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Biology-botany

Water availability and seasonality affect phytomass production and photosynthetic pigments of *Aloysia citrodora* Paláu

Disponibilidade hídrica e sazonalidade afetam a fitomassa e pigmentos fotossintéticos de *Aloysia citrodora* Paláu

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ABSTRACT

Vegetative growth can be affected by different environmental factors. The objective of this work was to evaluate phytomass production and photosynthetic pigments of *Aloysia citrodora* plants submitted to different irrigation levels and seasons. The experiment was carried out in a protected environment from August 2015 to March 2017, in an experimental randomized blocks design, 4x4 factorial scheme, with four blocks with four plants each. The irrigation levels tested were 25%, 50%, 75% and 100% of the field capacity, in the four seasons of the year (autumn, winter, spring and summer). Plant height, fresh and dry mass of branches, fresh and dry mass of leaves, leaf area and photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) were evaluated. It was observed that water availability and seasons influence phytomass production as well as the photosynthetic pigment analyzes. Low irrigation levels and cultivation during autumn and winter adversely affect vegetative growth. Furthermore, our results indicate that for higher phytomass production, it is recommended to cultivate *Aloysia citrodora* during spring and summer under irrigation levels of 75% and 100% of field capacity. Spring season promotes higher content of photosynthetic pigments. This study will open new avenues and perspectives to elucidate possible changes in secondary metabolites of *Aloysia citrodora*, a medicinal plant, in the presence of changes in the growing seasons and different stress conditions, including water stress.

Keywords: Lemon verbena; Aromatic plant; Chlorophyll; Environmental factors

RESUMO

O crescimento vegetativo pode ser afetado por diferentes fatores ambientais. O objetivo deste trabalho foi avaliar a produção de fitomassa e pigmentos fotossintéticos de plantas de *Aloysia citrodora* submetidas a diferentes níveis de irrigação e estações do ano. O experimento foi realizado em ambiente protegido, no período de agosto de 2015 a março de 2017, em delineamento experimental de blocos casualizados, em esquema fatorial 4x4, com quatro blocos com quatro plantas cada. Os níveis de irrigação testados foram de 25%, 50%, 75% e 100% da capacidade de campo, nas quatro estações do ano (outono, inverno, primavera e verão). Foram avaliadas a altura das plantas, massa fresca e seca de ramos, massa fresca e seca de folhas, área foliar e pigmentos fotossintéticos (clorofila *a*, clorofila *b* e carotenoides). Observou-se que a disponibilidade hídrica e as estações do ano influenciam a produção de fitomassa e as análises de pigmentos fotossintéticos. Baixos níveis de irrigação e cultivo durante o outono e inverno afetam adversamente o crescimento vegetativo. Portanto, nossos resultados indicam que para maior produção de fitomassa, recomenda-se o cultivo de *Aloysia citrodora* durante a primavera e o verão sob níveis de irrigação de 75% e 100% da capacidade de campo. A estação da primavera promove maior conteúdo de pigmentos fotossintéticos. Este estudo abrirá novos caminhos e perspectivas para elucidar possíveis alterações nos metabólitos secundários de *Aloysia citrodora*, uma planta medicinal, quando submetida a mudanças nas estações de cultivo e nas diferentes condições de estresse, como o estresse hídrico.

Palavras-chave: Cidró; Planta aromática; Clorofila; Fatores ambientais

1 INTRODUCTION

Aloysia citrodora Paláu, originated in South America, is a perennial medicinal and aromatic species that belongs to the family Verbenaceae. The genus *Aloysia* contains about 30 species distributed throughout the Americas from the United States to Patagonia (ROJAS *et al*., 2012). Several species have been introduced in Europe and North Africa where there are pharmacological research related to their medicinal properties (EL-HAWARY *et al*., 2012; FELGINES *et al*., 2014). Its essential oil has several chemical components with emphasis on citral which presents great importance to the pharmaceutical and cosmetic industries (ROJAS *et al*., 2012), since it presents antimicrobial (SOUZA *et al*., 2017) and anesthetics properties (BECKER *et al*., 2017) as well as anxiolytic effect (JIMÉNEZ-FERRER *et al*., 2017). In addition, the species has been attracting interest in the agricultural chemical industry since it produces secondary metabolites with antifungal, insecticidal and repellent properties (HABER and CLEMENTE, 2013).

Phytomass production of plants is dependent on genetic and environmental factors. Agronomic performance and productive potential of each species depend on the genotype-environment interaction, which directly influences plant growth and development (PRAVUSCHI *et al*., 2010; FERREIRA *et al*., 2015). Among the environmental factors, seasonality directly affects phytomass production mainly due to the variation of climatic elements throughout the year, being a limiting factor for several crops (SCHWERZ *et al*., 2015; FERREIRA *et al*., 2016).

Seasonal variation is associated with other environmental factors that result in abiotic stress to the plants such as water availability which can cause stress due to deficiency or excess, limiting the growth and productivity of plants, and reducing crops yield (FARROQ *et al*., 2012; OZ *et al*., 2015) due to the decrease of photosynthetic rates (JOHARI-PIREIVATLOU, 2010; OSAKABE *et al*., 2014). This reduction occurs due to a direct influence on the plant metabolism that causes several morphological, biochemical and physiological responses such as production of reactive oxygen species (ROS), capable of damaging cellular structures (MISRA *et al*., 2011; RAO and CHAITANYA, 2016), causing stomatal closure and limiting the entry of carbon dioxide (CO_2) in the leaves, which inhibits photosynthesis and, consequently, the production of photoassimilates which will affect phytomass production (reduction of shoot, acceleration of senescence and foliar abscission) (WANG *et al*., 2008; FERRARI *et al*., 2015).

The increased demand for raw material originating from natural products and attractive prices, when compared to other agricultural products, arouses the interest of producers in the cultivation of aromatic plants. In the cultivation of these species, such as *Aloysia citrodora*, there is a need to consider the production of biomass, considering that this factor directly affects the yield of active principles in essential oils. Therefore, several factors influence the final quantity and quality of the product, such as the genetic characteristics of the plant, climatic variations, growing season, among others. Therefore, it is essential to determine the ideal growing conditions for the plants to reach high phytomass production and, at the same time, reach a higher essential oil yield per unit area.

Thus, the objective of this work was to evaluate the phytomass production and the content of photosynthetic pigments of *Aloysia citrodora* plants subjected to different levels of irrigation and seasonality.

2 MATERIALS AND METHODS

2.1 Crop conditions and species propagation

The work was carried out in a protected environment in the experimental area of the Federal University of Santa Maria, Frederico Westphalen Campus, located at 27º 23' S, 53º 25' O and 493 m altitude, from August 2015 to March 2017. According to Köppen's classification, the climate of the region is Cfa-temperate type, humid with hot summer and maximum air temperatures higher than 22 °C in the warmer months (ALVARES *et al*., 2013). The protected environment consisted of a galvanized steel arch structure, arranged in the East-West direction with dimensions of 10 x 20 m and 3.0 m on the ceiling height, covered with a 150 μm thick transparent low-density polyethylene film of, treated with ultraviolet radiation with 87% non-selective transmittance.

Aloysia citrodora seedlings were propagated by mini-cutting. Phenolic foam plates (2x2x5 cm) were washed in running water to eliminate probable compounds resulting from the industrialization process. The mini-cutting procedure consisted of the removal of small cuttings with three buds (approximately 10 cm long) from the parent plants, disinfested in sodium hypochlorite solution (1% active chlorine) for one minute, followed by washing in distilled water. A phenolic foam cell was used for each mini-cutting, by introducing a bud into the plate and two outwards. The cuttings were kept on a bench for 68 days with irrigation shifts controlled by timer, 15 minutes on and 60 minutes off. At night, only two irrigation periods were performed (15 minutes each). After passing through the phenolic foam, the solution returned to the reservoir.

After 13 days, the cuttings received nutrient solution with 25% of the recommendation (0.5 g L^{-1} of Calcinit®, 0.4 g L^{-1} of Hidrogood® and 0.03 g L^{-1} of chelated iron), maintaining the electrical conductivity and pH at approximately 300 µS and 6.0, respectively. After 68 days, the mini-cuttings were transplanted to five-liter pots containing a commercial substrate Carolina®, remaining for more 84 days for the complete formation of seedlings.

2.2 Experimental conditions and growth parameters

The 152 days old seedlings were transplanted to 14.3-liter plastic pots painted white on the external wall to promote greater reflection and less absorption of solar radiation, avoiding heating and excessive water loss by soil evaporation. Each pot was filled with fine gravel layer (3 kg) and sieved soil mix + 10% tanned cattle manure. Sugarcane bagasse (100 g) was added to form a cover in each pot to avoid the evaporation process.

The experiment was performed in a randomized complete block design (RCB), 4x4 factorial scheme with four blocks. The experimental unit was composed of four plants. The four irrigation levels tested were 25%, 50%, 75% and 100% of the field capacity, and the experiment was carried out during the four seasons of the year (autumn, winter and spring of 2016 and summer of 2016/2017).

Water restriction begun 45 days before the plants were collected, that is, from the period that corresponds to the half of each season. Before the beginning of water restriction, all the plants were irrigated with 100% of the field capacity. Water management was based on soil moisture, determined by the daily weighing of the pots, using a digital scale. The replacement of evapotranspiration water was performed whenever the variation between the initial pot mass and the mass obtained on the day of the evaluation was equal to or greater than 2%. Thus, the difference between the masses corresponded to the quantity of water to be completed, assuming a 1:1 weight/volume ratio.

At the end of each season, the following variables were evaluated: plant height, fresh and dry mass of branches, fresh and dry mass of leaves, leaf area and photosynthetic pigments (chlorophyll *a*, *b* and carotenoids).

Plant height was determined from the distance between the ground level and the highest meristematic apex. To determine branches and leaves fresh and dry mass, pruning was performed on the plants, approximately 10 cm above the ground, leaving buds for regrowth. The material was taken to the laboratory for separation and weighing. To determine dry mass, the branches and leaves were taken to a greenhouse for drying at 60 ºC until reaching a constant mass. The leaf area was determined by the "leaf disc method" (BENINCASA, 1988), which consists of removing leaf blade discs in a known amount and fixed area. The discs were also kept in a drying oven at 60 °C to obtain the dry mass. Subsequently, the leaf area was estimated through equation 1:

$$\mathrm{LF} = (\mathrm{ND} * \mathrm{DA} * \mathrm{LDM})/\mathrm{DDM} \qquad (01)$$

Where: LF = Leaf area (cm^2); ND = Number of discs; DA = Discs area (cm^2); LDM = Leaf dry mass (g); DDM= Disc dry mass (g).

2.3 Quantification of photosynthetic pigments

Leaf discs from leaves located at the fourth node of the apex to the base of the highest branch of all plants were used for the quantification of photosynthetic pigments (chlorophyll *a*, *b* and carotenoids). The experimental unit was composed of four test tubes for pigment extraction, which corresponded to the plants of each block. Five leaf discs of fresh material with 5 mm in diameter were taken and transferred to test tubes containing 5 mL of dimethylsulfoxide - DMSO solution (saturated with 5 g L^{-1} of calcium carbonate, $CaCO_3$), following the modified methodology proposed by Santos *et al*. (2008). The discs were covered with foil and incubated in a dark place for 48 hours. After this period, the absorbance of the samples was determined in a spectrophotometer (BEL Photonics model SP 1105), using 10 mm cuvettes at 665, 645 and 480 nm wavelengths for chlorophyll *a*, *b* and carotenoids, respectively, in µg cm^{-2}.

The variables corresponded to chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids, total chlorophyll (Chl *a*), chlorophyll *a*/*b* ratio (Chl *a*/*b*) and total chlorophyll/carotenoids (Total/carot). Total chlorophyll (Chl total) was determined by the sum of chlorophyll *a* and chlorophyll *b*; the ratio *a*/*b*, by dividing the chlorophyll *a* by *b*; and total chlorophyll/carotenoids calculated by the sum of chlorophyll *a* and *b* and subsequent division by carotenoids.

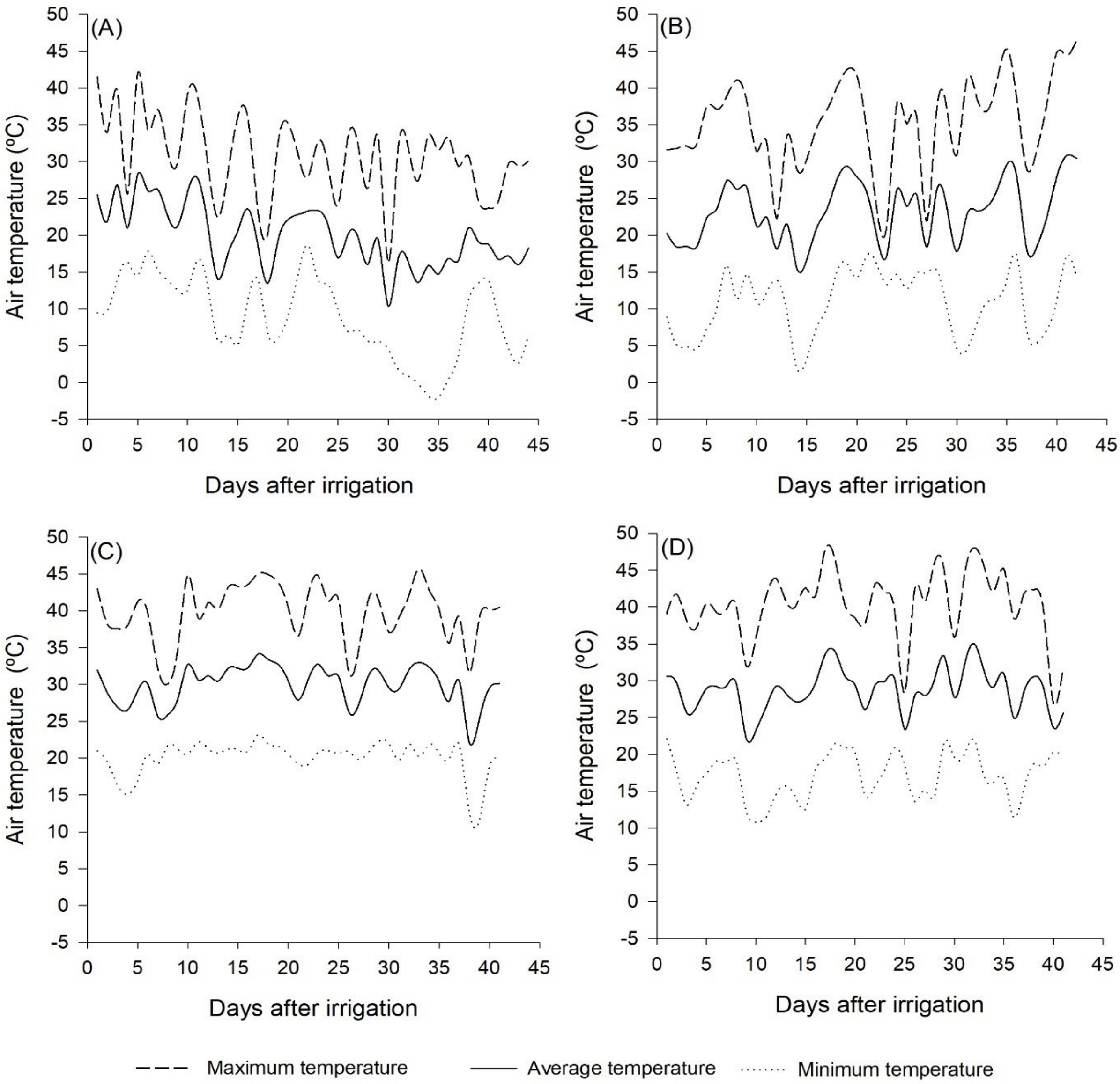
2.4 Statistical Analysis

Results were submitted to analysis of variance to evaluate the effect of different irrigation levels and seasonality. When significant, regression analysis was performed for quantitative data and means comparison for qualitative data by the Scott-Knott test at 5 % probability, using the statistical program Genes (CRUZ, 2013).

3 RESULTS

The meteorological conditions in the protected environment presented a high thermal amplitude. The effect of seasonality showed periods with high temperatures, reaching values near 50 ºC in the spring and others with low temperatures, reaching negative values during autumn (-0.2, -1.8 and -2.1 ºC). In the autumn it was possible to observe maximum and minimum temperatures ranging from 41.5 and -2.1 ºC and the average temperature of the period was 19.9 ºC (Figure 1A). For the winter season, the average temperature was higher than in the autumn (23.2 °C), but this period presented a high thermal amplitude with maximum and minimum temperatures of 46.6 and 2 °C, respectively (Figure 1B). In the spring, temperatures were between 47.9 and 10.8 °C with an average temperature of 28.7 °C (Figure 1C). The average temperature for summer was 30 °C, with maximum and minimum temperatures of 45.8 and 11.6 °C, respectively (Figure 1).

Figure 1 - Maximum, minimum and average air temperatures recorded inside the protected environment in autumn (A), winter (B) and spring (C) of 2016 and summer (D) 2016/2017, when *Aloysia citrodora* was under different irrigation levels



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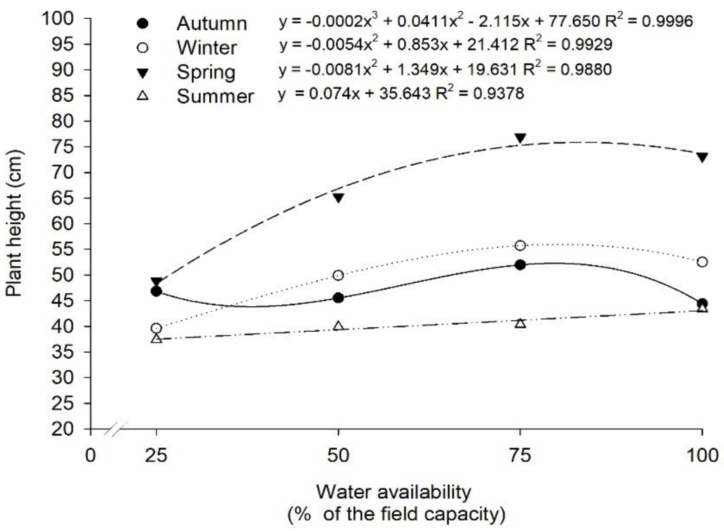
By the analysis of variance, it was possible to observe that plant height, fresh and dry mass of branches, fresh and dry mass of leaves and leaf area were significant for the interaction seasonality x water availability by the F test at 5% probability. For chlorophyll *b*, total chlorophyll/carotenoid ratio and chlorophyll *a*/*b* ratio, there was a significant difference for seasonality; and for chlorophyll *a*, carotenoids and total chlorophyll, there was a significant only for the two individual factors.

The greatest plant heights were observed during the spring season, while the lowest were verified during the summer and with irrigation level of 25% of the field capacity for most seasons. The regression equations presented cubic response for autumn and quadratic response for winter and spring. For these three seasons, irrigation level of 75% of the field capacity provided greater plant height (51.9, 55.7 and 76.9 cm, respectively). In the summer, there was a linear increase in height with increasing soil humidity, until 100% of field capacity, with an average height of 43.4 cm (Figure 2).

Figure 2 - Plant height of *Aloysia citrodora* under different irrigation levels during the four seasons of the year



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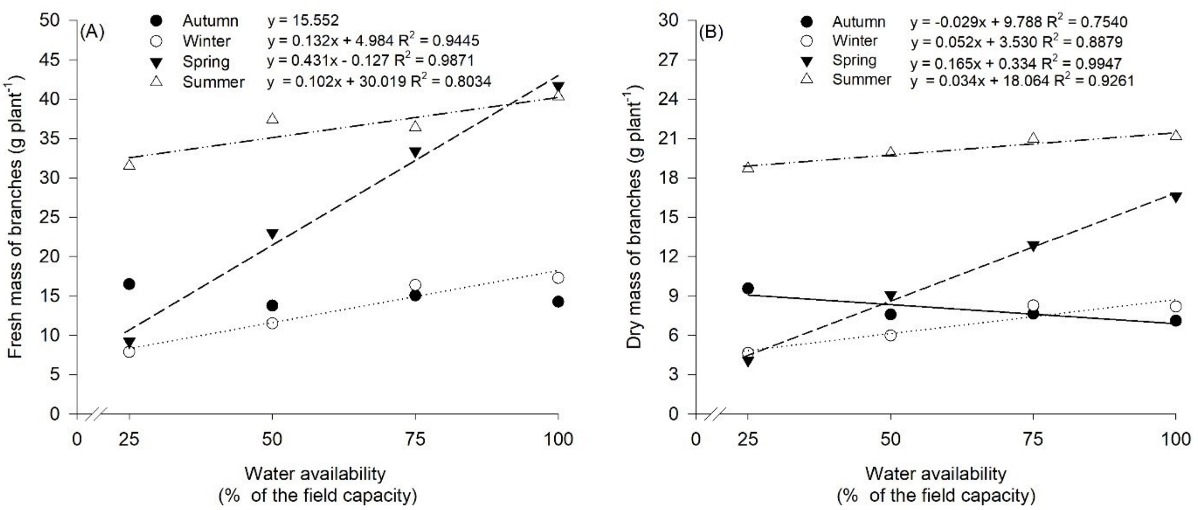
For fresh mass of branches, summer was superior to the other seasons, followed by spring. However, for these two seasons, at 100% of the field capacity, similar values were observed (41.7 and 40.3 g plant^{-1}, respectively) (Figure 3A). For the summer, there was an increasing linear response due to the increase in water availability with the lowest mean being 31.5 g plant^{-1}. The same trend was observed for winter, with the lowest mean of 7.9 g plant^{-1} and the highest of 17.3 g plant^{-1}. For the autumn season, no regression equation was significant (p>0.05) with a mean of 14.9 g plant^{-1}. For the spring season, the best-fitting regression was linear, with higher results being found for 100% field capacity water availability (Figure 3A).

Figure 3 - Fresh mass (A) and dry mass of branches (B) of *Aloysia citrodora* under different irrigation levels during the four seasons of the year



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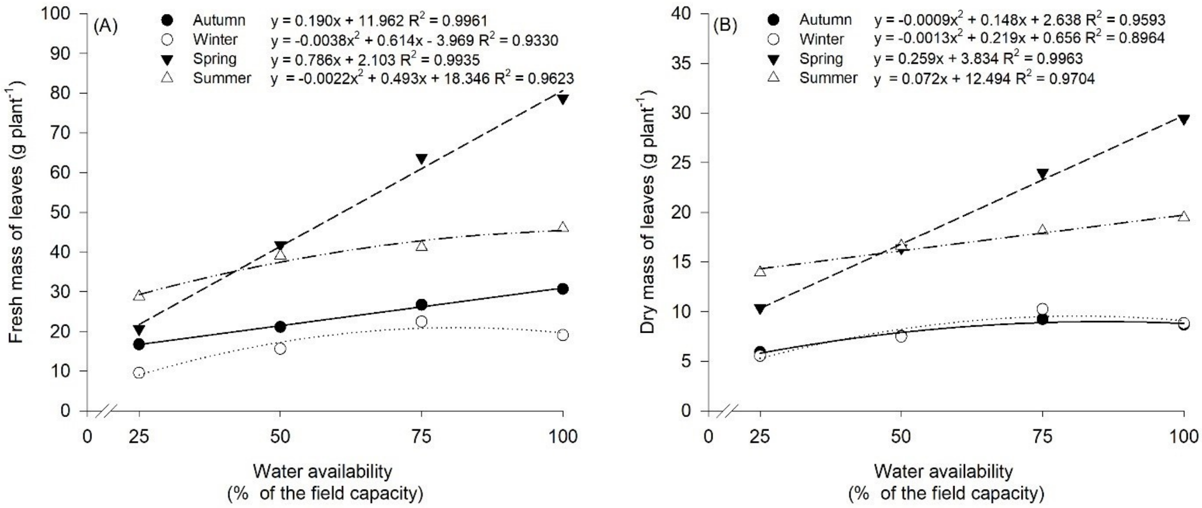
For dry mass of branches, there was an increasing linear response as a function of water availability for all the seasons, except for autumn. There was superiority in the summer in all irrigation conditions, with means of 18.698, 19.886, 20.971 and 21.153 g plant^{-1} for 25%, 50%, 75% and 100% of the field capacity, respectively. During autumn, there was a decreasing linear response, with 25% of the field capacity presenting the greatest accumulation of dry mass (9.552 g $plant^{-1}$) (Figure 3B).

Regarding fresh mass of leaves, there were higher means in the spring and lower ones in the winter. For the autumn and spring seasons, there was an increasing linear response due to the increase in water availability, in which the superior result was found with irrigation level of 100% of the field capacity (30.689 and 78.752 g $plant^{-1}$, respectively). For the other seasons, the best-fitting trend line was quadratic and in winter the fresh mass of leaves was higher with the irrigation level of 75% (22.456 g $plant^{-1}$), while in summer with 100% of the field capacity (45.981 g $plant^{-1}$) (Figure 4A).

Figure 4 - Fresh mass of leaves of *Aloysia citrodora* under different irrigation levels during the four seasons of the year



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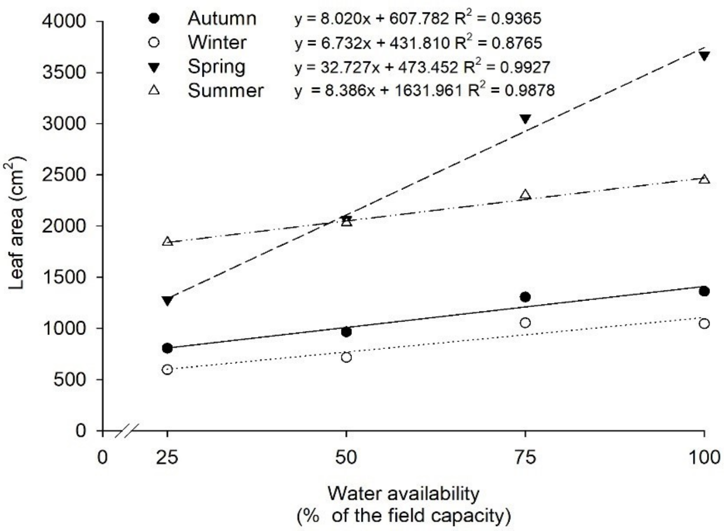
Dry mass of leaves showed a similar tendency to the fresh mass. The highest means were observed during spring and the lowest ones in autumn and winter. For the autumn and winter seasons, the best-fitting trend line was quadratic with greater accumulations in the irrigation level of 75%. For spring and summer, there was an increasing linear response in the accumulation of leaf dry mass with means of 29.438 and 19.453 g plant^{-1} with 100% of the field capacity in spring and summer, respectively. The irrigation level of 25% and cultivation during the autumn and winter seasons adversely affected the accumulation of fresh and dry mass in leaves (Figure 4B).

According to the regression equations, there was an increasing linear response of leaf area as a function of water availability for all seasons. There was superiority in spring and summer and the leaf area with irrigation level of 25% of the field capacity was greater than with irrigation level of 100% in the autumn and winter seasons. The leaf area was intensely reduced during autumn and winter and under low water availability (Figure 5).

Figure 5 - Leaf area of *Aloysia citrodora* under different irrigation levels during the four seasons of the year



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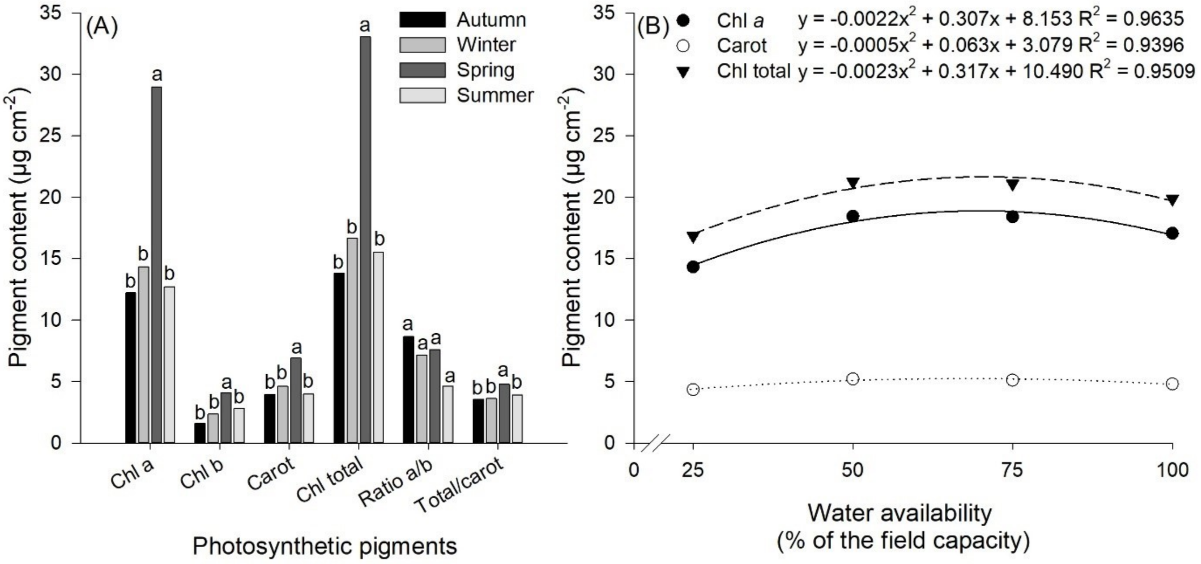
It was possible to observe that *Aloysia citrodora* presented variations in photosynthetic pigments due to the seasons of the year and irrigation levels. In the spring, there was significant superiority for chlorophyll *a*, chlorophyll *b*, carotenoids, total chlorophyll and carotenoid ratio (28.96, 4.08, 6.90, 33.05 and

4.80 µg cm^{-2}, respectively). There were no significant differences among the seasons for chlorophyll *a*/*b* ratio (Figure 6A).

Figure 6 - Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Carot), total chlorophyll (Chl total), chlorophyll *a/b* ratio (Ratio *a/b*) and total chlorophyll/carotenoid ratio (Total/carot) of *Aloysia citrodora* significant for the seasonality factor (A) and chlorophyll *a*, carotenoids and total chlorophyll significant for water availability factor (B)



*Means followed by the same letters within each variable do not differ significantly from each other by the Scott-Knott test at 5% probability.
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According to the regression equation for chlorophyll *a*, carotenoids and total chlorophyll, the quadratic tendency line of these variables was observed as a function of the increase in irrigation levels. The highest contents of chlorophyll *a* and total chlorophyll were obtained with water availability of 50% and 75% of the field capacity, and lower contents with 25% and 100% of the field capacity. For carotenoids, there was a small variation among the different water availabilities, with the highest values found in 50% of the field capacity (Figure 6B).

4 DISCUSSION

Water availability and seasonality influence and directly affect plants growth and development. The cultivation of *Aloysia citrodora* in stations with intense luminosity, higher temperatures (spring and summer) and greater water availability favored growth and phytomass accumulation. This result was confirmed due to the responses to photosynthetic pigments, since it is through chlorophyll and/or carotenoids in the foliar structures that the light energy is transformed in carbohydrates and in dry matter.

The high thermal amplitude, as observed during the cultivation of *Aloysia citrodora,* can affect physiological processes in plants. At high temperatures, plants present a reduction in stomatal conductance and, consequently, in photosynthesis which may justify the results in the variables related to leaf mass and leaf area during summer. On the other hand, low temperatures associated with the presence of light, reduce the activity of enzymes responsible for photosynthesis reactions, leading to the inhibition of photosystem I and sometimes of photosystem II, allowing the formation of ROS (ZHANG and SCHELLER, 2004; YANG *et al*., 2017; AWASTHI *et al*., 2015), as well as reducing membrane flexibility and fluidity (RAJU *et al*., 2018). Besides temperature, there are other meteorological factors (such as solar radiation and relative air humidity) which affect plant growth and development, mainly in species that present dormancy (PINTO and BERTOLUCCI, 2002; CORRÊA JUNIOR and SCHEFFER, 2013). Brant *et al*. (2008) confirms that *Aloysia triphylla* (LHér.) Britton (synonym of *Aloysia citrodora*) presents the highest vegetative growth in the middle of October (spring season), reaching maximum growth in the middle of summer (around February) when flower differentiation begins. These affirmations justify the greater phytomass and leaf area during spring, since it is a tropical and subtropical climate species, providing better responses on photosynthetic pigments, and consequently greater crop growth. On the other hand, plants can lose all the leaves during winter since they present dormancy at low temperatures (PROCHNOW *et al*., 2016), reducing leaf area and phytomass production.

Plant height presented lower results during summer. However, this reduction was compensated by the increase in lateral branches, justified by the greater number of prunings performed after the end of this season. Another factor that may justify the lower height of plants during summer is the intense incidence of solar radiation, so the plants do not need to grow searching luminosity to produce a high amount of phytomass. The radiation limit refers to the minimum amount of solar radiation required to produce photoassimilates and promote plant growth (PERIN *et al*., 2018). Therefore, in the autumn and winter seasons, the low radiation incidence affected plants height and caused less accumulation of dry matter.

Under conditions of water stress, there is a negative impact for all growth variables since water deficiency affects plant metabolic processes such as reduction of division, multiplication and cellular elongation in the apical meristems. Plants growth and development occur through mitosis, *i.e*., through cell multiplication with subsequent elongation and expansion of the plant body and increase of phytomass. This process is affected by external factors such as luminosity, temperature and water availability (ALMEIDA and RODRIGUES, 2016).

The highest increment of branches leaves and leaf area with higher irrigation levels during summer and spring is related to the higher temperatures of each period (Figure 1C and 1D). In a study with the same species, Prochnow *et al*. (2016) found greater results of fresh and dry mass of branches and leaf dry mass during summer, followed by autumn, spring and winter, regardless water stress. In addition, Schmidt *et al*. (2017) evaluating the physiological response of *Aloysia triphylla* (synonymy of *Aloysia citrodora*) in two seasons of the year observed that during summer there were better conditions of radiation, foliar temperature and transpiration when compared to the winter. These results corroborate with the present study in which the summer season presented superiority for most of all the growth variables when compared to winter, but with variations in different irrigation levels. This is because water stress promotes changes in plant such as reduction of water potential, stomatal closure, acceleration of senescence and foliar abscission (FERRARI *et al*., 2015). These changes may justify the response of the plants submitted to the lowest irrigation level, since water stress promoted foliar abscission and, consequently, reduction of phytomass and leaf area.

The reduction in plants growth is directly related to the reduction of leaf area during the vegetative phase, because plants submitted to water deficiency conditions and to the reduction of stomatal opening tend to present wilt and roll leaf in order to reduce the photosynthetic area exposure, which affects photosynthesis (SOUZA *et al*., 2013; FERRARI *et al*., 2015). In the present work, the plants showed leaf roll and, consequently, reduction in the exposure area of the leaf, resulting in a lower absorption of solar radiation. The increase of the leaf area increases the capacity of the plant to absorb photosynthetically active radiation for the photosynthetic process and to use it in photoassimilates to increase productivity (GONZALEZ-SANPEDRO *et al*., 2008).

In water deficit conditions, the main defense mechanism of the plant is the stomatal closure, due to the production of abscisic acid (ABA) which acts by decreasing the turgor pressure of the guard cells (PATAKAS *et al*., 2010; ZHAO *et al*., 2001), reducing water loss by transpiration. In addition, ABA also performs functions that involve water absorption and hydraulic control of the plant to withstand water stress since it is synthesized in the roots, and guard cells can also work as a source of this plant hormone (KUROMORI *et al*., 2018). On the other hand, the stomatal closure reduces CO_2 entry in the leaves, reducing the photosynthetic process and consequently the dry matter production. Duarte and Peil (2010) claim that the production and accumulation of dry matter by plants is directly related to the plant's ability to fix carbon through the photosynthetic process. In this work, it was verified that plants cultivated during autumn and winter and with low water availability (25% and 50% of the field capacity) showed a reduction in the production of leaves fresh and dry mass and leaf area. Due to water stress and seasonality, they promote stomatal closure and foliar abscission as a response to hormonal signaling.

Since it is a C_3 plant, *Aloysia citrodora* presents the photorespiration process, which negatively affects growth and accumulation of phytomass. Photorespiration may occur at very high rates, especially under conditions of high temperature and low water availability, once the rubisco enzyme, present in chloroplasts, uses the oxygen molecule (O_2) instead of CO_2, favoring the oxygenase activity (HAGEMANN and BAUWE, 2016) which may have been detrimental to the growth and production of *Aloysia citrodora* phytomass.

In general, the plants presented positive responses with high irrigation levels for plant height, branches fresh and dry mass, leaves fresh and dry mass and leaf area. Similar responses were observed by Silva *et al*. (2002) with *Melaleuca alternifolia*, Carvalho *et al*. (2003) with *Artemisia* and Schwerz *et al*. (2015) with *Aloysia triphylla* (synonymy of *Aloysia citrodora*), in which high irrigation levels favored the production of fresh and dry mass of plants, while water deficiency affected these parameters negatively.

Plants responses to the conditions of water availability and seasonality are related to photosynthetic pigments. The greatest content of chlorophyll was found during spring and it demonstrates that in this season the plants presented the best climatic conditions for growth and development as a function of the increase in the photosynthetic capacity. Hendry and Price (1993) and Blum (2005) reported that higher contents of chlorophyll in leaves indicate greater photosynthetic efficiency by the plants and higher production of the crop. Therefore, higher content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total and carotenoids) found during spring associated with high temperatures, increased light intensity and water availability, favoring the photosynthetic process and, consequently, phytomass production.

Growth and yield are defined by photosynthesis, which depends on the interception and spectral characteristics of the light absorbed by the leaf area (MALDANER *et al*., 2009). Therefore, chlorophyll content is an important parameter to evaluate the effects of water deficiency and seasonality, since leaves are the most responsible organs for the photosynthetic process. Besides, the foliar pigmentation tends to vary depending on the photosynthetic pigment content, internal structures and intrinsic characteristics of each plant species (SANTOS *et al*., 2014).

Solar radiation energy reaches the external structures of the leaf and diffuses to the leaf mesophyll, being absorbed by the chloroplasts and finally used for the photosynthetic process (PONZONI *et al*., 2012). The highest concentration of chloroplasts occurs in the upper portion of leaf mesophyll, where the chlorophylls and carotenoids are, since this region receives the highest amount of solar radiation (CHEN *et al*., 2015). Then, it can be inferred that lower pigment content can result in lower photosynthetic rate and phytomass production, as observed in those plants grown during autumn and winter and under water stress conditions.

The reduction of chlorophyll content in the leaf may also be related to the acceleration of leaf senescence caused by water deficiency (EFEOĞLU *et al*., 2009). Plants submitted to lower levels of irrigation showed greater leaf senescence as a way of tolerating the lack of water, reducing transpiration and the drain-source ratio. Before senescence, leaves show yellowing symptoms, *i.e*., the chlorophylls begin to get degraded and the green pigmentation is reduced (CHAVES and OLIVEIRA, 2004). Therefore, the process of chlorophyll degradation and leaf senescence justifies the lowest pigment content, since senescence occurs first in the basal part of the plant and the determination of chlorophyll was carried out to the leaves near the apical region of the stem (young leaves). In addition, chlorophylls degradation may also justify the inferior results found in the plants submitted to water availability of 25% of the field capacity.

The lowest content of pigments found in autumn and winter can be justified by the beginning of leaf loss in colder periods (usually in May and June) since the plant presents dormancy due to low temperatures (PAULUS *et al*., 2013). Leaf loss can occur even under protected environment conditions but with less severity. Therefore, the photosynthetic pigments are directly related to phytomass and leaf area, demonstrating that cultivation of *Aloysia citrodora* during spring with enough water availability allows plants to present greater photosynthetic pigmentation, which reflects in greater production of fresh and dry mass of leaves and leaf area.

These results highlight the best seasons of the year and water availability to achieve high phytomass production for the species. In general, the content and composition of secondary metabolites are varied with changes in the growing seasons, growth years, and environment, increase or decreases under developmental processes or in the presence of different stresses conditions (Li *et al*., 2020). Therefore, our results are important for future studies as well as establishing whether a condition that promotes greater phytomass production is the same one that allows greater production and quality of essential oil, which is the main secondary metabolite produced by the plants. The new technologies used to study genomics, transcriptomics, and metabolomics can be used to reveal active constituents of medicinal plants at different growth stages and stress conditions (Li *et al*., 2020).

5 CONCLUSIONS

It is concluded that even under protected environment conditions, low water availability and cultivation during autumn and winter adversely affect the vegetative growth of *Aloysia citrodora*. The cultivation of this species is recommended during spring and summer under water levels of 75% and 100% of the field capacity because they favor phytomass production. Spring provides higher content of photosynthetic pigments, indicating better physiological responses by plants.

This study will open new avenues and perspectives to elucidate possible changes in secondary metabolites of *Aloysia citrodora* in the different seasons of the year and water stress.

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