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Effect of Cattle Manure on Young Plants of *Khaya senegalensis* Submitted to Different Irrigation Intervals

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Abstract

This study aimed to evaluate the effect of cattle manure on young plants of *Khaya senegalensis* submitted to different irrigation intervals. A greenhouse experiment was carried out during 2017 cold season, based in a 5x3 factorial arrangement – cattle manure fertilizing with 0, 10, 20, 30 and 40% doses, and 3-day, 6-day and 12-day irrigation intervals. Water potential, relative water content (RWC), water consumption (WC), water use efficiency (WUE), chlorophyll content estimation (SPAD index), plant height, stem diameter, plant total dry mass and free amino acid and proline content were measured. Irrigation intervals had no significant effect on physiological traits and plant water relationships, except for WUE, which was higher under 12-day irrigation interval. This was assumed as an indication of non-occurrence of water stress, even under the longest irrigation interval. Cattle manure, in turn, played a significant effect on these traits, except for water potential, irrespective of irrigation intervals. Increasing cattle manure doses improved RWC (just doses up to 20%), reduced WC and increased WUE, SPAD index, and both free amino acid and proline contents. On the other hand, growth-related parameters were not affected by cattle manure, but a significant effect of irrigation intervals was observed. Both plant height and total dry mass were lower under 3-day and 6-day irrigation intervals. We suspect a possibly low evapotranspiration, given the relatively low temperatures of that season (14-16 ℃), which may have reduced the demand for irrigation in the experimental period. Thus, soil moisture under the shorter irrigation intervals may have increased until reach to a close to saturation level, limiting the amount of soil oxygen and hampers nutrient uptake.

Keywords: woody plants, water stress, organic fertilizing

1. Introduction

African mahogany stands out in the noble wood market and is referred one of the most valuable tropical woody species in the world (Nikiema and Pasternak, 2008). *Khaya senegalensis* is the African mahogany species most cultivated in Brazil, due to its rusticity and relative adaptability for cultivation under adverse conditions, especially in low rainfall regions (Pinheiro et al., 2011).

Water is one of the most limiting abiotic factor for plants, since its availability reflects on many morphological and physiological features, as well as on metabolic processes, which are essential for plant growth and productivity. Regarding to young plants, eventual water restrictions may be determinant for survival, either during seedlings production or at initial development after seedlings transplanting on the definitive site.

Water deficit inhibits cell division and elongation, leading to decrease on plant growth rate. The cell division is affected by enzyme inhibitors and microRNAs which regulate cell division and differentiation (Claeys and Inzé, 2013). The cell elongation is a consequence of cell wall extensibility and turgor pressure, which are gibberelins-signaling dependent. Gibberelins act in proteins synthesis that control the reactive oxygen species levels, regulating the Ca^{2+} content, which influences the cell wall stiffness and play direct control on its resistance of turgor pressure (Achard et al., 2008; Dupuy et al., 2010).

Water stress also negatively affect growth due to reducing photosynthesis rates, which leads to a decreasing on primary carbohydrate synthesis. Since stomatal closure is among the first physiological events to occur in response to water deficiency, and since stomatal conductance is always strongly correlated with CO_2 net assimilation, it is assumed that a reduced stomatal opening reduces CO_2 absorption in leaves under water stress (Aroca, 2012). Water stress, on the other hand, induces the reactive oxygen species synthesis, which oxidize chlorophylls, causing damage to the photosynthetic apparatus (Van Breusegem and Dat, 2006). If stress is severe, there may be a decrease on Rubisco activity and difficulty to regenerate RuBP, negatively affecting photosynthesis (Bota et al., 2004).

Cattle manure is an organic fertilizer that, in addition to providing nutrients, can improve other soil chemical and physical attributes by altering its water retention ability. Cattle manure aggregates soil coloids, affecting the porosity (macro, micro and total), density and resistance to root penetration (Tormena et al., 2002; Galvão et al., 2008; Guareschi et al., 2012). The pore size is linked to capillarity and adsorption, which, in turn, are related to water retention. For these reasons, organic matter is considered as one of the factors that influence the water retention and, consequently, the soil water availability (Tuller and Or, 2004).

In addition to the effects on soil attributes, the application of manure may improve conditions for plant growth under water stress. Studies on [*Glycine max* (L.) Merr.] leaves fertilized with cattle manure showed that the increase in chlorophyll content may be an indication of decreased activity of glutathione peroxidase and superoxide dismutase (Nozari et al., 2013; Taherianfar et al., 2013). Oregano plants (*Origanum vulgare* L.) submitted to irrigation intervals of up to three weeks showed increases in number of stems, plant propagation, stem diameter, leaf area and fresh and dry plant yield, with increasing levels of cattle manure (Gerami et al., 2016).

This study aimed to investigate the effect of cattle manure on morphophysiological and biochemical changes of *Khaya senegalensis* young plants submitted to different irrigation intervals in order to better understanding the tolerance mechanisms to eventual water deficit.

2. Material and Method

The experiment was carried out in a greenhouse at the Southwest Bahia State University, in Vitória da Conquista, Bahia, Brazil (14°53' S 40°48' W), 881 m a.s.l. and Tropical Altitude climate (Cwa), according to Köppen classification. The experimental period lasted from March to August 2017 (cold season), in order to simulate the weather conditions of a typical season with low temperatures and shortage rainfall.

2.1 Plant Material and Experimental Design

Khaya senegalensis seedlings 120 days old were produced in 280 cm^3 tubes and were transplanted in 20 L pots, whose substrate was a yellow dystrophic Tb latosol, sandy-clay texture, to which the cattle manure was added and mixed in different proportions.

The experimental design was randomized blocks, arranged in factorial 5x3, three blocks, one plant per replication, totaling 45 plants. One factor was the cattle manure in percentages 0, 10, 20, 30 and 40% of the total volume of the substrate (16 L). The other factor was the irrigation intervals, that is, 3, 6 and 12 days. At each irrigation, the amount of water supply was equivalent to the difference between the weight of each pot and the average weight of three pots kept permanently close to the field capacity.

2.2 Water Potential and Relative Water Content

The plant water status was evaluated based on water potential and relative water content data of mature leaves of the middle part of the canopy, measured at the pre-dawn. For water potential measurements, a pressure chamber (Model 1000, PMS) was used, according to Scholander et al. (1965). For relative water content (RWC) determination, leaf discs were collected, and their fresh (FM), dry (DM) and turgid (TM) masses were checked. RWC was obtained by the formula RWC = [(FM-DM) / (TM-DM)].100, according to Weatherley (1950).

2.3 Water Consumption and Water Use Efficiency

For water consumption (WC) measurement per plant, the amount of water supplied at each irrigation required to restore the substrate moisture close the field capacity, was considered. The water use efficiency (WUE) determination was obtained by the stem volume (SV) and WC ratio, that is: WUE = SV / WC.

2.4 Chlorophyll Content Estimation (Spad Index)

For SPAD index measurements, mature leaves of the middle part of the canopy were chosen, and a SPAD-502 Plus chlorophyllometer (Minolta Corporation, Ltda., Osaka, Japan) was used. Measurements were carried out from 12 a.m. to 1 p.m., and data were assumed as the average of seven measurements per plant.

2.5 Morphological and Physiological Evaluations

Plant height and stem diameter were measured by using a tape measure and digital caliper, respectively. After the experimental period, plants were removed from the pots, submitted to kiln-drying, and dry mass was checked.

2.6 Biochemical Analysis

After the experimental period, mature leaves from the middle part of the canopy were collected and submitted to kiln-drying. 15 mL of potassium phosphate solution 0.1 M were used to aminoacid (total) and proline extraction, from 200 g of dried leaves.The mixture was filtered and centrifuged at 8,000 rpm for 15 minutes, collecting the supernatant, in a replicated procedure three times, until the final extract was obtained.

For amino acid content determination, the method proposed by Yemm and Coccking (1955) was followed. Aliquots from the extract were added to a medium containing 0.5 mL of 0.2 M sodium citrate buffer (pH 5.0), 0.2 mL of 5% ninhydrin in methyl cellosolve, 1.0 mL of 2% KCN, in methyl celosolve and 0.94 mL of water. At the end, the amino acid content was analyzed by a spectrophotometer at 570 nm.

For proline content determination, the method proposed by Bates et al. (1973) was followed. Aliquots from the extract were added to a medium containing 2.0 mL of acidic ninhydrin solution, 2.0 mL of glacial acetic acid and 1.5 mL of water. The medium was stirred and heated in a 100 ℃ water bath, for 1 hour. After cooling to ambient temperature, 4 mL of toluene was added to the medium, for complete proline extraction, followed by stirring, thereby forming a supernatant. At the end, proline content was analyzed by a spectrophotometer at 520 nm.

2.7 Statistical Analysis

Liliefort and Cochran tests were used for data analysis, in terms of normality and homogeneity, respectively. The analysis of variance, followed by the means test, was used to compare the effects of irrigation intervals. Regression analysis was applied to cattle manure doses data. These procedures were performed by using SAEG 9.1 and ASSISTAT programs, edit 7.7 pt.

3. Results and Discussion

The irrigation intervals had no significant effect on almost all traits, except for WUE. Inversely, the cattle manure doses affected almost all traits, except the water potential. Interaction between the factors had significant effect on RWC, WC, SPAD index and proline content (Table 1).

Table 1. Analysis of variance and coefficient of variation (CV) of predawn leaf water potential (Ψw), leaf relative water content (RWC), water consumption (WC), water use efficiency (WUE), SPAD index (SPAD), and amino acid (AA) and proline (P) leaf contents in young plants of *Khaya senegalensis* fertilized with different cattle manure (CM) doses and submitted to three irrigation (I) intervals.

Sources of Variation	Degrees of Freedom	Mean Squares			
		Ψw	RWC	WC	WUE
(CM) doses	4	0.01	46.29**	45.78**	33780.73*
(I) intervals	2	0.02	4.85	5.75	98044.01**
(CM) x (I)	8	0.01	20.77*	7.49**	5057.26
Blocks	2	0.03*	8.55	0.01*	291.82
Error	28	0.01	8.05	1.95	8373.17
CV (%)		17.88	3.43	7.26	23.50

Sources of Variation	Degrees of Freedom	Mean Squares		
		SPAD	AA	P
(CM) doses	4	107.88**	0.01**	0.93**
(I) intervals	2	4.58	0.00	0.03
(CM) x (I)	8	23.11**	0.00	0.15**
Blocks	2	2.57	0.01	0.01
Error	28	3.67	0.00	0.03
CV (%)		2.99	19.17	15.95

(*) significant ($p \leq 0.05$); (**) significant ($p \leq 0.01$)

Leaf water potential was not affected by both factors. This can be interpreted that there was no significant reduction on substrate water availability, even after the largest irrigation interval. The predawn leaf water potential, besides representing the plant water status, is generally assumed to represent the mean soil water potential next to the roots, being closely corelated to the relative transpiration rate (Améglio et al., 1999; Williams and Araujo, 2002; Chastain et al., 2016).

The fact that there was no water potential change, even after 12-day irrigation interval, can be related to a probable low evapotranspiration rate, due to the meteorological conditions of that period, since the experiment was carried out during the winter, when temperature and solar radiation are reduced.

Field conditions researches on both brazilian mahogany (*Swietenia macrophylla*) (Morais et al., 2015) and african mahogany (*Khaya senegalensis*) (Arndt et al., 2015) showed that predawn leaf water potential varies according to the meteorological conditions, since the mean values in dry season (winter) were lower than in rainy season (summer).

Irrigation intervals had no effect on RWC, which corroborates the understanding that the substrate water availability did not decrease significantly even after the longest irrigation interval. On the other hand, RWC was affected by cattle manure doses, and the interaction between this factor and the irrigation intervals was significant (Table 1).

Cattle manure doses had a quadratic effect on RWC, although this was occurred just on the 12-day irrigation interval. In this irrigation interval, the RWC increased, with increasing cattle manure doses up to the estimated value of 20.87%, followed by a decrease at higher doses (Figure 1). At the shortest irrigation intervals, a regression equation to express the cattle manure effect on RWC became unpracticable. Researchers have reported variable effects of organic manures on leaf RWC in plants under water deficit (Khadem et al., 2010; Rahbarian et al., 2014), but this relationship remains not clear.

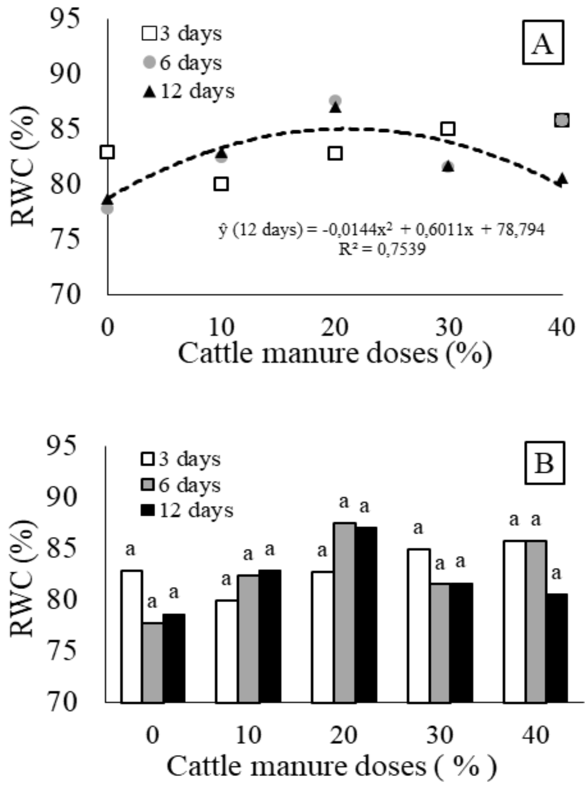

Figure 1. Leaf relative water content (RWC) in young plants of *Khaya senegalensis* fertilized with different cattle manure doses and submitted to three irrigation intervals. Effect of cattle manure doses on RWC in each irrigation interval [A]. RWC mean values of irrigation intervals, in each cattle manure dose [B]. Within each cattle manure dose, RWC mean values having the same letters indicate that there is no significant difference among the irrigation intervals. Mean values were compared by Tukey's test ($p \leq 0.05$)

Irrigation intervals had no significant effect on WC. However, it was affected by the cattle manure doses, and there was significant interaction between this factor and the irrigation intervals (Table 1). WC was slightly lower, with increasing cattle manure doses (Figure 2A). In plants fertilized with 30% cattle manure, WC in the 12-day irrigation interval was lower than in the shorter irrigation intervals (Figure 2B).

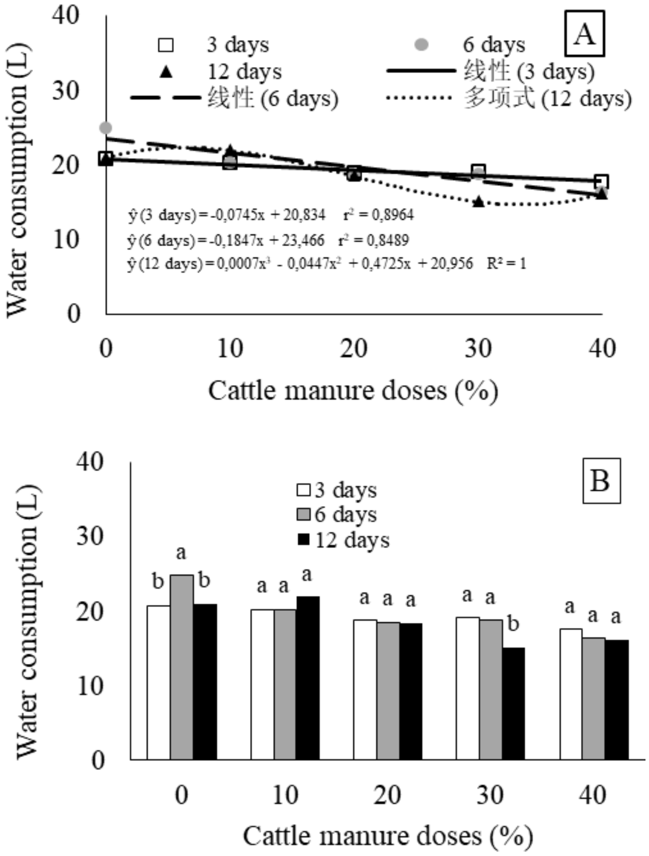

Figure 2. Water consumption in plant-soil system, in which young plants of *Khaya senegalensis* were fertilized with different cattle manure doses and submitted to three irrigation intervals. Effect of cattle manure doses on water consumption in each irrigation interval [A]. Water consumption mean values of irrigation intervals, in each cattle manure dose [B]. Within each cattle manure dose, water consumption mean values having the same letters indicate no significant difference among the irrigation intervals. Mean values were compared by Tukey's test ($p \leq 0.05$)

Changes on WC can be attributed to the beneficial effects of manure fertilization on soil physical properties. With the addition of organic matter, the soil specific surface area increases resulting in increased water holding capacity at higher tensions (Haynes and Naydu, 1998; Vengadaramana and Thairiyanathan, 2012).

Both cattle manure doses and irrigation intervals had significant effect on WUE. Nevertheless, there was no significant interaction between these factors (Table 1). Increasing cattle manure doses led to a greater WUE (Figure 3A). There was no difference on WUE between 3-day and 6-day irrigation intervals, but WUE was higher in the 12-day irrigation interval (Figure 3B).

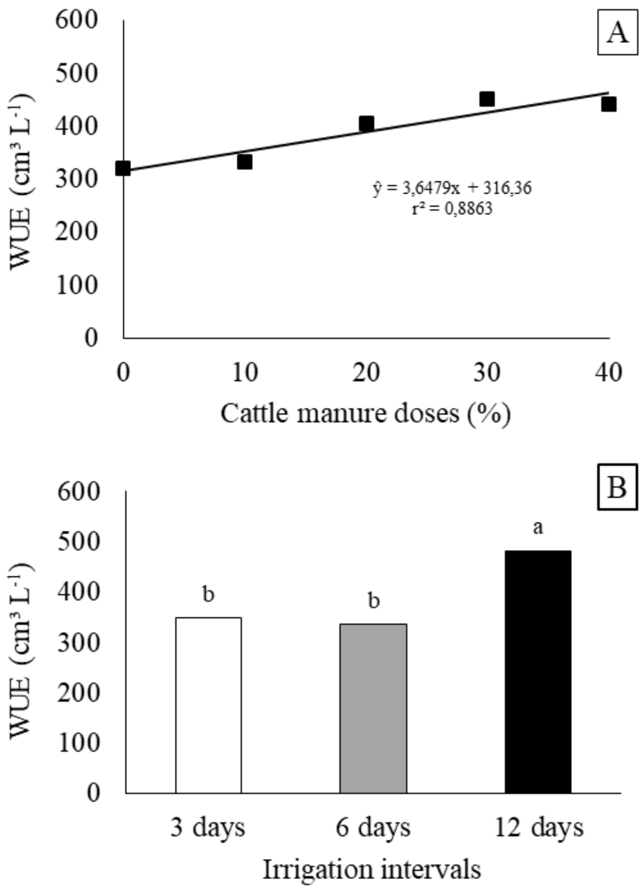

Figure 3. Water use efficiency (WUE) in young plants of *Khaya senegalensis* fertilized with different cattle manure doses and submitted to three irrigation intervals. Effect of cattle manure doses on WUE [A]. Effect of irrigation intervals on WUE [B]. WUE mean values having the same letters above the columns indicate no significant difference among the irrigation intervals. Mean values were compared by Tukey's test ($p \leq 0.05$)

Previous studies have shown that organic fertilizers application can increase the soil water-holding capacity and improving WUE (Fan et al., 2005; Wang et al., 2017; Zhang et al., 2017). Liu et al. (2013) found that organic fertilizer increased the soil water-holding capacity by increasing the percentage of macro-aggregates (> 0.25 mm) and soil nutrients, which ultimately improved WUE. As for the increased water use efficiency under 12-day irrigation interval, this physiological response has been attributed by some researchers to a decreased stomatal conductance more pronounced than carbon assimilation, which results in greater instant water use efficiency (Pita et al., 2005; Perez et al., 2016), as well as to a decreased water deep percolation and evaporation (Gholamhoseini et al., 2013).

Irrigation intervals had no effect on SPAD index. On the other hand, SPAD index was affected by cattle manure doses, and there was significant interaction between this factor and irrigation intervals (Table 1). Increasing cattle manure doses increased SPAD index, irrespective of irrigation intervals (Figure 4). This is similar to the findings of a number of researchers (Njoku et al., 2008; Jahromi et al., 2012; Yamika et al., 2018), who observed higher chlorophyll levels in plants under organic fertilization than in no fertilized plants.

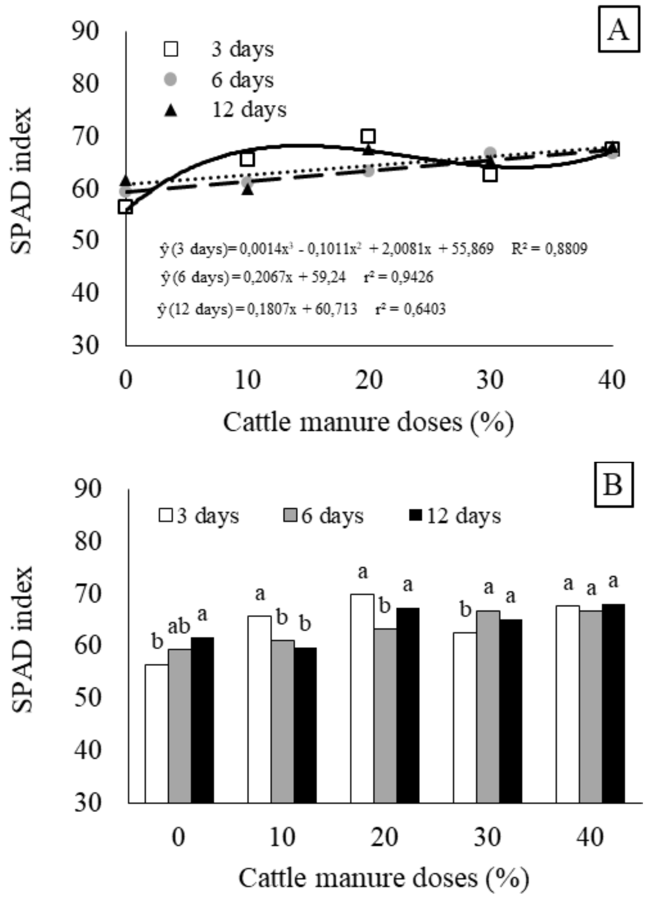

Figure 4. Leaf chlorophyll content estimation (SPAD index) in young plants of *Khaya senegalensis* fertilized with different cattle manure doses and submitted to three irrigation intervals. Effect of cattle manure doses on SPAD index in each irrigation interval [A]. SPAD index mean values of irrigation intervals, in each cattle manure dose [B]. Within each cattle manure dose, SPAD index mean values having the same letters indicate no significant difference among the irrigation intervals. Mean values were compared by Tukey's test ($p \leq 0.05$)

Increasing SPAD index related to increased cattle manure doses was probably due to the role of organic matter in improving availability of soil nutrients that can be absorbed by *Khaya senegalensis* young plants. Decreasing leaf chlorophyll content in plants under limited water supply is well known (Aroca, 2012). Thus, the greater values of SPAD index, related to increasing doses of cattle manure, are assumed to be important because they signal to a possible mitigation of the negative effects of water stress, since the photosynthetic activity and carbohydrate yield increase with increased leaf chlorophyll content (Ramesh et al., 2002).

Irrigation intervals had no effect on free amino acid content in leaf tissues. Nevertheless, amino acids were affected by cattle manure doses, although there was no significant interaction between this factor and irrigation intervals (Table 1). Increasing cattle manure doses increased amino acid content, irrespective of irrigation intervals (Figure 5). Similar results were previously reported by Abou El-Khair et al. (2011), and this is probably due to the known cattle manure effect on improving availability of soil nutrients, specially the nitrogen (Haynes and Naydu, 1998).

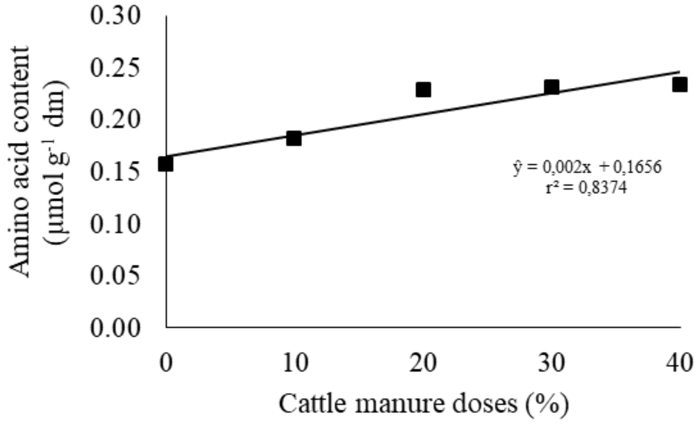

Figure 5. Cattle manure effect on leaf amino acid content in young plants of *Khaya senegalensis* fertilized with different cattle manure doses and submitted to three irrigation intervals. Data were transformed using $\sqrt{(x)}$, to attain a normal distribution, according to the Lilliefort test

Irrigation intervals had no effect on proline content. On the other hand, proline was affected by cattle manure doses, and there was significant interaction between this factor and irrigation intervals (Table 1). Cattle manure effect was similar to that on total amino acids, this is, a positive correlation between increasing doses and proline content (Figure 6A). Nevertheless, there was no standard response among the irrigation intervals on each cattle manure dose (Figure 6B).

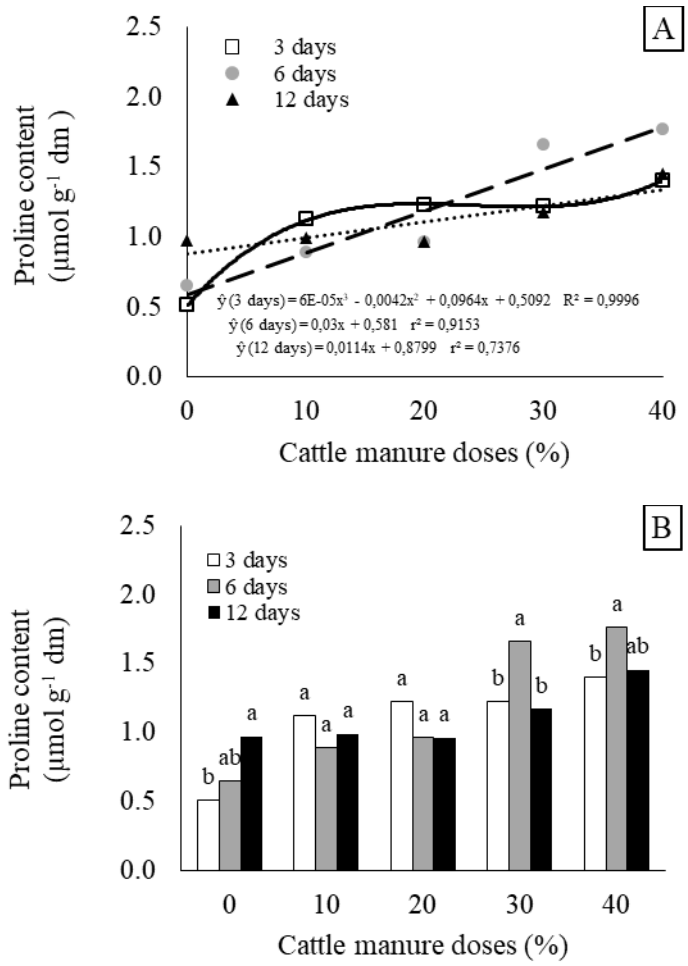

Figure 6. Cattle manure effect on leaf proline content in young plants of *Khaya senegalensis* fertilized with different cattle manure doses and submitted to three irrigation intervals. Effect of cattle manure doses on proline content in each irrigation interval [A]. Proline content mean values of irrigation intervals, in each cattle manure dose [B]. Within each cattle manure dose, proline content mean values having the same letters indicate no significant difference among the irrigation intervals. Data were transformed using log(x), to attain a normal distribution, according to the Lilliefort test. Mean values were compared by Tukey's test ($p \leq 0.05$)

Cattle manure had no significant effect on growth parameters. Nevertheless, irrigation intervals reflected significant differences on plant height, stem diameter and total dry mass (Table 2). All growth parameters were positively correlated with irrigation intervals. In general, the mean values of these parameters were higher in plants under a 12-day irrigation interval, with little or no difference between the 3-day and 6-day irrigation intervals (Figures 7 and 8).

Table 2. Analysis of variance and coefficient of variation (CV) of plant height, stem diameter and total dry mass in young plants of *Khaya senegalensis* fertilized with different cattle manure (CM) doses and submitted to three irrigation (I) intervals

Sources of Variation	Degrees of Freedom	Mean Squares		
		Plant height	Stem diameter	Total dry mass
(CM) doses	4	9.45	2.17	190.57
(I) intervals	2	349.27**	8.05*	1046.72**
CM x I interation	8	24.98	1.29	228.51
Blocks	2	0.54*	0.64	37.54
Error	28	36.89	2.32	130.25
CV (%)		11.45	9.70	24.10

(*) F significant ($p \leq 0.05$); (**) F significant ($p \leq 0.01$)

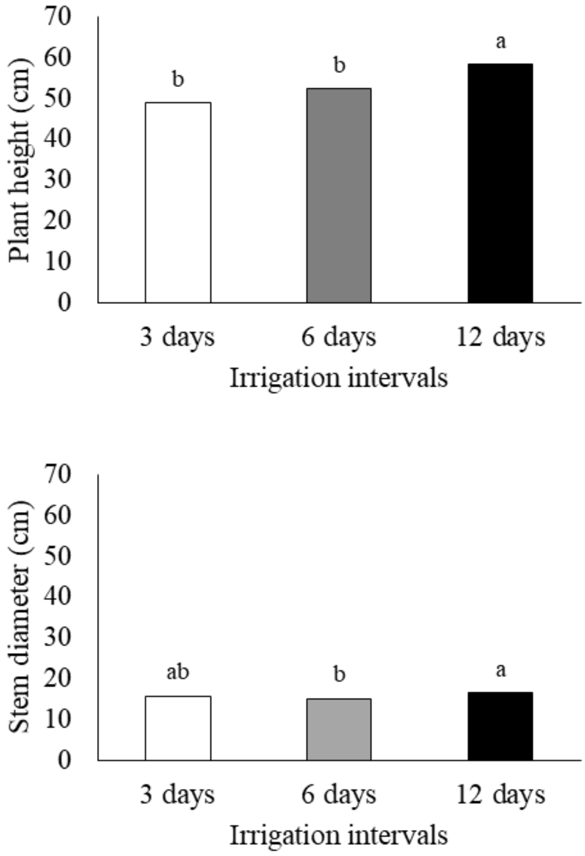

Figure 7. Irrigation intervals effect on plant height and stem diameter in young plants of *Khaya senegalensis* fertilized with different cattle manure doses. Mean values having the same letters above the columns indicate no significant difference among the irrigation intervals. Mean values were compared by Tukey's test ($p \leq 0.05$)

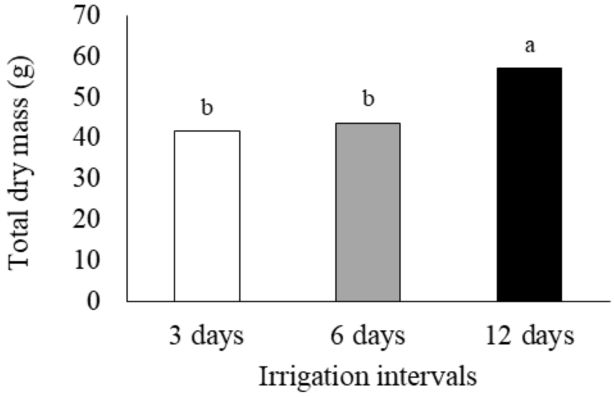

Figure 8. Irrigation intervals effect on total dry mass in young plants of *Khaya senegalensis* fertilized with different cattle manure doses. Mean values having the same letters above the columns indicate no significant difference among the irrigation intervals. Mean values were compared by Tukey's test ($p \leq 0.05$)

In general, growth-related parameters, such as plant height, stem diameter and dry mass accumulation, tend to be negatively affected by longer irrigation intervals, given the less frequent water supply (Bagheri et al., 2012; Asadi and Alimohamadi, 2019). In this study, however, lower values of plant height and total dry mass were found in 3-day and 6-day irrigation intervals, compared to 12-day interval.

We suspect some factors that may have influenced these performances on growth-related parameters. First, a supposedly low evapotranspiration, given the winter mean temperatures (14-16 ℃), may have reduced the demand for irrigation in the experimental period. No difference in water potential among the irrigation intervals corroborates an indication that there was no soil water deficit. Thus, both in 3-day and 6-day irrigation interval, soil moisture may have increased until reach to a close to saturation level. This could create a condition which greatly limits the amount of oxygen roots can obtain and hampers nutrient uptake.

The biology of plant tolerance to saturated soils is still poorly understood, and studies conducted to determine flood tolerance have resulted in contradictory conclusions. Still, it is generally accepted a decline in growth as an usual plant response to saturated soils. Restricted tree and root growth was observed when air capacity was reduced to level less than 15% (Nel and Bennie, 1984). Soil saturation up to close to flooding negativelly affecting growth-related parameters in tree species has also been reported (Sakio, 2005; Oliveira and Joly, 2010; Allen et al., 2019).

4. Conclusion

Irrigation intervals had no significant effect on both physiological traits and plant water relationships, except for WUE, which was higher under 12-day irrigation interval. This was assumed as an indication of non-occurrence of water stress, even under the longest irrigation interval. Cattle manure, in turn, had a significant effect on these traits, except for water potential, irrespective of irrigation intervals. Increasing cattle manure doses improved RWC (just doses up to 20%), reduced WC and increased WUE, SPAD index, and both free amino acid and proline contents. On the other hand, growth-related parameters were not affected by cattle manure, but a significant effect of irrigation intervals was observed. Both plant height and total dry mass were lower under 3-day and 6-day irrigation intervals. A probable reduced demand for irrigation during experimental period may have occurred, related to a possible low evapotranspiration, given the relatively low temperatures of that season (14-16 ℃). We suspect that shorter irrigation intervals may have increased the soil moisture until reach to a close to saturation level, limiting the amount of soil oxygen and hampering the nutrient uptake.

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