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Thermotherapy In The Control Of Pathogens In Seeds Of Cedrela Fissilis

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Abstract:
This research explores the impact of thermotherapy on the physiological and sanitary qualities of Cedrela fissilis seeds, aiming to determine the optimal exposure duration through germination and sanity tests. Seeds underwent heat treatment via water immersion at 60°C for varying durations (five, 10, 15, and 20 minutes), followed by germination tests in vermiculite and sanity tests using the "Blotter test" method. Each test utilized 100 seeds, divided into four sub-samples of 25 seeds each. Seeds were then placed in a BOD chamber for seven days, exposed to a 12-hour photoperiod at 25°C, and subsequently evaluated for microbial presence using microscopy. The study employed a completely randomized experimental design, with results analyzed through polynomial regression. The findings indicate that moist heat treatment at 60°C for 10 minutes effectively controlled fungi associated with Cedrela fissilis seeds.

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

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I. Introduction

Forest seeds can be susceptible to pathogenic fungi throughout all stages of their production cycle. Such fungal associations have been observed to negatively impact seed quality, diminish germination rates, and contribute to issues like damping-off and rotting of seedlings (Santos and Parisi, 2011). The presence of pathogens linked with seeds is recognized as a potential factor contributing to reduced germination rates in seed batches (Carneiro, 1987). Moreover, this symbiosis between pathogens and seeds frequently facilitates the long-range dissemination of pathogens and serves as a means for pathogen transmission from seeds to plants.

Cedrela fissilis, commonly known as cedar or pink cedar, is a species of tree belonging to the Meliaceae family. It is native to South America, found mainly in countries such as Brazil, Argentina, Paraguay and Uruguay (Kageyama et al, 2003). Cedar is known for its high-quality wood and is widely used in the furniture industry and construction (Silva et al, 2017). Considering the vulnerable state of the specie (Conde et al, 2020), is crucial that its seeds are previously sanitized, avoiding in-situ contamination.

Efficient eradication of fungal inoculum from seeds has been achieved through various treatments including chemical, biological, and physical methods. While chemical treatments have been commonly employed for control purposes, they pose environmental risks and may lead to pathogen resistance to specific compounds (Mendes et al., 2001). In addition to chemical methods, seed treatment options encompass physical, biological, and combined approaches (Machado, 2000). Thermotherapy stands out as one of the most effective methods due to its ability to eradicate deep infections without environmental contamination. However, it lacks residual protection post-treatment and carries the risk of seed damage (Coutinho et al., 2007).

Heat treatment is a physical method involving the application of moist or dry heat to reduce pathogen presence in seeds while preserving germination and vigor. Although moist heat is considered the most effective, it may result in seed damage (Machado, 2000). It's important to note that seed damage occurs due to the combination of temperature and exposure duration required to eliminate pathogens, which can sometimes be detrimental to the seeds themselves.

Hence, the efficacy of the control approach hinges primarily on understanding the optimal combination of thermotherapy parameters, which may vary depending on factors such as species, cultivar, and seed lot. Additionally, to precisely evaluate the impact of this combination, germination tests are imperative.

In light of these considerations, the study aimed to explore the correlation between thermotherapy and the physiological and sanitary characteristics *C. fissilis* seeds, pinpointing the most effective exposure duration through sanity and germination assessments.

II. Material and methods

The seeds utilized in this study were sourced from the Seed Exchange Program of the UFSM Forest Nursery. These seeds had been collected and processed by seed banks before being stored in a cold chamber (maintained at temperatures between 5-10°C and low relative humidity) until they were dispatched for our research.

To begin, the seeds underwent heat treatment through immersion in water heated to 60°C for durations of 5, 10, 15, and 20 minutes. These treated seeds were then placed in nylon mesh bags, labeled according to their respective treatments, and randomly positioned in a water bath at the predetermined treatment temperature and durations.

Following 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 with distilled water until reaching 60% of its water retention capacity. The seeds were then incubated in a BOD chamber, maintaining a 12-hour photoperiod of direct light and a constant temperature of 25°C. Evaluations were conducted weekly over a span of 30 days, assessing the emergence of healthy, morphologically intact seedlings devoid of any cracks or lesions, as well as identifying any nonviable seeds.

The assessment of sanitary quality utilized the "blotter-test" method. Each sample consisted of 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. They were then incubated in a BOD chamber at a temperature of 25°C, under a 12-hour photoperiod, for a period of seven days. Following incubation, the microorganisms present in the seeds were examined using stereoscopic and optical microscopes. Fungi were identified in accordance with the guidelines outlined by Barnett & Hunter (1999).

The experimental design followed a completely randomized arrangement, with four replications. The assessment of both sanity and germination considered the exposure duration of seeds to hot water as the treatment variable. The obtained results underwent polynomial regression analysis, wherein linear, quadratic, and cubic models were examined to determine the optimal fit. Additionally, a linear correlation analysis between various variables was conducted using the Assistat 7.6 software (Silva, 2011).

III. Results and Discussion

The main pathogens identified in the seed sanity test of *C. fissilis* were *Rhizoctonia* sp. and *Penicillium* sp. In a study by Lazarotto et al. (2012), the presence of *Ascochyta* sp., *Colletotrichum* sp., *Fusarium* sp., *Penicillium* sp., *Pestalotia* sp., *Phomopsis* sp., and *Rhizoctonia* sp. in *C. fissilis* seeds was identified. In another study by Pinheiro et al. (2016) attempting to test four superficial seed asepsis treatments, the germination test revealed the incidence of *Penicillium* sp. and *Fusarium* sp.

For *Rhizoctonia* sp., it was observed that as the time of heat treatment at 60 °C increased, there was a significant reduction in the percentage of *Penicillium* sp. In the control, the percentage of *Penicillium* sp. was approximately 90%, and its incidence was reduced to 0% when using heat treatment at 60 °C for 20 min.

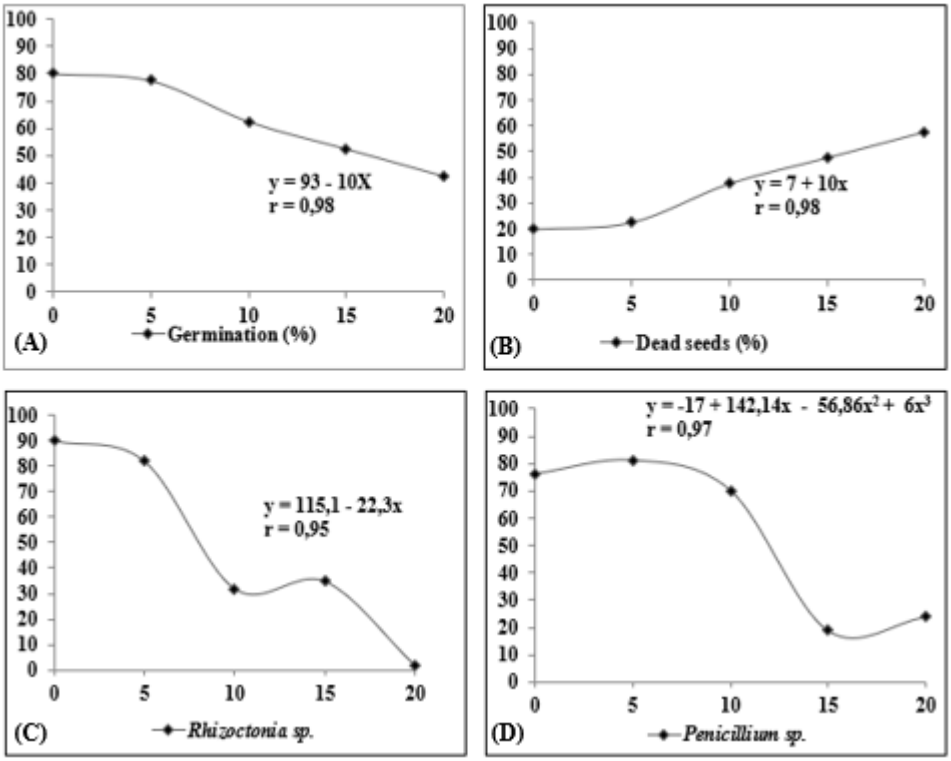
The percentage of *Penicillium* sp. (Figure 1-D) was reduced with the increased time of seed exposure to heat treatment at 60 °C. The percentage of *Penicillium* increased at 5 minutes of exposure to heat treatment at 60 °C. Its reduction was only observed when using a duration of 15 minutes.

The highest percentage of healthy seeds occurred in the treatment with maximum exposure to moist heat for 20 min. However, it should be noted that this treatment did not yield satisfactory results when observing the variable percentage of germinated seeds (Figure 1-A).

Lazarotto et al. (2009), using moist heat at 50 °C for 30 min on *C. fissilis* seeds, verified the eradication of *Pestalotia* sp., *Ascochyta* sp., *Rhizoctonia* sp., and *Colletotrichum* sp. from the seeds; however, such treatment favored the incidence of *Penicillium* sp. and *Aspergillus niger*. The same authors obtained a satisfactory germination percentage of *C. fissilis* seeds, i.e., germination was higher than the control, thus demonstrating the efficiency of using moist heat at 50 °C for 30 min on cedar seeds.

In Figure 1, data from the germination test and seed sanity test of *C. fissilis* are presented. For germination percentage, significant effects ($P \leq 0.05$) were observed in relation to the duration of heat treatment, with a mean linear regression equation being adjusted (Figure 1-A). As the time of heat treatment at 60 °C increased, a significant reduction in the percentage of germinated seeds was noted, especially in the 10-20 minute durations, increasing the percentage of dead seeds (Figure 1-B).

Figure 1: Equations of the changes in the studied variables with *Cedrela fissilis*: (A) abnormal seedlings from the germination test; (B) dead seeds from the germination test; (C) incidence of *Rhizoctonia* sp. in the sanity test; (D) incidence of *Penicillium* sp.



On the horizontal axis are the treatments, represented in minutes, being 0, 5, 10, 15, and 20 respectively, on the vertical axis is the percentage of incidence.

In Table 1, the Pearson correlation coefficient is presented between the different fungi identified in the seed health test and dead seeds in the *C. fissilis* seed germination test. The correlation was not significant for the variables analyzed. However, it was observed that there is a positive correlation between dead seeds and Rhizoctonia sp. for 20 minutes in moist heat, this result demonstrates that *Rhizoctonia* sp., together with the exposure of the seeds to moist heat, can be responsible for the increase in dead seeds in the germination test. A similar result was also observed when evaluating the incidence of *Penicillium* sp. in moist heat at 60 °C for five minutes. According to Duarte et al. (2008), these are associated with seed deterioration, and their action is dependent on the physical and physiological conditions of the seeds at the time of storage, and the prevailing environmental factors during this period.

Table 1: Pearson correlation coefficient (r) between dead seeds in the germination test and the different fungi present in the health test in *Cedrela fissilis* seeds.

	Time				
Pathogens	0	5 min.	10 min.	15 min.	20 min.
Rhizoctonia sp.	0.0	0.2071	-0.2190	0.1578	0.8281
Penicillium sp.	0.0	0.8140	0.3384	-0.5173	0.1952

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

IV. Conclusion

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

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