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Seed vigor of *Piptadenia moniliformis* Benth. subjected to thermal stress

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ABSTRACT. The objective of this work is to evaluate the influence of accelerated aging test at 41ºC on the germination and seed vigor of *Piptadenia moniliformis* Benth. Two experiments were conducted. Experiment 1: seeds were submitted to dormancy overcoming treatments. They were immersed into sulfuric acid for 0 (intact seeds), 5, 10, 15, 20 and 25 minutes. Experiment 2: two methods were evaluated for the accelerated aging test at 41ºC, that is, the traditional method and the saturated NaCl solution method with exposure periods of 0, 24, 48, 72 and 96 hours. After each treatment, the seeds were subjected to electrical conductivity and germination tests. Seeds overcame their dormancy when immersed into concentrated sulfuric acid for 5, 10, 15, 20 or 25 minutes, but the germination was faster when immersed for 20 minutes. The different methods for the accelerated aging test, decreased the germination potential and seed vigor of *P. moniliformis* after 24 hours of exposure. Regarding the method used, the saturated solution provided, in general, the best results since the seed water content was lower, thus reducing the proliferation of fungi. The electrical conductivity test showed that, by increasing the aging period regardless of the method, there is an increase in seed deterioration, corroborating with germination results, which showed that the correlation was moderate and negative and indicated that the higher the conductivity, the lower the percentage and the germination speed index. The immersion of seeds of *P. moniliformis* into concentrated sulfuric acid for 20 minutes provides a fast and increased germination. The methods for accelerated aging at 41ºC, traditional and saturated solution, can be used as vigor tests to evaluate the physiological quality of seeds.

Keywords: deterioration; accelerated aging; forest species; germination.

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Introduction

The duration and intensity of the seed deterioration process during storage are determined mainly by the interaction between genetic inheritance, water content/relative humidity and ambient temperature, causing physiological, biochemical, physical and cytological changes. Thus, there is a reduction of vigor, culminating in the death of the seed (Marcos Filho, 2015). In order to simulate what occurs during storage, the seeds are submitted to the accelerated aging test, considered a stress vigor test.

Accelerated aging is one of the most widespread vigor tests due to its accuracy and sensitivity in detecting quality differences among seed lots with a similar germination (Pereira, Torres, & Linhares, 2015). This test aims to evaluate seed resistance after a period of exposure to high temperatures and relative humidity (Marcos Filho, 2015). Temperatures above 40°C are known to cause a decreased capacity for RNA and protein biosynthesis, mitochondrial degeneration, increased chromosome fragmentation, membrane lipid peroxidation, and enzymatic changes such as reduced activity of lipases, amylases, proteases, peroxidases, among others (Dotto & Silva, 2017; Bewley, Bradford, Hilhorst, & Nonogaki, 2013; Mahjabin & Abidi, 2015). This results in a decreased germination speed and percentage, as well as in an increased formation of abnormal seedlings (Marcos Filho, 2015). Thus, seed lots that maintain a high germination rate after accelerated aging are considered of high vigor when compared to lots with a reduced viability (Pereira, Martins Filho, & Laviola, 2012).

The species *Piptadenia moniliformis* Benth., belonging to the Fabaceae family, is a native species to the Northeastern Brazil. It has a height of four to nine meters, and its wood is heavy, of a medium texture, a medium mechanical resistance and a good natural durability (Lorenzi, 2002). This species presents

dormancy due to impermeability of the integument to water, characteristic of this family and others such as Malvaceae, Geraniaceae, Chenopodiaceae, Convolvulaceae, Solanaceae, and Liliaceae (Carvalho & Nakagawa, 2012). Due to its small size, Lorenzi (2002) emphasized that this species can be used in small works of civil construction, in light joinery and in coal production. It is also indicated for heterogeneous reforestation for preservation purposes due to its rusticity and fast growth.

Considering the increased demand for seeds of high-quality native forest species, mainly to support mixed reforestation works, it is essential that seed quality assessment methods be efficient. The objective of this work is to evaluate the influence of accelerated aging test on the physiological quality of *P. moniliformis* seeds.

Material and methods

Experiment site

The experiments were carried out at the *Universidade Federal Rural de Pernambuco* - Serra Talhada Academic Unit, Brazil. The seeds of *P. moniliformis* were granted by the Ecology and Environmental Monitoring Center (NEMA) of the city of Petrolina, PE, Brazil.

Overcoming the integument dormancy of *P. moniliformis* seeds

To overcome dormancy, the seeds were submitted to different periods of immersion into concentrated sulfuric acid (95-97%), *i.e.*, 0 (intact seeds), 5, 10, 15, 20 and 25 minutes, totaling six treatments and five replications of 20 seeds per treatment. After immersion, the seeds remained for ten minutes under running water to eliminate any acid residues.

Subsequent to dormancy overcoming treatments, the seeds were sown on two sheets of blotting paper arranged in Gerbox plastic boxes previously moistened at 2.5 times their dry weight. The germination test was conducted in a climate-controlled room at an average temperature of 24ºC and an average RH of 35%, constant light, for ten days. Daily counts of the number of germinated seeds were performed until germination stabilization, adopting as a criterion seeds with a primary root protrusion of 2 mm. During the conduction of the germination test, the germination speed index (GSI) (Maguire, 1962) and the mean germination time (MGT) were obtained (Labouriau, 1983). At the end of the test, the germinated seeds were counted to determine germination percentage (GP). The immersion period in the concentrated sulfuric acid that provided the best germination in the shortest time (20 minutes) was used for overcoming the dormancy of seeds submitted to the accelerated aging test.

Evaluation of the methodology of accelerated aging test of *P. moniliformis* seeds

After immersing seeds of *P. moniliformis* into sulfuric acid for 20 minutes, the seeds were subjected to two methods for the accelerated aging test at 41ºC and five aging periods (0, 24, 48, 72 and 96 hours) in a 2x5 factorial design.

Accelerated aging test (traditional method): it was conducted using Gerbox plastic boxes containing 40 mL of water inside and a suspended stainless-steel mesh tray, where the seeds were distributed forming a uniform layer. The boxes were kept in an incubator for four aging periods: 24, 48, 72 and 96 hours at 41ºC and 100% relative humidity, besides the control.

Accelerated aging test using saline solution: instead of using only H_2O inside the plastic boxes, 40 mL saline solution prepared with sodium chloride (40 g of NaCl in 100 mL of distilled water) were used to adjust the relative humidity to 76%, according to Jianhua and McDonald (1996).

Before and after each aging period, regardless of the method, the seed water content was determined by the greenhouse method at 105 ± 3ºC for 24 hours, according to the Seed Analysis Rules (RAS) (Brasil. Ministério da Agricultura, Pecuária e Abastecimento, 2009). After each treatment, the seeds were subjected to electrical conductivity and germination tests.

The electrical conductivity test was performed with four replicates of 25 seeds per treatment. Seeds were initially weighed and placed in plastic cups containing 75 mL of distilled water for 24 hours at 25°C. After this period, the electric conductivity of the imbibition solution was determined by a benchtop conductivity meter, and the results were expressed as $\mu S.cm^{-1}.g^{-1}$.

Germination test: four replications with 25 seeds from each treatment were sown in Gerbox plastic boxes on two sheets of blotting paper previously moistened with distilled water at 2.5 times their dry weight. The germination test was conducted in a climate-controlled room at an average temperature of 24ºC and an

average RH of 35%, constant light, for ten days. Daily counts were performed of the number of germinated seeds until germination stabilization. The seed that emitted a primary root of at least 2 mm was considered germinated. The germination speed index (GSI) and the mean germination time (MGT) were evaluated according to Maguire (1962) and Labouriau (1983), respectively. On the tenth day, all germinated seeds were counted to determine germination percentage (GP).

The Pearson's simple correlation coefficients (r) were also calculated among electrical conductivity (EC) and germination percentage (GP), and germination speed index (GSI) and mean germination time (MGT) for the different methods for the accelerated aging test using the Excel software.

Experimental design and statistical analysis

The experiment was completely randomized. Data were submitted to analysis of variance (ANOVA), and the means of quantitative factors were submitted to regression analysis using the software SISVAR (Ferreira, 2011).

Results and discussion

Overcoming the integument dormancy of *P. moniliformis* seeds

The analysis of variance of the germination percentage and germination speed index (Table 1) of *P. moniliformis* seeds showed significant effects ($p<0.05$) when seeds were submitted to different periods of immersion into sulfuric acid to overcome dormancy since the mean germination time was not influenced by the treatments.

Table 1. Analysis of variance of germination percentage (GP), germination speed index (GSI) and mean germination time (MGT) of *Piptadenia moniliformis* Benth. seeds submitted to different periods of sulfuric acid immersion to overcome dormancy.

Variation Factor	DF	Mean Square		
		GP (%)	MGT (days)	GSI
Treatment	5	1,214.0**	0.020^{ns}	6.107**
Residue	24	50.417	0.017	0.325
CV%		9.10	18.88	11.79

Significant effect at 1% (**), at 5% (*) and not significant effect (^{ns}); coefficient of variation (CV); degree of freedom (DF)

For seed germination percentage of *P. moniliformis* seeds submitted to different periods of immersion into sulfuric acid, a quadratic behavior was verified for the different times. The values obtained for 5, 10, 15, 20 and 25 minutes of acid immersion were 78, 86, 83, 88 and 86%, respectively, while intact seeds showed a germination of 47% (Figure 1A).

By using different pre-germination treatments for *P. moniliformis* seeds, Azeredo, Paula, Valeri, and Moro (2010) found that periods of immersion into sulfuric acid (20, 25 and 30 minutes) provided a higher germination (values higher than 80%), while the immersion for 15 minutes resulted in less than 50% germination, whereas for the control, seeds that were not submitted to any germination treatment presented a germination of 15%. In turn, Benedito, Torres, Ribeiro, and Nunes (2008), using sulfuric acid, observed a germination of 81 and 82% when seeds were immersed for 10 and 15 minutes, respectively, while the immersion for 20 minutes resulted in a germination of 58%. The variation in germination found in the present study and by Azeredo et al. (2010) and Benedito et al. (2008), using seeds immersed into sulfuric acid for the same period, may be related to the degree of dormancy in which the seed was, since the intensity of dormancy varies depending on seed age from plant to plant, or even among seeds of a same plant, besides the influence of environmental conditions in which the seeds formed.

The proportion of hard seeds is higher when the plant is exposed to relative water deficiency during the seed maturation process, more specifically during the dry mass accumulation period (Marcos Filho, 2015). In this context, in the Northeast region of Brazil, characterized by an irregular distribution of rainfalls and high temperatures, there are many native forest species whose seeds present integumentary dormancy as a survival strategy. Still, the degree of dormancy may vary depending on seed size, in which smaller seeds have a higher proportion and degree of dormancy.

Regarding vigor characterized by GSI, the control presented a lower germination speed for the other treatments, as the seed immersion period increased the speed index. It can be observed that seeds immersed for 20 minutes provided a higher germination speed.

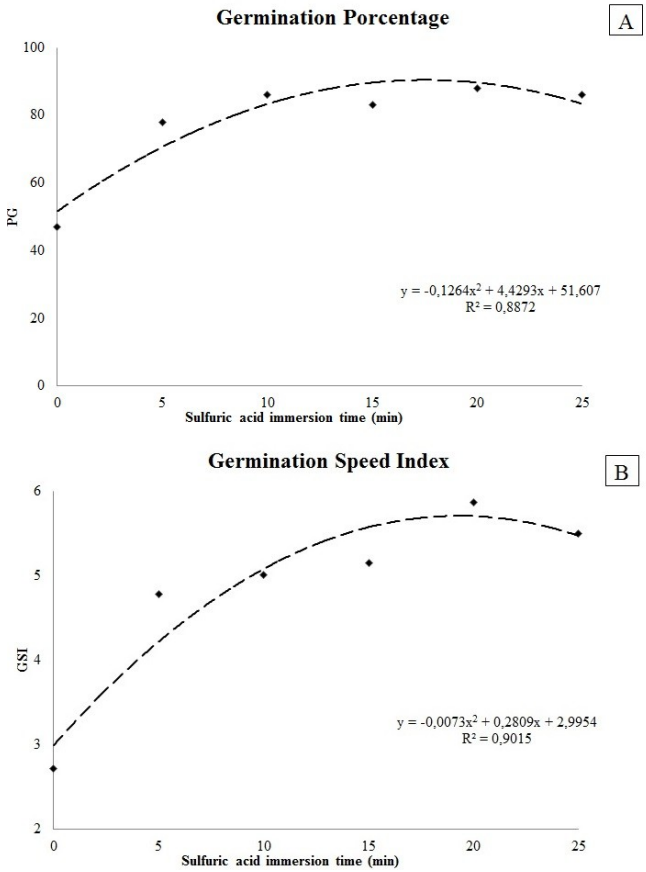

Figure 1. Germination percentage (GP) (A) and germination speed index (GSI) (B) of *Piptadenia moniliformis* Benth. seeds submitted to different periods of sulfuric acid immersion to overcome dormancy.

The use of sulfuric acid is one of the most common methods to overcome dormancy of species with an impermeable integument. It modifies the permeability of membranes, allowing the water to be absorbed, which is essential to germination. It thus starts the germination process (Dousseau, Alvarenga, Castro, Arantes, & Nery, 2007). In addition, ruptures in the integument allow gas exchanges and suppresses mechanical resistance to protrusion of the primary root. It also facilitates embryo expansion.

By overcoming dormancy, the immersion process into water accelerates. The seeds tend to germinate faster, as can be observed for seeds that were immersed into sulfuric acid for 20 minutes. Therefore, the consumption of energy reserves during the germination process will be lower. Such reserves thus contribute to the development of seedlings (Souza, Araújo, Pinto, & Brito, 2016).

Evaluation of the method for accelerated aging test of *P. moniliformis* seeds

Seeds after dormancy overcoming and subsequent submission to the accelerated aging test at 41ºC for different periods were evaluated as for water content, as it may interfere with the results. According to Bewley and Black (1994), drier seeds, because they have a low matric potential, absorb water quickly when placed in a humid atmosphere. The seed water content values obtained before and after the accelerated aging test (Table 2) show that, according to the traditional accelerated aging test method, there is a marked increase in seed moisture considering that water content was initially 9.06% and that after overcoming dormancy (immersion into sulfuric acid for 20 minutes), the observed content was 22.17%. After 24 hours of aging, a value of 44.49% was reached, reaching 54.77% after 96 hours of exposure to accelerated aging.

Table 2. Water content (%) of seeds of *Piptadenia moniliformis* Benth. before and after the accelerated aging test, traditional methods and saturated solution at 41ºC for 24, 48, 72 and 96 hours

Accelerated aging methods	Aging period (hours)				
	0	24	48	72	96
Traditional	9.06*	22.17**	44.49	52.39	54.86	54.77
Saturated solution	9.11*	22.19**	20.39	18.57	17.39	18.71

Initial water content *before and **after overcoming seed dormancy.

When the seeds aged using the saturated solution method, the initial water content was similar as the previous method (22.19% after overcoming dormancy). However, a decrease in water content was observed over the aging periods, reaching 20.39% after 24 hours and 18.71% after 96 hours. According to Marcos Filho (2015), the water content corresponds to the hygroscopic equilibrium point, which increases or decreases due to relative humidity. The salt present in the solution contributes to a lower evaporation of water molecules. Therefore, the seeds absorb less water, which reflects in a lower water content. Thus, seeds in contact with air, whose relative humidity is 100%, will present a higher water content than seeds in contact with air at 76% relative humidity. By subjecting seeds of *Tabernaemontana fuchsiaefolia* to accelerated aging using both traditional and saline methods, Moraes, Lopes, Farias, and Maciel (2016) observed a higher incidence of fungal attacks in traditional aging treatments, corroborating the observations of the present study. Carvalho and Nakagawa (2012) emphasized that the increase in water content favors the increase in seed temperature due to the activation of the respiratory process and the greater activity of microorganisms.

Due to the high humidity found in seeds exposed for 72 and 96 hours during the accelerated aging test using the traditional method (exclusive use of water inside the plastic box), there was proliferation of microorganisms. It is therefore a disadvantage to use the traditional method in comparison to the saturated solution method.

The results of analysis of variance of the variables germination percentage, germination speed index and electrical conductivity had significant effects ($p<0.05$) when the seeds were subjected to different methods and periods of exposure to accelerated aging (Table 3). Both factors evaluated interfered simultaneously with the seed vigor of *P. moniliformis* seeds. Mean germination time was not influenced by the treatments.

Table 3. Analysis of variance of the variables germination percentage (GP), germination speed index (GSI), mean germination time (MGT) and electrical conductivity (EC) of *Piptadenia moniliformis* Benth. seeds in function of methods (MEA) and periods (PEA) of exposure to accelerated aging at 41°C.

Variation Factor	DF	Mean Square			
		GP (%)	MGT (days)	GSI	EC ($\mu S.cm^{-1}.g^{-1}$)
MEA	1	193.6*	0.017^{ns}	0.769*	219,293.6**
PEA	4	6,686.6**	0.033^{ns}	20.8**	19,849.2**
MEA x PEA	4	150.60*	0.034^{ns}	0.509*	13,040.8**
Residue	30	40.0	0.626	0.148	198.6
Total	39				
CV%		18.17	66.30	20.43	7.74

Significant effect at 1% (**), at 5% (*) and not significant (^{ns}); coefficient of variation (CV); degree of freedom (DF)

By using the traditional accelerated aging test method, there was a marked decrease in germination between the 0- and 24-hour periods (Figure 2A). The germination was 84 and 44%, respectively. The aging periods 48, 72 and 96 hours provided a very low germination. The period of 96 hours provided 1% of germination, possibly because of the higher degree of deterioration. With the reduction in vigor, there is also a decrease in respiratory rates, indicating a decrease in the number and/or efficiency of mitochondria, which are organelles responsible for producing a great amount of ATP, thus culminating in deleterious interferences with vital processes of seeds (Marcos Filho, 2015).

A similar behavior was observed using the NaCl saturated solution method (Figure 2A). The seeds that did not age presented a germination of 74%. For the 24-hour aging period, the germination was 52%. Therefore, there was a reduction of 22% in relation to the control (absence of aging). For the periods 48, 72 and 96 hours, the germination was 26, 20 and 13%, respectively. Salt, by limiting the evaporation of water molecules, contributed to a lower water content, thus reflecting a lower degree of deterioration, *i.e.*, a greater vigor, as can be observed by germination values and germination speed index. Temperatures above 40°C are known to cause protein denaturation, both structural and enzymatic, causing changes in the degradation and mobilization of stored seed reserves, thus reducing the energy available for germination (Dotto & Silva, 2017).

Data regarding the germination speed index (GSI) of *P. moniliformis* seeds in function of the different treatments presented a decreasing linear behavior. Using the traditional methodology, the periods 0, 24, 48, 72 and 96 hours provided values of 4.68, 2.14, 0.88, 0.96 and 0.05, respectively, in relation to the means obtained using the saturated solution method: 4.18, 2.77, 1.45, 0.98 and 0.71, respectively (Figure 2B). Comparing both methodologies in the periods of 24, 48 and 96 hours, the saturated solution method provided a higher germination rate.

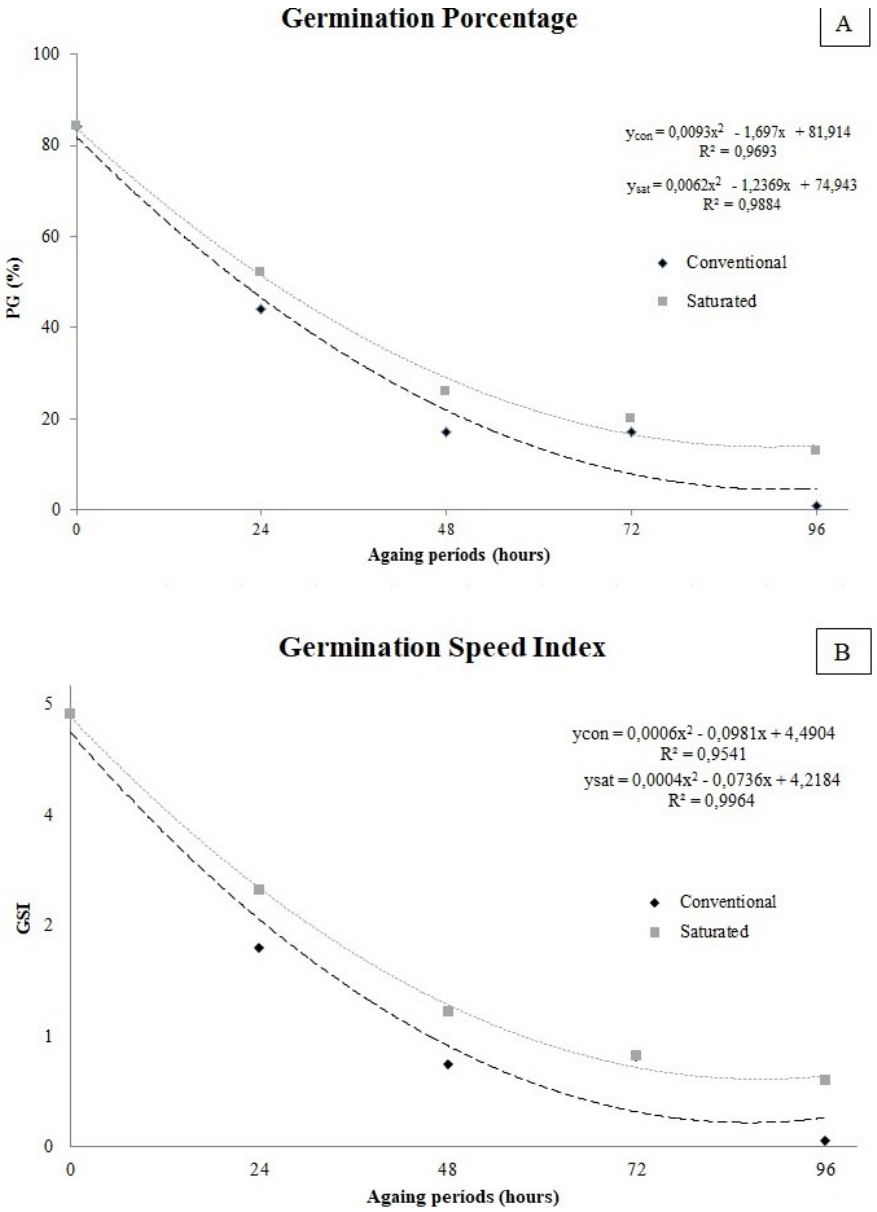

Figure 2. Polynomial regression of the mean values of germination percentage (PG) and germination speed index (GSI) of *Piptadenia moniliformis* Benth. seeds submitted to different methods for the accelerated aging test at 41°C for different periods.

As the seeds aged, there was a decrease in vigor determined by the electrical conductivity test (Figure 3). It detected a significant increase in the amount of leachate from seeds subjected to aging using the traditional method. The seeds that aged for 96 hours presented a higher amount of leachate compared to seeds that aged during the other periods. Regarding the methodology using saturated solution, the values did not oscillate much among aging periods. The membrane system, which delimits the organelles and other cellular components, gradually organizes itself during seed maturation, reaching its maximum organization near physiological maturity. The deterioration process causes the loss of integrity of this system, thus releasing essential constituents to germination (Marcos Filho, 2015). By using the electrical conductivity test on *Erythrina velutina* Willd seeds previously submitted to accelerated aging, Guedes et al. (2009) found that seed vigor was greatly affected during accelerated aging, as evidenced by the increase in the amount of leachate released by seeds.

When the accelerated aging test methodologies were compared, the saturated solution caused a lower leachate release compared to the traditional method for the 72- and 96-hour periods. This resulted in a lower intensity of deterioration due to a lower evaporation rate of water molecules obtained by adding NaCl to water until saturation.

When correlating electrical conductivity with the percentage and germination speed index (Table 4), it is possible to observe a negative correlation of using both accelerated aging test methodologies, *i.e.*, as there was an

increase in the values of electrical conductivity, germination and speed index decreased. For the mean germination time, there was a positive correlation when seeds aged by the traditional method because, as the release of exudates increased, there was an increase in the time required for seeds to germinate.

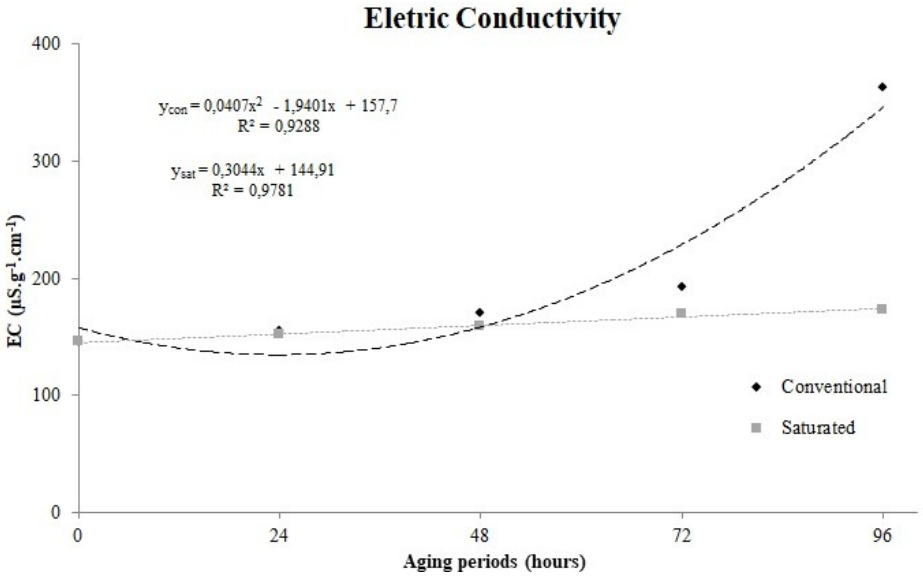

Figure 3. Polynomial regression of the mean values of electrical conductivity (EC) of *Piptadenia moniliformis* Benth. seeds submitted to different methods for accelerated aging test at 41°C for different periods.

Table 4. Simple Pearson's correlation (r) among electrical conductivity (EC) and the variables germination percentage (GP), germination speed index (GSI) and mean germination time (MGT) of *Piptadenia moniliformis* Benth seeds in function of methods for the accelerated aging at 41°C for different periods.

	Traditional accelerated aging		
	GP	GSI	MGT
EC	-0.649	-0.627	0.080
	Accelerated aging using saturated solution		
	GP	GSI	MGT
EC	-0.799	-0.802	-0.122

The disruption of the cell organelle membrane system, evidenced by the progressive increase in the amount of leachate, *i.e.*, higher EC values, makes seeds more susceptible to the deleterious effects of O_2. In view of a stress situation, there are increases in the concentration of reactive oxygen species (ROS) in cells, culminating in membrane damage from protein degradation and from DNA and RNA molecules, impaired enzymatic activity, carbohydrate oxidation, and lipid peroxidation (Mittler, 2002). Reductions in the activity of lipase, amylase, protease, peroxidase, among others, decrease the rate of digestion of reserve substances, consequently reducing both the rate and the percentage of germination. Therefore, there is a process called oxidative stress, which corresponds to the oxidative destruction of the cell (Choudhury, Rivero, Blumwald, & Mittler, 2017). According to Bewley and Black (1994), oxidation causes reserves to be consumed faster, reflecting in the reduction of seed germination potential, which can be verified in the present work. Furthermore, the researchers point out that unsaturated fatty acids are the main targets of free radicals. Because mitochondrial membranes are very rich in unsaturated fatty acids, there is an increased likelihood of lipid peroxidation. In addition to affecting membrane permeability, peroxidation acts on the respiratory activity, directly affecting seed performance. It is noteworthy that the visible mycelial growth in seeds aged for 72 and 96 hours using the traditional method, a growth caused by a higher water content, contributed to intensify the disorganization of the cell membrane system, consequently favoring a greater loss of leachate.

Conclusion

The immersion of *P. moniliformis* seeds into concentrated sulfuric acid for 20 minutes provides a fast and increased germination.

The methods for accelerated aging test at 41ºC, *i.e.*, traditional and saturated solution, affect the physiological quality of *P. moniliformis* seeds.

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