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Aspidosperma pyrifolium seeds treated with ultrasound and microwave

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Abstract - *Aspidosperma pyrifolium* Mart. & Zucc. (Apocynaceae) is a tree present in the Brazilian semiarid Region and it may be used as timber or as raw material for cosmetic and medicinal purposes. Sustainable methods of management are important to improve and to assure sustainable forest yield. Among these methods, we can note the physical treatment to improve vigor in forest seeds with the use of ultrasound (US) and microwave (MW). The aim of this research aimed was to test the use of US and MW to improve vigor to *A. pyrifolium* seeds. The seeds were submitted to the following treatments: 1) MW: 0, 5, 10, 15, and 20 s and 2) US: 0, 1, 2, 3, and 4 min. After treatment, the seeds were sown on paper rolls and incubated in BOD (25 ± 2 ºC, seven days, 12 h photoperiod). The seeds were evaluated for vigor's characters during 14 days. The results were analyzed by linear regression and Pearson correlation, using R program. The physical treatments US and MW did not show positive effects to enrich vigor in *A. pyrifolium* seeds.

Sementes de *Aspidosperma pyrifolium* tratadas com ultrassom e micro-ondas

Resumo - *Aspidosperma pyrifolium* Mart. & Zucc. (Apocynaceae) é uma árvore presente no semiárido brasileiro, utilizada para exploração madeireira, cosmética e medicinal. Métodos sustentáveis de manejo são importantes para melhorar o rendimento e garantir a produção florestal sustentável. Dentre esses métodos, podemos citar o tratamento físico para melhorar o vigor das sementes, com o uso de ultrassom (US) e micro-ondas (MW). Esta pesquisa teve como objetivo testar o uso de US e MW para melhorar o vigor de sementes de *A. pyrifolium*. Sementes desta espécie foram submetidas aos seguintes tratamentos: 1) MW: 0, 5, 10, 15 e 20 s e 2) US: 0, 1, 2, 3 e 4 min, sendo em seguida semeadas em rolos de papel e incubadas em câmara de germinação (BOD) (25 ± 2 ºC, sete dias, fotoperíodo de 12 h). As sementes foram avaliadas por 14 dias sobre variáveis de germinação e vigor e os resultados foram analisados por regressão linear e correlação de Pearson, com o programa R. Os tratamentos físicos US e MW não apresentaram efeitos positivos para enriquecimento do vigor de sementes de *A. pyrifolium*.

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Introduction

The germination is a physiological process inherent to plants by which the seeds absorb water from the environment, starting the process to produce biochemical composts that will start the embryo establishment. Latter, in this process, the embryo uses the biochemical reserve from cotyledons, protrudes the tegument and completes the germination process (Carvalho & Nakagawa, 2012).

Some forest seeds can be resistant to inhospitable environmental conditions and, for the evolutionary process, promote morphological or biochemical changes to accelerate or retard the germination process (Brasil, 2009).

Great examples of different mechanisms of germination can be seen in the Brazilian Semiarid area, where the Caatinga Biome is inscribed (Oliveira et al., 2015). This Biome is the Brazilian Dry Season Forest covering about 53.1% of the Northwest Brazilian region (882.081 km^2) and presenting population of over 20 million. It is characterized by the occurrence of dry seasons and xerophytic species (Carvalho, 2012; Correia et al., 2019) and it is rich in endemism (Pereira et al., 2016).The extractives of natural resources and predatory activity in this region is diminishing plant populations from different species of Caatinga Biome (Ferreira & Cunha, 2000; Petersen et al., 2012).

Aspidosperma pyrifolium Mart. & Zucc. (Apocynaceae) is an important plant species from Caatinga Biome. It is a tree species used for timber and as raw material for cosmetic and medicinal purposes (Ferreira & Cunha, 2000). The extractives and predatory activities have also diminished *A. pyrifolium* population in Caatinga Biome. Planted forests could be an alternative to guarantee conservation of this important tree species (Andrade Júnior et al., 2020). The seeds of *A. pyrifolium* loose their viability in a short period after antithesis, decreasing also quality and vigor after harvest (Oliveira et al., 2015). This characteristic is due to the short rainy season that occurs in Caatinga Biome and it represents a biological mechanism that does not contribute to the sowing and the production of seedlings of this species to commercial management (Felippi & Possenti, 2016; Correia et al., 2019).

Therefore, to improve the commercial management of forest species, the use of sustainable technologies is emergent to improve vigor to seeds and seedlings. Seeds treatment with physical agents is indicated to decrease the dormancy stage and to improve the quality or active processes (Knox et al., 2013; Gomes, 2019).

These techniques can be used in forest seeds to avoid or reduce the loss of viability caused by environmental issues, for example (Venâncio & Martins, 2019). The ultrasounds (US) and microwaves (MW) are important natural frequencies that can be used to seed treatment. These frequencies are recommended to some different authors to improve the seed quality in some plant species because they are sustainable, low cost, innovative, and they also present easy access (Miano et al., 2019).

Rifna et al. (2019) recommend the use of US and MW to improve vigor in rice, sorghum, millet, sugar beet, coffee, bean and eucalyptus seeds, for example. Consequently, this paper has aimed to test vigor improve of *A. pyrifolium* seeds with the use of US and MW.

Material and methods

The experiments were carried out in the Seed Technology Laboratory, in Semiarid Nacional Institute (Campina Grande, PB). The seeds of *Aspidosperma pyrifolium* were collected in Patos, Paraiba State, and they were stored for six months in a climate chamber (8 + 2 °C, in the dark) in plastic recipients.

We used two independent assays: microwave (MW) and ultrasound (US). For MW, the seeds were submitted to MW (model Piccolo Style; frequency of 400 W; Panasonic®) for 0, 5, 10, 15, and 20 s in Petri dishes. For US, the seeds were submitted to 0, 1, 2, 3 and 4 min, at 25 ± 2 ºC; we used ultrasound bath (model STD; frequency of 42 kHz; BioWash®), as described by Venâncio & Martins (2019). In each treatment, 200 seeds were treated, in both assays.

After the treatment procedure, the seeds were sowed on germitest paper and they were rolled. Each repetition was presented by one paper roll. The germitest paper was moistened with distilled water at proportion of 2.5 times the dry paper mass (Brasil, 2009; Carvalho & Nakagawa, 2012) and the roll papers were incubated at 25 ± 2 ºC, for 14 days, 12 h of photoperiod.

We evaluated the percentages of: final germination (G), normal seedlings (N), abnormal seedlings (A), and non-germinate seeds (NG), presetend as in Equations 1 to 4.

Both assays were in completely randomized design, with four repetitions of fifty seeds for each treatment. The data were analyzed using linear regression and Pearson correlation, in the R program.

$$G = \frac{Number\ of\ germinated\ seeds}{Number\ of\ sowing\ seeds} x\ 100 \quad (1)$$

$$N = \frac{Number\ of\ normal\ seedlings}{Number\ of\ germinated\ \ seeds} x\ 100 \quad (2)$$

$$A = \frac{Number\ of\ abnormal\ seedlings}{Number\ of\ germinated\ \ seeds} x\ 100 \quad (3)$$

$$NG = \frac{Number\ of\ non\ germinated\ seeds}{Number\ of\ sowing\ seeds} x\ 100 \quad (4)$$

Results

For US assay, we may visualize the results: final germination (G), normal seedlings (N), abnormal seedlings (A) and non-germination seeds (NG) have no difference between the treatments tested in this paper (Table 1).

The results from variable G% (F = 1.669; FD = 4; p = 0.2066) presented the equation y= 92.07 + 0.0001383 x (R^2 = 0.03). For the variable N (F = 6.924; FD = 4; p = 0.0019) we obtained the equation y = 85.995036 - 0.021878 x (R^2 = 2.16), showing no adjust. The results from variable A (F=4.452; FD = 4; p = 0.0123) presented the equation y = 6.591749 + 0.01622 x (R^2 = 2.13), and it also did not adjust. For the variable NG (F = 1.867; FD = 4; p = 0.1642) we obtained the equation y = 7.413215 - 0.005656 x (R^2 = 0.37).

Table 1. Means (%) to US treatment in *Aspidosperma pyrifoloium* seeds.

Treatments (min)	G	N	A	NG
0	94.50	92.00	4.50	5.5
1	90.50	85.50	5.00	9.5
2	86.60	78.66	8.00	12.40
3	94.50	80.50	14.00	5.50
4	94.00	92.50	2.5	6.00

G = final germination; N = normal seedlings; A = abnormal seedlings; NG = non-germinate seeds.

The Pearson correlations tests result in a few correlations between the parameters analyzed, maybe this fact explains the non-adjust to Linear Regression model used. However, for points between NG to G and N, there were the correlation coefficients -0.9753 and -0.6109, respectively, and to intercept between N and A there is 0,6845 of correlation (Table 2).

Table 2. Pearson correlation between the variables final germination (G), normal seedlings (N), abnormal seedlings (A), and non-germinate seeds (NG) of *Aspidosperma pyrifolium* seeds treated with different times to expose to Ultrasound.

	Treatment	G	N	A	D
Treatment	1	0.38229	0.2127	-0.007	-0.2805
G	0.38229	1	0.21276	0.19223	-0.9753
N	0.21276	0.56607	1	-0.6845	-0.6109
A	-0.007	0.19223	-0.6845	1	-0.1591
D	-0.2805	-0.9753	-0.6109	-0.1591	1

For the US assay, we may visualize that all variables have significant results, as observed for variable G (F = 32.50; FD = 4; p = 0.0319), N (F = 97.06; FD = 4; p = < 0.0001), A (F = 2116.70; FD = 4; p = < 0.0001) and NG (F = 9.88; FD = 4; p = 0.318). The medium values to G and N decrease due to longer exposure to US, while A and NG increases them (Figure 1).

About the MW assay, there were non-significatives results for all variables (p<0.05), the exposition of microwave caused death of *A. pyrifolium* seeds. For Pearson correlation, we can observe that treatments is a stronger correlation to G, N and D (-0.8186, -0.8196, and 0.8186, respectively). Also, G has a stronger correlation to N and D (0.8047 and -1, respectively). Otherwise, N is a stronger correlation to D (0.8047). However, the variables G, N and D have stronger correlation to each other (Table 3).

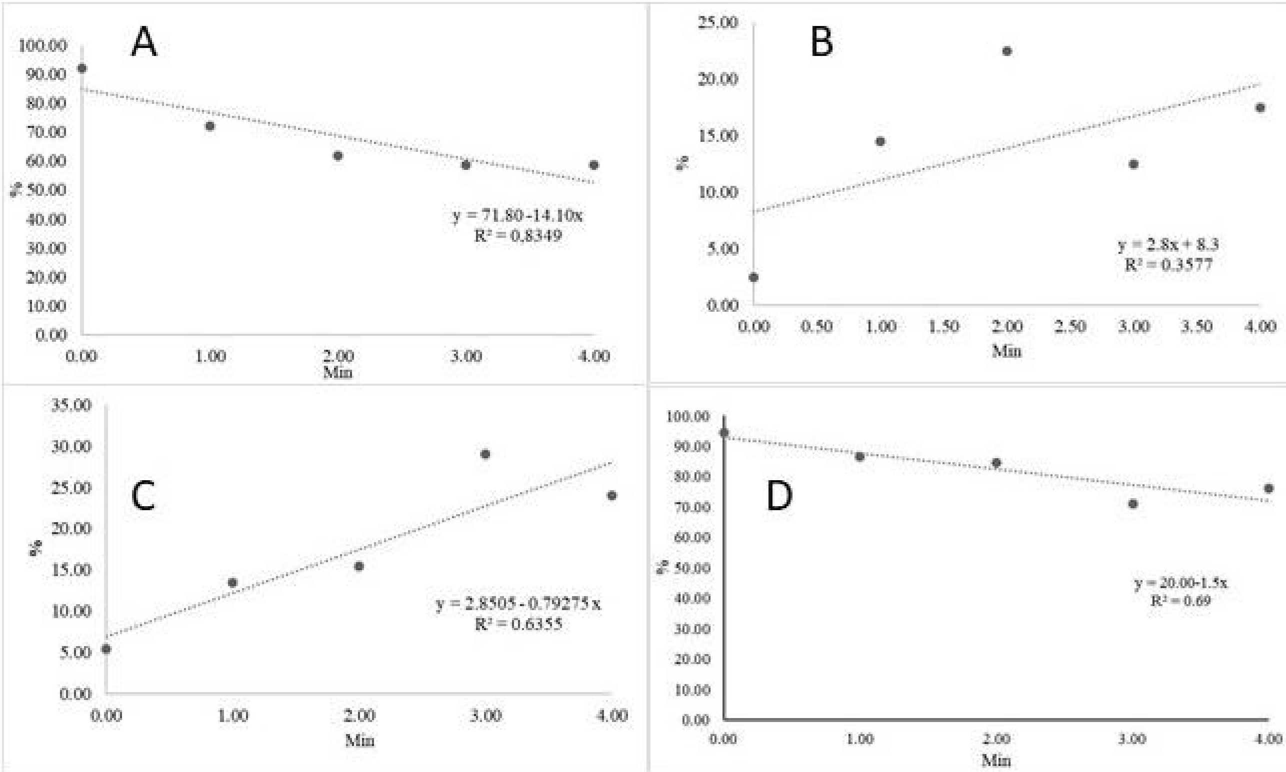

Figure 1. Linear regression to variables final germination (A), normal seedlings (B), abnormal seedlings (C), and non-germinate seeds (D) of *Aspidosperma pyrifolium* seeds treated with different times to expose to microwave.

Table 3. Pearson correlation between the variables final germination (G), normal seedlings (N), abnormal seedlings (A) and non-germinate seeds (NG) of *Aspidosperma pyrifolium* seeds treated with different times to expose to Microwave.

	Treatment	G	N	A	D
Treatment	1	-0.8186	-0.8196	0.06794	0.81863
G	-0.8186	1	0.80474	0,23836	-1
N	-0.8196	0.80474	1	-0.3847	-0.8047
A	0.06794	0.23836	-0.3847	1	-0.2384
D	0.81863	-1	-0.8047	-0.2384	1

Discussion

The results obtained in this research are very important to affirm knowledge about the germination and vigor of *Aspidosperma pyrifolium* seeds, because they decrease when exposed to microwave (MW) and ultrasound (US), in accordance with the conditions used in this research. Probably, the chemical and morphological characteristics inherent to *A. trifolium* seeds contribute to these results (Carvalho & Nakagawa, 2012; Venâncio & Martins., 2019).

The *A. pyrifolium* seeds, as some other seeds are composed of different layers of organic tissue (Miano et al., 2019) and miscellaneous substances, that protect and/or nurture the embryo (Ferreira & Cunha, 2000; Andrade Júnior et al., 2020). Although *A. pyrifolium* seeds can lose their normal viability by environmental factors (Pereira et al., 2016; Andrade Júnior et al., 2020). The treatment used in this paper can be considered a decreasing agent to *A. pyrifolium* seeds (Tables 1 and 2, Figure 1). Probably, it can be explained due to morphological characteristics of *A. pyrifolium* seeds (Ferreira & Cunha, 2000; Oliveira et al., 2015; Pereira et al., 2016). Nevertheless, the US and MW used in this paper can reach inside the embryo or other tissues and kill them (Oliveira et al., 2015; Pereira et al., 2016).

Similar to the results obtained in this paper, Oliveira et al. (2015) affirmed that *Aspidosperma subincanum* seeds are sensitive to high temperatures, decreasing the germination and vigor. Probably, *A. pyrifolium* seeds show similar sensitivity MW assay, when inside in the substance, allows an increase in the temperature of the bodies exposed to this type of radiation due to the agitation of their molecules and consequently their dehydration (Miano et al., 2019; Rifna et al., 2019; Venâncio & Martins, 2019; Andrade Júnior et al., 2020). Even with germination occurring in all treatments, the physiological quality of seedlings decreased and an increase in the amount of non-germinated seeds can still be seen, probably due to such biological action (Miano et al., 2019; Rifna et al., 2019; Venâncio & Martins, 2019).

Microwaves were used for drying *Curcubita melo* (Diniz et al., 2020), *Oryza sativa* (Rifna *et al.*, 2019), *Avena sativa* (Miano et al., 2019; Rifna et al., 2019) and *Parkia multijuga* (Miano et al., 2019), for example. All these authors described that the seeds when submitted to this process lose water by capillarity, from the inside to the outside of the tissues. They also described that temperatures above 70 °C can be recorded in these seeds if the exposure period and frequency are extended (Miano et al., 2019; Rifna et al., 2019; Venâncio & Martins, 2019).

Reddy et al. (1998), Banic et al. (2003) and Knox et al. (2013) described that there is a marked biological effect with the use of MW in seeds. For some species, according to them, the effect was positive in promoting germination (rice and wheat) and for others, it was harmful (spontaneous herbs). It has been written that the technology can be used as a technique for drying and making the seed embryo unfeasible for other purposes, such as its use in the cosmetic or medicine industry.

Linking the information previously described, we realized that the waves of MW could be used for drying *A. pyrifolium* seeds for different purposes. It is important to note that *A. pyrifolium* is already used for different purposes and that this technology can be tested as part of the production process of herbal medicines and their derivatives (Gomes, 2019).

Regarding the results obtained for the US test, we can describe that the increased exposure periods promoted the mortality of *A. pyrifolium* seeds (Figure 1; Table 2). In contrast, Miano et al. (2019) and Venâncio & Martins (2019) discribed that the US use improved the vigor and germination in *Phaseolus vulgaris* and *Senna multijuga* seeds, respectively. The use of US in *A. pyrifolium* seeds can be useful for the extraction of organic compounds present in the seeds, according to Rifna et al. (2019), that described the benefits of extracting organic components using US from plant tissues. Probably, for *A. pyrifolium* seeds, the US frequencies and the period of exposure promoted greater wear of all tissues (Miano et al., 2019; Venâncio & Martins, 2019).

Conclusions

The ultrasound (US) and microwave (MW) and treatments are not effective to improve the viability of *Aspidosperma pyrifolium* seeds, under the conditions studied in this research. We suggest tests with a combination of other factors to understand whether physical agents tested in this research can promote the germination of *A. pyrifolium* seeds.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

Thiago Costa Ferreira: Conceptualization, formal analysis, investigation, methodology, writing – original draft and writing – review & editing.

Fábia Shirley Silva Ribeiro: Methodology and supervision.

Manoel Rivelino Gomes de Oliveira: Methodology and supervision.

Aldrin Martin Pérez-Marin: Supervision

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