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Technical feasibility of minicutting of different branch portions to produce clonal seedlings of *Aloysia citrodora*

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Abstract

Seedling production using the minicutting technique enables quick and efficient plants formation. However, there are no reports of application for *Aloysia citrodora* (lemon verbena) and the ideal portion of the branch. This study aimed to evaluate the production of seedlings from minicuttings taken from different parts of the branch. The experiment was performed in a completely randomized design. Three treatments consisted of minicuttings taken from other branch portions (basal, median, and apical), divided into two experiments (Exp1 and Exp2). Exp1 was carried out in a sub-irrigation system in phenolic foam, evaluating rooting percentage, length of the longest root, root number, number of shoots, number of leaves, and total dry mass. Exp2 used the minicuttings from the previous experiment, kept for another 35 days in pots to assess the quality of the seedlings, evaluating plant length, number of shoots, and leaves. For Exp1, 35 days after cutting, there was a higher percentage of survival, size of the longest root, number of the root, number of shoots, number of leaves, and total dry mass, in those minicuttings produced from the basal and median portions, being the exact behavior of Exp2, 35 days after transplantation to pots. The minicutting proved to be an efficient method for producing *Aloysia citrodora* seedlings, when removed from the basal or median portion, standing out for a higher percentage of survival, better growth, and initial development and post-transplantation of the produced seedlings.

Keywords: Lemon verbena, cuttings, vegetative propagation

Introduction

Among the richness of the Brazilian flora, medicinal and aromatic species stand out, as they are sources of various compounds for pharmaceutical application. Among these, the essential oils stand out, substances extracted from the stem, leaves, roots, flowers, and fruits of plants (Ribeiro et al., 2018). The search for new natural and biologically active substances has encouraged the use of these plants by the pharmaceutical industry, as these molecules can have different therapeutic and pharmaceutical purposes (Moura & Silva, 2013), such as antimicrobial, antioxidant, and flavoring characteristics (Brant, 2010; Cutrim et al., 2019).

The *Aloysia citrodora* (Verbenaceae) is popularly known as lemon verbena or cidró. The plant is rich in volatile oil that has antimicrobial, neuropsychological, gastrointestinal, antioxidant, anti-inflammatory, analgesic, sedative, antispasmodic activities, among others, being cultivated in several countries due to its medicinal properties and high economic value (Bahramsoltani et al., 2018; Paulus et al., 2014), in addition to having proven bactericidal and insecticidal properties (Santos et al., 2015).

Lemon verbena propagates sexually hardly, as the species belonging to the Verbenaceae family have dormancy, and it is challenging to collect seeds. Furthermore, Brazilian climatic conditions do not allow the production of viable seeds with a fully developed embryo (Pimenta et al., 2007). For this, the production of seedlings of this species becomes possible from the vegetative propagation being performed mainly by cuttings, a method that induces stimulation of rooting and development of the aerial part from a segment removed from the parent plant, originating a new plant (Sousa et al., 2013).

The improvement of cutting can be achieved

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especially with the development of the minicutting technique, widely used in the forestry sector (Pessanha et al., 2018; Rodrigues et al., 2017), allowing considerable gains arising mainly from the increase in rates rooting, less use of propagating material and reduced time for new plant formation (Pires et al., 2015).

Several factors influence this propagation method, including the branch maturation stage. It generates a gradient in the concentration of hormones, accumulation of nutrients, and the number of reserve carbohydrates (Rosa et al., 2017). Younger cuttings taken from the apex of the branch tend to have less lignification, more active metabolism, and reduced nutrient reserve, which brings a greater propensity for tissue dehydration (Hartmann et al., 2011). Therefore, cuttings from different branch portions present different rooting potentials, modifying the plants' quality. However, studies on vegetative propagation from minicutting are scarce, especially those analyzing the different branch portions from which the minicuttings have been removed.

For this the objective was to evaluate the viability of producing *Aloysia citrodora* seedlings from clonal minicuttings taken from different portions of the parent plant branch.

Materials and Methods

Experiments performed in a protected environment in the experimental area of the Federal University of Santa Maria (UFSM), *Campus* Frederico Westphalen, geographically located at 27° 23' S and 53° 25' W and altitude of 490 meters, in the period from August 28 to October 02, 2015. According to the Köppen climate classification, the climate of the region is Cfa - humid subtropical with hot summer, with an average annual temperature of 19.1° C, ranging from a maximum of 38° C and a minimum of 0° C (Alvares et al., 2013).

The collection of branches was made on August 28, 2015, from mother plants located in the medicinal garden, separated into the basal, median, and apical portions. The minicuttings were excised with a straight cut at the base and beveled above the last axillary bud and patterned with three knots each (± 10 cm in length), and the leaves were carefully removed to avoid any damage to the bud. Subsequently, they were placed for one minute in a sodium hypochlorite solution (1% active chlorine) for disinfection and then washed with distilled water.

For fixing the minicuttings, an inert phenolic foam substrate was used (dimensions 2x2x5 cm). First, the phenolic foams were washed in running water to remove likely compounds arising from the industrialization process. Then, each phenolic foam cell received a stake, one of the yolks was introduced into the substrate and kept two out.

The minicuttings were kept in a protected environment on a bench with a sub-irrigation system and irrigation for 15 minutes every hour during the day and with two 15-minute shifts during the night period, using water for the first 13 days. After this period, a nutrient solution (25% of the recommendation) was added consisting of HidrogoodFert®, calcium nitrate, and chelated iron (Yara®). The electrical conductivity of the nutrient solution was sustained at approximately 300 µS, and the pH, between 6.0 and 6.5.

The maximum and minimum air temperature was recorded daily with a digital thermometer during the entire rooting period. The meteorological conditions inside the protected environment after the minicutting presented a high thermal amplitude. The maximum and minimum temperatures reached values between 49.5 and 5 °C, respectively, with an average of 25.4° C (Figure 1).

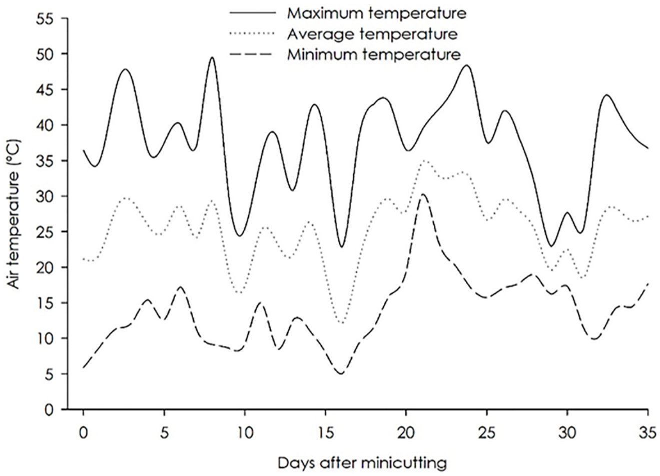

Figure 1. Maximum, average, and minimum air temperature recorded inside the protected environment during the rooting period of *Aloysia citrodora* minicuttings.

Experiment 1: Influence of the branch portion on the setting of Aloysia citrodora minicuttings.

The experiment was performed in a completely randomized design consisting of three treatments and four replications, and the experimental unit consisted of five plants, totaling 60 minicuttings. The treatments consisted of minicuttings from the branch's basal, median, and apical portions. Therefore, 90 mini-cuttings remained for conducting experiment 2.

At 35 days, the experiment evaluated the following: percentage of rooting and mortality of minicuttings (%), length of the longest root (cm), number of roots, number of shoots, number of developed leaves (more than 1.0 cm in length), and minicuttings total dry mass (mg).

Experiment 2: Influence of branch portion on the quality of potted plants.

Forty rooted seedlings of each treatment were used, transplanted into black polypropylene pots with a volume of 5L containing sieved soil (oxisol), and kept in a protected environment to verify the initial growth and development of the plants produced from minicuttings. Daily irrigation according to the needs of the seedlings. Evaluation after 35 days of transplanting evaluating: plant length (cm), number of shoots, and number of leaves.

The experiment was performed in completely randomized design, with three treatments (plants from minicutting of the branch's basal, median, or apical portion), four replications, and the experimental unit consisted of ten minicuttings each.

Variance analysis was performed on data from both experiments, and treatment averages were compared by Tukey's test at 5% significance using the SISVAR statistical program (Ferreira, 2011).

Results and Discussion

Experiment 1: Influence of the branch portion on the setting of Aloysia citrodora minicuttings.

The experiment highlighted that minicuttings removed from the basal and median branch portions presented a higher rooting percentage with 98.7% and 95.3%, respectively, with a mortality percentage of only 1.3 and 4.7%. For minicuttings removed from the apical portion, there was a higher percentage of mortality (25.3%) and a lower percentage of rooting (74.7%) when compared to the other portions (Figure 2). The survivability of cuttings taken from different portions of the plant branch tends to differ in terms of rooting potential, as throughout the branch, the content of carbohydrates and rooting promoting and inhibiting substances in the tissues vary (Hartmann et al., 2011).

The branch portion influenced the rooting percentage from the stemming minicutting. In other words, minicuttings taken from the basal and median portion presented a more significant increase for rooting. Cuttings of *Passiflora alata* and *Passiflora edulis* also presented increased results in cuttings rooting. In addition, cuttings from the same portions (basal and median) represented superior results than apical ones (Salomão et al., 2002). On the other hand, cuttings of *Hyptis suaveolens* removed from the basal portion represented reduced rooting (Maia et al., 2008).

More lignified branches, located at the base of the branches, are less favorable for root differentiation, as lignification negatively affects the auxin content of cuttings, considering that the primary place of synthesis

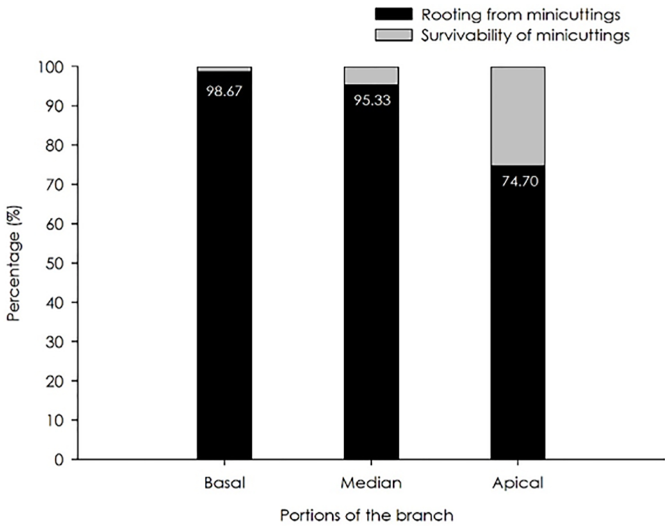

Figure 2. Rooting percentage and mortality of *Aloysia citrodora* minicuttings propagated from different portions of the branch (apical, median, and basal).

of this plant hormone is the young tissues, located at the shoot's apex (Taiz et al., 2017). Furthermore, peroxidase, the enzyme responsible for lignin synthesis, degrades auxin, affecting root formation (Hartmann et al., 2011). However, this fact did not occur in the present work, as basal minicuttings presented superior results. Thus, it appears that the best portion in the branch of a plant to obtain cuttings is variable and genotype-dependent.

Usually, basal cuttings have a larger diameter and are more lignified. These characteristics allow a more significant accumulation of reserves (carbohydrates) to be translocated to root formation and, therefore, have a lower risk of dehydration of the plant material (Hartmann et al., 2011; Ferreira et al., 2010). Thus, the superiority of rooting of cuttings in the basal and median portion of the branch, observed in the present study, may be related to the greater reserve of carbohydrates. On the other hand, the lower rooting of minicuttings in the apical portion is explained by the high-temperature conditions inside the protected environment during the experiment period (Figure 1), which may have favored the dehydration process of the plant tissue.

Minicutting becomes an efficient technique for vegetative propagation of *Aloysia citrodora*, as those removed from the basal and median portion of the branch showed rooting greater than 95%. In 10 cm cuttings of *Aloysia triphylla* (synonymy of *Aloysia citrodora*) with doses of indolebutyric acid, they presented rooting of approximately 90% (Paulus et al., 2014). However, for the present study, even not testing the use of exogenous rooting, the high rooting rate suggests that using these regulators for this species is unnecessary.

Through the analysis of variance, the F test

($p<0.05$) displayed significance for the variables length of the longest root, number of shoots, number of roots, number of leaves, and total dry mass of minicuttings.

For the variables length of the longest root, number of shoots, number of roots, and total dry mass, there was significant superiority for minicuttings of the basal and median portions of the branch (Figure 3A, 3B, 3C, and 3E, respectively). Only for the number of leaves, the basal branch was superior to the other branch portions (Figure 3D), with superiority always observed for cuttings from the basal portion and inferior for the apical portion. These results demonstrate that the closer the branch apex, the lower the cuttings' vigor and productive potential to produce a new plant. Basal and medium cuttings of *Hibiscus rosa-sinensis* displayed equivalent results, superior in root length, number of leaves, number of shoots, and dry mass of shoot and minicutting roots (Souza et al., 2015).

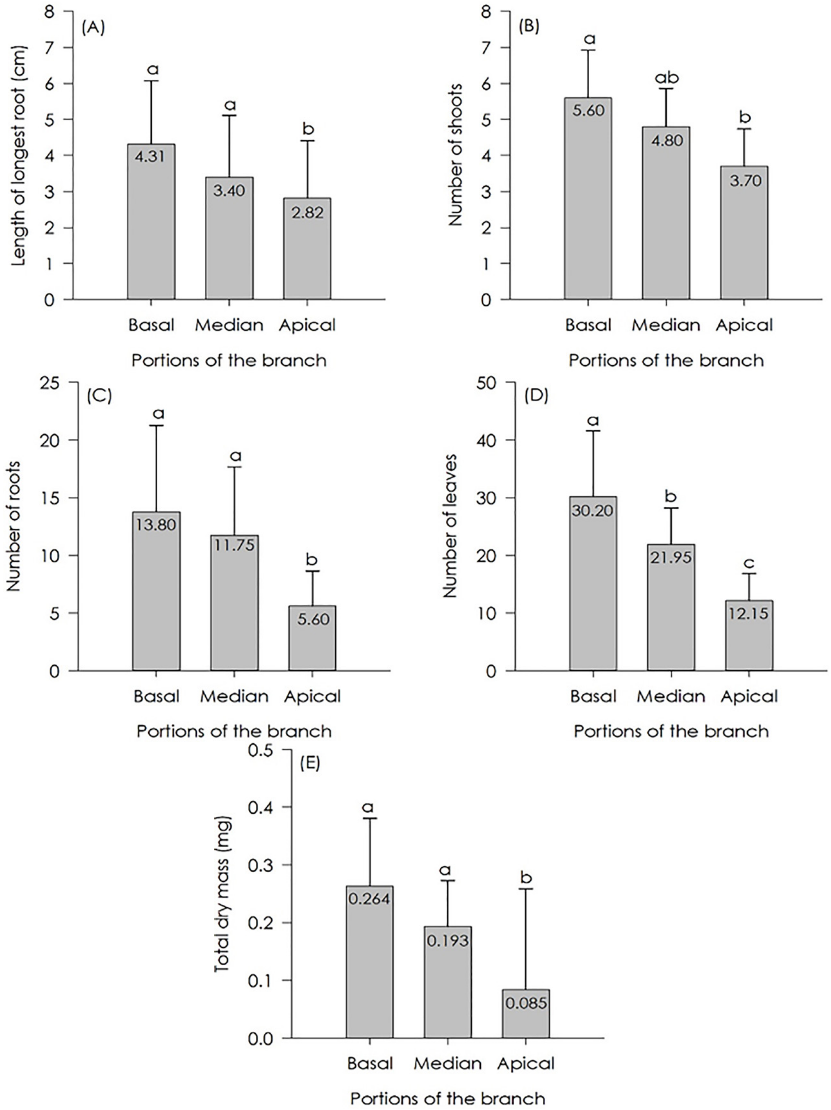

Figure 3. Length of the longest root (A), number of shoots (B), number of roots (C), number of leaves (D), and total dry mass (E) of *Aloysia citrodora* minicuttings propagated from different branch portions (apical, median and basal). *Averages followed by the same lowercase letters do not differ by Tukey's test, at 5% significance. Vertical lines represent standard deviation.

Dry mass is an essential parameter for assessing cutting vigor, and those with low reserves usually show low vigor (Silva, 2015). Cuttings from the median and basal portions of *Piper hispidum* demonstrated greater vigor (Cunha et al., 2015), which corroborates our data. The dependence between plants' physiological factors and the interaction with the external environment explained the superiority in the variables presented (Figure 3). Moreover, the greater the root system's quantity and quality, the greater the conditions for further plant development. The roots are the organs responsible for absorbing water and nutrients vital for plant growth

(Lucas et al., 2013). Therefore, the places with the highest degree of lignification and accumulation of reserves in the branches tend to reduce the rooting of cuttings of certain species (Marangon & Biasi, 2013). However, minicuttings of *Aloysia citrodora* did not present this fact. More significant formation of roots, number of leaves, and shoots obtained from basal branch cuttings positively impacted dry mass accumulation.

Experiment 2: Influence of branch portion on the quality of potted plants.

By analysis of variance, there was no significant difference for the variable length of plants from minicuttings taken from different portions of the branch. However, there was a significant difference in the number of shoots and leaves.

The seedlings showed 100% survival, regardless of the portion collected in the branch, corroborating the data observed in *Toona Ciliata* minicuttings, with 100% survival after initial rooting (Souza et al., 2009). In addition to the high percentage of rooting of the species, the results reflect the high capacity for acclimatization to environmental change.

For the number of shoots and number of leaves, there was a significant superiority for the minicuttings of the basal and median portions compared to the apical ones (Figure 4), maintaining the trend of the previous experiment.

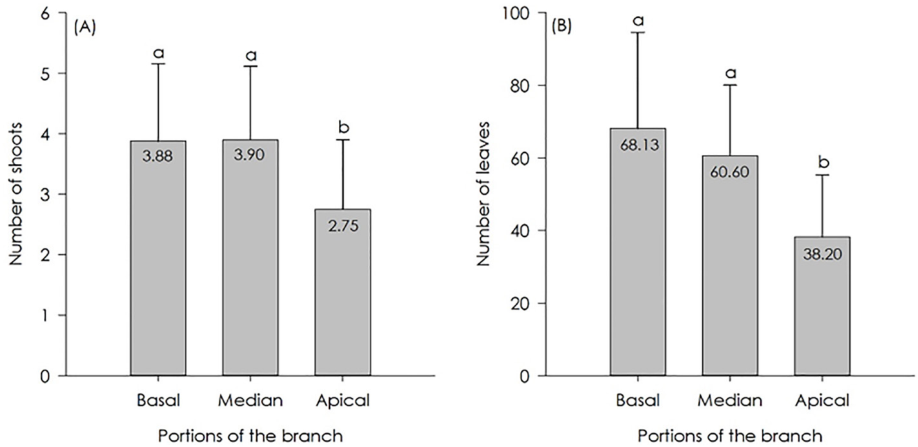

Figure 4. The number of shoots (A) and the number of leaves (B) of *Aloysia citrodora* plants propagated from minicutting of different portions on the branch. *Averages followed by the same lowercase letters do not differ by Tukey's test, at 5% significance. Vertical lines represent standard deviation.

The initial growth and vigor of seedlings after planting is an important characteristic and considered a limiting factor for the success of commercial planting. The accelerated growth of seedlings is essential because it reduces activities related to cultural treatments, favoring the initial growth of plants in the field, such as weeding, combating pests, and replanting seedlings, which generally increase the costs of implementing the culture.

Using the minicutting technique, the production of *Aloysia citrodora* seedlings took place in 70 days, from cutting until the seedling availability for planting in an ideal location, demonstrating that this technique is viable for the vegetative propagation of the species in a fast, easy, efficient and economical.

Conclusions

The outcomes of the performed experiments pointed out that minicutting from the basal or median portions of the *Aloysia citrodora* branch presented a higher percentage of rooting, better growth, and initial development, promoting the pot plants' most significant number of shoots and leaves.

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References

Alvares, C.A., Stape, J.L., Sentelhas, P.C., Moraes, G., Leonardo, J., Sparovek, G. 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711-728.

Bahramsoltani, R., Rostamiasrabadi, P., Shahpiri, Z., Marques, A.M., Rahimi, R., Farzaei, M.H. 2018. *Aloysia citrodora* Paláu (Lemon verbena): A review of phytochemistry and pharmacology. *Journal of ethnopharmacology* 222: 34-51.

Brant, R.D.S., Pinto, J.E.B., Bertolucci, S.K., Albuquerque, C.J.B. 2010. Produção de biomassa e teor do óleo essencial de cidrão em função da adubação orgânica. *Horticultura Brasileira* 28: 111-114.

Cunha, A.L.B., Chaves, F.C.M, Batista, A.C., Hidalgo, A.F. 2015. Propagação vegetativa de estacas de *Piper hispidum* Sw. em diferentes substratos. Revista Brasileira de Plantas Medicinais 17: 685-692.

Cutrim, E.S.M., Teles, A.M., Mouchrek, A.N., Mouchrek Filho, V.E., Everton, G.O. 2019. Avaliação da atividade antimicrobiana e antioxidante dos óleos essenciais e extratos hidroalcoólicos de *Zingiber officinale* (Gengibre) e *Rosmarinus officinalis* (Alecrim). *Revista Virtual de Química* 11: 60-81.

Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. *Ciência e agrotecnologia* 35: 1039-1042.

Ferreira, L.E., Andrade, L.A.D., Gonçalves, G.S., Souza, E.P.D., Ferreira, H.V. 2010. Diâmetro de estacas e substratos na propagação vegetativa de maniçoba, *Manihot glaziovii* Muell. Arg. *Revista Ciência Agronômica* 4: 393-402.

Hartmann, H.T., Kester, D.E, Davies Jr, F.T. Geneve, R.L. *Plant propagation: principles and practices*. Prentice Hall, Boston, EUA. 915 p.

Lucas, W.J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.R., Helariutta, Y., He, X.Q., Fukuda, H., Kang, J., Brady, S.M., Patrick, J.W., Sperry, J., Yoshida, A., López-Millán, A.F., Grusak, M.A., Kachroo, P. 2013. The plant vascular system: evolution, development and functions. *Journal of integrative plant biology* 55: 294-388.

Maia, S.S., Pinto, J.E., Silva, F.N., Oliveira, C. 2008. Enraizamento de *Hyptis suaveolens* (L.) Poit. (Lamiaceae) em função da posição da estaca no ramo. *Revista Brasileira de Ciências Agrárias* 3: 317-320.

Marangon, M.A., Biasi, L.A. 2013. Estaquia de mirtilo nas estações do ano com ácido indolbutirico e aquecimento do substrato. *Pesquisa Agropecuária Brasileira* 48: 25-35.

Moura, R.B., Silva, I.C. 2013. Espécies de Asteraceae e Lamiaceae usadas na medicina popular da região sudeste para problemas respiratórios: O que as evidências científicas indicam. *Revista Fitos* 6: 21-28.

Paulus, D., Valmorbida, R., Toffoli, E., Paulus, E. 2014. Propagação vegetativa de *Aloysia triphylla* (L'Hér.) Britton em função da concentração de AIB e do comprimento das estacas. *Revista Brasileira de Plantas Medicinais* 16: 25-31.

Pessanha, S.E.G.L., Barroso, D.G., Barros, T.C., Oliveira, T.P.F., Carvalho, G.C.M.W., Cunha, M. 2018. Limitations on vinatic production (*Plathymenia reticulata* Benth) for minicutting. *Ciência Florestal* 28: 1688-1703.

Pimenta, M.R., Fernandes, L.S., Pereira, U.J., Garcia, L.S., Leal, S.R., Leitão, S.G., Salimena, F.R.G., Viccini, L.F., Peixoto, P.H.P. 2007. Floração, germinação e estaquia em espécies de *Lippia* L. (Verbenaceae). *Brazilian Journal of Botany* 30: 211-220.

Pires, P., Wendling, I., Auer, C., Brondani, G. 2015. Sazonalidade e soluções nutritivas na miniestaquia de *Araucaria angustifolia* (Bertol.) Kuntze. *Revista Árvore* 39: 283-293.

Ribeiro, S.M., Bonilla, O.H., Lucena, E.M.P. 2018. Influência da sazonalidade e do ciclo circadiano no rendimento e composição química dos óleos essenciais de *Croton spp.* da Caatinga. *Iheringia. Série Botânica*, Porto Alegre 73: 31-38.

Rodrigues, M.B., Pimentel, N., Lencina, K. H., Kielse, P., Bisognin, D.A. 2017. Enraizamento de miniestacas de ipê-roxo (*Handroanthus heptaphyllus* Vell. Mattos). *Revista Brasileira de Plantas Medicinais* 19: 118-128.

Rosa, G.G., Zanandrea, I., Mayer, N.A., Bianchi, V.J. 2017. Propagação de porta-emxerto de *Prunus* spp. por estaquia: efeito do genótipo, do estádio de desenvolvimento do ramo e do tipo de estaca. *Ceres* 64: 90- 97.

Salomão, L.C.C., Perreira, W.E., Duarte, R.C.C., Siqueira, D. L. 2002. Propagation of *Passiflora alata* and *P. edulis* f. *flavicarpa* by cutting. *Revista Brasileira de Fruticultura* 24: 163-167.

Santos, A.C.B., Nunes, T.S., Coutinho, T.S., Silva, M.A.P. 2015. Uso popular de espécies medicinais da família Verbenaceae no Brasil. *Revista Brasileira de Plantas Medicinais* 17: 980-991.

Silva, K.N. 2015. *Propagação de potenciais porta-enxertos de pessegueiro por estacas lenhosas com auxilio de AIB.* 38f. (Dissertação de Mestrado) – Universidade Federal de Viçosa, Viçosa, Brasil.

Sousa, C.M., Busquet, R.N., Vasconcellos, M.A.S., Miranda, R.M. 2013. Effects of auxin and misting on the rooting of herbaceous and hardwood cuttings from the fig tree. *Revista Ciência Agronômica* 44: 334-338.

Souza, J.C.A.V., Barroso, D.G., Carneiro, J.G.A., Teixeira, S.L., Balbinot, E. 2009. Vegetative propagation of *Toona ciliata* M. Roemer by the minicutting technique. *Revista Árvore* 33: 205-213.

Souza, R.R., Cavalcante, M.Z.B., Lima, M.P.D., Alixandre, T.F., Nascimento, R.T. 2015. Propagação vegetativa de hibisco com diferentes tipos de estacas e concentrações de ácido indolbutírico. *Comunicata Scientiae* 6: 291-296.

Taiz, L., Zeiger, E., Møller, I.M., Murphy, A. 2017. *Fisiologia e desenvolvimento vegetal.* Artmed, Porto Alegre, Brazil, 888p.

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