

## RESEARCH ARTICLE

# Sanitary quality in seeds from species of Caatinga biome and control methods for fungi

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## ABSTRACT

Sanitary and quality of seeds of three native species (Aroeira, Jucá, and Mofumbo) from the Caatinga biome, Brazil, and different control methods of microorganisms in seeds were evaluated. We used 500 seeds of each species, 100 for each treatment: control, superficial disinfection, Enzimatic II, Captana and Mancozebe. Seeds were distributed in Petri dishes containing potato-dextrose-agar culture medium and stored in incubator type Biochemical Oxygen Demand at  $28 \pm 2$  °C for five days. For germination tests, 100 seeds were arranged in germitest paper previously sterilized and moistened with sterilized distilled water, and incubated for 19 (mofumbo), 20 (jucá), and 25 (aroeira) days in incubators at  $28 \pm 2$  °C. *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Lasiodiplodia* sp., *Penicillium* sp. and *Rhizopus* sp. are associated with the studied seeds. Captana reduced the incidence of *Aspergillus* and *Penicillium* in the seeds, being the most efficient in controlling the microfauna associated with these seeds. Enzimatic II also proved to be an efficient control for *Aspergillus* spp. There was no interference of the tested products on seed germination.

**Keywords:** Combretum leprosum; Libidibia ferrea; Myracrodruon urundeuva; Seed storage

## INTRODUCTION

The Caatinga biome integrates an important portion of the ecosystem in the Brazilian Northeast semiarid region. Comprehending a total area of 734,000 km<sup>2</sup>, this biome is present in all of nine states of the Northeast region, and a small part of the state of Minas Gerais. The rich and diverse biodiversity of Caatinga has been suffering from the impacts caused by deforestation and the exploitation of its natural resources. The intensive use of land with agricultural practices contributed to a loss of the productive areas due to erosion and nutrient withdrawal; the land use for pastures with exotic species, and the use of plants for firewood reduced the native vegetation and decreased the diversity of the forest (Moura et al., 2010).

Some of Caatinga's native species present great economic potential and may be used for wood supply and as a food source. Aroeira (*Myracrodruon urundeuva* Allemão) is

known for its wood and medicinal properties (Gomes et al., 2013). Jucá (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz) is widely used for landscaping of urban sites. Its leaves may serve for animal feeding and the wood is used in construction and joinery works. In addition to that, its extract has presented healing and antiseptic properties (Negri et al., 2009; Mota et al., 2012). Mofumbo (*Combretum leprosum* Mart.) is widely used in programs of reforestation of degraded lands (Pacheco et al., 2014) and in folk medicine as anti-inflammatory, expectorant, and in hemorrhages and flu treatments (Horinouchi et al., 2013). The longevity and large biomass production of these native trees are important characteristics regarding the reduction of environmental impacts in degraded areas (Gomes et al., 2013).

A recent Brazilian law (No. 12,615) protecting native lands from exploratory practices is fueling the increasing demand of seeds of forest native species in the country, mostly by programs aiming to recover and restore

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Received: 11 September 2019; Accepted: 12 December 2019

degraded areas (Brancalion et al., 2016). However, there are not many methodologies to formalize the activities for commercialization and quality control of seeds from such species, especially due to a lack of knowledge of some biological aspects of most of them (Wielewicz et al., 2006).

Forest species have many seed-associated pathogens, among which the fungi group stands as the most important causal agent (Muniz et al., 2007). Studies on seed health are important tools to understand the present microorganisms and the sanitary quality of these seeds. These factors may interfere directly in germination, bringing losses through deterioration, abnormalities, and lesions in plantlets (Netto and Faiad, 1995), besides the reduction in storage time (Asdal et al., 2019).

For these aforementioned factors, the objective of our study was to evaluate the health and quality of seeds of three different native species (aroeira, jucá, and mofumbo) of the Caatinga, and control methods for fungi that were incident.

## MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Microbiology and Plant Pathology at the Universidade Federal Rural do Semi-Árido – UFERSA, Mossoró, state of Rio Grande do Norte (RN), Brazil. Sample seeds of aroeira and mofumbo were collected in the city of Lagoa Nova-RN, and jucá seeds in Assu-RN (Table 1).

### Sanitary quality and control of microorganisms

For sanitary evaluation and control of microorganisms in the seeds, the quantification and identification of fungi in the seeds were performed. Five hundred seeds of each species were used (100 per treatment): control treatment (no disinfestation); superficial disinfestation, Enzimatic II (Alltech do Brasil Agroindustrial Ltda, Maringá, Brazil), Captana (Adama Makhteshim LTD., Beer-Sheva, Israel),

and Mancozebe (Indofil Industries Limited, Thane, India). The product Enzimatic II, currently under testing process (registration process), was conceived by Alltech Crop Science™.

Superficial disinfestation of seeds was done with ethanol 70% (for 30 secs) and sodium hypochlorite 2.5% (60s), with posterior rising in sterile distilled water. Seed treatment with Enzimatic II, Captana and Mancozebe were performed as presented in Table 2. Treated seeds were placed in Petri dishes containing potato-dextrose-agar (PDA) culture medium (supplemented with tetracycline 0.05g/L) (Boughalleb et al., 2006). Plates were stored in *Biochemical Oxygen Demand* (BOD) incubators for five days at  $28 \pm 2$  °C (Fig. 1).

After the incubation period, fungi developing in the seed were identified by morphological characterization, using microscope and identification keys, and quantified. The incidence of each fungus (IF) was calculated with the following equation (a) (Boughalleb et al., 2006).

$$IF (\%) = \frac{\text{number of infected seeds}}{\text{total number of tested seeds}} \times 100$$

### Germination tests

Germination tests were conducted to assess the effects of the products used in the control trial on the germination of aroeira, jucá, and mofumbo seeds. Dormancy of aroeira and jucá seeds were overcome by immersion in neutral detergent and scarification, respectively (Brasil, 2013). The treatment of sampled seeds followed the same methodology explained in the control session. One hundred seeds were used per treatment, with 4 replication with 25 seeds each, placed in *germitest* paper, previously sterilized and moistened with sterile distilled water (2.5x of water by weight of dry paper), and stored for 19 (mofumbo), 20 (jucá), and 25 (aroeira) days in BOD

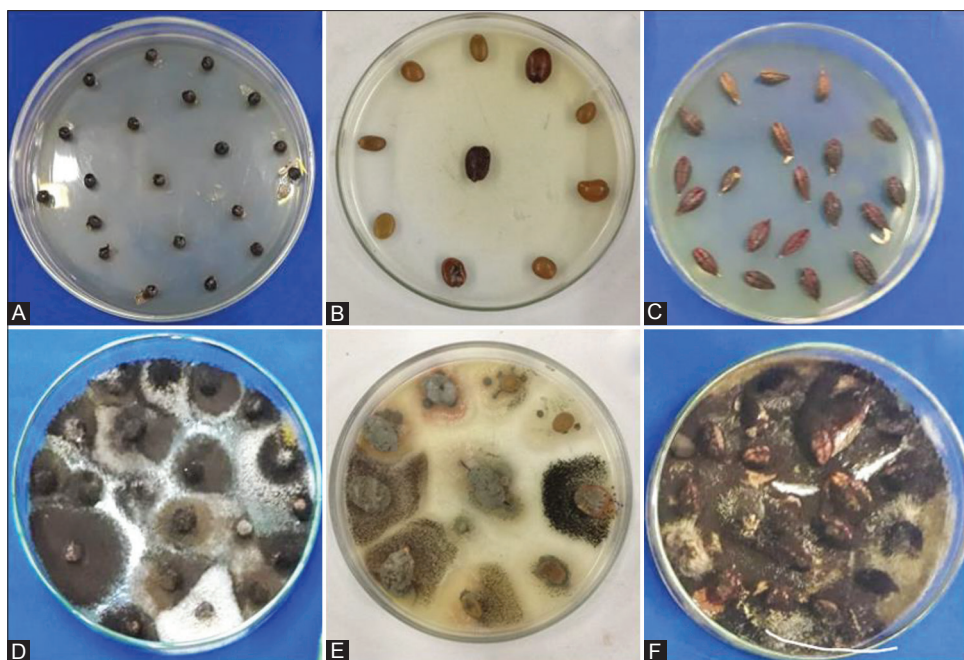
**Table 1: Detailed information of site of collection and mother trees for seeds of aroeira, jucá and mofumbo**

Plant species	Date of collection	Local of collection	Coordinates	Size of plant (m)
Aroeira	November 11, 2018	Lagoa Nova-RN	06° 07' 08.0" S 36° 26' 00.7" W	7.5
Jucá	July 23, 2018	Assu-RN	05° 31' 07.2" S 36° 54' 34.0" W	6.5
Mofumbo	August 11, 2017	Lagoa Nova-RN	05° 03' 52.1" S 37° 20' 29.2" W	4.5

**Table 2: Composition of tested products for control of fungi in aroeira, jucá, and mofumbo seeds**

Commercial product	Active ingrediente	Dose of c.p./kg of seed
Enzimatic II <sup>1</sup>	Secondary methabolites from the microbiological multiplication, copper, and enzymes (xylanase, Hemicellulase and Lignase)	2 mL
Captan SC™	Captana	3 mL
Manfil 800 WP™	Mancozebe	2 g

<sup>1</sup>Product from Alltech Crop Science™ is currently under testing process, awaiting registration to be commercialized



**Fig 1.** Distribution of seeds of aroeira (A, D), jucá (B, E) and mofumbo (C, F) in PDA for the sanitary/control assay, and representative plates at the end of incubation period of each species.

incubator at  $28 \pm 2$  °C. Germination seeds were first counted after 7 (mofumbo), 9 (jucá), and 14 (aroeira) days, and at the end of the incubation period the percentage of germinated seeds was calculated (Alves et al., 2009; Brasil, 2013; Pacheco et al., 2014).

### Statistical analysis

The completely randomized experimental design was used for the sanitary, control, and germination tests. Due to the non-normal distribution of the fungal incidence data, non-parametric Kruskal-Wallis test was carried out and means were compared using a multiple comparison test at 5% probability. Results from the seed germination assay were expressed as arithmetic means and subjected to analysis of variance and comparison of means using Tukey's test at 5% of probability (Ferreira et al., 2019; Matsoukis et al., 2015). All statistical analyses were performed in the R statistical software (R Development Core Team, 2013).

## RESULTS AND DISCUSSION

According to data from the phytosanitary evaluation, six fungi are associated with the studied seeds (*Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Lasiodiplodia* sp., *Penicillium* sp. e *Rhizopus* sp.). The genera *Aspergillus* was the most frequent in seeds of aroeira, jucá, and mofumbo, where the *A. niger* was the most frequent species in nondisinfected seeds, with averages of 92%, 45%, and 27%, respectively (Fig. 2). The *A. niger* was also present in all of the three species when seeds were treated for superficial disinfection with sodium hypochlorite (in 96% of aroeira seeds, 60% of

**Table 3: Fungi incidence in aroeira, jucá, and mofumbo seeds treated with different products and superficial disinfection**

Treatments	Aroeira	Jucá	Mofumbo
	Incidence (%) <sup>1</sup>		
Control	342.5 (100) <sup>a</sup>	273.5 (48) <sup>b</sup>	345.5 (68) <sup>a</sup>
Superficial disinfection	332.5 (96) <sup>a</sup>	353.5 (80) <sup>a</sup>	325.5 (60) <sup>a</sup>
Enzimatic II	142.5 (20) <sup>b</sup>	196.0 (17) <sup>c</sup>	178.0 (1) <sup>c</sup>
Captana	92.5 (0) <sup>c</sup>	206.0 (21) <sup>c</sup>	225.5 (20) <sup>c</sup>
Mancozebe	342.5 (100) <sup>a</sup>	223.5 (28) <sup>c</sup>	178.0 (1) <sup>b</sup>

Values followed by the same letter within columns are not statistically different (non-parametric multiple comparison test,  $p > 0.05$ ). <sup>1</sup>Values for percentage of seeds with incidence of fungi are average "ranks" from the nonparametric analysis (real values are in parentheses)

mofumbo, and 51% of jucá). Jucá and mofumbo seeds disinfected with sodium hypochlorite also presented *A. flavus* (12 % and 10%, respectively). *Aspergillus* is one of the main representants of the "storage fungi", which invade seeds during the postharvest and storage processes causing seed decay and deterioration (Bala, 2017; Vechiato, 2010), reducing the seed germination and vigor. *Rhizopus* sp., another typical fungus of storage (Bhattacharya and Raha, 2002), occurred in nondisinfected seeds of mofumbo (48%) and aroeira (20%). In seeds of jucá disinfected with sodium hypochlorite, we also observed the presence of *Lasiodiplodia* sp., a fungus commonly found in seeds from native forest species in Brazil (Botelho et al., 2008). To a lesser extent, the nondisinfected seeds also presented *A. ochraceus* (in aroeira, 2%), *A. flavus* (3% of jucá and 2% of mofumbo), and *Penicillium* sp. (7% of mofumbo) (Fig. 2).

Overall, the seed treatment with Captana, Mancozebe, and Enzimatic II presented satisfactory results in reducing

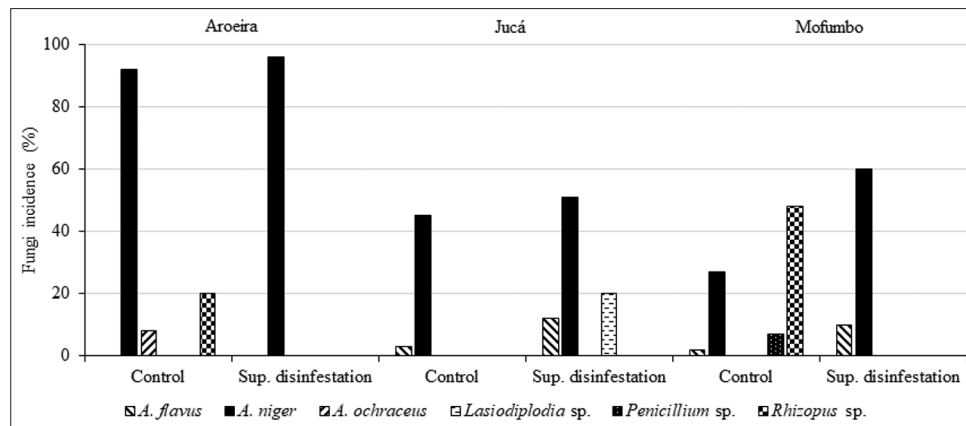


Fig 2. Fungi incidence (%) in aroeira, jucá e mofumbo seeds in the sanitary test.

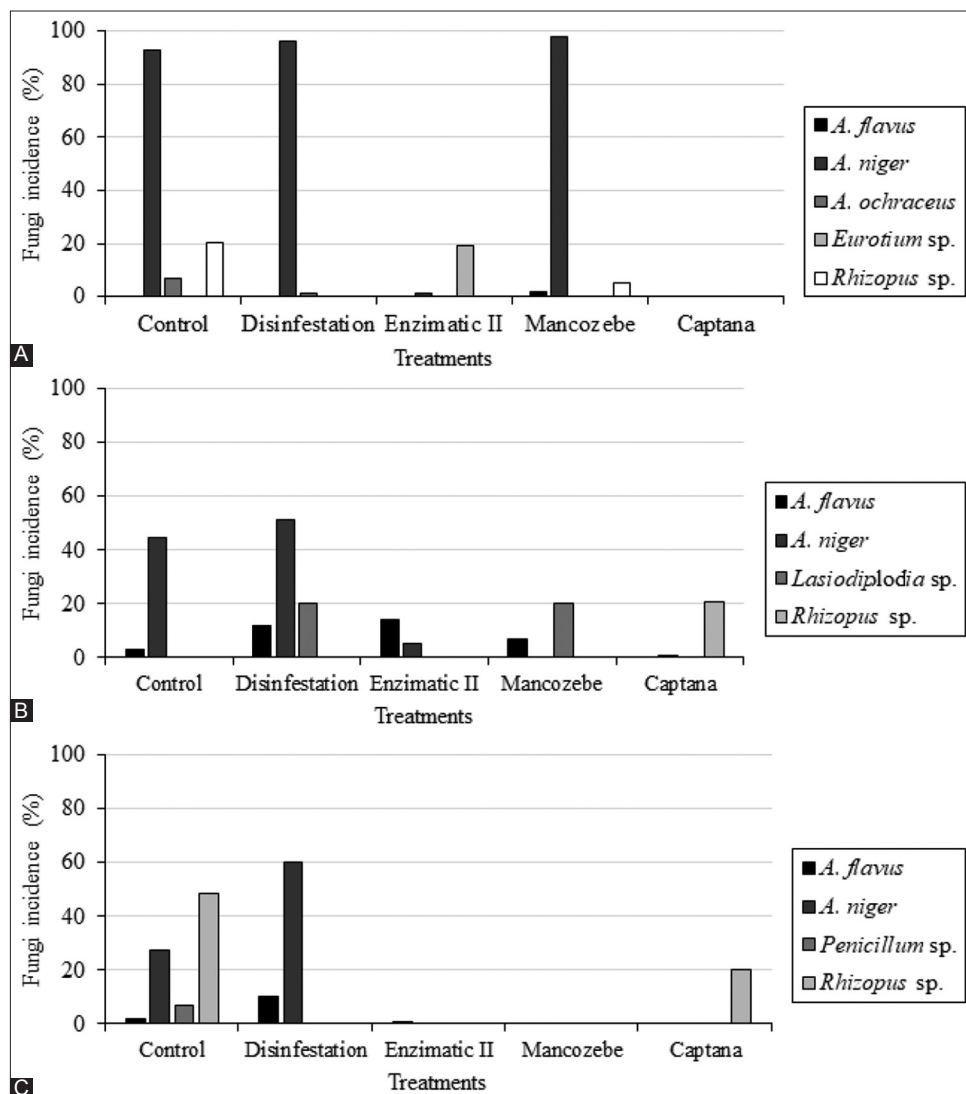


Fig 3. Incidence of fungi (%) in seeds of aroeira (A), jucá (B), and mofumbo (C) treated with different products and superficial disinfection.

the incidence of the main fungi in seeds of aroeira, jucá, and mofumbo (Table 3), showing significant differences ( $p < 0.05$ ) when compared to the control and superficial disinfection.

The treatment with Captana reduced in almost 100% the incidence of *Aspergillus* in all three species (Fig. 3). Silva et al. (2011) also reported the efficiency of Captana in controlling *Aspergillus* in seeds of five different seeds from



**Table 4 : First and final count of germination of aroeira, jucá, and mofumbo seeds treated with different products and superficial disinfection**

Tratamentos	First count (%)			Final germination (%)		
	Aroeira	Jucá	Mofumbo	Aroeira	Jucá	Mofumbo
Control	53 <sup>a</sup>	83 <sup>a</sup>	90 <sup>a</sup>	53 <sup>a</sup>	83 <sup>a</sup>	92 <sup>a</sup>
Superficial disinfection	54 <sup>a</sup>	79 <sup>a</sup>	85 <sup>a</sup>	54 <sup>a</sup>	79 <sup>a</sup>	85 <sup>a</sup>
Enzimatic II	54 <sup>a</sup>	81 <sup>a</sup>	92 <sup>a</sup>	54 <sup>a</sup>	82 <sup>a</sup>	93 <sup>a</sup>
Captana	69 <sup>a</sup>	86 <sup>a</sup>	90 <sup>a</sup>	70 <sup>a</sup>	86 <sup>a</sup>	95 <sup>a</sup>
Mancozebe	57 <sup>a</sup>	88 <sup>a</sup>	87 <sup>a</sup>	58 <sup>a</sup>	89 <sup>a</sup>	88 <sup>a</sup>
CV (%)	15.50	13.65	6.67	15.45	14.6	7.41

Values followed by the same letter within columns are not significantly different (Tukey's test,  $p > 0.05$ ). CV: coefficient of variation. First count: 7 (mofumbo), 9 (jucá), and 14 (aroeira) days after start of trial. Final germination: 19 (mofumbo), 20 (jucá), and 25 (aroeira) days after start of trial

the Mata Atlântica (*Anadenanthera macrocarpa*, *Dalbergia nigra*, *Tabebuia chrysotricha*, *T. heptaphylla*, and *Senna siamea*). The Captana also inhibited *Penicillium* sp. and reduced the incidence of *Rhizopus* sp. in seeds of mofumbo and jucá. Classified as a nonspecific fungicide (Parisi and Santos, 2011), the Captana presents a wide range of action, acting against many fungi from the Oomycota division (known as not true fungi) until the true fungi (Ascomycota and Basidiomycota) (Blaney and Kotanen, 2001). Other studies also report the efficiency of Captana on *Aspergillus* spp. (Souza et al., 2003; Gallo et al., 2013) and other genera of seed fungi (Medeiros et al., 2012).

Mancozebe inhibited the development of fungi in seeds of mofumbo, but it was not efficient in controlling *Aspergillus* spp. in seeds of aroeira and *Lasiodiplodia* sp. in jucá (Fig. 3). However, other studies have reported the efficiency of Mancozebe in the control of *Aspergillus* spp. in forest species (Gallo et al., 2013) and cultivated crops (Saleem et al., 2012). Several studies in the literature report that different active ingredients behaved in different ways regarding their efficiency in controlling fungi in seeds, what may be related to factors such as the seed, form of application of the product, microorganism diversity in the seed, among others (Coutinho et al., 1999).

The Enzimatic II presented promising results, reducing the overall incidence of fungi (Table 3) and, especially, the incidence of *Aspergillus* spp. in all three species (Fig. 3). The *Eurotium* genera was not observed in the control group nor in seeds disinfected with sodium hypochlorite but was present in aroeira seeds treated with Enzimatic II (Fig. 3), what may be due to the action of the product on some fungal species that inhibited the development of such species by competition (Coutinho et al., 1999) or other antagonism mechanisms. *Eurotium* sp. has already been reported as an endophytic fungus in seeds (Mota et al., 2017).

Thus, with the lack of chemical products registered for treatment of forest seed (Parisi et al., 2019) and the scarcity of information on the use of alternative productions in

these species, our results bring a promising option to assist on this processing stage, reducing the incidence of microorganisms in seeds and helping to moderate the use of harmful chemicals to the environment and human health.

The use of different methods of control did not interfere in the seed germination of any of the assessed species, presenting no statistical difference by the Tukey test ( $p < 0.05$ ), showing that there was no phytotoxic effect of the products on the seeds (Table 4).

## CONCLUSIONS

The fungi associated with seeds of aroeira, mofumbo, and jucá collected in areas of Caatinga forest, in the state of Rio Grande do Norte, are *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Lasiodiplodia* sp., *Penicillium* sp., and *Rhizopus* sp. Captana reduced the incidence of *Aspergillus* and *Penicillium* in the assessed seeds, showing the greatest efficiency in controlling the microfauna associated with these seeds. Enzimatic II showed efficiency in controlling *Aspergillus* spp. in seeds of all tree native species. Treatment of aroeira, mofumbo, and jucá seeds with Captana, Mancozebe or Enzimatic II did not cause any damage to the seeds nor affected the germination.

## ACKNOWLEDGMENTS

To the Centro de Pesquisa Leopoldo Américo Miguez de Mello (CENPES/PETROBRAS) for the technical and financial support provided for our study.

### Authors' contributions

All authors contributed effectively to this study. NASCIMENTO conducted and evaluated the experiments, and wrote the paper; NOGUEIRA did the literature search and wrote the paper; ALVES and ARAÚJO conducted and evaluated the experiments; DOMBROSKI and MACHADO coordinated the research project and analyzed the data; AMBRÓSIO coordinated the research project

and the students, designed the study, and gave valuable contributions to the paper.

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