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Evaluation of pre-germinative treatments in seeds of *Cereus jamacaru* DC subsp. *jamacaru* (Cactaceae)

Avaliação de tratamentos pré-germinativos em sementes de *Cereus jamacaru* DC subsp. *jamacaru* (Cactaceae)

Evaluación de tratamientos pre-germinativos en semillas de *Cereus jamacaru* DC subsp. *jamacaru* (Cactaceae)

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Abstract

Cereus jamacaru DC subsp. *jamacaru* (mandacaru) is an endemic species in Brazil belonging to Cactaceae of great ecological importance, as it is a pioneer in the colonization of arid and inhospitable environments, and contributes directly to the food chain for its fruits highly appreciated by fauna. Therefore, this work aimed to evaluate, through chemical scarification, the germinative behavior of *C. jamacaru* subsp. *jamacaru*. The cactus seeds were collected in May 2016 in the city of Quixelô-CE-Brazil. The acids used in chemical scarification were acetic acid, hydrochloric acid, propionic acid and sulfuric acid for 5, 10 and 15 minutes. The seeds were placed in Petri dishes, and placed to germinate in a B.O.D. with a light/dark cycle of 12 hrs at 30 ºC. Daily readings were performed and analyzed: percentage of germination, Germination Speed Index (GSI) and mean germination time (Tm). The results indicate that acids negatively interfere in seed germination when compared to the control group, mainly acetic and propionic acid, since at the end of the experiment only 8% of the seeds germinated when subjected to acetic acid, and 4% to the propionic. In addition, there was also a significant increase in Tm. Thus, water is the best method of obtaining a higher percentage of germination of *C. jamacaru* subsp. *jamacaru*.

Keywords: Mandacaru; Caatinga; Germination; Acids.

Resumo

Cereus jamacaru DC subsp. *jamacaru* (mandacaru) é uma espécie endêmica do Brasil pertencente à Cactaceae de grande importância ecológica, por ser pioneira na colonização de ambientes áridos e inóspitos, e contribuir de forma direta na cadeia alimentar por seus frutos extremamente apreciados pela fauna. Sendo assim, este trabalho objetivou avaliar por meio de escarificação química o comportamento germinativo das sementes de *C. jamacaru* subsp.

jamacaru. As sementes do cacto foram coletadas em maio de 2016 na cidade de Quixelô-CE-Brasil. Os ácidos utilizados nas escarificações química foram o ácido acético, ácido clorídrico, ácido propiônico e ácido sulfúrico durante 5, 10 e 15 minutos. As sementes foram acondicionadas em placas de Petri, e postas para germinar em câmara do tipo B.O.D. com um ciclo claro/escuro de 12 hrs a 30 ºC. Foram realizadas leituras diárias e analisadas: porcentagem de germinação, Índice de Velocidade de Germinação (IVG) e tempo médio para germinação (Tm). Os resultados apontam que os ácidos interferem de modo negativo na germinabilidade das sementes quando comparados com o grupo controle, principalmente o ácido acético e o propiônico, visto que ao final do experimento apenas 8% das sementes germinaram quando submetidas ao ácido acético, e 4% ao propiônico. Além disso, verificou-se também um aumentou significativo do Tm. Sendo assim, a água é o melhor método de se obter uma maior porcentagem de germinação de sementes de *C. jamacaru* subsp. *jamacaru*.

Palavras-chave: Mandacaru; Caatinga; Germinação; Ácidos.

Resumen

Cereus jamacaru DC subsp. *jamacaru* (mandacaru) es una especie endémica en Brasil perteneciente a Cactaceae de gran importancia ecológica, ya que es pionera en la colonización de ambientes áridos e inhóspitos, y contribuye directamente a la cadena alimentaria por sus frutos muy apreciados por la fauna. Por tanto, este trabajo tuvo como objetivo evaluar, mediante escarificación química, el comportamiento germinativo de *C. jamacaru* subsp. *jamacaru*. Las semillas de cactus fueron recolectadas en mayo de 2016 en la ciudad de Quixelô-CE-Brasil. Los ácidos utilizados en la escarificación química fueron ácido acético, ácido clorhídrico, ácido propiónico y ácido sulfúrico durante 5, 10 y 15 minutos. Las semillas se colocaron en placas de Petri y se colocaron para germinar en un B.O.D. con un ciclo de luz/oscuridad de 12 ha 30 ºC. Se realizaron y analizaron lecturas diarias: porcentaje de germinación, índice de velocidad de germinación (IVG) y tiempo promedio de germinación (Tm). Los resultados indican que los ácidos interfieren negativamente en la germinación de las semillas en comparación con el grupo control, principalmente ácido acético y propiónico, ya que al final del experimento solo el 8% de las semillas germinaron al ser sometidas a ácido acético, y el 4% al propiónico. Además, también hubo un aumento significativo en Tm. Así, el agua es el mejor método para obtener un mayor porcentaje de germinación de *C. jamacaru* subsp. *jamacaru*.

Palabras clave: Mandacaru; Caatinga; Germinación; Ácidos.

1. Introduction

The Cactaceae family is native to the Americas, where a high species richness occurs. It is very important in maintaining ecosystems, especially in the Caatinga, given the production of various resources for the local fauna, such as fruits, nectar, pollen and water, thus participating directly in the food chain (Judd, Singer, & Singer, 2009; Cavalcante, Teles, & Machado, 2013; Gomes, Meiado, Quirino, & Machado, 2016).

One of the species of this botanical family is *Cereus jamacaru* DC. subsp. *jamacaru* endemic to Brazil and popularly known as “mandacaru” (Cavalcante et al., 2013; Menezes, Taylor, & Loiola, 2013). It is a pioneer in the colonization of arid and inhospitable environments, especially rocks. Its fruits are appreciated by birds, which are the main responsible for the dispersion of its seeds, through endozoocoria. In this process, the seeds pass through the birds' digestive tract and are scarified with the hydrochloric acid present in their proventricle, thus the seed's forehead becomes thin and more susceptible to the entry of water and oxygen. the seeds are released into the environment, they will be stored in the feces to which they provide water and substrate for the beginning of their germination (Traveset, Riera, & Mas, 2001; Gomes, Quirino, & Araujo, 2014).

However, when the seeds of *C. jamacaru* subsp. *jamacaru* are released into the environment, some fail to germinate due to several factors. One of these is the allelopathic action of volatile terpenes produced by the secondary metabolism of other plants as a defense mechanism against herbivory (Bezerra et al., 2018; Bezerra et al., 2017). This fact can be observed in the field, where most individuals of "mandacaru" occur in isolation from other vegetables, leading to believe that some species of the Caatinga release allelopathic compounds and inhibit the germination of "mandacaru" seeds, thus decreasing, the ecological succession of the species (Brito, Nascimento, Coelho, & Félix, 2010). These volatile terpenes are known as essential oils and can act in different ways on seeds, among them harming water assimilation, nutrient uptake, protein synthesis and on the biochemical processes of germination (Torquato et al., 2020).

As much as individuals of the species *C. jamacaru* subsp. *jamacaru* produce many fruits and seeds and have endozoochoric dispersion, unfortunately it is not very common to frequently observe the presence of seedlings of this species in the field, such absence may be related to one or more specific requirements (Meiado, Rocha, Rojas-Aréchiga, & Leal, 2008). Therefore, it is necessary to find methods that help in the maximum germination of seeds in a short period of time, since the time of complete development of the plant is long and,

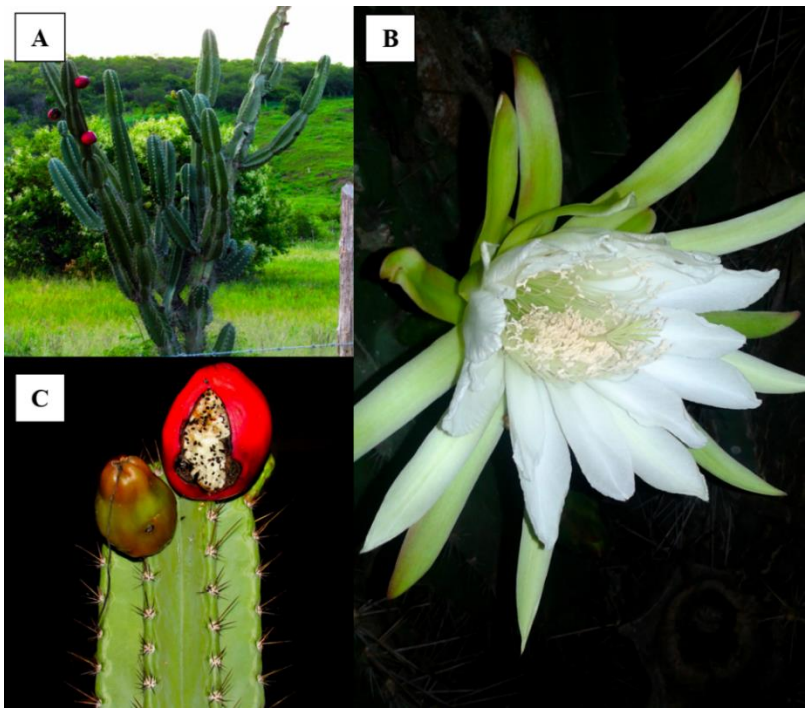
currently, the rate of use of this species as forage is high, which can compromise their survival (Cavalcanti, & Resende, 2006). In addition, representatives of *C. jamacaru* subsp. *jamacaru* are highly vulnerable in their early stages to anthropogenic disturbances or caused by the presence of other plants. Considering these aspects with this work, the objective was to evaluate, through chemical scarification, the germinative behavior of the seeds of *C. jamacaru* subsp. *jamacaru*.

2. Materials and Methods

2.1 Plant Material

The seeds of *C. jamacaru* subsp. *jamacaru* (Figure 1) were extracted from the open mature fruits of 15 different individuals in May 2016 in the city of Quixelô - CE (06º24'85.9 "S, 39º27'87.3" W). For the separation of the seeds and the pulp, a sieve and running water were used and later, the seeds were placed to dry the shade. The plant material was collected, pressed, identified and deposited at the Herbarium Caririense Dárdano de Andrade-Lima - HCDAL of the Regional University of Cariri - URCA under number #12.513.

Figure 1. *Cereus jamacaru* subsp. *jamacaru*. A= Adult organism in Caatinga area – Quixelô - CE. B= Flower. C= Mature fruit.



Source: Author (2017).

2.2 Pre-germination Methods

The methods adopted for the experiments were chemical scarification to assess whether the acids in a concentrated manner, and at different times of exposure, could modify some germ parameter. For sterilization the seeds of *C. jamacaru* subsp. *jamacaru*, were immersed in 5% hypochlorite for 5 minutes and washed in running water for the same time. After this procedure, they were submitted to the treatments described below.

2.3 Chemical scarification

For chemical scarifications, acetic acid (CH_3COOH), hydrochloric acid (HCl), propionic acid ($C_3H_6O_2$) and sulfuric acid (H_2SO_4) were used for 5, 10 and 15 minutes of exposure to the reagents, in their analytical purities. After such procedure, the seeds were washed in running water during the same time of exposure to acids to remove possible remains of reagents that could interfere in the tests.

Each treatment consisted of four repetitions of 25 seeds each, totaling 100 seeds per treatment. The tests were mounted on Petri dishes, lined with two sheets of germitest paper, moistened with 3 mL distilled water (Pereira, D. D., Pereira, M. D., & Bezerra, 2013). After packing the seeds in the plates, they were sealed with film paper in order to reduce the contact between the inside of the plate and the external environment. The plates were kept in a Biochemical oxygen demand (B.O.D) germination chamber with a 12 h light/dark photoperiod, at a constant temperature of 30 ºC according to Meiado et al. (2010). Germination readings were taken every 24 h for 7 days and germination was considered when the seeds had a radicle of at least 1 mm in length. For the control group, seeds without chemical scarification were used.

2.4 Analyzed Variables

2.4.1 Germination percentage

The germination percentage (GP) of seeds corresponds to the proportion of the number of seeds that produced seedlings classified as normal and present their essential structures, such as the root system (primary root), aerial part (hypocotyl or epicotyl), terminal buds and cotyledons. The germination percentage formula used was:

$$GP = \left(\frac{N}{Nt}\right) \cdot 100$$

Where N refers to the number of seeds germinated and Nt refers to the total number of seeds sown.

2.4.2 Germination speed index (GSI)

The Germination Speed Index (GSI) is used as an index to assess the speed of occupation of a given plant species in a given environment (Ferreira, & Borguetti, 2009). There are reports in the literature that rapid germination is characteristic of species whose evolutionary strategy consists of occupying an environment, as quickly as possible, whenever appropriate. To determine the GSI, the formula of Maguire (1962) was adopted:

$$\mathrm{GSI} = \frac{\mathrm{E}^1}{\mathrm{N}^1} + \frac{\mathrm{E}^2}{\mathrm{N}^2} + \cdots + \frac{\mathrm{E}^n}{\mathrm{N}^n}$$

Where, E^1, E^2 and E^n is the number of normal seedlings emerged computed in the first, second and last count, respectively; and N^1, N^2 and N^n, is the number of days from sowing to the first, second and last count.

2.4.3 Mean germination time (Tm)

To evaluate the mean germination time of emergency, the formula proposed by Edmond and Drapala (1958) was used:

$$\mathrm{Tm} = \frac{\mathrm{E}^1\mathrm{T}^1 + \mathrm{E}^2\mathrm{T}^2 + \cdots + \mathrm{E}^n\mathrm{T}^n}{\mathrm{E}^1 + \mathrm{E}^2 + \cdots + \mathrm{E}^n}$$

Where Tm is the mean germination time required for the species to reach maximum germination; E^1, E^2 and Em corresponds to the number of seedlings emerged in times T^1, T^2 and Tn. Subsequently, the seeds were classified as seeds with rapid germination (germination <5 days), or intermediate (> 5 and <10 days) or slow (> 10 days) for the control group. This classification is used to assess the speed of occupation of a species in a given environment (Ferreira et al., 2001).

2.5 Statistical Analysis

For the statistical analysis of the data, the mean (± standard deviation) was made using GraphPadPrism 6 with one-way analysis of variance (One-Way ANOVA), followed by the Tukey test ($p<0.05$). Germination tests were performed in quadruplicate.

3. Results

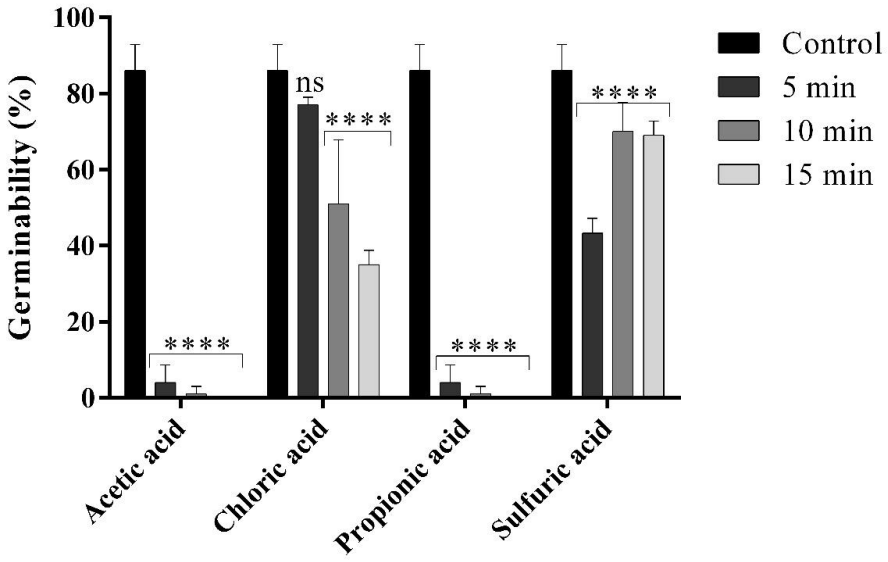
The results indicate that none of the examined acids significantly interfered in the germination of seeds of *C. jamacaru* subsp. *jamacaru*, and some made the seeds unfeasible by promoting damage to them, given the time of exposure. Acetic acid intervened negatively as to the germination percentage of *C. jamacaru* subsp. *jamacaru*, since on the 7th day the control group had 86% (±6.9) of germinated seeds, while for seeds submitted to Acetic acid the highest germination percentage was only 4% (±2.5), in 5 minutes of exposure. In addition, in the 15-minute group, the acid made seed germination unfeasible, since there was no germination over 7 days (Figure 2).

In the group of seeds submitted to hydrochloric acid, the same acid present in the birds' stomach, it was possible to observe that on the last day the germination of the group exposed for 5 minutes (77% ±6.9) did not differ statistically from the control group (86% ±6.9). However, the seeds exposed to a longer time interval to hydrochloric acid had the percentage of germination significantly decreased (Figure 2). Thus, if this acid is used as a pre-germinative method in seeds of *C. jamacaru* subsp. *jamacaru*, the exposure period should not exceed 5 minutes.

Propionic acid at all times of exposure negatively interfered with its germination. In addition, in the group of seeds that were exposed for 15 minutes, none of the seeds managed to germinate in a period of 7 days (Figure 2).

Sulfuric acid, on the other hand, showed a different performance from other acids, since the longer the exposure time of the seeds to it, the higher the percentage of germinated seeds. However, even with the longest exposure time (15 minutes) with sulfuric acid, the total germinated seeds 69% ±3.8, was lower than the control group (86% ±6.9), therefore not justifying the application of such treatment (Figure 2).

Figure 2. Germination percentage of seeds of *C. jamacaru* DC subsp. *jamacaru* subjected to different acids for 5, 10 and 15 minutes. Two-way analysis of variance (ANOVA). Mean (±standard deviation). ns: without statistical significance. **** $p<0.0001$ compared to the control group.



Source: Author (2018).

Regarding the germination speed index (GSI), the higher the value, the faster the seed germination in a short period of time. In this study it is possible to notice that the majority of the treatments with acids interfere negatively in the GSI of the seeds of *C. jamacaru* DC. ssp. *jamacaru*. Except hydrochloric acid at 5 minutes (3.37 ± 0.38) and sulfuric acid at 15 minutes (3.86 ± 0.17), which did not differ statistically from the control (4.47 ± 0.54) (Table 1).

It appears that propionic acid, followed by acetic acid, were the ones that negatively affected the IVG of the seeds with more intensity (Table 1). It was found that the seeds scarified with these two acids, suffered damages that made their germination almost impossible, consequently, the GSI values become close to zero.

Table 1. Germination speed index (GSI) of *C. jamacaru* DC subsp. *jamacaru* seeds scarified with acids at different times.

Exposure time	Aceti acid	Chloric acid	Propionic acid	Sulfuric acid
5’	0,15±0,13b	3,37±0,38ab	0±0b	1,50±0,71b
10’	0,04±0,02b	2,12±0,96bc	0±0b	2,29±1,45b
15’	0±0b	1,53±0,13c	0±0b	3,86±0,17a
Control (H_2O)	4,47±0,54a	4,47±0,54a	4,47±0,54a	4,47±0,54a

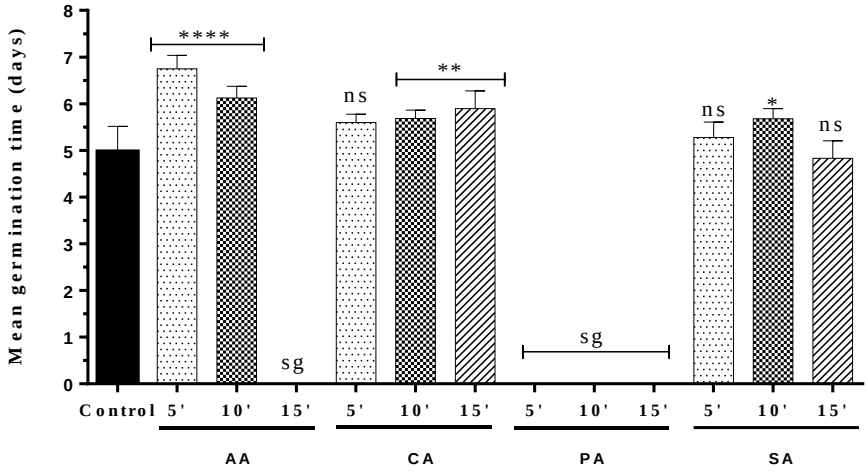
Means followed by the same letter in a column do not differ at 5% probability, using the Tukey test. Source: Author (2017).

In the present study, the shortest mean time was observed in the control group (H_2O) (4.97 ±0.54), that is, the seeds take around 4.9 days to germinate (Figure 3). Unlike acetic acid at 15 minutes, which inhibited 100% of germination. While the group exposed to 5 minutes had a Tm of 6.75 ± 0.28 days and that of 10 minutes 6.12 ± 0.25 days for the first seeds of *C. jamacaru* subsp. *jamacaru* would germinate.

Acetic acid did not interfere positively or negatively in the Tm of the seeds exposed for 5 minutes (5.59 ± 0.17) (Figure 3), however, in 10 minutes (5.68 ± 0.18) and 15 minutes (5.89 ± 0.37) of exposure, the interference was negative.

Propionic acid was the reagent that most interfered in the mean germination time, both in relation to the group of seeds exposed for the least time (5 minutes), and the largest (15 minutes), since the results were null, given the inhibition almost total seed germination (Figure 3). Therefore, this acid should not be used in “mandacaru” seeds. Sulfuric acid, on the other hand, did not significantly affect Tm on the group of seeds exposed to 5 and 15 minutes, however the group submitted for 10 minutes was negatively affected with a Tm of 5.67 ± 0.22.

Figure 3. Mean germination time of *C. jamacaru* DC subsp. *jamacaru* seeds submitted to acetic acid (AA), chloridric acid (AC), propionic acid (PA) and sulfuric acid (SA) for 5, 10 and 15 minutes. Mean (± standard deviation). One-way analysis of variance (ANOVA). ns: without statistical significance. sg: without germination. * p<0.05, ** p<0.01, **** p<0.0001 compared to the control.



Source: Author (2018).

4. Discussion

The objective of potentiating the germination of seeds of Cactaceae species is an important operation, as cacti are frequent targets of anthropic actions (Cavalcante et al., 2013) and, in the case of *C. jamacaru* DC. subsp. *jamacaru*, its diaspores do not form seed banks (Meiado, 2012). With this, the application of acids for greater germination of seeds of several botanical families has been used in laboratory tests, as is the case of Fabaceae species, such as *Bowdichia virgilioides* Kunth (sucupira-preta) (Smiderle, & Sousa, 2003; Albuquerque, Guimarães, Almeida, & Clemente, 2007), *Anadenanthera pavonina* L. (Costa, Lima, Zanella, F., & Freitas, 2010) and *Dimorphandra mollis* Benth. (buckwheat) (Hermansen, Duryea, & White, 2000). Meanwhile with *C. jamacaru* subsp. *jamacaru* there are no reports on the application of acids to enhance the germination of its seeds.

This study shows that the use of acids in analytical purity compared to “mandacaru” seeds are not efficient methods to accelerate, stimulate or increase the number of germinated seeds. This is explained by the seeds of *C. jamacaru* subsp. *jamacaru* have thin integuments and the acids may have corroded them and reached the embryo, consequently making the seeds unfeasible for germination. In addition, the seeds have a relatively large operculum,

which facilitates the entry and contact of the acid with the micropyle, and, depending on the exposure time, it can corrode it, destroying the hypocotyl-root axis (Rojas-Aréchiga, & Vázquez -Yanes, 2000; Abud, Pereira, Gonçalves, Pereira, & Bezerra, 2013).

Endozoocoria is an important mutualistic mechanism for Cactaceae, firstly by dispersing the seeds away from the mother plant and secondly by chemical scarification of the seeds (Gomes et al., 2014; Nascimento, Meiado, Nicola, & Pereira, 2015; Gomes et al., 2016). Although hydrochloric acid is found in the birds proventricle, this study shows that this acid in analytical purity negatively impacted all the analyzed parameters.

However, Gomes et al. (2014) in their study showed that seed germination, after passing through the birds' proventricle and being eliminated in the feces, had high germination (87%), and did not differ statistically from the control group. However, the seeds found in the feces had a shorter germination time, around 4 days, when compared to the control group, in which it took 6 days for germination to occur. Thus, the action of the acid accelerated the germinative process, this is justified by the fact that it is not found in pure form in the proventricle, in addition, the time of exposure of the seeds to it is enough to corrode part of the integument. With that, the endozoocoria assists in the ecological succession of *C. jamacaru* subsp. *jamacaru* in a semi-arid environment like the Caatinga, because with a shorter germination time, seedlings will be able to obtain more water for their metabolic activities (Leal, 2003).

As for acetic and propionic acid, these are low molecular weight aliphatic organic acids that are produced by anaerobic microorganisms through the fermentation of intermediate products found in the soil and that can be phytotoxic to some species (Kopp et al., 2007; Kopp et al., 2008). Thus, this study shows that the aforementioned acids have phytotoxicity compared to the seeds of *C. jamacaru* subsp. *jamacaru*. For Neves et al. (2014) this fact is justified by these acids inhibiting the mitochondrial functions of plant cells, resulting in the decoupling of oxidative phosphorylation, transport of glycolytic enzyme metabolites, in addition to inhibiting functions linked to membranes, such as polysaccharide synthesis and the ATPase of in order to compromise germination and development.

The presence in the soil of organic acids produced by microorganisms impair the viability of the seeds of *C. jamacaru* subsp. *jamacaru*, so for the planting of seeds of this species, physical-chemical and biological evaluations of the soil are necessary in order to identify the presence as well as concentrations of acetic and propionic acid. In the present study there was a small percentage of germination in some treatments with aliphatic organic acids, with the shorter time of exposure to acids, this is explained by some seeds having genes

that provide greater capacity for formation of cell membranes that tolerate these acids (Armstrong; Armstrong, 2001).

As for the average germination time of “mandacaru” seeds, the results are in line with those found by Meiado et al. (2010), Alencar, Gomes-Filho and Innecco (2012), Abud et al. (2013) and Gomes et al. (2014), in which the Tm was 6±2 days for seed germination to occur.

5. Conclusion

The immersion of seeds of *C. jamacaru* subsp. *jamacaru* in water is the best method of obtaining a greater number of germinated seeds as well as at a higher germination speed and shorter germination time;

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