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QUALITY OF SEEDS OF FOREST SPECIES

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ABSTRACT

The aim of the present study was to determine the incidence of fungi associated with the seeds of forest species. For such, we used seeds from *Anadenanthera macrocarpa*, *Ceiba speciosa*, *Caesalpinia ferrea* var. *leiostachya*, *Mimosa caesalpiniifolia*, *Myracrodruon urundeuva*, *Amburana cearenses*, *Schinopsis brasiliensis, Erythrina verna, Aspidosperma pyrifolium, Moringa oleifera, Guazuma ulmifolia* and *Enterolobium contortisiliquum*. Incubation on a substrate of filter paper (blotter test) was used for the identification of fungi. Twenty-four genera of fungi were associated with the seeds, the most frequent of which were *Alternaria* spp., *Aspergillus* spp., *Cladosporium* sp., *Colletotrichum* sp., *Fusarium* spp. and *Penicillium* spp.

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INTRODUCTION

Forest species have considerable ecological value, offering the potential for reforestation and the recovery of degraded areas (Carmo *et al*., 2017), for which the production of high-quality plantlets is of fundamental importance. The main form of propagation of these plantlets is through seeds. Therefore, the success of plantlet production and the establishment forest populations depend on the use of seeds of good quality (Mendes *et al*., 2005; Piveta *et al*., 2010). In recent years, the demand for seeds of forest species has increased, especially after Brazilian Law nº 12.651, which established the protection of native vegetation in the country as well as the determination of areas of permanent preservation and legal reserves (Brasil, 2012). To meet this demand, there has been an increase in the exchange of seeds among different regions, which has been accompanied by the dissemination of pathogens associated with seeds to new areas (Carmo *et al*., 2017). Numerous pathogens can infect the seeds of forest species. The most frequent harm is caused by fungi, leading to plantlets with abnormalities and lesions (Walker *et al*., 2016) as well as the discoloration, deterioration, deformation and rotting of the seeds, which results in the loss of germinative potential and a reduction in plant vigor (Guimarães & Carvalho, 2014). Despite advances in seed technology, little had been done to improve the quality of forest seeds and information on the occurrence of potentially pathogenic fungi is scarce.

Such information can provide insight into the actual situation of plantlet production for reforestation purposes and contribute to the treatment of seeds and plants in nurseries, ensuring the establishment and development of new crops and improving the management of forest systems. Therefore, the aim of the present study was to determine the incidence of fungi associated with the seeds of forest species.

METHODOLOGY

The experiment was conducted at the Phytopathology Lab of the Department of Phytotechnology and Environmental Sciences of the Federal University of Paraíba, Campus II, located in the municipality of Areia, state of Paraíba (PB), Brazil. Seeds were collected from 12 forest species in different locations: *Anadenanthera macrocarpa* Benth. Brenan, *Ceiba speciosa* A.St.-Hil., *Caesalpinia ferrea* var. *leiostachya* Benth. and *Mimosa caesalpiniifolia* Benth. from the city of Areia, PB; *Myracrodruon urundeuva* Allemão, *Amburana cearenses* Allemão A.C. Smith, *Schinopsis brasiliensis* Engl., *Erythrina verna* Vell. Conc. and *Aspidosperma pyrifolium* Mart. from the city of Boa Vista, PB; *Moringa oleifera* Lam. from the city of Pombal, PB; *Guazuma ulmifolia* Lam. and *Enterolobium contortisiliquum* Vell. Morong. from the city of Triunfo in the state of Pernambuco (PE). The samples were placed in hermetically sealed plastic bags and identified. The seeds were sorted manually by visual inspection. Malformed seeds and those attacked by pests (partially or

completely destroyed with perforations that reached the embryo) were discarded (Brasil, 2009). For the analysis, the seeds were incubated in Petri dishes containing a double layer of filter paper moistened with sterilized distilled water at a temperature of 25 ± 2ºC with a 12-h light/dark photoperiod for seven days (Brasil, 2009). Two hundred seeds from each species were divided into ten replicates of 20 seeds each. The seeds were then disinfected by immersion in a sodium hypochlorite solution (1%) for three minutes. After drying, the seeds were individually distributed in Petri dishes under aseptic conditions, followed by the morphological identification of fungi with the aid of an optical microscope and specialized literature (Seifert *et al*., 2011). The results were expressed as the percentage of seeds infected by each fungus identified.

RESULTS AND DISCUSSION

Twenty-four genera of fungi were detected and identified on the seeds of the twelve forest species (Table 1; Figure 1). The most incident fungi were considered potential field pathogens: *Alternaria* spp., *Cladosporium* sp., *Colletotrichum* sp., *Fusarium* sp. and

Table 1. Incidence (%) of fungi associated with seeds of *Anadenanthera macrocarpa* (1), *Myracrodruon urundeuva* (2), *Amburana cearenses* (3), *Schinopsis brasiliensis* (4), *Moringa oleifera* (5), *Erythrina verna* (6), *Guazuma ulmifolia* (7), *Ceiba speciosa* (8), *Caesalpinia ferrea* var. *leiostachya* (9), *Aspidosperma pyrifolium* (10), *Mimosa caesalpiniifolia* (11) and *Enterolobium contortisiliquum* (12)

	Forest species											
Fungi	1	2	3	4	5	6	7	8	9	10	11	12
Alternaria spp.	0.0	20.0	7.0	24.0	26.5	12.0	3.5	0.0	0.0	3.0	0.0	1.5
Aspergillus flavus	26.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus niger	3.0	0.0	3.5	0.0	0.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus sp.	9.0	0.0	2.5	4.0	2.0	8.5	3.0	3.5	0.0	7.5	10.5	10.0
Bipolaris sp.	0.0	0.0	0.0	0.0	11.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lasiodiplodia sp.	0.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cercospora sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Chaetomium sp.	0.0	3.5	0.0	3.0	0.0	0.0	7.0	3.5	0.0	0.0	0.0	1.0
Cladosporium sp.	20.0	0.0	5.0	6.0	12.5	8.5	3.5	13.0	7.5	6.0	17.0	9.0
Colletotrichum sp.	0.0	7.5	0.0	0.0	7.5	0.0	0.0	2.0	0.0	8.0	0.0	0.0
Curvularia sp.	3.0	11.0	1.0	1.0	25.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Exserohilum sp.	0.0	9.0	0.0	5.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fusarium spp.	48.0	11.0	3.5	40.0	20.0	15.5	0.0	10.5	0.0	18.5	7.5	6.0
Helminthosporium sp.	0.0	2.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nigrospora sp.	3.0	0.0	0.0	6.0	3.5	1.5	0.0	0.0	3.5	0.0	0.0	0.0
Penicillium sp.	50.0	5.0	1.5	0.0	1.5	0.0	5.5	9.0	3.0	4.5	0.0	4.5
Periconia sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
Pestalotiopsis sp.	0.0	1.0	0.0	9.0	1.5	0.0	1.5	2.0	0.0	0.0	0.0	0.0
Phoma sp.	0.0	0.0	0.0	0.0	14.5	0.0	0.0	0.5	0.0	0.0	0.0	0.0
Phomopsis sp.	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	2.5	0.0	0.0
Pithomyces sp.	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhizopus sp.	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0
Trichoconiella sp.	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trichoderma sp.	0.0	5.0	0.0	6.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

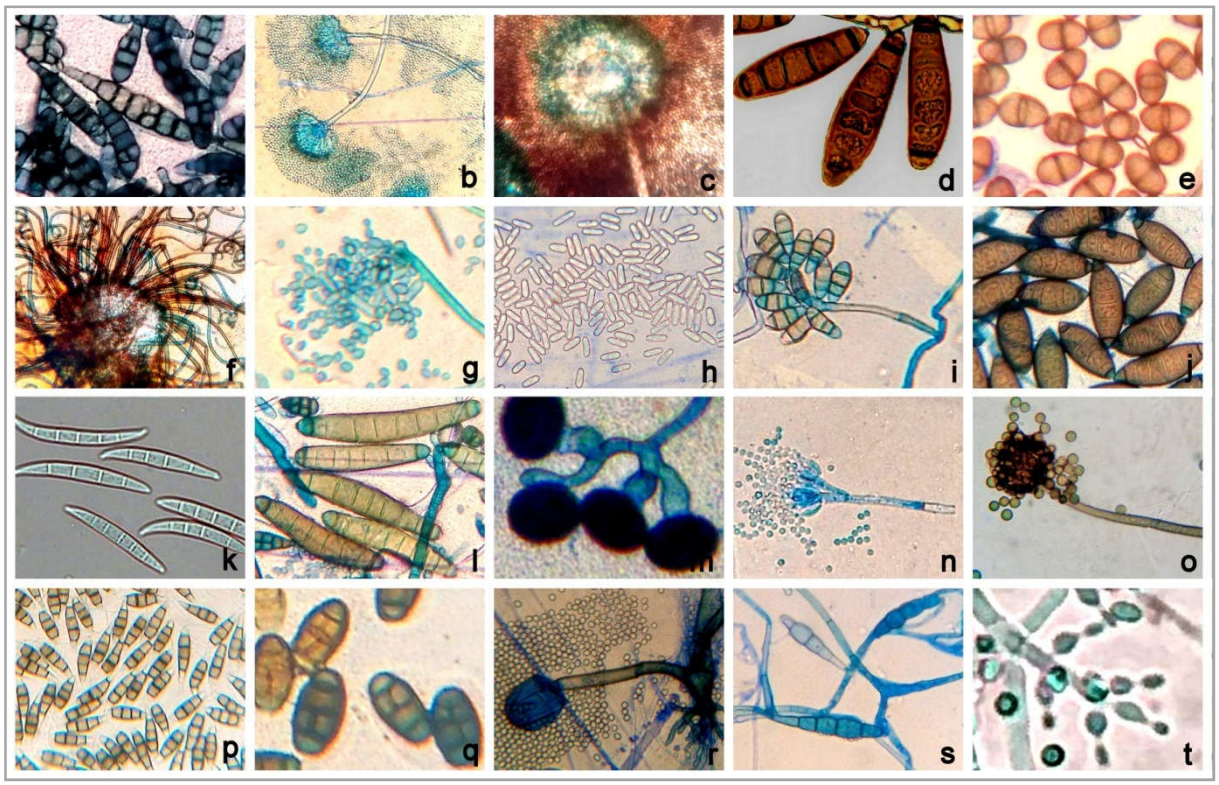

Figure 1. Fungi identified in the seeds of forest species. a. *Alternaria* sp., b and c. *Aspergillus* spp., d. *Bipolaris* sp., e. *Lasiodiplodia* sp., f. *Chaetomium* sp., g. *Cladosporium* sp., h. *Colletotrichum* sp., i. *Curvularia* sp., j. *Exserohilum* sp., k. *Fusarium* sp., l. *Helminthosporium* sp., m. *Nigrospora* sp., n. *Penicillium* sp., o. *Periconia* sp., p. *Pestalotiopsis* sp., q. *Pithomyces* sp., r. *Rhizopus* sp., s. *Trichoconiella* sp., t. *Trichoderma* sp.

Curvularia sp. The genus *Bipolaris* was only found on the seeds of *Moringa oleifera*. *Botrytis*, *Pithomyces* and *Trichoconiella* only occurred on the seeds of *Myracrodruon urundeuva*. *Cercospora* only occurred on the seeds of *Aspidosperma pyrifolium*. *Phoma* only occurred on the seeds of *Moringa oleifera* and *Ceiba speciose*. *Phomopsis* only occurred on the seeds of *Moringa oleifera* and *Aspidosperma pyrifolium*. *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp., *Penicillium* spp., *Chaetomium* sp. and *Rhizopus* spp. belong to a complex of "storage" fungi, which cause the deterioration of seeds, reducing the germinative potential of the seeds and the vigor of the plantlets (Guimarães & Carvalho, 2014). According to Carmo et al (2017), the high diversity of fungi on seeds is due to the fact that seeds are most often collected after falling to the ground, which increases the chance of infestation and infection by microorganisms in the soil. Indeed, we found a high incidence of *Fusarium* spp., which is a resident of the soil and was found on the seeds of ten of the 12 forest species analyzed in the present study: *Anadenanthera macrocarpa*, *Myracrodruon urundeuva*, *Amburana cearenses*, *Schinopsis brasiliensis, Moringa oleifera*, *Erythrina verna*, *Ceiba speciosa*, *Aspidosperma pyrifolium*, *Mimosa caesalpiniifolia* and *Enterolobium contortisiliquum* (Table 1).

Fusarium spp. has been associated with damping-off in the pre-emergence and post-emergence phase of different forest species, with the occurrence of seed-to-plantlet transmission (Walker et al., 2016). Evaluating the incidence, transmission and pathogenicity of fungi associated with the seeds of *Ceiba speciosa*, Lazarotto et al (2010) found the occurrence of *Fusarium* sp., *Alternaria* sp., *Colletotrichum* sp., *Curvularia* sp. and *Pestalotiopsis* sp., the most frequent of which was *Fusarium* sp., which was found on all samples. The same authors performed the pathogenicity test and found that *Fusarium* sp., *Alternaria* sp. and *Colletotrichum* sp. were pathogenic to plantlets. *Alternaria* sp. was the second most frequent fungus on the seeds (Table 1) and was found on the seeds of *Anadenanthera macrocarpa*, *Myracrodruon urundeuva*, *Amburana cearenses*, *Schinopsis brasiliensis, Moringa oleifera*, *Erythrina verna*, *Guazuma ulmifolia*, *Aspidosperma pyrifolium* and *Enterolobium contortisiliquum*. This fungus is generally associated with leaf disease, the symptoms of which are yellowish spots and the necrosis of cotyledons, as reported previously for *Ceiba speciosa* (Lazarotto et al., 2010). Other fungi found in this study cause leaf spots, such as *Colletotrichum* sp. (Santos et al., 2011), *Botrytis* sp., *Cladosporium* sp. (Alfenas et al., 2004), *Pestalotiopsis* sp. (Antunes, 2008) and *Cercospora* sp. (Mmbaga et al., 2015). Among the fungi identified on the seeds of the forest species, the genera *Phoma* sp. and *Phomopsis* sp. cause seed rot and damping-off in plantlets, significantly compromising the initial development phase and establishment of plants (Botelho et al., 2008). The present findings demonstrate a high incidence of fungi associated with the seeds of forest species, many of which can be a vehicle for the dissemination of these microorganisms under field conditions. As these seeds are used in nurseries, it is of fundamental importance to conduct studies on the harm caused by microorganisms in the pre-emergence and post-emergence phases of forest plantlets.

CONCLUSION

Twenty-four genera of fungi were associated with the seeds of 12 forest species, the most incident of which were *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium* and *Penicillium*. The seeds from *Anadenanthera macrocarpa*, *Myracrodruon urundeuva*, *Schinopsis brasiliensis, Moringa oleifera* and *Ceiba speciosa* had the highest incidence of fungi.

The findings of the present experiment underscore the need for tests of pathogenicity and physiological quality to quantify the harm caused by these pathogens.

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