



Do ultrasound, microwave and water immersion promote vigor to *Libidibia ferrea* seeds?

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Abstract - The aim of this paper was to test ultrasound (US) and microwave (MW), with/or not water immersion (WI) to infer their effect on promoting vigor to seeds of *Libidibia ferrea* (Mart. Ex Tul.) L.P. Queiroz. *L. ferrea* seeds were submitted to factorial design 2 x 5, where F1 = WI and F2 = physical treatments (MW - 0, 5, 10, 15 and 20 s or US - 0, 1, 2, 3 and 4 min). We analyzed according to Brazilian Rules of Seeds Analyses and statistical program R. The treatments were not effective to improve vigor to *L. ferrea* seeds.

Ultrassom, microondas e imersão em água promovem vigor em sementes de *Libidibia ferrea*?

Resumo - O objetivo deste trabalho foi avaliar o efeito no vigor de sementes de *Libidibia ferrea* (Mart. Ex Tul.) L.P. Queiroz por tratamentos com ultrassom (US) e microondas (MO), com ou sem imersão em água (IA). Os testes foram em esquema fatorial 2 x 5, onde F1 = WI e F2 = tratamentos físicos (MO - 0, 5, 10, 15 e 20 s ou US - 0, 1, 2, 3 e 4 min). As análises seguiram as Regras Brasileiras de Análise de Sementes, utilizando o programa R. Os tratamentos não foram eficientes para melhorar o vigor de sementes de *L. ferrea*.



Caatinga biome (Brazilian dry season forest) have unique characteristics of hard environmental conditions (high temperature and evapotranspiration's rate), and presence of xerophilous species (Medeiros Filho et al., 2005; Silva et al., 2017). This biome covers 844,453 km² of the Brazilian Northeast region (Embrapa Territorial, 2022). There are some forest species that produce seeds with dormancy (Medeiros Filho et al., 2005; Silva et al., 2017) and *Libidibia ferrea* (Mart. Ex Tul.) L.P. Queiroz (Fabaceae) [synonym: *Caesalpinia ferrea*] is one of those (Walter et al., 2019). This

species may be used for several purposes, as medicinal, pharmaceutical, and wood, forage and energy production (Urquiza et al., 2019).

The seed dormancy is a big problem to the establishment of *L. ferrea* seedlings in nursery (Walter et al., 2019), as *L. ferrea* seeds present physical dormancy (cutaneous) (Urquiza et al., 2019). Acid treatments are recommended to *L. ferrea* seeds, but this technique could cause serious damage to seeds, seedlings and to the environmental (Melo et al., 2017; Silva et al., 2017; Bragante et al., 2018). Alternative methods can be tested to *L.*

ferrea seeds to break dormancy. According to Rifna et al. (2019), ultrasound (US), microwaves (MW) and water immersion (WI) could be cited in promoting vigor to forest seeds. Thus, this work aims to test these treatments to promote vigor to *L. ferrea* seeds.

The experiments were carried out in Seed Technology Laboratory, in National Institute of Semi-Arid Region (Campina Grande, Paraíba State, Brazil).

We used seeds of *L. ferrea* stored under 10 °C in plastic bags for six months (Urquiza et al., 2019). The seed lots were evaluate by the Center for Ecology and Environmental Monitoring at the Federal University of Vale do São Francisco (NEMA/UNIVASF), reaching a germination percentage of 86% at harvest.

Two different and independent assays were carried out in this research: microwave (MW) and ultrasound (US). For MW assay, the seeds were submitted for 0, 5, 10, 15, and 20 s, in Petri dishes, in a Panasonic microwave, model Piccolo Style. For US assay, the seeds were submitted to 0, 1, 2, 3 and 4 min, at 25 ± 2 °C using ultrasound bath (model STD; frequency of 42 kHz; BioWash), as mentioned by Venâncio & Martins (2019). For each treatment, 200 seeds were used. In both cases, a half part of treated seeds were immersed in water for 24 h (20 °C temperature, in the dark).

After preparation, the seeds of each replication were sowed and rolled on germitest paper. The germitest paper was embedded in water in the proportion of 2.5:1 water/weight in dry paper (Brasil, 2009). The roll papers were incubated at 25 ± 2 °C, for 14 days, under 12 h of photoperiod. No acid treatment was carried out on the in *L. ferrea* seeds.

The evaluated variables were: percentage of first count (FC), germinated seeds (G), normal seedlings (NS), abnormal seedlings (AS), non-germinate seeds (NG) and the ratio G/NG (Brasil, 2009; Andrade Júnior et al., 2020). The assays were designed in a factorial scheme 5 x 2, where the F1 is the exposition period to MW or US (regression analysis), and F2 is immersion in water (immersion or non-immersion). Both assays were designed in completely randomized design (CRD) (Venâncio & Martins, 2019), in R program.

According to p-value results from ultrasound assay, for immersion factor, we observed that all analyzed variables were significant, except the G/M ratio. When considering the exposition periods, all variables were significant, and for the interaction between the analyzed factors only NS and NG were significant. The non-immersion treatments

showed better results than immersion by Tukey test. In relation to the exposition periods, the averages decreased when exposure increased, analyzed by quadratic models. And for interaction, the non-immersion treatments showed better results when compared to the others (Table 1).

We observed that for all analyzed variables the non-immersion treatment presented better results than immersion, except for AS and NG (Table 2). On the other hand, the variables presented worst results when the exposure increased (time of exposition), excepted to AS and NG.

The interaction between the studied factors results were important to point out that the non-immersion treatments are better than immersion treatments. According to p-values of microwave assay, and period of expositions all variables showed significant results, except FC, however this variable was the only one to show significant results when submitted to immersion. The interaction between the analyzed factors points out that only AS, NG and G/NG ratio variables were non-significant. There was no statistical difference between average and the equations obtained in this assay.

The protection to environmental factors (Walter et al., 2019) and acid treatments are recommended to break seeds dormancy (Melo et al., 2017; Silva et al., 2017). The physical treatments tested in this paper did not efficiently promote the decrease of this natural characteristic (Tables 1 and 2). Different theories can be used to explain these results. For example, Bracante et al. (2018) mentioned that *L. ferrea* seeds, when in contact with adverse environmental conditions decrease their normal physiology, may even deteriorate to death. Moreover, Albuquerque et al. (2019) and Santos et al. (2019) reported that *L. ferrea* seeds presented resistance even to non-extreme environmental conditions. Nevertheless, some dormancy break treatments, as acid scarification can be harmful to some *L. ferrea* seeds (Melo et al., 2017; Silva et al., 2017; Bragante et al., 2018; Lima et al., 2019). Lima et al. (2019) mentioned that *L. ferrea* seeds can be submitted to mechanical scarification with sandpaper, and this action is more effective than acid treatments and less invasive.

Using ultrasound exposure could be effective, as positive results are available in the literature. The ultrasound waves could be used to extract substance components in organic tissues (Rifna et al., 2019). Araújo et al. (2018) described that the US would lixiviate organic components from *L. ferrea* seeds

and the immersion in water would potentiate this fact (during or after treatment).

The lixiviate organic components are important to germination, because they can cause their inhibition, for example, Araújo et al. (2018) mentioned that gibberellin acid when present in *L. ferrea* seeds may inhibit their germination.

The MW assay, without the presence of water immersion, did not promote germination of *L. ferrea* seeds (Table 2). Probably, these waves were harmful

for *L. ferrea* seeds due to the high frequency and temperature (Melo et al., 2017; Silva et al., 2017). These results are similar to those described by Rifna et al. (2019) when affirmed that MW can cause lesions in seeds of *Beta vulgaris* and *Daucus carota*. Nevertheless, they were against those described by Lima et al. (2006), Melo et al. (2017) and Silva et al. (2017), that reported positive results with seeds of rice, sunflower, and soybeans.

Table 1. P-value, averages, Tukey test and regression equation to variables: percentages of first count (FC), germinated seeds (G), normal seedlings (NS), abnormal seedlings (AS), and non-germinate seeds (NG) and the ratio G/NG to *Libidibia ferrea* seeds treated with different periods of exposition to ultrasound (F1) and water immersion (F2), in factorial scheme.

	Variables					
	FC	G	NS	AS	NG	G/NG
P-value						
F1	**	**	**	**	**	NS
F2	*	**	**	*	**	**
F1 x F2	NS	NS	**	NS	**	NS
CV (%)	20.65	13.95	20.79	39.56	10.24	118.46
General average	13.40	31.75	20.60	11.35	43.25	0.99
Averages						
Immersion						
Non-immersion	17.00 b	43.60 b	27.40 b	6.50 a	30.10 a	1.26 a
Immersion	9.80 a	19.90 a	13.85 a	16.20 b	56.40 b	0.72 a
Standard Error	0.61	0.99	0.95	1.00	0.99	0.26
DMS	1.79	2.87	2.78	2.91	2.87	0.76
Averages						
Period of exposition (min)						
0.00	15.75	53.87	42.12	11.75	21.12	2.58
1.00	13.62	31.62	19.97	13.00	43.37	0.76
2.00	14.00	32.50	17.12	15.12	42.50	0.78
3.00	12.75	28.25	17.25	11.00	46.75	0.62
4.00	10.87	12.50	6.75	5.87	62.50	0.23
Equations	$Y = -0.08x^2 - 0.74x + 15.36$	$Y = 0.56x^2 - 10.86x + 50.09$	$Y = 1.88x^2 - 14.86x + 39.08$	$Y = -1.35x^2 + 4.05x + 11.39$	$Y = -0.56x^2 + 10.87x + 24.89$	$Y = 0.19x^2 - 1.25x + 2.34$
R ²	0.89	0.85	0.95	0.94	0.85	0.85
Error	0.97	1.56	0.95	1.58	1.56	0.26

F1 = Period of exposition to US (0, 1, 2, 3 and 4 min) and F2 = immersion in water (immersion or non-immersion). ** and * = significative at 1% and 5% level, respectively, NS = non-significative (p-value).

Table 2. P-value, averages, Tukey test and regression equation to variables: percentages of first count (FC), germinated seeds (G), normal seedlings (NS), abnormal seedlings (AS), and non-germinate seeds (NG) and the ratio G/NG to *Libidibia ferrea* seeds treated with deferment period of exposition to microwave (F1) and water immersion (F2), in factorial scheme.

	Variables					
	FC	G	NS	AS	NG	G/NG
	p-value					
F1	NS	**	**	*	*	*
F2	**	NS	NS	NS	NS	NS
F1 x F2	**	NS	NS	NS	NS	NS
CV (%)	16.35	11.36	16.59	39.56	56.40	118.46
General average	19.60	43.60	27.40	16.20	8.78	0.99
Immersion	Average					
Non-immersion	22.20	43.60 a	27.40 b	17.20 a	56.40 a	1.26 a
Immersion	17.00	43.60 a	13.85 a	16.20 a	57.00 a	1.31 a
Standard Error	0.71	1.10	0.95	1.43	1.10	0.35
DMS	2.06	3.19	2.78	4.13	3.19	1.03
Period of exposition (min)	Averages					
0.00	19.00	81.50	66.00	15.50	18.50	4.16
1.00	19.00	42.50	23.50	19.00	57.50	0.75
2.00	19.75	43.00	21.50	21.50	57.00	0.76
3.00	22.25	37.50	22.00	15.50	62.50	0.60
4.00	18.00	13.50	4.00	9.50	86.50	0.15
Equations	-	-	-	-	-	-
R ²	-	-	-	-	-	-
Error	1.13	1.75	1.60	2.26	1.75	0.56

F1 = period of exposition to MW (0, 5, 10, 15 e 20 s) and F2 = immersion in water (immersion or non-immersion). NS = non-significant (p-value). ** significant at 1%, level * significant at 5% level

Conclusions

The use of ultrasound, microwave, and water immersion treatments do not improve vigor to *Libidibia ferrea* 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; writing, review & editing. **Fábia Shirley Silva Ribeiro:** methodology. **Manoel Rivelino Gomes de Oliveira:** methodology **Aldrin Martin Pérez Marin:** supervision.

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