

# THERMOTHERAPY IN THE CONTROL OF *Fusarium* spp. IN ANGICO SEEDS [*Anadenanthera colubrina* (Vell.) Brenan] AND MAINTENANCE OF PHYSIOLOGICAL QUALITY

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## Resumo

*Termoterapia no controle de Fusarium sp. em sementes de angico [Anadenanthera colubrina (Vell.) Brenan] e manutenção da qualidade fisiológica.* O tratamento térmico é utilizado, frequentemente, para superação de dormência em sementes florestais; porém, este ainda possui potencial para controle de microrganismos veiculados pelas sementes. Objetivou-se neste trabalho avaliar a eficiência da termoterapia no controle de *Fusarium* sp. em sementes de angico [*Anadenanthera colubrina* (Vell.) Brenan] coletadas no estado da Paraíba e sua influência na qualidade fisiológica. Foram selecionadas nove matrizes, localizadas nos municípios de São João do Cariri - PB, Boa Vista - PB e Sumé - PB. Para o teste de sanidade os tratamentos utilizados foram compostos por sementes não tratadas (testemunha), tratamento químico e o tratamento térmico com temperaturas de 50, 60 e 70 °C por 10, 20, 30 e 40 minutos de imersão. No teste de germinação, utilizaram-se os mesmos tratamentos da sanidade sendo avaliados o percentual de germinação, primeira contagem, sementes mortas, comprimento de plântulas e índice de velocidade de germinação. Constatou-se que os tratamentos térmicos utilizados foram eficientes na redução de *Fusarium* sp. A termoterapia não prejudicou a qualidade fisiológica das sementes de angico e proporcionou um aumento no percentual de germinação.

*Palavras-chave:* Sementes florestais; Controle físico; Patologia de sementes.

## Abstract

Thermal treatment is frequently used to overcome dormancy in forest seeds; however, it can also be used to control seed-borne pathogens. This study aimed to evaluate the efficiency of thermotherapy in controlling *Fusarium* sp. in angico seeds [*Anadenanthera colubrina* (Vell.) Brenan] collected in the state of Paraíba, Brazil, and its effect on physiological quality. Nine seed sources located in the municipalities of São João do Cariri - PB, Boa Vista - PB, and Sumé - PB were selected for this study. For the sanitary test, the treatments were composed of untreated seeds (control), chemical treatment, and thermal treatment at temperatures of 50, 60, and 70 °C immersed for 10, 20, 30, and 40 minutes. The same treatments were used in the germination test, and the percentage of germination, first count, dead seeds, seedling length, and germination speed index were evaluated. The results showed that the thermal treatments effectively reduced *Fusarium* sp. in the seeds. Thermotherapy did not negatively affect the physiological quality of angico seeds and led to an increase in germination percentage.

*Keywords:* Forest seeds; Physical control; Seed pathology.

## INTRODUCTION

The unsustainable use of natural resources in the Caatinga biome has led to the loss of endemic species, the alteration of ecological processes, and desertification, which requires restoring activities for this biome (DANTAS *et al.*, 2014). The species occurring in the Caatinga have various uses, ranging from timber, food, and charcoal production to other forest products such as fruits, medicinal plants, fibers, beekeeping, and forage (PEREIRA JÚNIOR *et al.*, 2014).

The angico tree [*Anadenanthera colubrina* (Vell.) Brenan] – Fabaceae is a native species of the Brazilian flora, with a tree-like structure that can reach up to 10 to 20 meters (PIRES NETO *et al.*, 2016). The fast germination rate and high potential of its seeds enable the viability of reforestation programs due to the production of a higher number of seedlings in a short period (DORNELES *et al.*, 2013).

The demand for forest species seeds has become increasingly crucial for the production of seedlings intended for reforestation programs, such as the restoration of degraded areas and maintenance of native species threatened with extinction (VECHIATO; PARISI, 2013). However, it faces several challenges, particularly plant health issues, due to the seed-borne pathogens (ARAÚJO *et al.*, 2013).

Fungi are the main group of pathogens associated with seeds that can cause damage during field, post-harvest, and storage. The *Fusarium* genus comprises a group of fungi highly pathogenic to various plant species (DUAN *et al.*, 2016). This genus is composed of several species that have wide adaptation in different

environments, capable of surviving in the soil or crop residues in the form of chlamydospores (resistant structures) (HALL *et al.*, 2013).

The eradication or reduction of fungi associated with seeds can be achieved through chemical, biological, and physical treatments (SANTOS *et al.*, 2016). Physical treatments such as thermotherapy are an alternative method with great potential, especially for controlling pathogens attached to the surface or inside the seeds (PEREIRA *et al.*, 2015).

Therefore, this study aimed to evaluate the efficiency of thermotherapy in controlling *Fusarium* sp. in angico seeds [*Anadenanthera colubrina* (Vell.) Brenan] collected in the state of Paraíba and its effect on physiological quality.

## MATERIALS AND METHODS

The experiment was conducted at the Plant Pathology Laboratory at the Universidade Federal da Paraíba, Areia. Nine seed sources located in the state of Paraíba, Brazil, were selected. Three municipalities were chosen for the identification and georeferencing of trees, and five trees were selected in each municipality to monitor the selection criteria and phenological stages characterization. The trees that exhibited the best criteria and phenological stages were chosen. Only one tree source was used from each municipality, with the selection criteria based on the higher number of inflorescences, good canopy formation, and satisfactory phytosanitary appearance.

The municipalities from which the seed batches were taken were: São João do Cariri (S 7°23'27" W 36°32'7"), Boa Vista (S 7°14'34" W 36°14'11"), and Sumé (S 7°40'18" W 36°52'18"), comprising three batches.

### Seed harvesting and processing

Mature angico [*Anadenanthera colubrina* (Vell.) Brenan] fruits were harvested in October using pruning shears and then taken to the laboratory. The fruits were placed in trays to facilitate aeration until the processing, which occurred one day after harvesting. The seeds were extracted from the fruits that had opened spontaneously and processed on disinfected workbenches and manually selected by discarding any deformed seeds, whether due to mechanical damage or pests, ensuring visually homogeneous selection.

### Sanitary quality of seeds subjected to thermotherapy

A total of 100 seeds were used per treatment, distributed in ten replicates of ten seeds each. The seeds were placed in gauze bags and tied with cotton string for subsequent immersion in heated water using a Digital Water Bath Tecnal Te 184.

The treatments used were as follows: the control, which consisted of seeds immersed in sterile distilled water (DAS) at room temperature ( $25 \pm 2$  °C) for 5 minutes, as described by Schneider *et al.* (2015); the chemical treatment composed of the contact fungicide dicarboximide at the dose of 240 g/100 kg of seeds; and the thermal treatments at temperatures of 50, 60, and 70 °C for 10, 20, 30, and 40 minutes of immersion. After treatment, the seeds were incubated in Petri dishes (90 x 15 mm) on a double layer of sterilized filter paper moistened with SDW. The plates were kept at  $25 \pm 2$  °C for seven days. Fungal identification was performed using an optical stereoscopic microscope by comparing the fungi structures with specialized literature.

### Seed physiological quality

The same treatments described earlier were used using four replicates of twenty-five seeds for each treatment. After the application of the treatments, the seeds were distributed on sterilized germitest paper, previously sterilized at 120 °C for 2 hours. The volume of S.D.W. used for moistening the paper was equivalent to 2.5 times the weight of the substrate. The seeds were incubated in a B.O.D. (Biochemical Oxygen Demand) chamber at 25 °C and 12 hours photoperiod. Evaluations were performed from the 4th to the 10th day after sowing.

The germination percentage, first germination count, percentage of dead seeds, seedling length, and germination speed index were evaluated. For the germination speed index (G.S.I.), daily counts were made after germination of the first seed until the date when the stand remained constant, and the index was calculated according to the formula proposed by Maguire (1962).

The percentage of dead seeds was recorded at the end of the test. The length of normal seedlings from each replicate was measured using a ruler graduated in centimeters, and the results were expressed in centimeters per seedling.

### Experimental design and statistical analysis

A completely randomized experimental design was used. The treatments were arranged in a factorial scheme (3 x 4 x 3) + 2, consisting of three seed collection sites, four immersion periods of the seeds (10, 20, 30, and 40 minutes), three water immersion temperatures (50, 60, and 70 °C), and two additional control treatments

(SDW and fungicide). The data were submitted to analysis of variance. Regression analysis was performed for the quantitative data (immersion time), and the significance of the models was verified by the F-test ( $p \leq 0.05$ ).

## RESULTS

### Sanitary quality

*Fusarium* sp. incidence in angico seeds submitted to thermotherapy treatment and collected from different municipalities in the state of Paraíba are shown in Figure 1. The seeds collected in São João do Cariri - PB and Boa Vista - PB did not show significant differences on *Fusarium* spp. incidence. In general, the efficacy of the hydrothermal treatment for controlling *Fusarium* spp. in seeds was confirmed, as the percentage values, in most cases, were close to or equal to zero when comparing the incidence of the fungus in the control treatment.

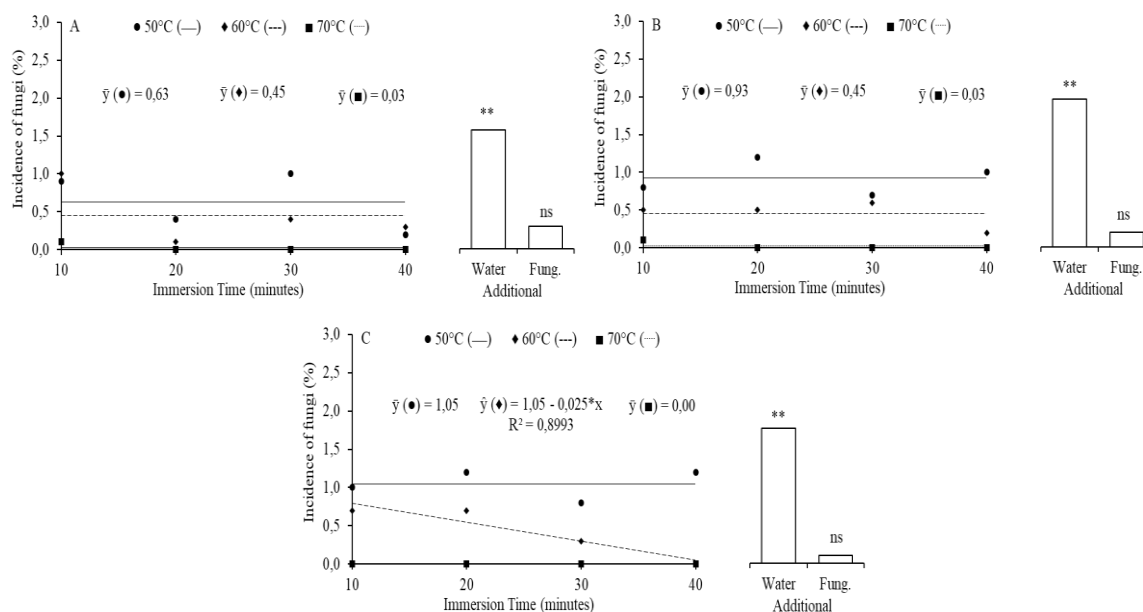


Figure 1. *Fusarium* spp. incidence in *Anadenanthera colubrina* seeds collected in São João do Cariri (A), Boa Vista (B), and Sumé (C), Paraíba state under thermotherapy treatment. ns, \*\*, and \*: not significant, significant at 1%, and significant at 5% probability by the F-test, respectively.

Figura 1. Incidência de *Fusarium* sp. em sementes de *Anadenanthera colubrina* coletadas em matrizes localizadas no estado da Paraíba: São João do Cariri (A), Boa Vista (B) e Sumé (C) sob os efeitos do tratamento hidrotérmico. ns, \*\* e \*: não significativo e significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.

### Physiological quality

The seed moisture content was 8.5%, 8.1%, and 8.6% for lots A (São João do Cariri), B (Boa Vista), and C (Sumé), respectively. The moisture content in forest seeds is influenced by physiological maturity at harvesting time (MEDEIROS; EIRA, 2006). Figure 2 shows the germination test results. It can be observed that temperatures of 50, 60, and 70 °C for 10, 20, 30, and 40 minutes of immersion resulted in increased germination in all analyzed lots. In lots A (São João do Cariri) and C (Sumé), the highest average germination values were obtained when seeds were treated at 60 °C, with values of 68.9% (lot A) and 65.8% (lot C). For lot B (Boa Vista), the temperature of 70 °C resulted in the highest germination percentage (80%).

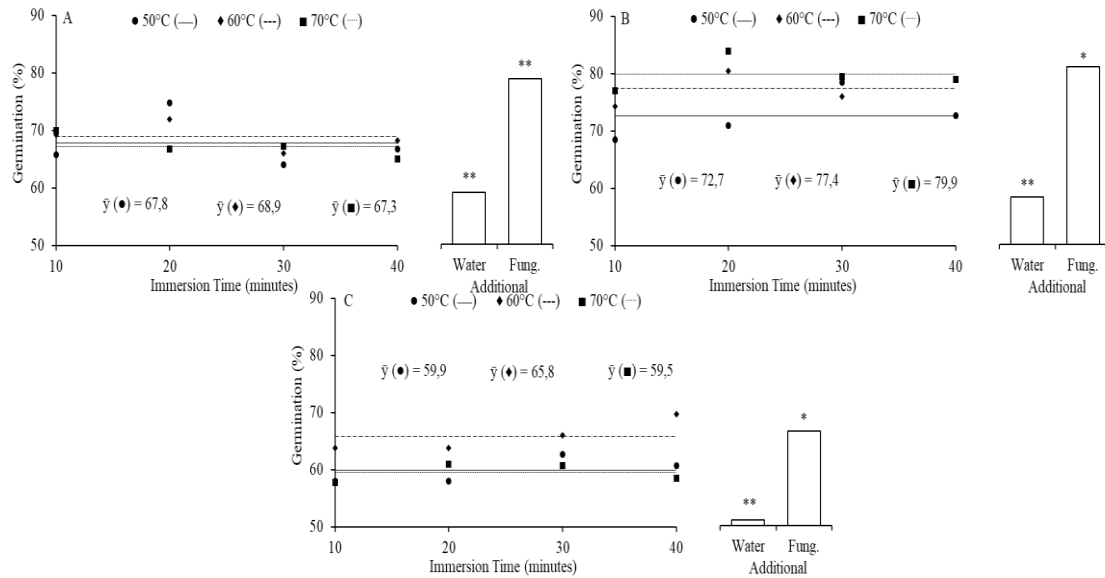


Figure 2. Germination of *Anadenanthera colubrina* seeds collected in São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C), Paraíba state, under thermotherapy treatment. ns, \*\*, and \*: not significant, significant at 1% and 5% probability by the F test, respectively.

Figura 2. Germinação de sementes de *Anadenanthera colubrina* coletadas em matrizes localizadas no estado da Paraíba: São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C) sob os efeitos do tratamento hidrotérmico. ns, \*\* e \*: não significativo e significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.

As seen in the first germination count (Figure 3), seeds from lots B (Boa Vista) and C (Sumé) had a higher germination percentage than those in the control treatments. Thus, temperatures of 50, 60, and 70 °C at all immersion times did not negatively affect this variable. For lot A (São João do Cariri), average values of 32%, 35%, and 34% were observed when submerged at temperatures of 50, 60, and 70 °C, respectively. These values were lower than those observed in the control (50%).

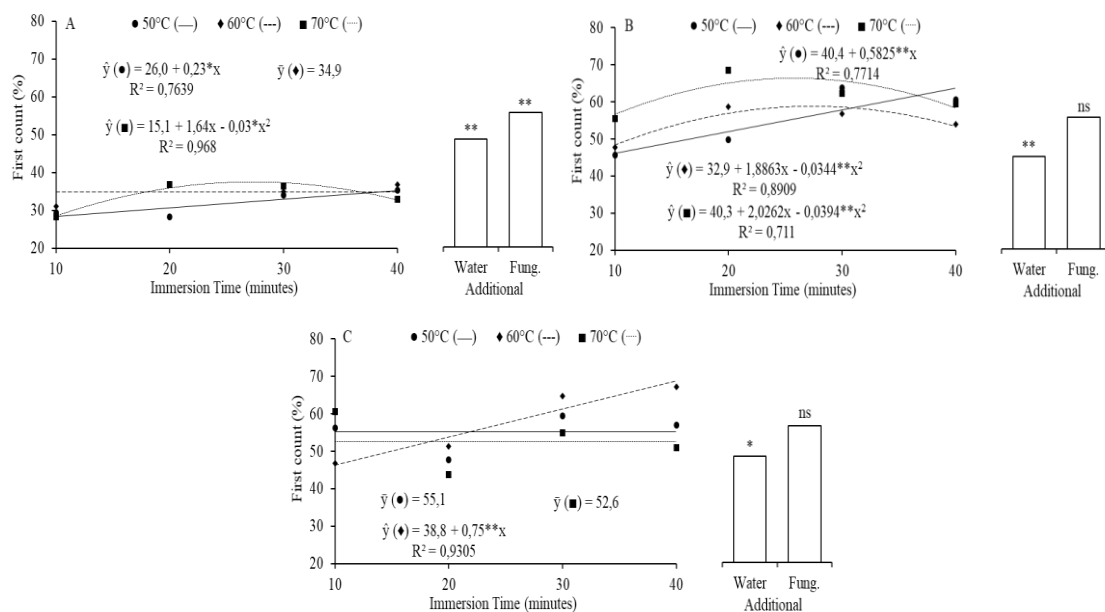


Figure 3. First germination count of *Anadenanthera colubrina* seeds collected from São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C), Paraíba state under thermotherapy treatment. ns, \*\*, and \*: not significant, significant at 1% and 5% probability by the F test, respectively.

Figura 3. Primeira contagem do teste de germinação de sementes de *Anadenanthera colubrina* coletada em matrizes localizadas no estado da Paraíba: São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C) sob os efeitos do tratamento hidrotérmico. ns, \*\* e \*: não significativo e significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.

Figure 4 shows the percentage of dead seeds. No correlation was observed between temperature and the increase in dead seeds percentage. For seeds from lot A (São João do Cariri), the temperature of 70 °C for 40 minutes of immersion resulted in the highest percentage of dead seeds (31%) compared to other treatments. In lots B (Boa Vista) and C (Sumé), the temperatures of 50 °C for 10 minutes and 70 °C for 40 minutes of immersion caused the highest number of dead seeds, with 27% and 34% respectively.

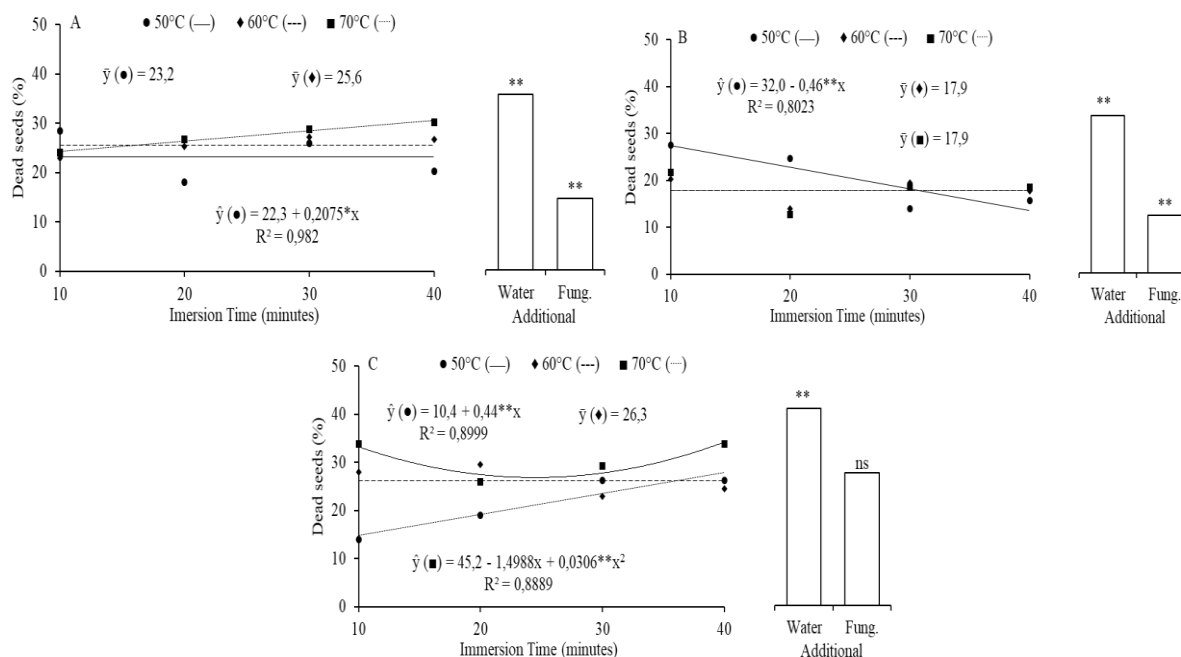


Figure 4. Dead seeds of *Anadenanthera colubrina* collected from São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C), Paraíba state under thermotherapy treatment. ns, \*\*, and \*: not significant, significant at 1% and 5% probability by the F test, respectively.

Figura 4. Sementes mortas de *Anadenanthera colubrina* coletadas em matrizes localizadas no estado da Paraíba: São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C) sob os efeitos do tratamento hidrotérmico. ns, \*\* e \*: não significativo e significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.

For seedling length (Figure 5), it was observed that the seedlings from lot A (São João do Cariri) had an average length of 12 cm when the seeds were immersed at 70 °C. There was no significant difference between the treatments for seeds collected from Boa Vista - PB and Sumé - PB. Generally, seeds treated with fungicide showed the highest seed length than the other treatments.

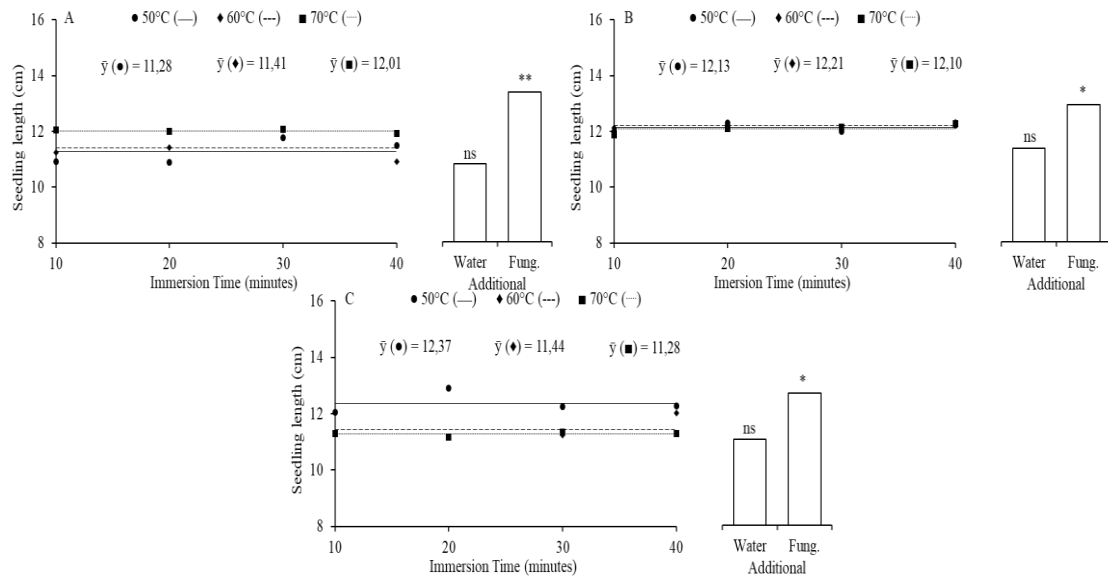


Figure 5: Seedling length of *Anadenanthera colubrina* collected from São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C), Paraíba state under thermotherapy treatment. ns, \*\*, and \*: not significant, significant at 1% and 5% probability by the F test, respectively.

Figura 5. Comprimento de plântulas de *Anadenanthera colubrina* coletadas em matrizes localizadas no estado da Paraíba: São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C) sob os efeitos do tratamento hidrotérmico. ns, \*\* e \*: não significativo e significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.

Figure 6 shows germination speed index (GSI) values. It was found that thermotherapy treatments resulted in a lower germination speed in the seeds collected in the municipality of São João do Cariri (lot A). For lots B (Boa Vista) and C (Sumé), a linear increase in GSI was observed as the immersion time increased, with a maximum value observed at 50°C (7.3) and 60°C (7.9) for 40 minutes, respectively.

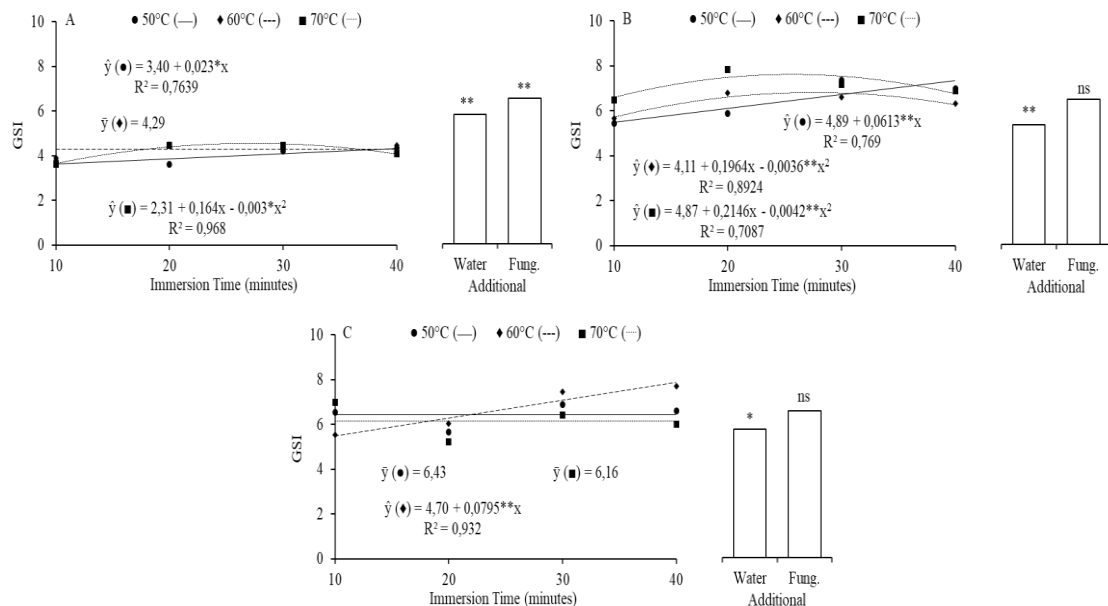


Figure 6: Germination speed index (GSI) of *Anadenanthera colubrina* seeds collected from São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C), Paraíba state under thermotherapy treatment. ns, \*\*, and \*: non-significant and significant at the 1% and 5% probability levels by the F test, respectively.

Figura 6. Índice de velocidade de germinação (IVG) de sementes de *Anadenanthera colubrina* coletadas em matrizes localizadas no estado da Paraíba: São João do Cariri-PB (A), Boa Vista-PB (B), Sumé-PB (C)

sob os efeitos do tratamento hidrotérmico. ns, \*\* e \*: não significativo e significativo a 1% e 5% de probabilidade pelo teste F, respectivamente.

## DISCUSSION

According to Marangoni *et al.* (2014), angico seeds germinate satisfactorily in water contents ranging from 8% to 21%, with no influence on fungal incidence. These results corroborate with our study, as the moisture contents in the seed lots from São João do Cariri and Sumé were analyzed.

Coutinho *et al.* (2007) tested the immersion of maize (*Zea mays* L.) - Poaceae seeds in water at 60°C for 5, 10, and 15 minutes and observed a reduction in *Fusarium verticilloides* incidence at 10 and 20 minutes. Lazarotto *et al.* (2013a) found that dry heat thermotherapy at 70°C for 48 hours eliminated fungi associated with cedar (*Cedrela fissilis* Vell.) - Meliaceae seeds without affecting their vigor. According to LAZAROTTO *et al.* 2013b, an ideal moist heat exposure period is when the fungi percentage is reduced without compromising seed viability.

Machado *et al.* (2013) discovered that thermotherapy at 70°C was effective in the pre-germination treatment of angico-de-bezerra (*Piptadenia moniliformis* Benth) seeds, resulting in an 80% germination rate. However, the authors mentioned that thermal treatment at temperatures above 100°C may lead to seed embryonic death.

Arruda *et al.* (2015) evaluated the germination of angico seeds under pre-germination treatments and found that seeds immersed at 70°C for 5 minutes had a first count germination value of 24%. According to Martins *et al.* (2014), the first count germination test is substantial to assess both seed vigor and seed physiology, due to its practicality and execution time. Moreira *et al.* (2014) evaluated the germination and vigor of angico seeds [*Anadenanthera peregrina* (L) var. *falcata* Benth.] and obtained a percentage of 80% in the first count test.

Medeiros *et al.* (2016) assessed the association of fungi with tambor seeds [*Enterolobium contortisiliquum* (Vell.) Morong] - Mimosaceae collected in four municipalities in Paraíba and found that the mortality rate of the seed lots varied between 7% and 21%. This result is consistent with the findings of this study, where the presence of fungi is directly related to the increase in dead seed percentage. Thus, it can be considered that, possibly for these analyzed lots, besides the presence of microorganisms in the seeds, exposure to high temperatures may have affected the embryo and caused the degradation of storage components. A correlation has been observed between the dead seeds and the percentage of fungi incidence in forest species, including angico-branco [*Anadenanthera colubrina* (Vell.) Brenan] - Fabaceae, leading to high losses in stands and compromising the sanitary quality of the lots (BEZERRA *et al.*, 2013). Bezerra *et al.* (2012) found that the association of fungi in angico seeds has resulted in the emergence of several diseases in the field, as seeds are the most viable plant propagules over time.

Moreira *et al.* (2014) evaluated the germination of angico-vermelho seeds (*Parapiptadenia rigida* Benth.) - Fabaceae under stress conditions and found an average value of 3.1 for the germination speed index, with a germination percentage of 54% and a first count value of 79.7%. Miranda *et al.* (2012) observed values of 14.3, 18.7, and 17.8 for the germination speed index in the germination test of angico-branco using the respective substrates: sand, filter paper, and vermiculite. Santos *et al.* (2012) studied the effect of temperature on the physiological quality of angico-branco seeds with different moisture contents and found that the germination speed index at room temperature (25°C) was 6.1, 11, and 13.2 when the seeds had water contents of 5%, 8%, and 16%, respectively.

Lopes *et al.* (2007) used a temperature of 70°C for 1 and 2 minutes of immersion to evaluate the germinative of pata-de-vaca seeds (*Bauhinia variegata* L.) - Fabaceae and obtained germination speed index values of 4.8 and 5.3, respectively, for the immersion times. These authors also stated that hot water significantly affected the seed coat, facilitating water absorption and contributing to germination. These factors may have influenced the data obtained in the present study, considering that a temperature of 70°C for 40 minutes may damage the seed.

## CONCLUSION

- Immersion of seeds at 70°C for 10 minutes, using moist heat, effectively reduced *Fusarium* spp. without compromising the physiological quality of *Anadenanthera colubrina* seeds.

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