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Isolamento de fungos fitopatogênicos em sementes da árvore Caatinga

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A qualidade sanitária é um parâmetro que avalia a incidência de patógenos em sementes, principalmente em espécies de produção agrícola, havendo poucos estudos sobre espécies florestais arbóreas. O presente trabalho tem como objetivo avaliar a qualidade sanitária em sementes de espécies arbóreas da Caatinga. O experimento foi conduzido no Laboratório de Fitopatologia do Centro de Ciências e Tecnologia Agroalimentar da UFCG, campus de Pombal-PB. Foram estudadas sementes de quatro espécies arbóreas: *Myracrodruon urundeuva* (Allemão): aroeira, *Poincianella pyramidalis* (Tul.): catingueira, *Mimosa tenuiflora* (Willd.)Poiret: jurema-preta e *Libidibia ferrea* (Mart) ex Tul.: pau-ferro, as mesmas foram divididas em duas subamostras, sendo uma submetida à desinfestação superficial por imersão em hipoclorito de sódio (NaClO) a 2% por 5 minutos . A sanidade das sementes foi avaliada pelo “blotter test”. Os gêneros fúngicos comumente detectados nas sementes das espécies estudadas foram: *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp. e *Trichoderma* spp.. A desinfestação com hipoclorito de sódio a 2% reduziu a incidência de fungos nas sementes das espécies estudadas. O gênero *Aspergillu*s apresentou o maior número de colônias e diversidade de espécies fúngicas nas sementes tratadas e não tratadas, estando presente em todas as espécies arbóreas. A menor incidência de colônias fúngicas ocorreu na espécie *Mimosa tenuiflora*.

Palavras-chave: sementes florestais, sanidade, desinfestação, micoflora, diversidade.

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The sanitary quality is a parameter that evaluates the incidence of pathogens in seeds, especially in species of agricultural production, so that there are few studies on forest tree species. This study aims to evaluate the sanitary quality and the effect on seeds of four species from the Caatinga region. The experiment was conducted at the Laboratory of Phytopathology from the Centre for Agri-Food Science and Technology at UFCG, Pombal - PB. Seeds from four species were studied: *Myracrodruon urundeuva* (Allemão): aroeira, *Poincianella pyramidalis* (Tul.) L.P. Queiroz: catingueira, *Mimosa tenuiflora* (Willd.) Poiret: jurema-preta and *Libidibia ferrea* (Mart ex Tul.) L.P. Queiroz: pau-ferro. The seed health was assessed using the “blotter test”. The genera of fungi commonly found in the seeds of the studied species were *Aspergillus* spp., *Cladosporium* spp., *Fusarium* spp., *Penicillium* spp. and *Trichoderma* spp. The disinfection with 2% sodium hypochlorite reduced the incidence of fungi on seeds of the studied species. The genus *Aspergillus* presented the highest number of colonies and diversity of fungal species in treated and untreated seeds, being present in all four species. The lowest incidence of fungal colonies occurred in the species *Mimosa tenuiflora*.

Keywords: forest seeds, seed health, disinfestation, micoflora, diversity.

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INTRODUCTION

The association of pathogens to seed implies a reduction of yield and impairment of crop quality. So, the assessment of sanitary quality of seeds plays a key role in the selection of resistant cultivars and possible pre-treatments (Martins et al., 2009).

In tropical regions, the environmental conditions of high humidity and temperature are favorable for the growth and development of pathogens so that the seeds of native species in these areas are prone to be attacked by them (Nascimento et al., 2006). In addition to the reduction in germination of a seed lot, the presence of fungi may bring about problems in interpreting the results of the germination tests under laboratory conditions (Santos et al., 2011a).

Fungi are considered a primary pathogen that causes damage when associated with seeds. Dhingra et al. (2002) report *Pestalotiopsis maculans* as potentially pathogenic to seeds and seedlings of *Anadenanthera macrocarpa* (mimosa).

In the forestry field, loss of native species seeds is common due to the attack of saprophytic and wood-decay fungi resulting from inadequate storage conditions. Alfenas and Mafia (2007) attributed this to two factors: (1) the fruits open in the field before harvesting, leaving the seeds exposed to contamination; and (2) due to the height of trees, the fruits are collected on the ground, where fungi may colonize them and reach the seeds.

The pathogens transmitted or not by seeds can also affect their force field, presenting a more pronounced effect when they consist of organisms that colonize the internal tissues of the seeds (Fagan et al., 2004). This type of information is essential to projects focusing on the reforestation with native tree species (Santos et al., 2011b).

In addition to the significant losses at the field level, other problems arise when infected seeds and kernels are used in both human and animal food, considering the fact that fungi can produce toxins and there are high incidences of fungal contaminants observed in many types of seed storage. These are concerning data since they directly affect the physiological quality of seeds (Silva et al., 2010).

Looking for tree seeds for reforestation with preservationists purposes or not has intensified in recent years. Thus, the exchange of seeds between regions has been expanded and may constitute a mean of pathogens movement since this allows their dissemination to the long distances (Santos et al., 2011b).

Currently, there are no products in Brazil to treat seeds of tree and forest species for control pathogens, nor any satisfactory result of research supports this method. So, the study of health quality of these species aims to contribute to knowledge and technologies for the production system of native and exotic tree seeds.

Studying the association of fungi with tree species may provide a basis for epidemiological models, ranging from the storage of seed to seedling production (Santos et al., 2011b). Therefore, this study aims to assess the health and the effect of surface disinfection on the sanitary quality of seeds of tree species from the Caatinga region.

MATERIAL AND METHODS

We carried out the experiment at the Laboratory of Phytopathology, Centre for Agrifood Science and Technology, Federal University of Campina Grande, Pombal – PB. Seeds or propagules with full physiological maturity were harvested from trees matrices located in the city of Aparecida, PB. After the crop, the seeds were extracted from fruits, and subjected to cleaning and manual selection to eliminate from those formed poorly or attacked by insects, put to dry and stored in glass pots with a laboratory temperature ranging from 18 to 25 °C for a year.

To assess the treatment effect of the surface disinfection on the incidence of fungi, seeds of four species – *Myracrodruon urundeuva* (Allemão): aroeira, *Poincianella pyramidalis* (Tul.): catingueira, *Mimosa tenuiflora* (Willd.) Poiret: jurema-preta and *Libidibia ferrea* (Mart) ex Tul.: pau-ferro – were sorted into two subsamples, one of them having been subjected to disinfection by immersion in 2% sodium hypochlorite (NaClO) remaining in contact with the seed for 5 minutes. Seeds were packed in bags of tulle, and then the excess was removed with their immersion in sterile distilled water, whereas the remaining subsample did not undergo any treatment. For health assessment, we applied the “blotter test” (Neegaard, 1979). Treated and untreated seeds were placed in Petri dishes (150 mm of diameter and 20 mm height) containing three sheets of filter paper moistened with 15 ml of sterile distilled water. The seeds were incubated at 23 ±2°C with 12 hours of photoperiod during ten days (Oliveira, 2009). After the incubation period, the seeds were individually examined under a stereoscopic microscope for the detection and identification of associated fungi therewith (Barnett and Hunter, 1998; Mapa, 2009; Santos et al., 2011b). The results of the tables refer to the raw data and are also expressed as percentages, the figures show the means and standard errors per repetition, that is, the "worked" data.

When we were unable to identify the pathogens on seeds, they were isolated in the culture medium PDA + A (potato-dextrose-agar + antibiotic) and incubated at 28°C with 12 hours photoperiod for ten days.

The complete randomized design was used with two treatments (with and without disinfection) and ten repetitions of 20 seeds per plate, totalizing 200 seeds per tree species. We applied the Kruskal-Wallis test comparing two or more paired groups (nonparametric ANOVA) to verify the occurrence of significant differences among species concerning the following variables: number of fungal colonies, number of species and fungal genera, and number of seeds germinated. The variables that showed significant differences were subjected to the multiple comparison tests. The Wilcoxon comparison test, which verifies the magnitude of the difference between two treatments, was applied to confirm if the treatment with sodium hypochlorite affected the variables above to show the efficiency of sodium hypochlorite for disinfestation of tree seeds. A Correspondence Analysis was used to investigate the occurrence of affinities between the studied tree species concerning their susceptibilities to a given set of fungal species. All analyses were performed using R 2.12.2

software (2017) with the statistical packages Vegan 1.17-8 and Pgirmess.

RESULTS AND DISCUSSION

After the incubation period of seeds of different tree species, 11 genera of fungi (Table 1) were found, nine genera were detected in untreated seeds and seven in treated seeds.

The number of fungal colonies developed in non-treated seeds was higher than the figure related to the treated seeds (Table 1.) The genera of fungi commonly found in the two samples of analyzed seeds (both untreated and treated) comprise *Aspergillus* spp*., Cladosporium* spp., *Fusarium* spp., *Penicillium* spp*.,* and *Trichoderma* spp. However, the number of these five fungal colonies was higher in untreated seeds. Despite the occurrence of these fungal genera in seeds subjected to the disinfection with sodium hypochlorite, the use of this compound has demonstrated a negative impact on the development of the colonies.

Similar results were obtained by Muniz et al. (2007), who evaluated the influence of laboratory asepsis in the incidence of pathogens associated with seeds and found that asepsis influenced seedlings of five forest species in the initial stage. According to these authors, the action of sodium hypochlorite in reducing the incidence of fungi was noticeable, indicating that they are on the surface of the seed.

According to Coutinho et al. (2000), one of the major forms of association between microorganisms and seeds is through their location on the external tissue, such as pericarp and tegument, so that seed treatment with sodium hypochlorite shows efficiency in reducing microorganisms associated superficially with the seeds.

Although the disinfection reduced the occurrence of species and genera of fungi, we recorded the genus *Aspergillus* in all forest species. However, the largest number of fungal colonies of this genus was found in the untreated seeds (Table 2). In addition to the occurrence of a higher number of colonies, this fungal genus showed high variability. In untreated *Myracrodruon urundeuva* seeds, five different species were detected, whereas in treated seeds of such a tree three species were identified in smaller amounts (Table 2).

The color of colonies allowed us to identify the species *Aspergillus niger*, *A. flavus*, *A. candidus*, *A. ochraceus* and *A. glaucus*, whereas in seeds submitted to surface disinfection it was observed the occurrence of *A. niger*, *A. flavus,* and *A. glaucus*. The latter being the only species with the highest number of colonies associated with the treated *Poincianella pyramidalis* seeds, as compared to the untreated ones.

According to Círio and Lima (2003) fungi belonging to the genus *Aspergillus* spp. are toxigenic, causing deterioration in grains and seeds, and are also cosmopolitan saprophytic, easily spread by its light and dry spores, they may grow in low humidity, facilitating the development of other genres that require more moisture.

Table 1. Fungal population associated with the seed of forestry species from the Caatinga region submitted or not to treatment of surface disinfection.

	Untreated Seeds		Treated Seeds	
Species and/or Genera	**Colony (No.)**	**Frequency (%)**	**Colony (No.)**	**Frequency (%)**
Aspergillus niger	97	32.44	12	26.09
Aspergillus flavus	85	28.43	4	8.70
Aspergillus glaucus	2	0.67	8	17.39
Aspergillus ochracus	3	1.00	0	0.00
Aspergillus candidus	10	3.34	0	0.00
Aspergillus spp	2	0.67	0	0.00
Penicillinum spp	80	26.76	12	26.09
Tricoderma spp	7	2.34	4	8.70
Fusarium spp	1	0.33	1	2.17
Alternaria spp	0	0.00	3	6.52
Chactomium spp	1	0.33	0	0.00
Rizoctonia spp	0	0.00	1	2.17
Colletotrichum spp	5	1.67	0	0.00
Cladosporium spp	2	0.67	1	2.17
Curvularia spp	2	0.67	0	0.00
Phytophthora spp	2	0.67	0	0.00
Total	299	100.00	46	100.00

Except for *Mimosa tenuiflora*, colonies of *Penicillium* spp. were found in seeds of all the other forest species, with the largest number of colonies observed in untreated seeds of *Libidibia ferrea*, followed by *Poincianella pyramidalis* and *Myracrodruon urundeuva* (Table 2). According to Freitas et al. (2000), fungi of the genera *Aspergillus* and *Penicillium* are the most found in the seeds during storage, which may impair their quality, due to its deterioration.

Piña-Rodrigues and Vieira (1988) argue that the occurrence of the genera *Aspergillus*, *Penicillium*, *Rhizopus,* and *Trichoderma* are common in tree seeds, transported directly from the place of collection to the laboratory.

Although the largest number of colonies of the species or genera of fungi were associated with untreated seeds *M. urundeuva* these had the lowest number of occurrence of fungi in seeds subjected to disinfection with *M. tenuiflora* (Table 2).

After analyzing the fungal population, it was possible to observe that forest species differed significantly ($p < 0.01$) concerning the number of fungal colonies, the number of species or genera of fungi, as well as to the number of germinated seeds for both the treated seeds and the untreated ones.

Figures 1A and 1B show that the average number of fungal colonies were smaller in the species *Mimosa tenuiflora*, both for treated and untreated seeds, differing from the other species. Among all species and genera detected in this study, *Aspergillus niger* was the only species found in the seeds of the forest species *Mimosa tenuiflora* (Table 2).

This result can be attributed to the presence or the effect of compounds with allelopathic activities existent in this species which may have inhibited the development of fungal colonies (Colpas et al., 2003).

As Silveira et al. (2011) state, currently *Mimosa tenuiflora* is widely distributed in the semi-arid region, and other native woody species fail to establish under its crown, suggesting an allelopathic effect on these species.

Studying the tannin potential of six tree species from the Brazilian semiarid region, Paes et al. (2006) found high potential of tannin production in plants of *Mimosa tenuiflora*. These compounds are produced in the leaves of tree species, increasing its concentration with age, making them less susceptible to the attack by pathogens (Bezerra, 2008). In addition to the leaves, tannins may be found in various plant parts, such as the bark, fruits, and seeds.

The most common phenols in plants are not considered toxic in normal amounts and conditions, except polymeric phenols referred to as tannins, which have the ability to complex and precipitate proteins from aqueous solutions (Salunkhe et al., 1990). Substances such as these protect plants from attack by herbivores, invertebrates, and vertebrates, by presenting astringent flavor, hindering their intake.

Table 2. Number of colonies for each fungal species and genus associated with seeds of four forest species from the Caatinga region submitted or not to surface disinfection.

	Forest species subjected to disinfestations							
	M. urundeuva		**P. pyramidalis**		**M. tenuiflora**		**L. ferrea**	
Species and Genus	Yes	No	Yes	No	Yes	No	Yes	No
Aspergillus niger	8	65	1	6	1	1	2	25
Aspergillus flavus	3	13	0	48	-	-	2	23
Aspergillus glaucus	1	2	7	-	-	-	-	-
Aspergillus ochraceus	-	3	-	-	-	-	-	-
Aspergillus candidus	-	9	1	-	-	-	-	-
Aspergillus spp	-	2	-	-	-	-	-	-
Penicillium spp	1	23	6	45	-	-	-	17
Trichoderma spp	2	-	1	1	-	-	-	7
Fusarium spp	1	1	-	-	-	-	-	-
Alternaria spp	1	-	2	-	-	-	-	-
Chaetomium spp	-	1	-	-	-	-	-	-
Rizoctonia spp	1	-	-	-	-	-	-	-
Colletotrichum spp	1	1	1	1	-	-	-	1
Cladosporium spp	-	1	-	1	-	-	-	1
Curvularia spp	-	1	-	-	-	-	-	1
Phytophthora spp	-	1	-	-	-	-	-	1
Total number of colonies	19	123	19	102	1	1	4	76

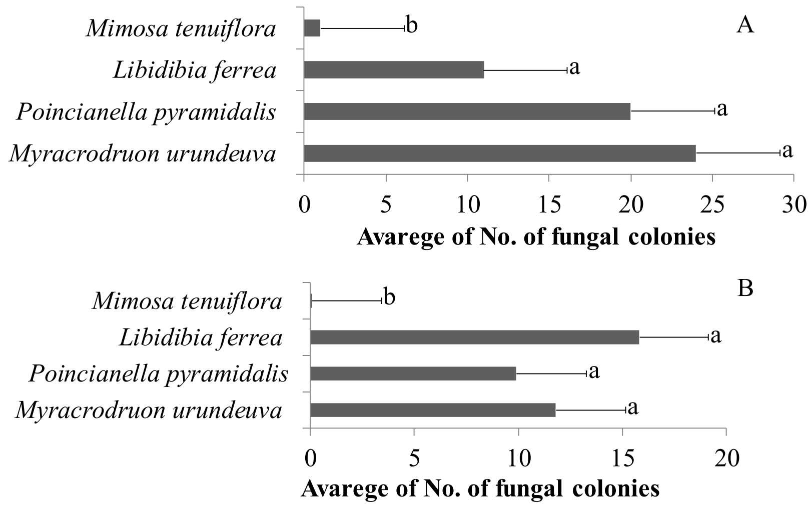

Figure 1. Mean number of fungal colonies from seeds treated (A) and untreated forest species (B). Means represented by columns with the same letter do not differ by Kruskal-Wallis test for multiple comparisons.

Mimosa tenuiflora is a tree species that can be useful for agricultural production. According to Brass (2009), the allelopathic effects have various uses that could contribute to the search for pesticides, to understand the antagonism of syndicated or successive crops; to reduce the use of synthetic herbicides, replacing them with allelopathy processes.

Similarly to the result of the average number of fungal colonies, forest species *Mimosa tenuiflora* presented the lowest values as the mean number of species and genera of fungi, regardless of the treatment used. Conversely, the highest values were found for the species *Myracrodruon urundeuva*, even though this species did not differ from *Poincianella pyramidalis* e *Libidibia ferrea* (Figure 2). Still, the values for the untreated seeds were higher (Figure 2B) then treated seeds (Figure 2A), demonstrating that the use of active chlorine as a disinfection agent reduces the fungal contamination, although it did not prevent the development of most resistant colonies, particularly those located internally in the seeds.

These results indicate that the species *Myracrodruon urundeuva* is more susceptible to pathogens and reinforce the importance of studies involving the use of other products (plant extracts, essential oils) combined with methods of pathogen control. As pointed out by Silva et al. (2003), seed treatment is an important tool to prevent the spread of these pathogens in the field.

Another aspect analyzed was the average of germinated seeds, which varied among species, with the highest values recorded for the species *Poincianella pyramidalis*, submitted or not to disinfestation (Figures 3A and 3B), although these species do not differ from *Myracrodruon urundeuva* and *Mimosa tenuiflora*. On the other hand, the species which showed the lowest values in the means of germinated seeds was *Libidibia ferrea*, both for the treated and untreated seeds. In general, treatment with the surface disinfection with sodium hypochlorite significantly reduced the incidence of these fungi, regardless of forest species. Studies conducted with seeds of forest species using seed disinfected with NaClO decrease the occurrence of fungi associated with the same (Muniz et al., 2007).

Castellani et al. (1996) observed that seed contamination could affect in a severe form the seed physiological quality and, in some cases, it completely inhibit the germination of seeds. However, Oliveira et al. (2006) compared methods of seeds disinfection of golden shower trees (*Peltophorum dubium*) and detected mainly *Trichoderma* sp., *Penicillium* sp., *Aspergillus niger*, and *Fusarium* sp. They also found that the percentage of seeds infested did not interfere with germination.

According to Carvalho and Nakagawa (2000), the composition of fungal flora also depends on the water content of the seed, because changes in humidity will affect the change in the microflora, both quantitatively and qualitatively. Therefore, the association of fungi with the seeds sometimes will not cause illness or loss of physiological quality, although this association may favor the proliferation and survival of fungi.

Correspondence analysis shows three distinct areas where forest species were separated due to the distribution of species and genera of fungi (Figure 4), with the predominance of the genus *Aspergillus* which occurred in five different species and was detected in four forest species. The tree species that showed more species and genera of fungi was the mastic tree. *Mimosa tenuiflora*, unlike other species, does not appear in the chart. We attribute this results to the occurrence of only a single colony of fungal species, *Aspergillus niger*, in the two treatments evaluated for this tree species.

Using the health test on filter paper, Cherobini et al. (2008) found in cedar seeds only the following fungi: *Aspergillu*s spp., *Chaetomium* spp., *Penicillium* spp., and *Trichoderma* spp.

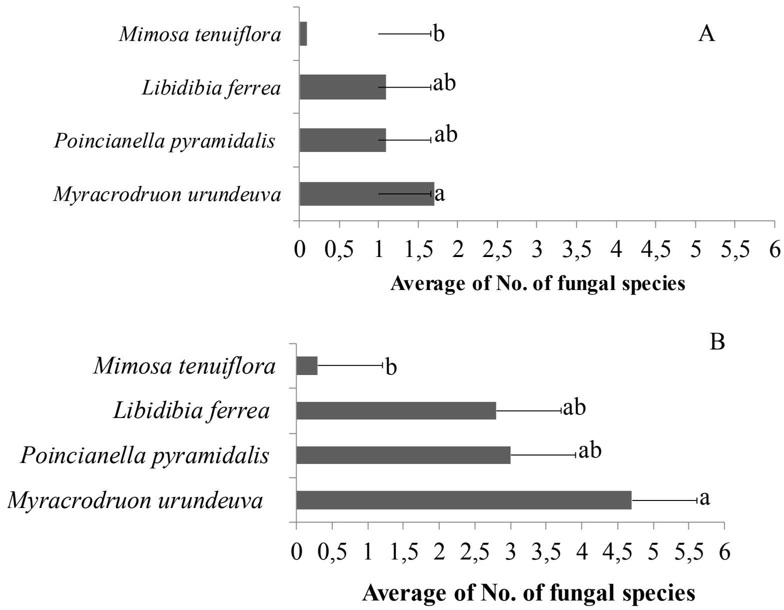

Figure 2. Average number of fungal species/genera in forest species seeds which were subjected to treatment (A) and which were not subjected to treatment (B). Means represented by the columns of the same lowercase letter do not differ from one another by Kruskal-Wallis multiple comparison tests.

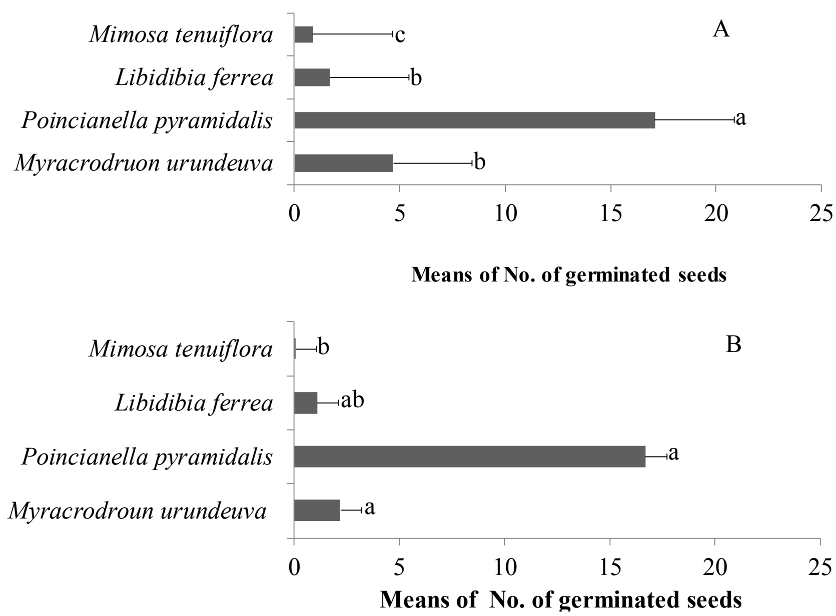

Figure 3. Means of germinated seeds among forest species seeds which were subjected to treatment (A) and which were not subjected to treatment (B). Means represented by the columns of the same lowercase letter do not differ from one another by Kruskal-Wallis multiple comparison tests.

Oliveira et al. (2012) verified that prior disinfection with 1.5% sodium hypochlorite for five minutes reduces fungal contamination, facilitating the evaluation of the health test, and the compound does not affect seedling emergence, being beneficial for the establishment of the seedling *Schizolobium amazonicum*.

According to Ruiz-Filho et al. (2004), fungi such as *Chaetomium*, *Trichoderma*, *Penicillium*, *Aspergillus,* and *Rhizopus* are associated with seed deterioration and its action is dependent on their physical and physiological conditions, at the beginning of storage, and on the dominant environmental factors during this period.

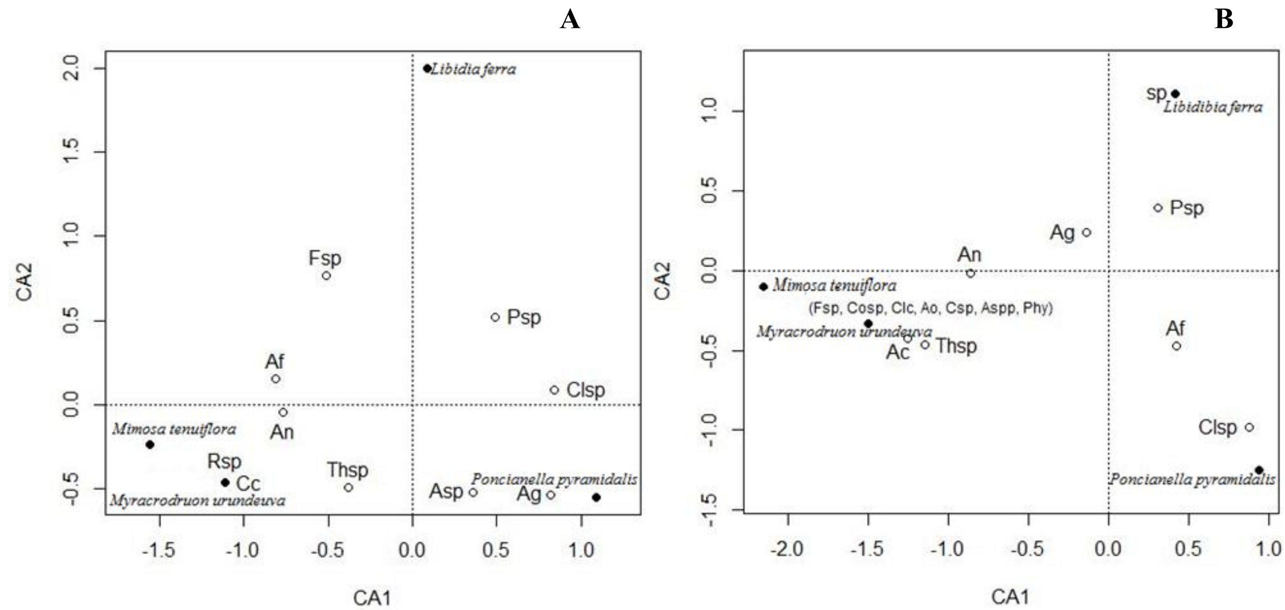

Figure 4. Fungal species identified in forest species in treated (A) and untreated (B) seeds. Aspergillus niger (An); Aspergillus glaucus (Ag); Aspergillus ochracceus (Ao); Aspergillus candidus (Ac); Aspergillus flavus (Af); Aspergillus sp (Asp); Penicillium (P); Fusarium (F); Colletotrichum (Cl); Cladosporium (Cla); Curvularia (Cv); Phytophthora (Phy); Trichoderma (T); Cladosporium spp. (Clsp); Rhizoctonia (R); Chaetomium (Ch), Numbers of unidentified fungal species (N)

In Figure 4, it can be observed that the species *Myracrodruon urundeuva* presented the highest micro-flora associated with untreated seeds related to treated seeds of the remaining species.

The incidences of the fungi *Aspergillus niger* (An), *Aspergillus glaucus* (Ag), *Penicillium* sp (P), *Fusarium* sp. (F), *Trichoderma* sp. (T), *Aspergillus flavus* (Af), and *Cladosporium* sp. (Cla) was significantly reduced in the treated seeds, indicating that they were located in the external part of the seeds or diaspores of *Myracrodruon urundeuva* (Allemão), *Libidibia ferrea*, and *Poincianella pyramidalis* (Tul.), and that the use of sodium hypochlorite was efficient.

According to Botelho et al. (2008), the disinfection with 1% sodium hypochlorite for 3 minutes reduced significantly the incidence of the fungi *Aspergillus* spp., *Curvularia* sp. and *Trichothecium* sp. associated with seeds of yellow and purple trumpet trees, in almost all samples.

CONCLUSION

The genera of fungi commonly found in the seeds of the studied tree species were: *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium,* and *Trichoderma*.

The incidences of fungi on seeds of the studied tree species were reduced by the disinfection using 2% sodium hypochlorite.

The genus *Aspergillu*s presented the highest number of colonies and diversity of fungal species in treated and untreated seeds, being present in all tree species.

The seeds *Mimosa tenuiflora* have the lowest fungal incidence.

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