	IM36	IF385	IF386
0	61,6 ns	68,0 ns	75,2 ns	18,3 a	19,7 ns	13,1 a	4,5 c	4,9 ns	4,9 b
25	70,4	64,8	68,0	14,7 a	14,8	12,1 ab	5,1 b	5,8	4,8 b
50	49,6	63,2	66,4	10,9 ab	13,1	10,6 ab	5,5 ab	5,3	5,0 ab
75	52,8	63,2	51,2	7,2 b	14,4	8,2 b	5,8 a	5,1	5,1 ab
100	68,0	52,8	55,2	7,1 b	15,1	8,0 b	5,7 a	4,9	5,3 a
CV (%)	18,1	25,9	21,4	20,4	38,4	23,0	5,2	4,8	4,2

Means followed by the same letter in the column do not differ statistically using the Tukey test (P<0.05). ns = not significant. CV = coefficient of variation.

The GP and GSI of *B. pilosa* seeds were not affected in the doses of metabolites evaluated. However, the MGT increased with higher doses. *U. brizantha* cv. Piatã seeds did not show a reduction in GP with the *Phomopsis* sp. metabolites, but reduced GP in the presence of increasing doses of bacterial metabolites. The GP directly impacted the GSI. Nevertheless, MGT did not differ between treatments using bacterial metabolites, but there was a reduction in MGT in the treatment with 75% of the metabolite from *Phomopsis* sp. The GP of *Stylosanthes* sp. seeds was not influenced by fungal and bacterial metabolites. Only doses of 75 and 100% of *Bacillus* sp. metabolites and *Phomopsis* sp. reduced the GSI of *Stylosanthes* sp. Similarly, these two doses increased the MGT of these seeds (Table 2).

All germinated seeds showed compromised radicle and shoot development at least in one dose.

Exceptions were the *Stylosanthes* sp. seedlings exposed to *Brevibacillus* sp. and *Phomopsis* sp. metabolites, which did not interfere with SL and RL, respectively (Table 3). Higher doses of metabolites reduced the length of both roots and shoots in the seedlings.

The most significant suppression of RL was observed in *U. brizantha* cv. Piatã seedlings (monocotyledon). The metabolite from *Bacillus* sp. at 50% reduced the RL of this species by 74%. For the *B. pilosa* and *Stylosanthes* sp, the greatest reductions in RL were observed at doses of 75 and 100% of the metabolites. The SL responded similarly to the CR.

Table 3. Shoot length (SL), and rootlet length (RL) of *Urochloa brizantha* cv. Piatã, *Bidens pilosa* and *Stylosanthes* sp. seedlings in the presence of metabolites from *Phomopsis* sp. (IM36), *Bacillus* sp. (IF385) and *Brevibacillus* sp. (IF386).

Tabela 3. Comprimento da parte aérea (CPA) e comprimento de radícula (CR) das plântulas de *Urochloa brizantha* cv. Piatã, *Bidens pilosa* e *Stylosanthes* sp. na presença de metabólitos de *Phomopsis* sp. (IM36), *Bacillus* sp. (IF385) e *Brevibacillus* sp. (IF386).

Urochloa brizantha cv. Piatã						
	SL (mm)			RL (mm)		
Dose (%)	IM36	IF385	IF386	IM36	IF385	IF386
0	20,4 a	15,2 a	18,7 a	43,2 a	36,3 a	32,9 a
25	17,6 a	13,5 ab	17,7 ab	36,7 ab	20,1 b	22,4 a
50	14,7 a	11,8 bc	16,7 bc	30,2 bc	9,5 bc	14,0 b
75	11,8 a	10,1 bc	14,9 bc	23,7 bc	5,0 bc	7,7 bc
100	8,9 b	8,4 c	13,7 c	17,2 c	4,5 c	3,0 c
CV (%)	28,6	22,1	21,5	24,6	33,4	21,9
Bidens pilosa						
	SL (mm)			RL (mm)		
Dose (%)	IM36	IF385	IF386	IM36	IF385	IF386
0	31,2 a	19,7 a	18,7 a	20,40 a	15,15 a	18,67 a
25	29,5 ab	15,5 b	16,4 b	17,6 ab	13,5 ab	17,4 b
50	27,9 b	11,3 b	14,1 b	14,7 b	11,8 ab	16,7 b
75	26,8 b	7,7 bc	11,8 b	11,8 bc	10,1 ab	14,9 b
100	24,5 b	2,9 c	9,4 b	9,0 c	8,4 b	13,7 b
CV (%)	19,0	38,3	25,9	18,7	32,1	24,5
Stylosanthes sp.						
	SL (mm)			RL (mm)		
Dose (%)	IM36	IF385	IF386	IM36	IF385	IF386
0	11,6 a	17,5 a	21,4 ^{ns}	21,3 ^{ns}	17,0 a	26,3 a
25	9,5 ab	14,5 ab	19,2	19,9	13,9 b	20,5 ab
50	7,5 ab	11,8 b	17,2	18,4	10,9 c	14,7 bc
75	5,5 b	8,4 b	14,2	17,0	7,8 c	8,8 c
100	3,4 b	5,4 b	12,6	15,5	4,8 c	3,4 c
CV(%)	43,1	29,3	34,4	25,3	23,2	30,7

Means followed by the same letter in the column do not differ statistically using the Tukey test (P<0.05). ns = not significant. CV = coefficient of variation.

DISCUSSION

The seasonally deciduous forests encompass a unique environment regarding soil, climate, and plant species. The soil is of limestone origin, the rainy season is well-defined and concentrated in a few months of the year, and the plants are xerophytes adapted to the semi-arid environment. As part of this singularity, it is expected to find endophytic microorganisms associated with plants having diverse biological action from the molecules originating from their metabolism. The discussion presents the potential of endophytic microorganisms to serve as sources of bioactive compounds in several areas.

Molecularly identifying fungi at the species level often requires a multilocus approach. Thus, the analysis of only the ITS region through the BLAST tool needed to be more robust for reliable determination at the species level. This point is why most isolated microorganisms were identified at the genus level.

One of the most common genera of endophytic fungi is *Diaporthe* (anamorph *Phomopsis*), which is also recognized as a phytopathogen, saprophyte, and pathogen in humans (GOMES *et al.*, 2013). In the present study, it was the most abundant endophytic fungus in the Aroeira tree. A review of the genus *Phomopsis* reported that from 2010 to 2019, more than 240 compounds from the secondary metabolism of endophytes from different species of *Phomopsis* were discovered, which have cytotoxic, antioxidant, antifungal, antiviral, bactericidal, zootoxic, phytotoxic and enzyme inhibitory activities (XU *et al.*, 2021).

The genus *Dothiorella* was identified in only one of the samples. This genus is related to phytopathogens and remains relatively unexplored. As a phytopathogen, it shows host specificity in many pathosystems. In the plant species *Bauhinia brevipes* Vogel, the genus *Dothiorella* was the most abundant endophyte after *Phomopsis* in leaf samples (HILARINO *et al.*, 2011).

Cytospora sp. was observed in only one sample. The genus belongs to the Order Diaporthales, like *Phomopsis*. These fungi produce metabolites such as cytosporone and dothiorelone (ABREU *et al.*, 2012), whose synthetic derivatives are used in the pharmaceutical industry. The cytosporone A has already been reported to have allelopathic action on lettuce, inhibiting seed germination and reducing plant development (ZAMBERLAM *et al.*, 2012).

The potential for endophytes to become pathogens is a recurring question. This change in the microorganism-plant relationship can cause harm to the host and allow the pathogen to spread to other plants of different species. The shift from endophyte to pathogen has already been observed in *Alternaria alternata*, which can remain asymptomatic in native plants (DE MERS, 2022).

The fungus *P. brasiliense* is an endophyte that produces compounds such as cytotoxic sesquiterpenes called brasilamides (LING *et al.*, 2015). The species demonstrates antagonistic activity against phytopathogens, produces enzymes such as lactase and amylase, has few phytopathogenic interactions, and is a producer of taxol used in cancer drug formulations (WANG *et al.*, 2021). Eight sesquiterpene compounds have already been isolated from Aroeira tree (WANG *et al.*, 2021), and this synthesis may be related to the endophytic community in the plant, as occurs with *P. brasiliense* in *Taxus* sp. in the production of taxol.

The basidiomycete genus *Xylaria* includes endophytic species such as *Xylaria cubensis* (Mont.) Fr. and *Xylaria feejeensis* (Berk.) Fr., producing bioactive compounds of interest to agriculture. *X. cubensis*, an endophyte of *Eugenia brasiliensis* Lam., had an herbicidal effect on wheat (*Triticum aestivum* L. variety Pizon) by elongating the seed coleoptile, possibly through the action of the cytochalasin molecule (BIASETTO *et al.*, 2019), which inhibitor of actin filament polymerization.

The basidiomycete *Eutypella* sp. is a genus of species with endophytic specimens, antibiotic sesquiterpene-producing properties, and steroids and triterpenes (ZHANG *et al.*, 2021).

There is a limited specialized literature on the topic of bioherbicides derived from microbial metabolites. Additionally, it is an area of science of recent exploration. It is assumed that there is a vast list of bioactive compounds with herbicidal effects to be known and plant endophytes with recognized allelopathic effects are good indicators for prospecting these molecules.

For an efficient pre-emergent herbicide, the inhibition of seed germination is the most important characteristic, as it reduces weed seedlings in the field. None of the concentrations of metabolites from *Phomopsis* sp. reduced the germination of the evaluated weeds. Pes *et al.* (2016) observed 100% inhibition of the germination of *Lolium multiflorum* L., *Conyza* sp., and *Echinochloa* sp. seeds with a concentrated metabolite from *Diaporthe* sp. achieved through centrifugation. Centrifugation of the metabolite to concentrate bioactive compounds is a good method to achieve better biological responses.

The GSI of the metabolite from *Phomopsis* sp. only affected *Stylosanthes* sp. seeds at doses of 75 and 100%. In these situations, the GSI was reduced by 61% compared to the control. Therefore, reducing GSI results in non-uniform germination and consequent irregular establishment in the field (PARIZ *et al.*, 2010).

Diaporthe gulyae is a phytopathogen that causes canker in sunflowers and produces a bioactive compound identified as phomentrioloxin that has herbicidal action on *Carthamus lanatus* (CIMMINO *et al.*, 2013). The authors identified at least seven phomentrioloxin derivatives with herbicidal, antimicrobial, and zootoxic activities. Among the bioactive compounds, gulypirone A and B, phomentrioloxin B and C and nitropropionic acid showed herbicidal activity (ANDOLFI *et al.*, 2015) with great potential for exploration.

The MGT indicates the time required for a particular portion of seeds to germinate, depending on the species under study and the environment to which they were exposed. In general, doses of 100% increased MGT, reflecting the loss of seed vigor. Additionally, increasing doses of microbial metabolites led to a reduction in root and shoot length of different weed species, which may be an effect caused by a disturbance in protein synthesis.

The bacterial metabolites used in this study had inhibitory effects on *U. brizantha* cv. Piatã germination.

Metabolites from *Bacillus subtilis* with herbicidal effects have been reported to inhibit the germination of seeds of *Amaranthus hybridus* L., *Sorghum halepense* (L.) Pers. and *Parthenium* sp. (JAVAID; ADREES, 2009).

B. pilosa seeds were the least sensitive to the metabolites when evaluating GP and GSI, as no dose of metabolites from the three microorganisms interfered with these indices. However, there was impairment in seedling development, with a reduction in RL and SL of the *Bacillus* sp. and *Phomopsis* sp. metabolites.

While the bioactive compounds in the metabolites utilized in this study remain unidentified, the results demonstrate characteristics similar to the mode of action of herbicides that inhibit initial growth from Group K, where seeds are capable of germination, but the emerging seedlings experience hindered development, displaying twisted and poorly formed leaves (SILVA *et al.*, 2011). Thus, the metabolites of *Phomopsis* sp., *Bacillus* sp., and *Brevibacillus* sp. interfered with the vigor of the seeds, resulting in malformed seedlings with non-uniform germination.

CONCLUSIONS

- The genus *Diaporthe*/*Phomopsis* was the most common cultivable endophyte in Aroeira tree;
- The basidiomycetes *Xylaria* sp. and *Eutypella* sp. were present as endophytes in Aroeira tree;
- The metabolites from microorganisms reduced the vigor of *U. brizantha* Stapf cv. Piatã, *B. pilosa*, and *Stylosanthes* sp seeds;
- The application of other methodologies for obtaining secondary metabolites from microorganisms may optimize the selection of bioactive herbicidal molecules;
- The isolation and identification of fungal and bacterial bioactive compounds are important for understanding the effect on the vigor of weed seeds;
- As far as we know, this is the first report evaluating metabolites of endophytic microorganisms from Aroeira tree as bioherbicides.

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