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Rodrigues Meira, Messulan; Ronie Martins, Ernane; Vilela Resende, Luciane
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Physical and physiological quality of the *Lippia rotundifolia* seeds according to the osmotic conditioning and electrical conductivity

Qualidade física e fisiológica das sementes de *Lippia rotundifolia* em função do condicionamento osmótico e condutividade elétrica

Messulan Rodrigues Meira[I] Ernane Ronie Martins[II] Luciane Vilela Resende[III]

ABSTRACT

Lippia rotundifolia *is a native species of the Cerrado, endemic to the chain backbone and highly aromatic, whose essential oil has medicinal properties of high pharmacological value, but because it is a non-domesticated species, little is known about its spread. Due to its importance, the objective was to evaluate the effect of the osmotic conditioning on the physical and physiological quality of seeds. The experimental design was completely randomized with four replications of 100 seeds, being treatments consisted of two kinds of seeds (with and without tegument) subjected to ten days of immersion in water (0, 2, 4, 6, 12, 24, 48, 72, 96 and 120 hours). After each period, the seeds were submitted to the electrical conductivity test and germination. Electrical conductivity and germination there is a directly proportional relationship, while the best osmotic conditioning time is achieved with the seed husk and immersed in water for 48 hours.*

Key words: *tea-of-pedestrian, germination, vigor test, electrical conductivity.*

RESUMO

Lippia rotundifolia *é uma espécie nativa do Cerrado, endêmica da cadeia do espinhaço e altamente aromática, cujo óleo essencial possui propriedades medicinais de alto valor farmacológico, mas, por tratar-se de uma espécie não domesticada, pouco se sabe quanto a sua propagação. Devido a sua importância, objetivou-se avaliar o efeito do condicionamento osmótico sobre a qualidade física e fisiológica de suas sementes. O delineamento experimental foi o inteiramente casualizado com quatro repetições de 100 sementes, sendo que os tratamentos consistiram em dois tipos de sementes (com e sem tegumento) submetidas a dez tempos de embebição em água (0, 2, 4, 6, 12, 24, 48, 72, 96 e 120 horas). Após cada período, as sementes foram submetidas ao teste de condutividade elétrica e de germinação. Entre a condutividade elétrica e a porcentagem de germinação há relação diretamente proporcional, enquanto o melhor tempo de condicionamento osmótico é alcançado nas sementes com tegumento e imersas em água, durante 48 horas.*

Palavras-chave: *chá-de-pedestre, germinação, teste de vigor, condutividade elétrica.*

INTRODUCTION

The ***Lippia rotundifolia***, known as tea-of-pedestrian, is a shrub 0.5 to 2m height, erect stems, alternate leaves, leathery and flowers with pink-lilac color (SALIMENA & SILVA, 2009). The species is native of Cerrado, endemic to the backbone chain and highly aromatic, whose essential oil present in the leaves and flowers have medicinal properties, which give them high pharmacological value, being β-myrcene, farnesol, limonene and the myrcene, the primary responsibility for their healing potential (LEITÃO et al., 2006; GOMIDE et al., 2013).

In the environment of occurrence of the specie there is a strong anthropic action, in which predatory extraction and intense mining activity in the backbone chain, added to lack of information about their *in loco* reproduction, hampers the conservation of this species in its natural environment (GASTAUER et al., 2012). However, there are several technologies

[I]Pós-graduação em Plantas Medicinais, Aromáticas e Condimentares, Universidade Federal de Lavras (UFLA), Campus Universitário, Rua Dezesseis, n° 150, Bairro Universitário, 39404-548, Montes Claros, MG, Brasil. E-mail: messulan.meira@gmail.com. Corresponding author.
[II]Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais (UFMG), Montes Claros, MG, Brasil.
[III]Departamento de Agricultura, Universidade Federal de Lavras (UFLA), Lavras, MG, Brasil.
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for the conservation of plant resources, but to native species, especially as regards to propagation methods, the existing techniques are still incipient compared to the methodology directed to domesticated plants, where many questions assigned to them, have already been answered by genetic improvement (LIMA et al., 2013; TAIZ & ZEIGER, 2013).

The initial contribution to the conservation of native species is the adjustment of the germination method for the production of quality seedlings aiming to adaptat them to field. To succeed in this step, the osmotic conditioning, it is a good example of treatment applied to the seed as an initial step in this process. It is an imbibition technique in solutions of different osmotic agents in order to control the hydration of seeds to obtain a uniform and synchronous germination (MARCOS FILHO, 2005). Vigor test is one of the parameters evaluated to check the state of equilibrium of the osmotic potential of seeds. Among methods applied to the test, it can be cited the electrical conductivity, first tested in pea seeds and known by its efficiency, speed, objectivity, simplicity, low cost and reproducibility (VIEIRA & KZANOWSKI, 1999; MASETTO et al., 2014; ISTA, 2015). The relevance attributed to the test is that it aims to assess indirectly the intensity of damage to the membrane system according to the reading of the amount of exudates released to the osmotic solution. Thus, the evaluation of the method in response to the seed deterioration process takes place from the biggest release of amount of leachate, being then interpreted as the lowest seed vigor (VIEIRA et al., 2002; DALANHOL et al., 2014).

Colorimetric tests are also recommended in the evaluation of seed vigor. As an example of this method, it can be mentioned the biochemical test of tetrazolium (chloride -2,3,5- triphenyl of tetrazolium). This test may be applied at the end of experiment germination, especially when there is suspected dormancy; thus, reduced -antioxidant activity, reacting with the enzyme involved in the respiratory process during the germination period, highlighting the seeds with vigorous endosperm who did not respond to the germination test of empty and dead seeds (DIAS & ALVES, 2008; NERLING et al., 2014).

Thereby, the aim of the present study was to evaluate the effect of the osmotic conditioning on the physical and physiological quality of the ***Lippia rotundifolia*** seeds.

MATERIALS AND METHODS

The experiment was conducted in November and December of 2014, at the Seed Analysis Laboratory of the Instituto de Ciências Agrárias (ICA) - Institute of Agricultural Sciences - of the UFMG, in the city of Montes Claros, MG. Seeds used were freshly picked from the garden of medicinal plants of the ICA, located in the geographic coordinates: latitude 16°40'50.92''S and longitude 43°50'22.36"W.

The standardization of seed size was from a fine mesh sieve and biometrics was determined using digital calipers from the width, length, and thickness measured in four replications with 25 seeds each. Weight of 1000 seeds was determined in analytical scale with a precision of 0,001g, as established by seed testing rule with eight repetitions of 100 units each and degree of humidity, with greenhouse method of 95±3°C, during 17 hours, using two subsamples, according to the recommendations of RAS, being the results expressed as a percentage (BRASIL, 2009).

The electrical conductivity (EC) was performed according to the methodology proposed by AOSA (1983), with four subsamples of 100 seeds devoid tegument and 100 seeds with tegument respectively. These were weighed accurately with two decimal points and then, with the aid of a sieve to catch and soak the seeds were placed in a plastic cup with 200mL of deionized water. The period of immersion seeds were zero to 120 hours, staying in incubator type of Biochemical Oxygen Demand (BOD) with cool white light 20W to 25°C until the end of test. Ranges of conductivity readings conducted in multiple periods (0, 2, 4, 6, 12, 24, 48, 72, 96 and 120 hours), performed in digital conductivity meter (GEHAKA brand, CG1800 model), with constant of conductivity cell K=1,0. The result of reading was divided by the weight (w) and initial conductivity of the respective sample, and results expressed as μScm^{-1} g^{-1} and w of seeds.

To evaluate the physiological potential of the seeds within each soaking period, these were submitted to the germination test. The substrate of this stage consisted in three paper sheets germitest® in Petri plates, which were moistened with distilled water with a volume equivalent to 2.5 times the weight of the substrate germinating to 25° and photoperiod of 8 hours. The first count (FC) was performed on the eighth day after sowing and germination speed index (GSI) was obtained from daily notes, at the same time, of the number of seedlings with roots protrusion during the 28-day evaluation. The SGI calculation was performed according to MAGUIRE (1962), in which: SGI= $\sum(n/t)$, where n is the number of germinated seeds per day and, t, the number of days on which the germination until the day of the experiment.

At the end of the germination test, the normal seedlings were measured and weighed to determine the length and fresh weight of shoot (CPA and MFPA) and root (CR and MFR), and results of these two variables expressed as average values in cm and g. Dry weight of the aerial part (MSPA) and root (MSR) were determined from drying in a forced-air oven at 45°C, to constant weight. The viability of the ungerminated seeds was evaluated by the tetrazolium test to 0.5% (p/v) with pH adjusted to 6.5. After time staining of embryos, solution was removed, seeds were rinsed in distilled water and the evaluation performed according to the methodology RAS (BRASIL, 2009), being classified as dead seeds (DS) and hard (HS).

The experimental design was completely randomized (CRD) with four replications, and, for statistical analysis, percentage data were transformed into sine arc √(X/100). Average of the EC test was related to the germination percentage by Pearson correlation. It was conducted by the Cochran test to test the variances of treatments for homogeneity, those that showed homogeneous, were subjected to analysis of variance by F test and, the means were compared by Tukey test at 5% probability. Averages which were significant were subjected to quantitative analysis by polynomial regression with the help of the program SAEG 9.1 (BANZATTO & KRONKA, 2006).

RESULTS AND DISCUSSION

The seeds of the ***Lippia rotundifolia*** have biometrics with standard deviation (SD), on average, 2.33mm (sd=7.33) of length, 0.74mm (dp=7.36) in width and 0.03 (dp=0.29) surrounded by thick hairy shell, weight of one thousand seeds (W_{1000}) was of 3.21g and degree of moisture of 8.54%. These values allowed estimating a kind of grass of seeds contains on average 311 units, being this important estimate to calculate the seeding rate, especially when it comes to small seeds (BRASIL, 2009).

The correlation between the electrical conductivity and the percentage of germination was highly significant with the value (r=0.89) to 1% of significance (P<0.01), but by the t test showed no significant difference between the data collected in the test compared to data collected in the retest. This demonstrates that, in addition to the correlation between two variables, Student's t test also pointed out the trust in the similarity between distribution of their linear association measures from the share of variance between the two variables.

Results obtained for the test vigor and germination potential due to the water absorption period, only the variable moisture content (U), electrical conductivity (EC), germination (GERM), germination speed index (GSI), and first count (PQ), there were significant differences. For these variables set up cubic equations, since variables fresh weight and dry shoot and root (MFPA, MSPA, MFR, MSR), aerial part length and radicle length (APL, RL) didn't show difference at 5% significance level (P<0.05).

For the water absorption due to the immersion period, it is observed a triphasic curve (Figure 1A), being the initial moisture content of seeds, prior to immersion of 8.54% and 2.25% of water respectively, for seeds with and without tegument. After it emerged of the seed tegument achieved with maximum absorption of 85% and the ones without tegument 56.25% at the first two hours of imbibition. At this time, it started the metabolism of substances in the reserve tissue, converting them into simpler compounds, low molecular mass and osmotically active, is characterized as phase I (TAIZ & ZEIGER, 2013).

After this quick imbibition, the moisture content reduced gradually until it reaches 50.5% and 26.7% of water in a longer period of imbibition, in the seeds with and without tegument respectively. It was observed that the loss of water was constant in the seeds without tegument, while the seeds with tegument had a gradual water loss after the maximal imbibition and later there was stabilization of the moisture in the periods of 48 (61.1%), 72 (64.07%) and 96:00 (60.5%). Variation of water within these periods corresponded to approximately 0.015mm, where moisture remained virtually unchanged, returning to lose water in the range of 96 to 120h. This indicates that in the period in which there was a decline in moisture, in four hours, began the phase II (MARCOS FILHO, 2005; ZUCHI et al., 2012). At this stage, the endosperm, influenced by respiration, accelerates the metabolism, bringing the substances in reserve tissues meristematic tissue until to use them during germination.

After this moment, the transition occurs to the phase III where the product metabolized in the second stage is rearranged to form the cell walls, allowing the embryonic axis to develop. This phase should be followed by rapid gain of moisture due to the need for water by new seedling cells in process of formation (BRADFORD, 1995); however, this did not occur, which explains this fact is the tiny size of the ***L. rotundifolia*** seed in which it has little reserve material.

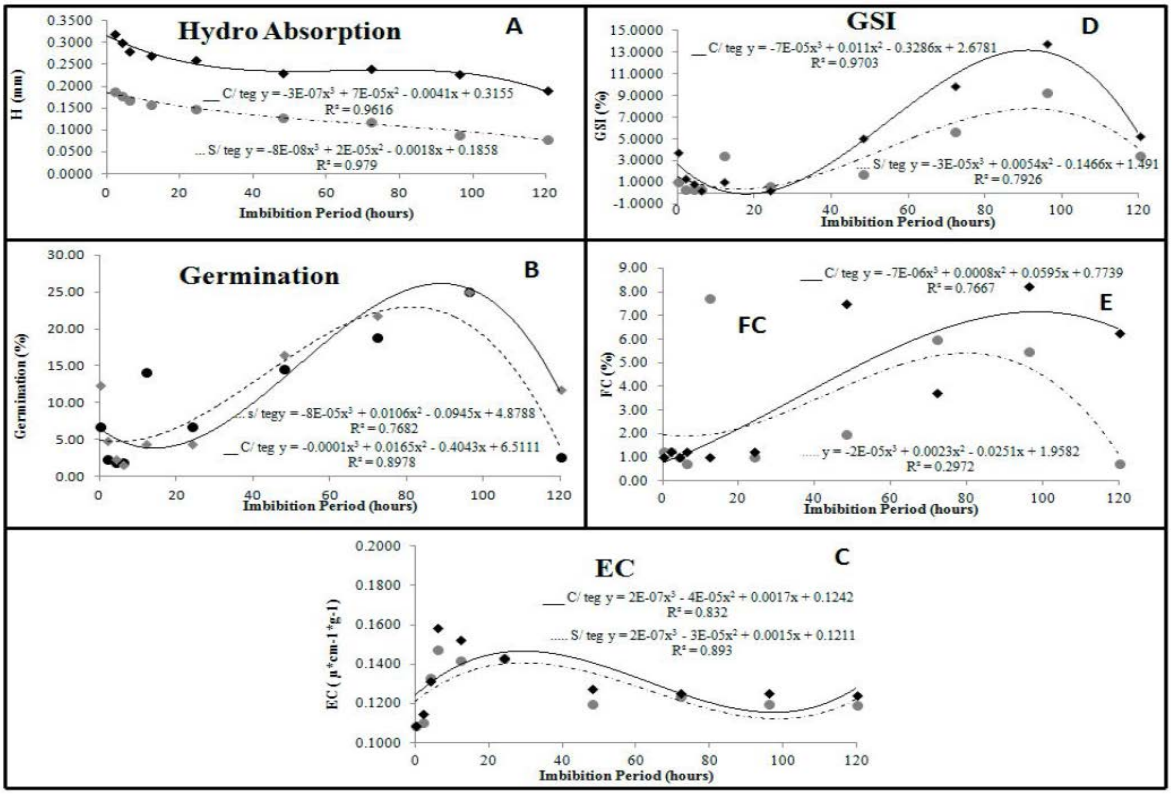

Figure 1 - Germinative behavior as a function of the imbibition curve and electrical conductivity of seed of ***Lippia rotundifolia*** Cham. OBS: The curves represent polynomial regression of third order adjusted to the significant variables. In that the axis y= humidity (a = H), germination percentage (b = GERM), electrical conductivity (c = EC), germination speed index (d = GSI) and first count (e = FC). The values of 'a' and 'b'= constants determined by regression calculated from the cubic equation. The continuing trend line represents the seeds with tegument and the dotted trend line represents the seeds without tegument.

When comparing the germination as a function of moisture content it was observed that, as the seeds have reached the maximum level in the first two hours it was not enough to overcome dormancy, once these values grouped to a set of worst percentage germinal (Figure 1B). The observed in this study, according to CARVALHO & NAKAGAWA (2012), may be due to the low water potential of the cell walls; therefore, the authors stated that this physical process can take place both in living seeds, though dormant, as for dead seeds.

The tetrazolium test confirmed this observation, whose dead seeds (without staining) were more significant at 6 and 12 hours of soaking. It can be said that the low germination percentage is due to the non-occurrence of post-ripening period because many seeds do not germinate immediately after dispersal. Seeds with this characteristic need a storage period in dry conditions to increase the germination capacity (BEWLEY & BLACK, 1994; TAIZ & ZEIGER, 2013). Thus, a suitable time of storage of the inflorescence of the ***Lippia rotundifolia*** it is necessary for the seed to reach higher percentage germination in the early hours of soaking.

Regarding to the vigor physiological test of the seeds using the electrical conductivity (EC) for the ten periods of soaking, there was constant initial increase in the first three periods, 2, 4 and 6 hours, respectively. After this interval, the conductivity was reduced gradually increased as the time for imbibition (Figure 1C). The highest recorded in the EC was in the 6h period and a drastic reduction occurred, approximately from 48 hours imbibition in both treatments. For DIAS et al. (1997), this fact is due to the increased release of solutes to the imbibition solution. MARTINI NETO et al. (2014) corroborated the authors, when they emphasized that the seeds with low physiological quality releases high amounts of

leached electrolytes in the early hours of contact with the solution; thus, compromising the germination percentage. The presented results confirmed the reported by the authors, because the excess release of electrolytes showed negative effect on germination, where low germination percentage was observed for the first few hours of imbibition.

The best percentage for this variable was presented in the 96 hours imbibing time with 25% of germination for both treatments (with or without tegument) (Figures 1C and C). During this period, many seeds showed long endosperm with the root system already well developed. This result was confirmed by lower mortality appointed by tetrazolium test, with 4.6 and 2% of dead seeds for 96h, followed by 5.5 and 3% in 48h and 8.25 and 5.33% in 72h, respectively, for the seeds with and without tegument. Results observed were lower than germination observed by PIMENTA et al. (2007), where values were recorded with 35%. This percentage may be related to conditions in which the species is reported, the time of collection and the population source, because the authors collected the seeds in the dry season in the natural environment, while in the present study, the collection occurred in the rainy season in cultivated plants.

Already the seed germination in the 120 period was low, with 11.75% for the seeds with tegument and 2.5% for seeds without tegument, the roots showed brittle and glassy in appearance. This observation was confirmed by the tetrazolium test, which showed that treatment as the highest rate of dead seeds (soft and empty). The low germination as well as the fragility of the seeds without tegument, is due to the damage caused to membrane system as a function of direct impact of the water devoid of the seed tegument, which led to the extravasation of the cell electrolyte, resulting in irreversible damage of the seeds (MARCOS FILHO, 2005; DERRÉ et al., 2011). However, in the best rates of germination observed (48 to 96h) there was also loss of solutes. However, at that moment that it happened the EC's stability also occurred repair of mechanical damage in the seeds (Figure 1C) (MARCOS FILHO, 2005). ROSA et al. (2000) corroborated the observation in this study by claiming this synchronization between reorganization of the membranes and the reduction in the release of ions. The response between germination and imbibition time higher water absorption, with repair membrane system, which can be explained by its occurring environment, because as *in loco* observation, the species occurs in more humid places such as river beds, springs and paths, keeping the reproductive viability under high humidity.

As for GSI, the best indices were achieved in the later soaking period, while the worst ratio was observed around 6 hours of imbibition (Figure 1D). The first count of the germination test did not vary significantly between treatments with and without tegument. From the obtained results, the first hours of water absorption were similar, differing from later periods, in which the top indexes occurred during the periods 48, 96 and 120 hours for seed with tegument and 12, 48, 72 and 96 hours in the seeds without tegument (Figure 1e). These were the indexes for the time needed for reorganization of tissues and the end of the mobilization of processes for germination of seeds of ***L. rotundifolia*** (CARVALHO & NACAGAWA, 2012).

Despite the low coefficient of determination for the first count, the R^2 is in accordance with the coefficients of GSI, and even as low value, the measurement of such rates is important to ascertain the distribution of germination processes. Therefore, the best index is related to higher daily germination, which are the best vigor, so the lower average of germination time, whose distribution is concentrated in the time and in the space (GONÇALVES et al., 2008; GOMES et al., 2010). These rates are in accordance with the imbibition curve as well as the germination and conductivity in which the negative correlation between these variables indicated that the higher conductivity is related to the lower germination and reduced in the vigor of the seed (VIEIRA et al., 2002). Similar behavior was also observed by ATAÍDE et al. (2012), indicating that the correlation result is as expected for the physiological and physical viability test in the seeds.

Finally, the results evidenced the importance of electrical conductivity test in determining the viability of seeds of ***L. rotundifolia***, so that it may be inferred that the average electrical conductivity 12.0μS cm^{-1} g^{-1} of seeds established a percentage of vigor of approximately 25% of normal seedlings from 48:00 of imbibitions. The water immersion up to 24 hours is not enough to overcome dormancy and imbibe the seeds for times longer than 96 hours because causes decay and loss of the vigor. During the experiment, it was observed the presence of fungi, but its effect was mitigated by formation of a mycelial inhibition halo around the seeds. This defense mechanism can be explained by the presence of the essential oil, since this metabolite is present throughout the plant. Therefore, the information regarding the natural defense are pioneering, which

may be inferred that for the ***L. rotundifolia*** the plant preconditioning is not necessary, since the presence of fungi did not damage the germination, being an indication that the essential oil served as fungicidal agent (LEITÃO et al., 2006; GOMIDE et al., 2013). However, from this observation, it was created an expectation of research in the plant pathology area to identify the fungi as well as the cause of the infestation and the degree of commitment to seeds of this species.

CONCLUSION

The osmotic conditioning is related to the physical and physiological quality of the ***Lippia rotundifolia*** seeds and the germination is best achieved by seed tegument immersed in water for 48 hours.

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