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Impact Of Thermotherapy On The Incidence Of Phytopathogenic Fungi In Lafoensia Pacari Seeds

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Abstract:
This study investigates the effects of thermotherapy on the physiological and sanitary attributes of Lafoensia pacari seeds, with the goal of determining the optimal exposure duration through germination and sanity assessments. Seeds were subjected to heat treatment via immersion in water at 60°C for varying durations (five, 10, 15, and 20 minutes), followed by germination tests in vermiculite and sanity tests using the "Blottertest" method. Each test involved 100 seeds, divided into four sub-samples of 25 seeds each. After incubating the seeds in a BOD chamber for seven days, exposed to a 12-hour photoperiod at 25°C, microbial presence was assessed using microscopy. Employing a completely randomized experimental design, the study analyzed results through polynomial regression. The findings highlight that moist heat treatment at 60°C for 10 minutes effectively controlled fungi associated with L. pacari seeds.

Keywords*: forest pathology, seed treatment, seeds, phytosanitary*

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I. Introduction

Fungal pathogens have the potential to colonize forest seeds throughout various stages of production, posing risks to seed quality, germination rates, and resulting in seedling damping-off and rot (Santos and Parisi, 2011). The presence of seed-associated pathogens is recognized as a contributing factor to diminished germination rates in seed batches (Carneiro, 1987). Furthermore, this symbiotic relationship between pathogens and seeds is often implicated in the long-range dispersal of pathogens and their transmission from seeds to plants.

Dedaleiro, the common name of *Lafoensia pacari*, is a tree specie who belong to the Lythraceae family. Native from South America, it is a moderate growing tree species (Azevedo et al, 2004) known for its value pharmacons due to chemical properties (Porfírio et al, 2009; Firmo et al, 2014), also known as a potential specie for urban forests (Martini et al, 2017). This is specie is relatively rare in the nature (Backes and Irgang, 2002), therefore, phytosanitary treatments on seeds may be necessary for the propagation of individuals *in situ* and *ex situ*.

Various methods, including chemical, biological, and physical treatments, have proven effective in eliminating infectious fungal inoculum from seeds. While chemical treatments have been widely utilized for control purposes, they come with drawbacks such as potential environmental impacts and the development of pathogen resistance to specific compounds (Mendes et al., 2001). In addition to chemical approaches, seed treatment can incorporate other control methods, such as physical and biological methods, either independently or in combination (Machado, 2000). Among these alternatives, thermotherapy stands out as particularly efficient, as it effectively eradicates deep-seated infections without causing environmental pollution. However, it should be noted that thermotherapy does not provide residual protection post-treatment and carries the risk of damaging the seeds (Coutinho et al., 2007).

Heat treatment involves the application of moist or dry heat as a physical method to diminish pathogen levels in seeds while preserving germination and vigor. Moist heat is recognized as the most effective, although it can occasionally result in seed damage (Machado, 2000). It's important to acknowledge that any damage to seeds is typically a consequence of the combination of temperature and exposure duration required to eradicate pathogens, which can sometimes be detrimental to the seeds themselves.

Therefore, achieving effective control relies heavily on understanding the nuanced interplay of thermotherapy parameters, which are subject to variability based on factors such as species, cultivar, and seed lot.

Furthermore, conducting comprehensive germination tests is crucial for accurately assessing the consequences of these parameter combinations.

Given these pivotal considerations, the study was designed to delve into the intricate relationship between thermotherapy and the physiological as well as sanitary characteristics of *L. pacari* seeds. The primary objective was to pinpoint the optimal duration of exposure through meticulous evaluations of seed sanity and germination.

II. Material and methods

The *L. pacari* seeds employed in this study were obtained from the Seed Exchange Program of the UFSM Forest Nursery. Prior to their use, these seeds were gathered and processed by seed banks, then stored in a cold chamber maintained at temperatures ranging from 5 to 10°C, along with low relative humidity, until they were acquired for our research.

Initially, the seeds were subjected to heat treatment by immersing them in water heated to 60°C for durations of 5, 10, 15, and 20 minutes. Subsequently, these treated seeds were placed in labeled nylon mesh bags and randomly positioned in a water bath at the specified treatment temperature and durations.

After the heat treatment, the germination test was initiated, comprising four sets of 25 seeds each, placed in vermiculite substrate. Autoclaved vermiculite with fine granules was utilized, moistened until reaching 60% of its water retention capacity. The seeds were then incubated in a BOD chamber under a 12-hour photoperiod of direct light and a constant temperature of 25°C. Evaluations were conducted weekly over a 30-day period, assessing the emergence of healthy, morphologically intact seedlings, and identifying any nonviable seeds.

For assessing sanitary quality, the "blottertest" method was employed. Each sample comprised 100 seeds, divided into four sub-samples of 25 seeds each. These sub-samples were placed onto sterile Petri dishes atop three sterilized and moistened filter paper sheets. Incubation in a BOD chamber at 25°C, under a 12-hour photoperiod, was conducted for seven days. Following incubation, microorganisms present in the seeds were examined using stereoscopic and optical microscopes, with fungi identified according to Barnett & Hunter's (1999) guidelines.

The experimental design adopted a completely randomized arrangement with four replications. Assessment of both sanity and germination considered the exposure duration of seeds to hot water as the treatment variable. Results underwent polynomial regression analysis, exploring linear, quadratic, and cubic models to determine optimal fit. Additionally, linear correlation analysis between variables was performed using Assistat 7.6 software (Silva, 2011).

III. Results and Discussion

The results regarding the germination test, dead seeds, and seed health of *L. pacari* are presented. The use of heat treatment did not reduce the germination percentage; it was also observed that using moist heat for 10 minutes increased the percentage of germinated seeds from 68 to 79%. However, 15 minutes of exposure to moist heat causes deterioration of *L. pacari* seeds. Oliveira and Lemes (2014), studying *L. pacari* germination, described that germination varied between 59 and 68%. In another study by Piveta et al. (2009), it was also concluded that the peak of germination occurred at 10 minutes of moist heat at 60 °C.

The fungi *Rhizoctonia* sp., *Penicillium* sp., and *Cladosporium* sp. occurred in all evaluated treatments (Figure 1-C, D, and E). Seneme et al. (2010), studying the sanitary quality of seeds, found the following fungi in *L. pacari* seeds: *Aspergillus niger*, *Fusarium semitectum*, *Penicillium* sp.

Piveta et al. (2009) identified the following fungi: *Aspergillus* sp., *Penicillium* sp., *Alternaria* sp., and in lesser incidence, *Cladosporium* sp., *Mucor* sp., *Epicoccum* sp., *Chaetomium* sp., *Fusarium* sp., *Rhizoctonia* sp.

The percentage of *Rhizoctonia* sp. fit a cubic model, and its incidence was reduced from 84 to 31% when using moist heat at 60 °C for 20 minutes. The quadratic model fit the fungi *Penicillium* sp. and *Cladosporium* sp. (Figure 1 D and E). The minimum incidence of *Penicillium* sp. occurred with 20 minutes of seed exposure to moist heat at 60 °C, and for *Cladosporium* sp., the time of 10 minutes resulted in the reduction of its incidence from 52 to 10%.

Figure 1: Representative equations of the changes in the studied variables with *L. pacari* seeds: (A) abnormal seedlings in the germination test; (B) dead seeds in the germination test; (C) incidence of *Rhizoctonia* sp. in the health test; (D) incidence of *Penicillium* sp; (E) incidence of *Cladosporium* sp.

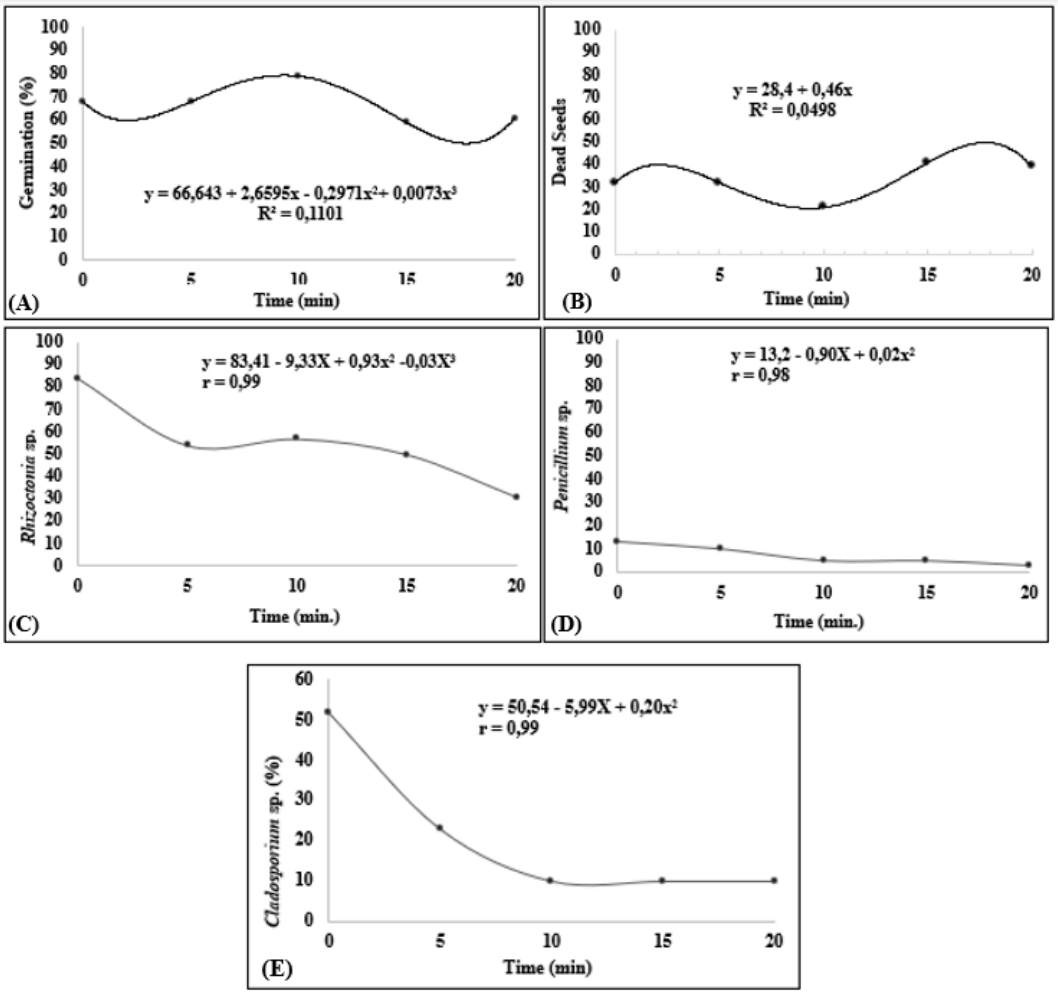

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Table 3: Pearson correlation coefficient (r) between dead seeds in the germination test and the different fungi present in the seed health test of *Lafoensia pacari*.

	Time				
Pathogens	0	5 min.	10 min.	15 min.	20 min.
Rhizoctonia sp.	-0.1054	0.8847	-0.3410	-0.7265	-0.4801
Penicillium sp.	-0.2542	-0.3297	0.0993	-0.0921	0.1822
Cladosporium sp.	0.9245	-0.8266	0.0000	0.0000	0.0000

* Significant at 5% probability by the F-test.

The correlation between dead seeds and *Rhizoctonia* sp. was positive when a duration of five minutes was used, demonstrating that Rhizoctonia sp. might have been responsible for the deterioration of *L. pacari*. seeds. According to Resende et al. (2008), *Rhizoctonia* sp. has resistant structures that enable it to inhabit soil for long periods, and it is known for causing seedling damping-off in nurseries. *Penicillium* sp. is considered a storage fungus and a cause of seed deterioration. It was observed that at 10 and 20 minutes, the correlation was positive, suggesting that *Penicillium* sp. might also have been responsible for the deterioration of the seeds.

IV. Conclusion

Moist heat therapy at 60 °C for 10 minutes is effective for controlling fungi associated with *L. pacari* seeds.

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