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The Role Of Thermotherapy In Disease Prevention In Sesbania Virgata Seeds

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Abstract:
This study reports on the relationship between thermotherapy and the physiological and sanitary potential of Sesbania virgata seeds, identifying the efficient exposure period through sanity and germination tests. The seeds were subjected to heat treatment (immersion in water at 60 °C for five, 10, 15, and 20 minutes), followed by a germination test, where they were sown in vermiculite, and a sanity test using the "Blottertest" method. Both tests used samples of 100 seeds, divided into four sub-samples of 25 each. The seeds were incubated in a BOD chamber for seven days, under a 12-hour photoperiod at 25 °C. Subsequently, the presence of microorganisms was assessed using a microscope. The experimental design was completely randomized, and the results were subjected to polynomial regression analysis. These conditions proved to be efficient for the control of fungi associated with sesbania virgata seeds by thermotherapy.

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

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I. Introduction

Pathogenic fungi can associate with forest seeds at all stages of production. This association can affect seed quality, reduce germination capacity, as well as cause seedling damping-off and rot (Santos and Parisi, 2011). The interference of pathogens associated with seeds can be one of the causes of low germination in seed lots (Carneiro, 1987). This pathogen-seed association is often responsible for the long-distance spread of pathogens and also for the transmission of pathogens from seed to plant. In this way, several species are subject to the risk of contamination by phytopathogens, which can drastically reduce the germination of certain individuals.

Sesbania virgata (Fabaceae) is a bush specie that naturally occurs in Brazil and have an important role in many Brazilian ecosystems, due to capacity of fixating nitrogen in the soil (Potomati and Buckeridge, 2002). Has a great potential to be used in degraded areas specially in flooded spaces, due to the fact that is a rustified and tolerant of long periods submerged (Buckeridge et al, 2007).

The elimination of infectious fungal inoculum in seeds has been efficiently achieved through chemical, biological, and physical treatments. The use of agrochemicals has been widely used for control but presents some disadvantages, due to potential environmental effects and the resistance developed by pathogens to certain compounds (Mendes et al., 2001). Besides the chemical method, seed treatment can also use other control methods, such as physical, biological, and combined (Machado, 2000). Among these, thermotherapy is one of the most efficient, as it has an eradicating action on deep infections and does not pollute the environment; however, it does not provide residual protection after treatment, in addition to the risk of causing damage to the seeds (Coutinho et al. 2007).

The heat treatment is a physical procedure based on the use of moist or dry heat to reduce the incidence of pathogens in seeds without losing germination and vigor. The use of moist heat is the most effective, but can, however, cause damage to the seeds (Machado, 2000). It should be noted that the damage caused to seeds comes from the combination of temperature and exposure period necessary to eliminate the pathogen, which, at times, is also lethal to the seeds.

Therefore, the success of the control method requires, above all, knowledge of the appropriate combination of these thermotherapy parameters, which can vary with the species, cultivar, lot, and other important factors. In parallel, to accurately assess the effects of this combination, germination tests are necessary.

In view of the above, the study aimed to investigate the relationship between thermotherapy and the physiological and sanitary potential of *S. virgata* seeds, identifying the efficient exposure period through sanity and germination tests.

II. Material and methods

The seeds of *Sesbania virgata* were obtained from the Seed Exchange Program of the UFSM Forest Nursery, which were collected and processed by seed banks and stored in a cold chamber (temperatures between 5-10°C and low relative humidity) until they were sent for this study.

Firstly, the sesbania seeds underwent dormancy breaking methods, which consisted of making a cut opposite to the embryo. Subsequently, they were subjected to heat treatment by immersion in water heated to 60 °C for 5, 10, 15, and 20 minutes. The seeds were placed in nylon mesh bags, identified according to the treatments, and randomly arranged in a water bath with water heated to the pre-determined treatment temperature and times.

Next, the germination test was conducted, consisting of four replications of 25 seeds, in a substrate of vermiculite. Fine granulometry autoclaved vermiculite was used, moistened with distilled water until reaching 60% of its water retention capacity. The seeds were kept in a BOD chamber, under a 12-hour photoperiod of direct light and a constant temperature of 25 °C. Evaluations were carried out weekly for a period of 30 days. Normal seedlings (well-developed and morphologically perfect, without cracks or lesions) and dead seeds were assessed.

The sanitary quality evaluation was carried out using the "blotter-test" method. Samples of 100 seeds were used, divided into four sub-samples of 25 seeds each, which were placed on sterile Petri dishes, over three sterilized and moistened filter paper sheets. They were then incubated in a BOD chamber at a temperature of 25 °C, with a 12-hour photoperiod, for seven days. Subsequently, the microorganisms present in the seeds were evaluated with the aid of stereoscopic and optical microscopes. Fungi identification was carried out according to the description of Barnett & Hunter (1999).

The experimental design was completely randomized, with four replications. The evaluation of sanity and germination was considered as treatment the exposure period of the seeds to hot water, the results were subjected to polynomial regression, where linear, quadratic, and cubic models were tested, choosing the best model. A linear correlation analysis was performed between the different variables, using the Assistat 7.6 software (Silva, 2011).

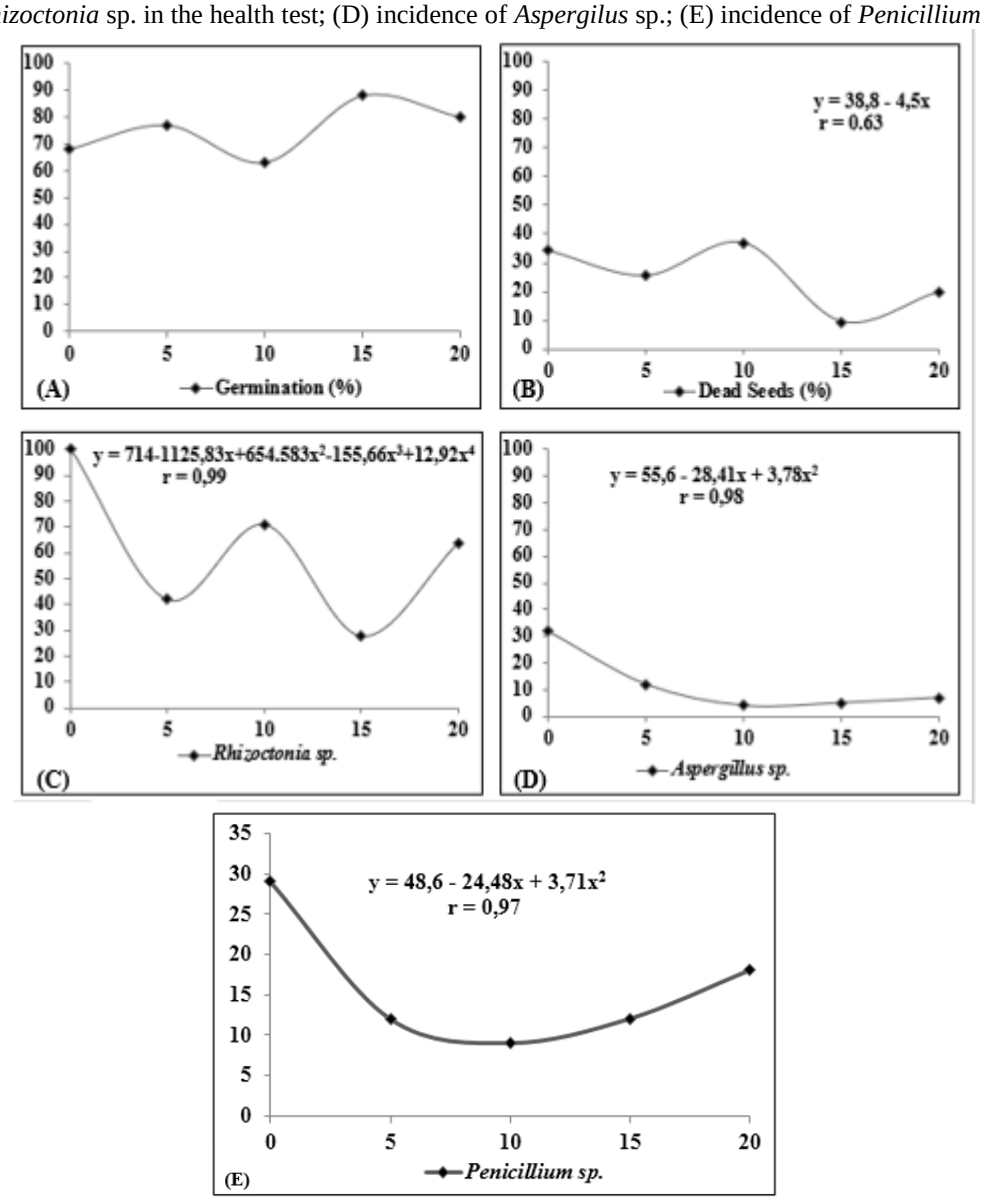
III. Results and Discussion

In the evaluation of the germination percentage of Sesbania virgata seeds, an analysis of variance of the data was not performed, due to the lack of normality and homogeneity of variance (Figure 1-A). However, it was observed that the use of heat treatment at 60 °C did not interfere with seed germination, but it increased the percentage of germinated seeds when the exposure of the seeds to heat treatment was extended. In Figure 1-B, it was generally observed that exposure to heat treatment at 60 °C for 15 minutes reduced the percentage of dead seeds in the germination test, but a duration of 20 minutes increased the percentage of dead seeds.

In the evaluation of the germination percentage of *Sesbania virgata* seeds, an analysis of variance of the data was not performed, due to the lack of normality and homogeneity of variance (Figure 1-A). However, it was observed that the use of heat treatment at 60 °C did not interfere with seed germination, but it increased the percentage of germinated seeds when the exposure of the seeds to heat treatment was extended. In Figure 1-B, it was generally observed that exposure to heat treatment at 60 °C for 15 minutes reduced the percentage of dead seeds in the germination test, but a duration of 20 minutes increased the percentage of dead seeds.

This result is likely associated with the incidence of pathogens associated with seeds, as an increase in dead seeds was also accompanied by an increase in the incidence of *Rhizoctonia* sp.. According to Cherobini et al. (2007), studying the germination of seeds of *S. virgata* from different origins, they reported that germination was between 47 and 22% depending on the batch studied. In another study by Souza et al. (2010), testing the temperature for germination, they concluded that intact seeds had a germination percentage of 24% and 46.88% for scarified seeds when germinated at 25°C; but when testing at 30°C, scarified seeds showed 57% germination, while intact seeds showed 11.5% germination.

Figure 1: Representative equations of the changes occurred in the studied variables with *Sesbania virgata* 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 *Aspergilus* sp.; (E) incidence of *Penicillium* sp.



The incidence of *Rhizoctonia* sp., *Aspergilus* sp., and *Penicillium* sp. in *S. virgata* seeds was verified. Cherobini et al. (2007), assessing the quality of sesbania seeds, identified the presence of *Penicillium* sp., *Alternaria* sp., *Cladosporium* sp., and *Nigrospora* sp.

In Figures 1-D-E, the evaluation of the incidence of *Aspergilus* sp. and *Penicillium* sp. had significant effects ($P \leq 0.05$) in relation to the duration of the heat treatment, with a mean quadratic regression equation being adjusted for both fungi. The incidence of *Aspergilus* sp. was reduced from 32% to near zero when using 10 minutes of moist heat, as well as when using temperatures of 15 and 20 minutes. For the fungus Penicillium sp., a 10-minute heat treatment at 60 °C drastically reduced its incidence from 29% in the control to 9%. These results demonstrate that the use of moist heat treatment at 60 °C for 10 minutes is effective in reducing pathogens in sesbania seeds, as the germination was not affected by the exposure time of *S. virgata* seeds to moist heat treatment at 60 °C.

Table 2: Pearson correlation coefficient (r) between dead seeds from the germination test and the different fungi present in the health test of *Sesbania virgata* seeds.

	Time				
Pathogens	0	5 min.	10 min.	15 min.	20 min.
Rhizoctonia sp.	0.00	0.2571	0.8448	-0.8233	-0.7862
Aspergillus sp.	-0.8903	0.9753*	0.7063	-0.5120	-0.4518
Penicillium sp.	0.7815	0.9753*	0.8377	-0.5094	0.6482

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

There was a positive and significant correlation between dead seeds in the germination test and the incidence of Aspergillus sp. and Penicillium sp. (r = 0.9753 and r = 0.9753, respectively), when using moist heat of 60 °C for five minutes (Table 2). Probably, the pathogens *Aspergillus* sp. and *Penicillium* sp. are responsible for the dead seeds in the germination test of *S. virgata* seeds.

IV. Conclusion

Moist heat therapy at 60 °C for 15 minutes is effective for controlling fungi associated with *S. virgata* seeds.

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