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Sanity and Physiology of *Ceiba speciosa* Seeds Treated With Essential Oils

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Abstract

Since the propagation of forest species is by seeds, the success of population establishment depends on the use of good quality seeds. And the use of alternative controls with essential oils for fungal control is ecologically important. The present work aimed to evaluate the influence of essential oils of Citronella (Cymbopogon winterianus), Palmarosa (*Cymbopogon martini*), Lemongrass (*Cymbopogon citratus*), Thyme (*Thymus vulgaris*), *Eucalyptus globulus* and Rosemary (*Salvia rosmarinus*) in reducing the incidence of fungus and physiological quality of painera—*Ceiba speciosa* seeds. The seeds were treated with the essential oils of Citronella, Palmarosa, Lemongrass, thyme, eucalyptus, and rosemary at a concentration of 2%. Captan fungicide (240 g/100 kg^{-1}) was used as a control treatment. After the treatments, the seeds were evaluated by the sanity test, using the "Blotter test" filter paper method. For physiological quality, germination, first count, germination speed index, and seedling length were evaluated. The following genera of phytopathogenic fungi have been identified on Paineira seeds: *Cladosporium* sp., *Fusarium* spp., *Penicillium* sp., *Colletotrichum* sp. and *Pestalotipsis* sp. Treating the seeds with essential oils of Citronella, Palmarosa, Lemongrass, and Thyme controlled the fungi on the Paineira seeds. However, changes in the physiological quality of the seeds were associated with the use of the essential oils, compromising seedling vigor.

Keywords: Barriguda, forest seeds, eco-friendly measures, Painera

1. Introduction

Deforestation in recent decades has left areas in a critical need of reforestation. Native species of Brazilian flora, like *Ceiba speciosa* (A.St.-Hil.) Ravenna popularly known as paineira or barriguda it is a species widely used in landscaping and reforestation programs for degraded areas (Sabonaro et al., 2015). It is also used in the wood industry for charcoal production, in folk medicine, and for making pillows and mattresses, which are made from the slat that comes from its fruits (Perez & Jardim, 2005; Vale et al., 2005; Lazarotto et al., 2011).

Propagation through seeds is the most common method of multiplication of its species. Knowledge about factors that interfere with seed quality is necessary. The quality required is expressed by the genetic, physical, physiological, and sanitary attributes, which are responsible for originating plants with high vigor (Peske et al., 2012).

The sanitary quality of seeds is one of the main obstacles to obtaining healthy seedlings (Medeiros et al., 2016). Various pathogens can infect seeds of forest species, especially fungi. Fungal infection can cause seed discoloration, deterioration, deformation and rotting. It may also result in loss of germination potential and vigor (Goulart, 2018). Chemical treatment is the most used in the control of fungal diseases due to its high efficiency

(Oliveira et al., 2011). However, with the growing concern about environmental impacts, the use of alternative methods has been explored (Manssouri et al., 2016; Starović et al., 2017).

The use of natural products such as essential oils has shown promising results in the treatment of forest seeds species (Pereira et al., 2016; Gomes et al., 2016; Medeiros et al., 2016). Essential oils are aromatic and volatile products of the secondary metabolism of some plants, and usually exhibit antimicrobial properties (Bajpai et al., 2011; Costa et al., 2015). The questions we asked in conducting this study are: Can Paineira seeds without any treatment germinate? Could alternative treatments with essential oils replace the use of industrialized products? Which is the best essential oil?

The aim of this study was to evaluate the influence of essential oils of Citronella, Palmarosa, Thyme, Eucalyptus and Rosemary in reducing the incidence of fungi and in the physiological quality of seeds of *C. speciosa*.

2. Method

2.1 Collection of seeds from Ceiba speciosa

Seeds of *C. speciosa* were collected directly from a representative matrix tree located at the Centro de Ciências Agrárias (CCA) of the Federal University of Paraíba (UFPB), Areia-PB, Brazil. Later, they were manually processed, discarding the malformed and attacked by pests, and then stored in a cold chamber at 5±2 °C, for 30 days.

2.2 Control of Fungi Associated With Ceiba speciosa Seeds With Essential Oils

The *Ceiba speciosa* seeds were subjected to the following treatments: T1-Control treatment, composed of seeds immersed in sterilized distilled water (ADE); T2-Citronella essential oil (*Cymbopogon winterianus*); T3-Palmarosa essential oil (*Cymbopogon martini*); T4-Lemongrass essential oil (*Cymbopogon citratus*); T5-Thyme essential oil (*Thymus vulgaris*); T6-Eucalyptus essential oil (*Eucalyptus globulus*); T7-Rosemary essential oil (*Salvia rosmarinus*). Essential oils treatments had a concentration of 2%. And were diluted in 100 mL of sterile distilled water (ADE); T8-Captana fungicide treatment (240 g/100 kg of seeds).

For the analysis and identification of fungi, seeds were incubated on filter paper substrate "Blotter test" (Brasil, 2009). A total of 100 seeds were used per treatment (10 repetitions of 10 seeds per treatment), immersed for 5 minutes in the corresponding treatments. Seeds treated with the essential oils were individually distributed under aseptic conditions in Petri dishes on a double layer of sterile filter paper moistened with ADE. Plates were incubated in a Biochemical Oxygen Demand (BOD) incubator, during 7 days at 25±2°C and 12 h light period.

After the incubation period, the microorganisms on the seeds were evaluated with the aid of optical and stereomicroscopic microscopes, which were identified to the genus level by means of specialized literature (Seifert et al., 2011). The results obtained were expressed as a percentage of infected seeds.

2.3 Physiological Quality of Ceiba speciosa Seeds Treated With Essential Oils

Physiological quality tests were carried out on paineira seeds, with the same treatments as described above and then the following tests were performed:

Germination test: 100 seeds were used, distributed in four repetitions of 25 seeds, placed in paper "germitest", moistened with 2.5 times the weight of dry paper and distributed in a BOD germination chamber at 25±2°C and 8 h light period. After sowing, the rolls were placed in transparent plastic bags to avoid water loss by evaporation. The germinated seeds were counted on the 7th and 10th day after sowing, counting the normal seedlings (Brasil, 2013).

First germination count: conducted together with the germination test, in which the germinated seeds on the 7th day after sowing were counted (Brasil, 2013). Germination speed index (GSI): conducted in conjunction with the germination test, where the number of germinated seeds was noted daily. The index was determined according to the formula proposed by Maguire (1962).

The length and dry mass of seedlings were measured after the germination test, with the help of a ruler graduated in millimeters. The length of the aerial part and root of the normal seedlings was evaluated. Afterwards, the aerial part and the root system of the seedlings were separated and placed in Kraft paper bags and taken to an oven with forced air circulation at a temperature of 65 °C until they reached a constant weight, for 48 hours. After this period, they were weighed on analytical scales with a precision of 0.001 g and the results expressed in g seedling^{-1}.

2.4 Statistical Analyses

The treatments were arranged in a completely randomized design. The normality of the sample means was determined by the Shapiro-Wilk test. The fungal infection data were transformed into $\sqrt{y + 0.5}$. Health and physiological quality data were compared using Tukey's test ($p \geq 0.05$) in Sisvar® software was used for statistical analysis (Ferreira, 2010).

3. Results

The fungi identified in *Ceiba speciosa* seeds (Table 1) were, *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., *Colletotrichum* sp. and *Pestalotiopsis* sp. (Table 1). For *Fusarium* sp. all treatments were effective except for eucalyptus oil (Table 1).

For *Penicillium* sp. the treatments with eucalyptus and Rosemary essential oils and Dicarboximide fungicide did not inhibit the growth of fungus present on the seeds.

Table 1. Incidence (%) of fungi in seeds of Paineira (*Ceiba speciosa*) treated with essential oils (0.2%)

Treatment	*Cladosporium* sp.	*Fusarium* sp.	*Penicillium* sp.	*Colletotrichum* sp.	*Pestalotiopsis* sp.
Witness	13.0a*	10.0a	8.0ab	3.0a	3.0a
Citronella	0.0b	0.0c	0.0b	0.0b	0.0b
Palmarosa	0.0b	0.0c	0.0b	0.0b	0.0b
Lemongrass	0.0b	0.0c	0.0b	0.0b	0.0b
Thyme	0.0b	0.0c	0.0b	0.0b	0.0b
Eucalyptus	2.0b	9.0a	8.0ab	0.0b	0.0b
Rosemary	0.0b	1.0b	11.0a	0.0b	0.0b
Fungicide	0.0b	1.0b	7.0ab	0.0b	0.0b
CV (%)	27.04	31.81	34.06	14.89	14.89

Note. * Means with the same letter in the column do not differ statistically from each other Tukey ($P \geq 0.05$).

The treatments affected the physiology of Paineira seeds; inefficient results were obtained for germination percentage (G), first count germination (FGC), and for the germination speed index (GSI) (Table 2). However, there was an exception with the Palmarosa oil treatment, for (FGC), as there was no significant difference between it and the control and fungicide (Table 2).

Table 2. First germination count (FGC), Germination (G), and Germination Speed Index (GSI) of Paineira (*Ceiba speciosa*) seeds treated with essential oils

Treatment	FGC (%)	G (%)	GSI
Witness	16.75a*	21.75a	20.13a
Citronella	2.50b	12.00b	7.17c
Palmarosa	13.00a	14.00b	11.78b
Lemongrass	3.50b	12.25b	6.17c
Thyme	3.00b	11.00b	5.74c
Eucalyptus	6.00b	13.25b	12.32b
Rosemary	3.50b	10.50b	5.42c
Fungicide	17.75a	20.00a	20.18a
CV (%)	26.01	12.51	11.99

Note. * Means with the same letter in the column do not differ statistically from each other Tukey ($P \geq 0.05$).

For the variables of aerial part length (APC), most treatments did not differ from the control and the fungicide, indicating that they were efficient, except for Lemongrass and Eucalyptus oils (Table 3). For root length (ARC), only the Palmarosa oil treatment did not influence root growth. These oils, along with the fungicide treatment, showed higher averages than the control (Table 3).

Table 3. Shoot length (APC), root length (ARC) of seedlings of Paineira (*Ceiba speciosa*) treated with essential oils

Treatment	APC (cm)	ARC (cm)
Witness	3.42a*	3.15b
Citronella	2.40abc	2.22b
Palmarosa	3.02abc	3.30ab
Lemongrass	2.92bc	2.42b
Thyme	3.00abc	2.20b
Eucalyptus	2.05c	2.25b
Rosemary	2.75abc	2.66b
Fungicide	3.30ab	4.32a
CV (%)	16.54	17.60

Note. * Means with the same letter in the column do not differ statistically from each other Tukey ($P \geq 0.05$).

4. Discussion

This work aims to analyze the influence of essential oils on the physiology and health of Paineira seeds. It is very likely that all essential oils have antifungal compounds that can cause damage to the structures of the fungi. However, essential oils can also cause physiological interference in seeds, as evidenced in this work. Interfering with its development. Thus, studies with alternative, ecologically favorable treatments face difficulties in finding oils that are efficient and do not cause physiological damage to the seeds. Studies that could evaluate each compound present in the essential oils, become necessary to elucidate if it is the same compound that prevents the development of fungus and seeds, simultaneously.

The presence of pathogens found by us, were also found by Seneme et al., (2018) with studies with *Ceiba speciosa* seeds. Seneme et al. (2018) found *Aspergillus* sp. (1%), *Rhizoctonia solani* (1%), *Alternaria* sp. (1%), *Cladosporium* sp. (1%), *Fusarium* sp. (5%), *Phytium* sp. (1%), *Rhizopus stolonifer* (2%) and *Curvularia* sp. (1%). The presence of the fungi: *Aspergillus* sp., *Fusarium* sp., *Colletotrichum* sp. and *Penicillium* sp. among others, were also found by Lazarotto et al. (2010).

The similar microflora found by Medeiros et al. (2011) analyzing sanitary quality of Flamboyant-mirim (*Caesalpinia pulcherrima* L.) seeds. It is evident that these fungi are also associated with other seeds of forest species. According to Carvalho and Muchovej (1991), fungi of the genera *Fusarium* and *Colletotrichum* are seed pathogens of many plant species. This is quite important, because it can be noted that the same fungi infest and/or infect several forest species in common. In this way, it could propose an oil that is efficient against all or most of these phytopathogens.

The essential oils of Eucalyptus and Rosemary were the least effective. Consequently, they presented similarities in inhibiting seed development. Both oils possess allelopathic activities (Pelegrini & Cruz-Silva, 2012; Meinerz et al., 2015; Lanzoni et al., 2018). These fungi (*Fusarium* sp., *Penicillium* sp.) possibly both developed some structure or mechanism of resistance to these compounds. Because, in addition to resistance to rosemary oil, *Penicillium* sp. was not inhibited with the industrialized fungicide.

The same fungicide based on dicarboximide promoted significant reduction of the incidence of fungi, F. oxysporum, *Penicillium* sp. in several forest species in works of Medeiros et al. (2013), Machado et al. (2004), and Mertz et al. (2009). Thus, possibly due to storage conditions, as well as the shelf life of this fungicide may have influenced these results, as the chemical product lost its efficiency over time.

The treatments with Citronella, Palmarosa, Lemongrass, and Thyme essential oils showed satisfactory results. Considering that these promoted total inhibition of all pathogens observed in *C. speciosa* seeds. Similar results with Lemongrass oil were found by Souza Junior et al. (2009) with a concentration of 1 $\mu L\ mL^{-1}$ totally inhibited the mycelial growth of the fungus *C. gloeosporioides*. Work with alternative control using oils and plant extracts has brought satisfactory results in the control of seed pathogenic fungi (Costa et al., 2015; Gomes et al., 2016; Medeiros et al., 2016). The essential oil is one of the most promising groups of natural compounds for the development of safer antifungal agents (Manssouri et al., 2016; Starović et al., 2017).

The treatments affected the physiology of the Paineira seeds, it can be seen that inefficient results were obtained for germination percentage (G), first count germination (FGC) and for the germination speed index (GSI). However, there was an exception with the Palmarosa oil treatment, for (FGC), as there was no significant difference between it and the control and fungicide (Table 2). Consequently, these results as (PCG) according to Nakagawa (1994), the first count makes it possible to determine seed vigor. Thus, estimating how germination and seedling quality will be. Palmarosa oil was more effective than the others, as it did not inhibit seed development and promoted fungus control.

For the variables of aerial part length (APC), most treatments did not differ from the control and the fungicide, indicating that they were efficient, with the exception of Lemongrass and Eucalyptus oils (Table 3). For root length (ARC), only the Palmarosa oil treatment did not influence root growth. These oils showed, along with the fungicide treatment, higher averages than the control (Table 3). However, the fungicide promoted root growth compared to the oil treatments and the control. This may be due to the efficiency of the chemical compound in protecting the seed during germination. It provides favorable conditions for the seed to germinate and develop. For, even the control was only treated with superficial sanitization with 70% alcohol, sodium hypochlorite and distilled water. Seeds need favorable conditions to germinate. However, seeds of some species may develop normally even if damaged (Souza et al., 2021)

The expected germination for *Ceiba speciosa* species is approximately 80%, according to Lorenzi (2002). In the same environmental conditions, Seneme et al. (2018) found germination of 58%. However, Lorenzi (2002) reported a lot of variation that can range from 30-100%. Lazarotto et al. (2010) also found this variation ranged from 0-59% by studing seeds from 7 different locations. Thus, the percentage of 21% that we found is within the expected germination rate for the species. However, factors such as storage may have some influence on this result. Thus necessitating a germination work with seeds collected on the same day.

The effect of applying certain products on the surface of the seeds can be expressed both in the speed of germination and also in the final percentage of germinated seeds (Labouriau, 1983). According to Ferreira & Áquila (2000), this effect varies according to the reaction of the seed to the product, such as the effect on membrane permeability, oxygen utilization, enzyme and receptor conformation. Further studies with the chemical composition of essential oils need to be done. To enable a better understanding of the action of these products on seed physiology.

5. Conclusion

Citronella, Palmarosa, Lemongrass and Thyme oils were efficient against all the fungi found in the study. However, these treatments interfered with the physiology of *Ceiba speciosa* seeds compromising their vigor and quality. Palmarosa essential oil was the best treatment.

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