

**Effect of using ultrasound to break dormancy and germination of xylopia emarginata
mart seeds**

**Efeito do uso de ultrassom na quebra de dormência e germinação de sementes xylopia
emarginata mart**

**Efecto del uso de ultrasonido para romper la latencia y la germinación de semillas de
xylopia emarginata mart**

Received: 24/08/2020 | Reviewed: 02/09/2020 | Accept: 06/09/2020 | Published: 07/09/2020

Gênesis Alves de Azevedo

ORCID: <https://orcid.org/0000-0002-4786-9278>

Universidade Estadual Paulista, Brasil

E-mail: azevedogenesis21@gmail.com

Andressa Aparecida Rodrigues de Melo

ORCID: <https://orcid.org/0000-0003-3593-6039>

Universidade Estadual Paulista, Brasil

E-mail: andressa.a.r.melo@gmail.com

Luan Dionnes Kaiber Moreira

ORCID: <https://orcid.org/0000-0002-7258-4792>

Universidade Estadual Paulista, Brasil

E-mail: luan.moreira@unesp.br

Rafael dos Santos Silva

ORCID: <https://orcid.org/0000-0003-1316-9844>

Universidade Estadual Paulista, Brasil

E-mail: rsantos.s@hotmail.com

Douglas Enrique Juárez Sánchez

ORCID: <https://orcid.org/0000-0002-6672-4541>

Universidade Estadual Paulista, Brasil

E-mail: douglas.juarez@unesp.br

Caio Botelho Ribeiro

ORCID: <https://orcid.org/0000-0002-6623-6804>

Universidade Federal do Maranhão, Brasil

E-mail: caiobotelhoribeiro@gmail.com

Valdrickson Costa Garreto

ORCID: <https://orcid.org/0000-0003-4245-8194>

Universidade Federal do Maranhão, Brasil

E-mail: valdrickson30@gmail.com

Igor Alves da Silva

ORCID: <https://orcid.org/0000-0002-8118-976X>

Universidade Federal do Maranhão, Brasil

E-mail: igortoprio@hotmail.com

Thalles Eduardo Rodrigues de Araújo

ORCID: <https://orcid.org/0000-0001-5173-9815>

Universidade Federal do Maranhão, Brasil

E-mail: thalleseduardorod@gmail.com

Raimundo Cleidson Oliveira Evangelista

ORCID: <https://orcid.org/0000-0002-1877-1714>

Universidade Federal do Maranhão, Brasil

E-mail: drnetobz@gmail.com

Carlos Alberto Monteles Carneiro

ORCID: <https://orcid.org/0000-0001-9526-3230>

Universidade Federal do Maranhão, Brasil

E-mail: carlos_monteles@hotmail.com

Abstract

Xylopia emarginata Mart. is a typical species of cerrado areas, characterized by its tolerance to strong water deficiencies, making it a potential species for the recovery of ecosystems. Thus, evaluating the germination process of this species is of great importance, as it encompasses technical knowledge- scientific research on the production of seedlings used in the revegetation of degraded areas, however, the seeds present dormancy, requiring techniques to overcome this physical characteristic. The research aimed to evaluate the effect of different periods of bathing in low-frequency ultrasound on the electrical conductivity and germination of *Xylopia emarginata* Mart. as an indicator plant. The research was carried out in simple DIC, with 6 replications containing 30 seeds. The seeds were subjected to different immersion times: 0, 3, 6, 9, 12, 15 and 18 minutes respectively in an ultrasonic bath in distilled water, and then the electrical conductivity of the solution was evaluated. Subsequently, the seeds were dried at room temperature and placed to germinate in trays with vermiculite for two

months, to evaluate the percentage of final germination. Statistical analyzes showed a significant difference between the evaluated treatments, not being enough to confer statistical significance at 1 or 5% probability. Therefore, immersing *Xylópia aromatica* seeds in low-frequency ultrasound can be dispensed with and can be replaced by another method of breaking seed dormancy.

Keywords: Pindaíba-do-brejo; Germinative potential; Revegetation.

Resumo

A *Xylophia emarginata* Mart. é uma espécie típica de áreas de cerrado, caracterizada pela sua tolerância às fortes deficiências hídricas, tomando-se uma espécie em potencial para a recuperação dos ecossistemas, assim, avaliar o processo germinativo dessa espécie de tem grande importância, pois abrange o conhecimento técnico-científico acerca da produção de mudas utilizadas na revegetação de áreas degradadas, porém, as sementes apresentam dormência, necessitando de técnicas para superação dessa característica física. A pesquisa teve o objetivo de avaliar o efeito de diferentes períodos de banho em ultrassom de baixa frequência na condutividade elétrica e germinação de *Xylophia emarginata* Mart. como planta indicadora. A pesquisa foi realizada em DIC simples, com 6 repetições contendo 30 sementes. As sementes foram submetidas a diferentes tempos de imersão: 0, 3, 6, 9, 12, 15 e 18 minutos respectivamente em banho ultrassônico em água destilada, e em seguida avaliou-se a condutividade elétrica da solução. Posteriormente as sementes foram secas em temperatura ambiente e colocadas para germinar em bandejas com vermiculita por dois meses, para avaliação da porcentagem de germinação final. As análises estatísticas apresentaram diferença sensível entre os tratamentos avaliados, não sendo suficiente para conferir significância estatística à 1 ou 5% de probabilidade. Desse modo, a imersão das sementes de *Xylópia aromática* em ultrassom de baixa frequência pode ser dispensado, podendo ser substituído por outro método de quebra de dormência das sementes.

Palavras-chave: Pindaíba-do-brejo; Potencial germinativa; Revegetação.

Resumen

Xylophia emarginata Mart. es una especie típica de áreas del cerrado, caracterizada por su tolerancia a fuertes deficiencias hídricas, lo que la convierte en una especie potencial para la recuperación de ecosistemas. Por lo tanto, evaluar el proceso de germinación de esta especie es de gran importancia, ya que abarca conocimiento técnico-investigación científica. en la producción de plántulas utilizadas en la revegetación de áreas degradadas, sin embargo, las

semillas presentan latencia, requiriendo técnicas para superar esta característica física. La investigación tuvo como objetivo evaluar el efecto de diferentes períodos de baño en ultrasonido de baja frecuencia sobre la conductividad eléctrica y la germinación de *Xylopia emarginata* Mart. como planta indicadora. La investigación se realizó en DIC simple, con 6 repeticiones que contenían 30 semillas. Las semillas fueron sometidas a diferentes tiempos de inmersión: 0, 3, 6, 9, 12, 15 y 18 minutos respectivamente en un baño de ultrasonidos en agua destilada, y luego se evaluó la conductividad eléctrica de la solución. Posteriormente, las semillas se secaron a temperatura ambiente y se colocaron a germinar en bandejas con vermiculita durante dos meses, para evaluar el porcentaje de germinación final. Los análisis estadísticos mostraron una diferencia significativa entre los tratamientos evaluados, no siendo suficiente para conferir significancia estadística al 1 o 5% de probabilidad. Por lo tanto, se puede prescindir de sumergir las semillas de *Xylópi*a aromática en ultrasonidos de baja frecuencia y se puede reemplazar por otro método para romper la latencia de las semillas.

Palabras clave: Pindaíba-do-brejo; Potencial germinativo; Revegetación.

1. Introduction

Xylopia emarginata Mart. is a species belonging to the family Annonaceae, popularly known as water pindaí, pindaíüa-do-brejo or pepper-do-brejo. According to Paula (1997), morphologically, it has a straight shaft, thin, aromatic bark and with the development of lichens on the surface; oblong or oblong-elliptical leaf, short petiole; axillary flower, in pairs or isolated; glabrous, apocarpous, bacifonnes fruits, consisting of 3 to 5 carpels each, which open through a longitudinal slit in the carpel suture; black seed with aryl. Lorenzi (1992) adds that the height is between 10 and 20 meters and the trunk is 20 to 30 cm in diameter. Its crown is small and pyramidal, with narrow, shiny and glabrous leaves, with 4 to 6 cm in length.

Considering the ecological aspects, *Xylópi*a *emarginata* Mart. It is a perennial plant, heliophyte, pioneer, characteristic of teneno swamps where it forms homogeneous massifs. It occurs mainly in secondary formations of almost all forest formations, however, always in wetlands by riverside. Its presence inside the dense primary forest is less common. It generally occurs in large groups, reaching almost all populations. Ratter (1971) describes *X. emarginata* as a tall, often emergent species, with a thin, straight trunk and a crown. narrow, and with the characteristic appearance of many gallery forests. Still, in swampy areas tabular roots (sapopemas) and superficial roots are abundant and characterize several species.

Young *X. emarginata* individuals have anchor roots, such roots leave the trunk up to 3 m above ground level. As the trunk grows, the roots join with it until they form tabular roots in large individuals. Despite its scarce production of viable seeds, it is cited as a potential species for the recovery of degraded riparian areas (Lorenzi, 1992). The natural occurrence of *X. emarginata* is recorded from Bahia to São Paulo, in Brasília (Ratter, 1971), in the Mineiro Triangle (Schiavini, 1992) and in Mato Grosso (Oliveira-Filho, 1989).

In recent years, there has been an increase in the interest of researchers in the propagation of native forest species, mainly due to environmental problems and the need to recover degraded areas. However, with regard to the management and analysis of the seeds of most of these species, there is still little knowledge available, mainly related to the appropriate conditions for obtaining maximum germination (Alves et al., 2008). Knowledge of the appropriate conditions for seed germination of a given species is of great importance, since the responses are differentiated and depend on several factors, such as those related to seed dormancy mechanisms and environmental conditions, such as water, light, oxygen and temperature (Carvalho; Nakagawa, 2000).

The importance of studying seeds is related to the maintenance and improvement of cultivated plants, the maintenance of genetic variability, production of resources for revegetation and restoration of vegetation, to maintenance mechanisms, so it is important to seek new techniques for the renewal of forest populations. There are different methods of overcoming seed dormancy, among which the following can be highlighted: mechanical scarification; the incisions in the integument; exposures to high temperatures, and chemical scarification by strong acids. These methods provide for the rupture or weakening of the integument, a condition that allows the entry of water and oxygen, triggering the beginning of the germination process (Carvalho; Nakagawa, 2000).

Ultrasound is a form of mechanical, vibrational energy, which can have a deleterious or development-inducing action on living tissues depending on the intensity, the exposure time, the frequency of application and the distance from the transducer to the target (Hebling, 1995).

The objective of this work was to evaluate the effect of ultrasound on germination and conductivity of *Xylopia emarginata* Mart. seeds.

2. Methodology

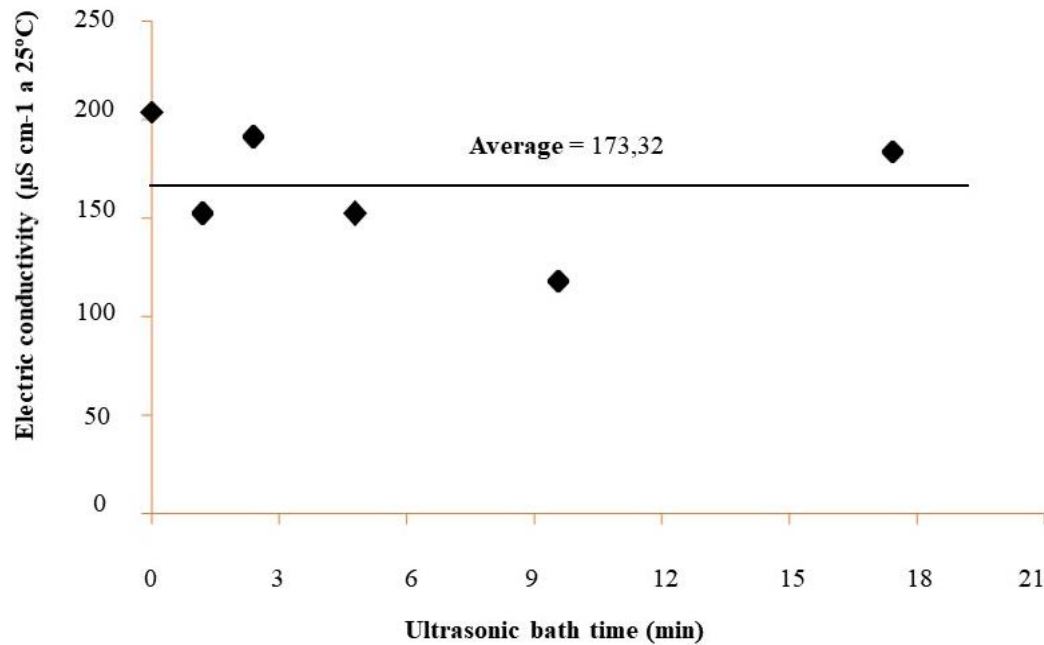
The experiment was conducted at the Laboratory of Seed Analysis and Plant Physiology and in a protected environment, belonging to the State University of Mato Grosso do Sul (UEMS), at the University Unit of Cassilândia (UUC), located in the municipality of Cassilândia - MS (19°07'21" S; 51°43'15" W; 516 m altitude) in the period from January to July 2018. Using seeds of *Xylopia emarginata* Mart. The tests were performed in 30 plots of six repetitions at different times (0, 3, 6, 9, 12, 15 and 18 minutes) of immersion in the ultrasound, the ultrasound used is of constant frequency at 45 KHz, and each plot had 30 seeds totaling 900 seeds for the experiment, all were kept soaked in sodium hypochlorite (NaClO) for 10 minutes, then added 500 ml of distilled water to the ultrasound together with two Beckers with 55 ml of distilled water, each seed parcel were placed in the 5 ml beakers respecting the different times, then after the ultrasonic bath, the beakers' contents were transferred to plastic cups and then placed in the BOD incubator chamber at 25 °C, for 24 h. Afterwards, the electrical conductivity of the solution with the seeds was evaluated. Then the seeds were placed to germinate in trays with vermiculite, in a laboratory environment (temperature, humidity and light). Seedling emergence was evaluated 60 days after sowing, obtaining the germination percentage (PG).

The data were submitted to Grubbs outlier tests, Anderson-Darling normality test and Barlett's homoscedasticity test. Then, the analysis of variance (ANOVA) was carried out to verify the effect of the treatments, considering the significance level of 5%.

3. Results and Discussion

Observing the statistical analysis for electrical conductivity, it is noted that there was no significant difference between the electrical conductivity averages depending on the ultrasound bath, presenting an average conductivity of $166.44 \mu\text{S cm}^{-1}$, in which a noticeable variation is noticed, not being sufficient to detect a statistical difference at a level of 1 or 5% probability (Figure 1).

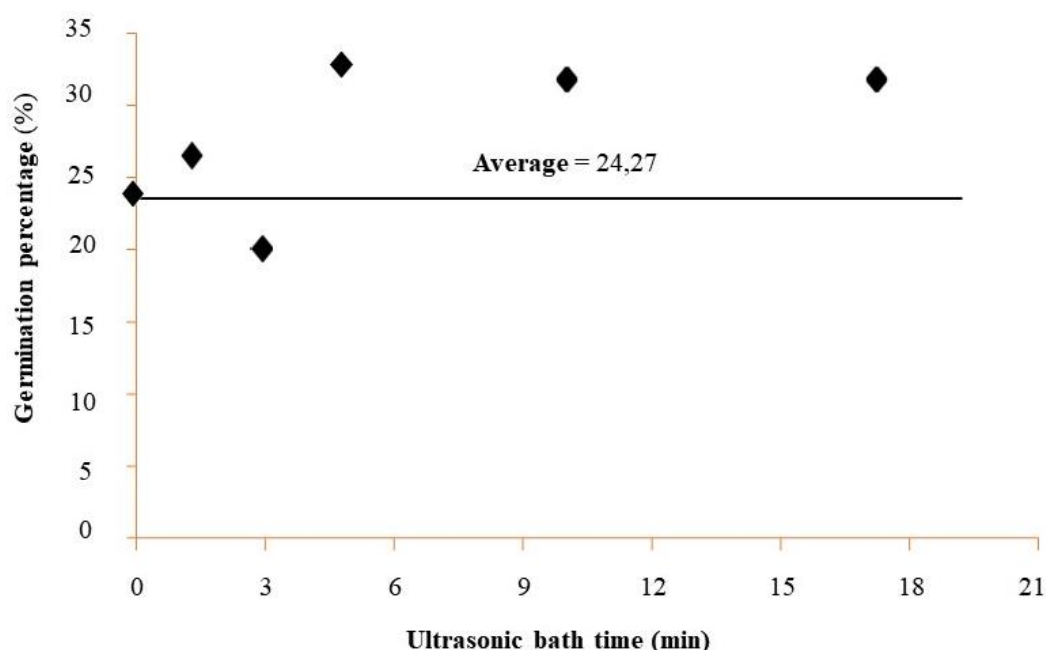
Figure 1. Electrical conductivity in relation to time in the ultrasonic bath.



Source: Adaptado de Baptista et al., 2017.

Figure 2 shows the same behavior for the germination percentage results (%), in which the analysis of variance did not determine a significant difference between the germination averages in relation to the different ultrasound bath times tested in the research, with an average result of 28.13%, a similar result that can be observed in the unadapted graphs of Baptista et al., (2017), in which a significant difference between the results is also observed, not being sufficient to express a statistical difference with different ultrasound times (Figure 2).

Figure 2. Germination percentage in relation to the time in the ultrasonic bath.



Source: Adaptado de Baptista et al., 2017.

In the study by Brinck, (2020), studying the overcoming of dormancy in okra seeds using low-frequency ultrasound, he observed that treatments that used ultrasound for 9 minutes presented a brief advantage in relation to other treatments in the evaluation of the seed speed index. germination, these results not being significant. Venâncio, (2020), working with the influence of low-frequency ultrasound on the germination of senna multijuga (rich.) h. s. irwin & barneby, also observe that there was a very small difference between the times evaluated in relation to the percentage of seed germination.

According to Baptista et al., (2017), the explanation for the equality of treatment averages would be that the seeds would need a higher frequency of kHz or a longer time in ultrasound with the same frequency and the temperature of the distilled water should be higher. According to studies by Franzin, (2006), for the germination of rice seeds, ultrasound was used, exposed to ultrasonic waves for periods of 5, 10, 15 and 20 minutes and temperatures of 20, 30 and 40°C. Studies demonstrate that the application of low-frequency ultrasound in a liquid medium can increase the germination and growth rate by facilitating the absorption of water by the seeds (Yaldagard et al., 2008).

Roossi, (2008) states that *Xylopi*a seeds present dormancy, caused by the deposition of coumarin, a substance that inhibits germination, in the seed coat, which is why it is advisable to collect young fruits, with a reddish color, as they have a lower coumarin content. in the seeds, for later ripening during storage (Barbosa et al, 1992).

Xylopia seeds present exogenous dormancy caused by the presence of germination inhibitors (Lima et al, 2006), in addition to integumentary dormancy (Fowler; Bianchetti, 2000). It was observed in the treatments of this experiment that the germination of *Xylópi*a seeds in the nursery germinated 40% of the total batch, with no need to break dormancy and use low frequency ultrasound at different times.

Contrary to the observed results, in studies carried out on bean seeds using low-intensity ultrasonic radiation (Berents apud Nagy, 1980 apud Frazin, 2006) they found an increase in seed germination speed and (Attaullaev apud Nagy, 1980 apud Frazin, 2006) in corn seeds, this demonstrates that ultrasound can also be effective in seeds that do not present dormancy, a result that can be explained by the morphophysiological difference of corn and beans in relation to *Xylópi*a Aromática seeds.

Exogenous seed dormancy is associated with physical, chemical or mechanical factors (Menezes et al., 2009). When associated with physical factors, it is caused by the presence of tissues located on the outside of the embryo, which are impermeable to water and gases (Baskin et al., 2004). This impermeability is caused by a layer of elongated and juxtaposed cells with different degrees of thickening, called macrosclereids, which may or may not be impregnated with lignin (Corner, 1976; Ferreira et al., 2004) and other hydrophobic substances (ROLSTON, 1978; MORRISON et al., 1998; BASKIN, 2003). In nature, living beings present in the soil, such as fungi and bacteria, can minimize this type of dormancy by degrading the seed coat of some species.

4. Final Considerations

It was concluded that the low-frequency ultrasound bath, up to 18 min, did not interfere with the electrical conductivity and germination of *Xylopia emarginata* Mart. seeds, and could be replaced by another method of breaking dormancy, since the results were not statistical significance between the treatments tested.

References

Alves, E. U., Nascimento, C. D. L., Bruno, R. L. A., Alves, A. U., Braga Júnior, J. M., Cardoso, E. A., Galindo, E. A., & Silva, K. B. Germinação e vigor de sementes de *Bauhinia divaricata* L. *Ciência Rural*, 38(4), 960 – 966, 2008.

Alves, J. M. Efeitos da energia ultrassônica na regeneração de pele animal com queimadura por calor. São Carlos, 1988. 79p. Dissertação de Mestrado - Escola de Engenharia de São Carlos, Universidade de São Paulo.

Baptista, I. S.; Medeiros Junior, G.; Santos, Vieira, A. H.; Cipriani, H. N. & Passos, A. M. A. Efeito do ultrassom de baixa frequência na germinação de sementes de *Copaifera langsdorffii* desf. 4º Encontro de Ciência e Tecnologia, Porto Velho, 2017.

Barbosa, J. M., Aguiar, I. B., & Santos, S. R. G. dos. Maturação de sementes de *Xylopia emarginata* Mart. In: Congresso nacional sobre essências nativas, 1992, São Paulo. *Anais*. São Paulo: Instituto Florestal, 1992. 665-674. *Revista do Instituto Florestal*, v. 4, parte 3, edição especial, 1992.

Baskin, C. C. Breaking physical dormancy in seeds – focussing on the lens. *New Phytologist*, v. 158, n. 2, p. 227 – 238, 2003.

Baskin, J. M., Baskin, C. C. A classification system for seed dormancy. *Seed Science Research*, v. 14, n. 1, p. 1–16, 2004.

Brinck, R. R. L. Superação da Dormência em Sementes de Quiabo. *Cadernos de Agroecologia*, v. 15, n. 4, 2020.

Carvalho, N. M., & Nakagawa, J. Sementes: ciência, tecnologia e produção. Jaboticabal: FUNEP, 2000. 588 p.

Catellani, E. D., Damião Filho, C. F., & Aguiar I. B. Sementes de *Xylopia emarginata* Mart.: *aspectos morfológicos*. Jaboticabal: FAPESP/CNPq/UNESP, 2001.

Corner, E. J. H. The seeds of Dicotyledons. London: Cambridge University Press, 1976.

Duarte, L. R. The stimulation of bone growth by ultrasound. *Archives of Orthopaedic and Traumatic Surgery*, 101, 153, 1983.

Ferreira, R. A., Davide, A. C., Motta, M. S. Vigor e viabilidade de sementes de *Senna multijuga* (Rich.) Irwin et Barn. e *Senna macranthera* (Collad.) Irwin et Barn., num banco de sementes em solo de viveiro. *Revista Brasileira de Sementes*, v. 26, p. 24-31, 2004.

Fowler, J. A. P., & Bianchetti, A. Dormência em sementes florestais Colombo: Embrapa Florestas. Documentos 40, 2000. 27 p.

Franzin, S. M. Dormência e pré-germinação de sementes de arroz. Santa Maria: [s.n.], 2006. 122 p.

Hebling, S. A. W. R. Efeitos do ultrassom de baixa intensidade na germinação de sementes de milho (*zea mays l.*) sob diferentes condições de disponibilidade hídrica. Piracicaba, 1995.

Lima, J. A. D., et al. Maturação e inibidores de germinação na emergência de plântulas de copaíba (*Xylopia emarginata* Mart.). In: Congresso Brasileiro De Olericultura, 2006, Associação Brasileira de Horticultura.

Lorenzi, H. *Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil*. Nova Odessa: Instituto Plantarum, 1992.

Lorenzi, H. *Árvores brasileiras: manual para identificação e cultivo de plantas arbóreas do Brasil*. Nova Odessa: Editora Plantarum, 1992.

Menezes, N. L., Franzin, S. M., Bortolotto, R. P. Dormência em sementes de arroz: causas e métodos de superação. *Revista de Ciências Agro-Ambientais*, v.7, n. 1, p. 35-44, 2009.

Morrison, D. A., Mcclay, K., Potew, C., Rish, S. The role of the lens in controlling heat induced breakdown of testa-imposed dormancy in native Australian legumes. *Annals of Botany*, v. 82, p. 35 – 40, 1998.

Oliveira-filho, A. T. Composição florística e estrutura comunitária da floresta de galeria do córrego da Paciência, Cuiabá (MT). *Aeta Botânica Brasflica*, São Paulo, 3(1), 91-112.

Paula, J. E., Imana-Encinas, J., & Pereira, B. A. S. Parâmetros volumétricos e da biomassa da mata ripária do córrego dos macacos. *Cerne*, Lavras, 2(2), 091-105, 1997.

Ratter, J. A. Notas sobre a vegetação da Fazenda Água Limpa (Brasília - DF). Brasília: Editora da Universidade de Brasília, 1971. (Coleção Textos Universitários n.3).

Rolston, M. P. Water impermeable seed dormancy. *The Botanical Review*, v. 44, p. 365-396, 1978.

Rossi, T. *Xylopia emarginata* Mart. Piracicaba, 2008.

Schiavini, I. S. Estrutura de comunidades arbóreas de mata de galeria da Estação Ecológica do Panga (Uberlândia-MG). Tese. (Doutorado). Universidade Federal de Campinas, Campinas, SP, 1992.

Venâncio, R. S. S. V., et al. Técnicas alternativas de quebra de dormência: uso do ultrassom de baixa frequência em sementes de senna multijuga (*rich.*) *h. s. irwin & barneby*. São Carlos: [s.n.], 2020. 15 p.

Yaldagard, M., Mortazavi, S. A., Tabatabaie, F. Influence of ultrasonic stimulation on the germination of barley seed and its alpha-amylase activity. *African Journal of Biotechnology*, v. 7, n. 14, p. 2465–2471, 2008.

Percentage of contribution of each author in the manuscript

Gênesis Alves de Azevedo – 30 %

Andressa Aparecida Rodrigues de Melo – 10 %

Luan Dionnes Kaiber Moreira – 10 %

Rafael dos Santos Silva – 10 %

Douglas Enrique Juárez Sánchez – 10 %

Caio Botelho Ribeiro – 5 %

Valdrickson Costa Garreto – 5 %

Igor Alves da Silva – 5 %

Thalles Eduardo Rodrigues de Araújo – 5 %

Raimundo Cleidson Oliveira Evangelista – 5 %

Carlos Alberto Monteles Carneiro – 5 %