Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2023, Volume 14, Issue 7, Page 838-851

http://www.jmaterenvironsci.com

Morphophysiological and biochemical responses to water stress in the juvenile and adult stages of *Salvia officinalis* L under a glass greenhouse

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Received *23 April 2023,*
Revised *29 June 2023,*
Accepted *10 July 2023*

Keywords:
- ✓ *water stress,*
- ✓ *morphological,*
- ✓ *physiological,*
- ✓ *biochemical parameters,*
- ✓ *treatments 100, 60 and 20% ET0.*

Citation*: Chetouani M., Chetouani R. (2023) Morpho-physiological and biochemical responses to water stress in the juvenile and adult stages of Salvia officinalis L under a glass greenhouse, J. Mater. Environ. Sci., 14(7), 838-851.*

Abstract: The Mediterranean vegetation is rich in plants adapted to climate change and irregular rainfall, which makes water one of the most frightening resources as a result, the Mediterranean region is classified as particularly sensitive to global warming. The scarcity of water resources is therefore considered a limiting climatic factor that affects agricultural production on a global scale and in particular in the Mediterranean basin. It is estimated that more than 800 million hectares of land in the world suffer from abiotic stress, which has allowed the development of a flora rich in medicinal and aromatic plants adapted to the climate such as Sage. As part of the evaluation of the morphophysiological and biochemical responses to the effect of water stress on plants in order to enhance their ability to resist water changes, we were interested in *Salvia officinalis. L* taking into account its medicinal, aromatic and socio-economic by applying three water regimes (moderate and severe: 100, 60 and 20% ET0). The *Salvia officinalis.L* showed that this species is relatively tolerant to water stress at moderate doses in the adult stage.

1. Introduction

Mediterranean vegetation is composed of plants adapted to climatic changes and irregular rainfall. The combination of summer droughts and irregular precipitation make water one of the most frightening resources (Joffre et al, 1999; Iglesias et al, 2006). Thus, Thuiller (2005) classified the Mediterranean region as particularly sensitive to global warming and allows the development of a rich flora of medicinal and aromatic plants adapted to the climate such as Sage. Survey of literature on Mediterranean climate indicated that the interval of water supply affected the trunk diameter, branch length, fruit weight, flesh firmness, flesh/seed ratio, and acidity of the fruit (Durgar *et al*, 2017; Nasri *et al*, 2021; Medda , 2022).

Indeed, studies on the effects of drought on certain species have been elucidated for many years. However, information on the relationship between plants and ecophysiological perfor mance in response to drought is still relatively scarce with a few exceptions (Chetouani *et al,* 2022). Therefore, understanding the behavior and how plants respond to water stress is paramount for the implementation of practices management under severe water stress. Among the direct consequence of the rising soil temperature caused by climate change, there are : soil texture, moisture properties, degree of insulation by snowpack or vegetation, latitude, and season (Abouatallah *et al.,* 2011; Jungvist *et al.,* 2014; Beer *et al.,* 2020). The water stress linked to climate change is responsible to the orientation of local economy. The current linear economic model focuses on products, which are produced, used, and then thrown away as waste. The circular model focuses on services instead, offering a single service that can be used by many instead of the same product replicated for multiple individuals season (Hernández-Chover *et al.,* 2023; Burg *et al.,* 2023; Beer *et al.,* 2020).

Among the wide cultivated and studied natural plant in morocco, Salvia officinalis L. presents great commercial importance due to its flavouring and seasonings properties, this plant was widely used in the preparation of many foods (Al-Mijalli *et al.,* 2022; Khiya *et al.,* 2019; Fakchich & Elachouri, 2021). *Salvia officinalis L* becomes popular in all part of the world, and has been recommended for the treatment of different types of disorders, including seizure, ulcers, gout, rheumatism, inflammation, dizziness, tremors, paralysis, the diarrhea, and hyperglycemia (Rasouli *et al.,* 2020). The commercial importance of the Salvia officinalis L. plant is due to its richness of phenolic and volatile compounds (such as essential oils). *Sage* is also as antioxidant and inhibiteur of steel corrosion (Maliki *et al.,* 2021; El Ouadi *et al.,* 2018; El Ouadi *et al.,* 2014; El Ouadi *et al.,* 2015. The important place in cosmetic and food industries because of its biological properties may be called bioeconomy (Greco *et al.* 2020; D'Amato *et al.* (2020).

In this study, we will focus on the behavior of *Salvia officinalis L* known also *Sage* at two stages (juvenile and adult) as a function of water stress intensity to assess its endurance in order to evaluate its morphophysiological and biochemical performance under water stress.

2. Materials and Methods

2.1. Experimental site.

The tests were carried out under glass greenhouse (average temperature of 24.5±0.2) at the experimental station of the Faculty of Science of Oujda at an altitude of 661 m, a latitude of 34 ° 39 '07' 'North and a longitude of 01 ° 53' 01 'West (GPS Back Track Bushnell).

2.2. Vegetal material

The 10 cm head cuttings were cut in July 2015 for plants in the juvenile stage and in April 2016 for plants in the adult stage on mother plants of sage, planted at the experimental station of the Faculty of Sciences of Oujda.

1.3. Conduct of the trial.

Three water modes T0, T1 and T2 corresponding to 100; 60 and 20% ET0 respectively were tested in this experiment (ET0= reference evaporation).

All the plants in the trial were watered with 100% of the ET0 (reference evapotranspiration) calculated by referring to the values of the city of Oujda (MARA, 1978).

The tests were conducted throughout the period from 01/12/2015 to 30/3/2016 (4 months) for juvenile plants and from 01/11/2016 to 30/04/2017 (6 months) for adult plants. Juvenile plants are transplanted individually into cups, filled with a peat/sand mixture (2V/V).

T0: Control treatment (water pumped from the well of the Faculty of Science of Oujda) with an electrical conductivity EC0 = 0.57 ms/cm.

1.4. Chemical analysis of the irrigation water used.

Each value represents an average of 3 samples ± the standard deviation. (Source: Oriental Center for Water Sciences and Technologies of the Faculty of Sciences of Oujda).

Table I: Chemical analysis of the irrigation water used.

pH	7.18±0,12
Electrical conductivity (ms/cm)	0.57±0.012
Nitrates mg/l	14.06±2.00

1.5. Morphological parameters.

1.5.1 Growth in height (in cm).

The height of the aerial part is evaluated, each month using a graduated tape in centimeters (cm) at the base of the collar at the top.

1.5.2. Leaf area (cm²).

Leaf area is measured each month by taking three leaves from each treatment using AUTOCAD 2010 software.

1.5.3. Determination of the root biomass Br, aerial biomass Ba and the ratio root biomass / aerial biomass BR/BA.

The ratio of dry root biomass to dry aerial biomass BR/BA, is considered a good indicator of the action of water stress on plants, (M O. LY et al 2014). Cumulative above-ground and root biomass expressed in grams were determined by weighing using a precision balance type AND GF300.

1.6. Physiological and biochemical parameters.

1.6.1. Relative water content (RWC%).

The relative water content of the leaf was determined monthly by the method described by Barrs, (1968). The relative water content is calculated by the formula of Clark and Mac-Caig, 1982):

$$RWC= (FP-S) / (TP-S)*100$$

RWC: Relative water content (%), FW: Fresh weight (g), SW: Turgid weight (g) & PS: Dry weight (g)

1.6.2. Basic leaf water pressure.

The measurements of the leaf water potential expressed in MPA, are carried out thanks to the Scholander pressure chamber (Scholander et al. 1965). The measurements were made at a rate of three repetitions per treatment.

1.6.3. Measurement of PSII quantum yield (ΦPSII).

The quantum yield of PSII is measured using a portable Fluorometer model FMS (FMS2 Pulse-Modulated Chlorophyll Fluorescence Monitoring System, Hansatech, England).

1.6.4. Chlorophyll determination.

Chlorophyll is extracted according to the procedure described by Tran et al (1995). It consists in grinding in a mortar 100 mg of fresh material taken from the leaf blade of the middle part of the leaf in acetone diluted to 80%. After determining the total volume of the extract, the optical density of the supernatant obtained is measured at 663 and 646 nm using a standard spectrophotometer (RAYLEIGH VIS-7220G). The total chlorophyll expressed in mg/g of fresh material is determined by the following formula: Total Chlorophyll = (7.15 x OD663 + 18.71 x OD646) x V/M; where V is the volume of the total extract in liter and M is the mass of the freshly ground material in grams.

1.6.5. Determination of proline.

The determination of this amino acid is determined by the method of Trolls and Lindsey, (1955), simplified and developed by Rasio et al, (1987). Two phases are separated, the upper phase is recovered and its optical density is determined at a wavelength λ = 528 nm using a typical spectrophotometer (RAYLEIGH VIS-7220G).

1.6.6. Determination of soluble sugars.

The determination was made by referring to the method of Yemn and Willis (1954) reported by Sidari et al, (2008). After cooling for 30mn in the dark, OD reading at λ =530nm using a spectrophotometer (RAYLEIGH VIS-7220G).

1.7 Experimental set-up for water stress.

The set-up includes 3 blocks with a total of 45 plants and each stage (juvenile and adult) 5 plants/treatment * 3 treatments * 3 replications.

1.8. Statistical analysis

The results obtained were submitted to the analysis of variance (ANOVA) with one factor. In the case of significant differences, multiple comparisons were made by the Tuckey test at a probability level of (5%, 1% and 0.1%). Each mean is assigned a letter, with means followed by the same letter not being significantly different.

3. Results and Discussion

3.1. Morphological parameters.

3.1.1. The height of the plant

- ***Juvenile stage:***

The results of plant height, shown in Figure 1 show that this parameter is slightly influenced by stress intensity. The lowest growth in height was observed in the treatment (60% ET0) which marked a reduction of 12% and 8% respectively for the months of February and March compared to the plants under normal water supply conditions (100% ET0). The results are verified by statistical test using analysis of variance, which reveals a significant difference ($P \leq 0.05$) on plant height.

- ***Adult stage***

The results in Figure 2 show that the effect of water stress on height growth was not very pronounced on plants in the adult stage of sage. The most significant decreases were seen during December and January. While for the moderate stress during the last months of the experiment there was an increase in plant height that was comparable to the control ($p \geq 0.05$).

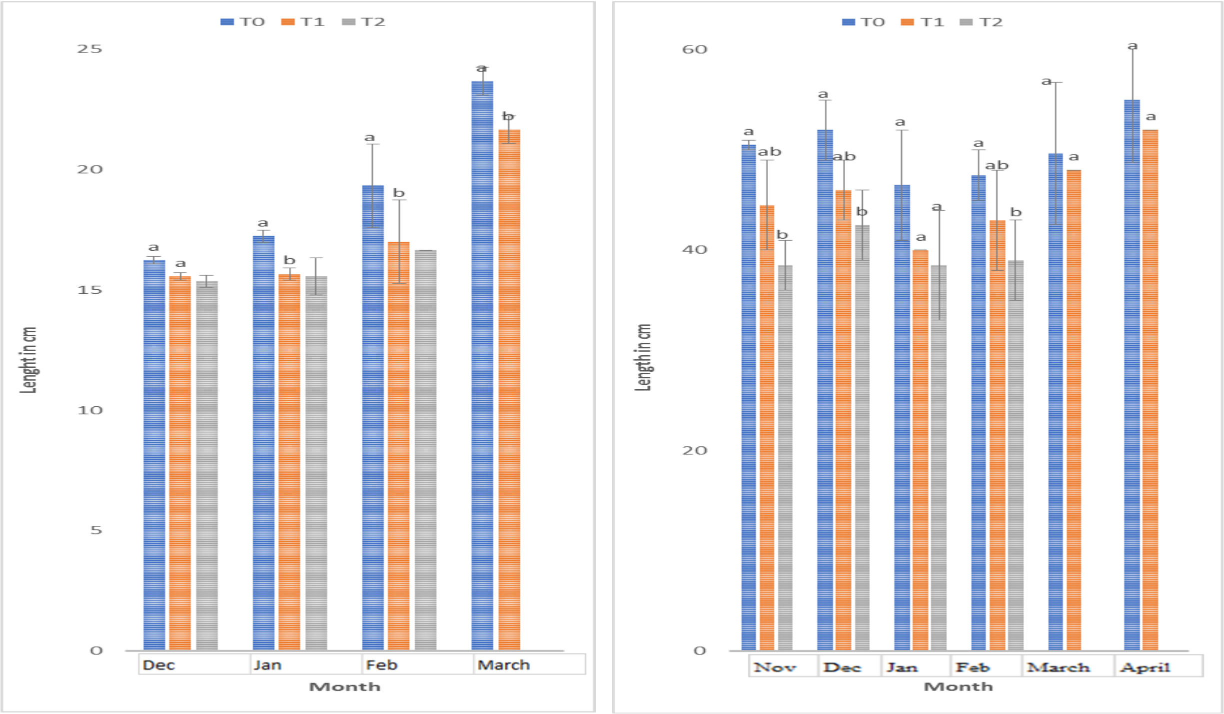
3.1.2 Leaf surface

- ***Juvenile stage.***

The results illustrated in Figure 3, show a significant decrease in total leaf area according to the degree of water stress applied. It goes from $5.03cm^2$ in the control to 4.5 cm^2 for the moderate treatment and to $3.85cm^2$ for the severe treatment, i.e., respective reductions of 11% and 24%. The analysis of variance shows that there is a significant difference ($P<0.05$) between the levels of water stress.

➢ ***Adult stage:***

The results, illustrated in Figure 4, the average leaf area of the adult sage plants increased according to the months to reach its maximum in the month of March 8.5cm2 and 6.6cm2 corresponding to the 100 and 60% ET0 treatments respectively. Concerning the effect of stress, the figure shows that the leaf area decreased according to the intensity of the applied water stress, with the most important decrease observed in the 60% ET0 treatment during the months of March and April with a total exhaustion of the plant. Single factor analysis of variance shows that the variation in leaf area was significant (P≤0.05) between stress levels.



Figures 1, 2: Effect of different levels of water stress (100, 60, and 20% ET0) on mean stem height growth of juvenile and adult sage.

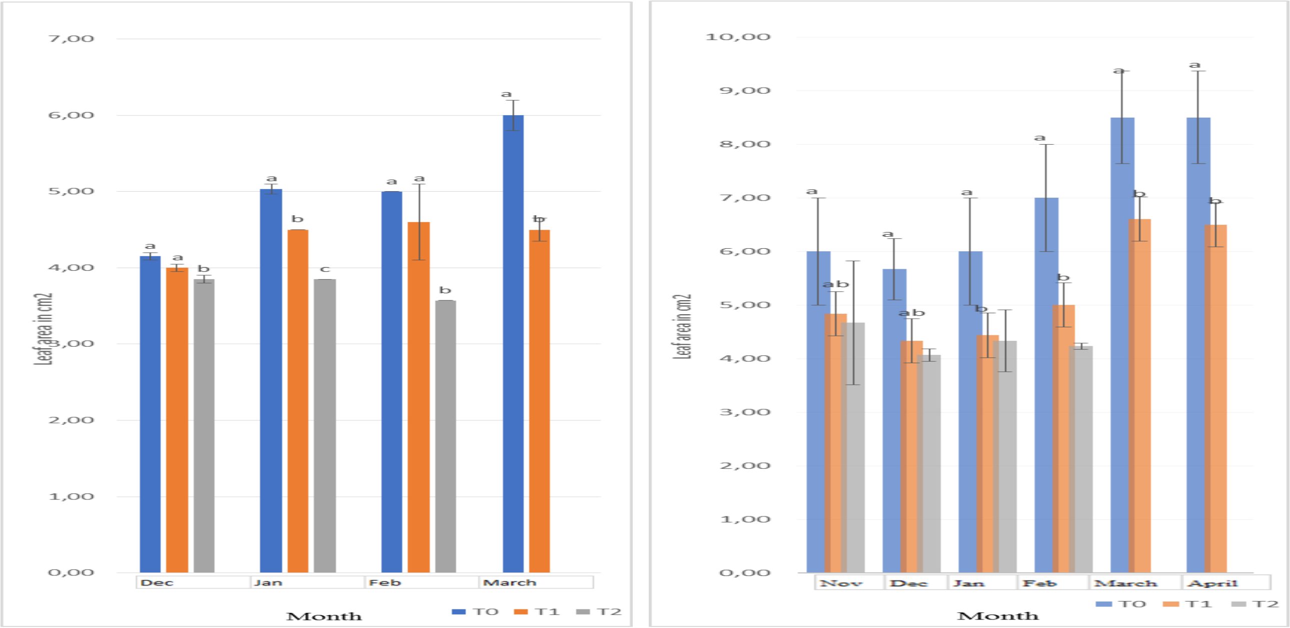


Figure 3, 4: variation of leaf area according to different water treatments (100, 60 and 20% ETO) in juvenile and adult sage.

3.1.3. The ratio of root biomass to aboveground biomass Br/Ba.

The results of the variation of the ratio of root biomass to aboveground biomass with different water treatments of sage are shown in Figures 5 & 6. The results showed a variation in the ratio with increasing water stress intensity from moderate to severe stress. Indeed, the application of water stress induced a decrease in this parameter in sage regardless of the plant Stage. However, statistical analysis showed that these observed decreases were not statistically significant ($p \geq 0.05$).

3.2. Physiological and biochemical parameters.

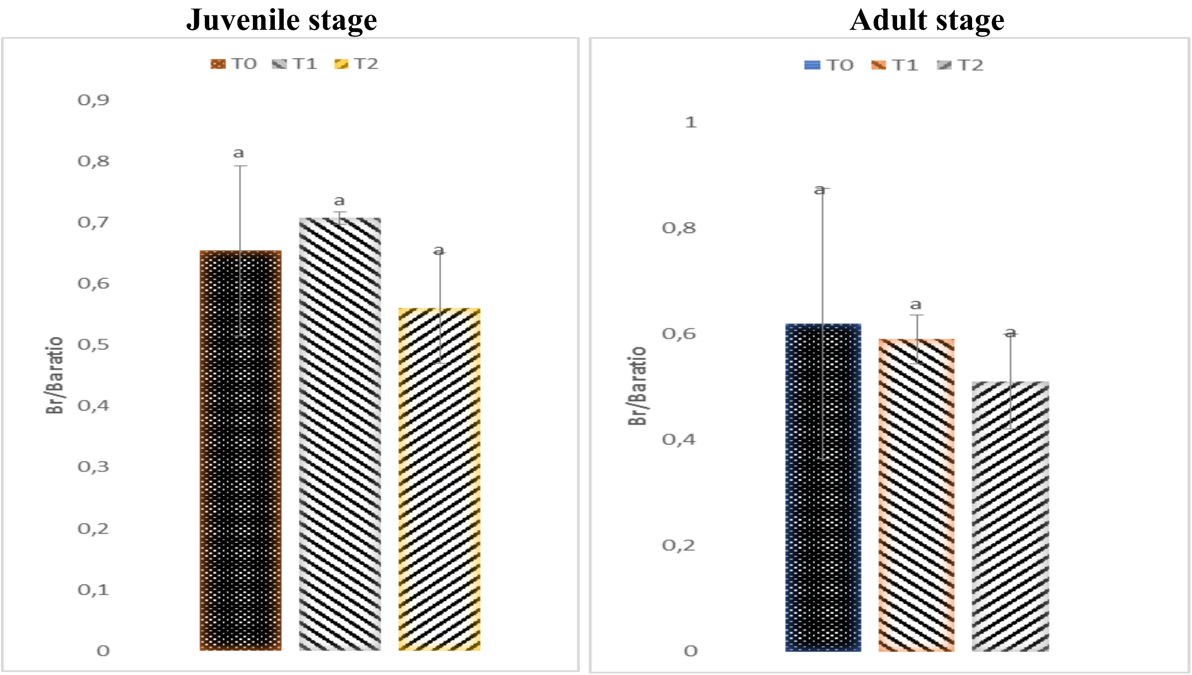
3.2.1. The relative water content (RWC).

- ***Juvenile stage.***

The water status of the leaves illustrated in Figure 7, shows that the RWC decreased proportionally to the intensity of the applied water stress. It decreased from 46% in the control in January to 37% in the moderate treatment and 30% in the severe treatment, reductions of 20% and 35% respectively. The analysis of variance (ANOVA) shows a significant difference between the different treatments applied ($p \leq 0.05$).

- ***Adult stage.***

Figure 8 shows that the RWC of adult stage sage plants decreased in proportion to the intensity of applied water stress. It decreased in February from 42% in the control to 36% in the moderate treatment and 32% in the severe treatment, reductions of 14% and 23%, respectively. Analysis of variance (ANOVA I) shows a significant difference for the severe treatment ($p \leq 0.05$).



Figures 5, 6: Effect of water stress on the ratio of root biomass to aerial biomass Br / Ba for sage at the juvenile and adult stage.

3.2.2. Leaf water potential (Ψf)

- ***Juvenile stage.***

The results of the variation of leaf water potential according to the water treatments are presented in Figure 9. This Figure shows that, the increase in stress intensity is accompanied by a decrease in leaf water potential compared to the control. Indeed, the water potential dropped by 19% in February when moving from the control to the moderate treatment (60% ET0) and by 25% during January when applying a severe stress (20% ET0).

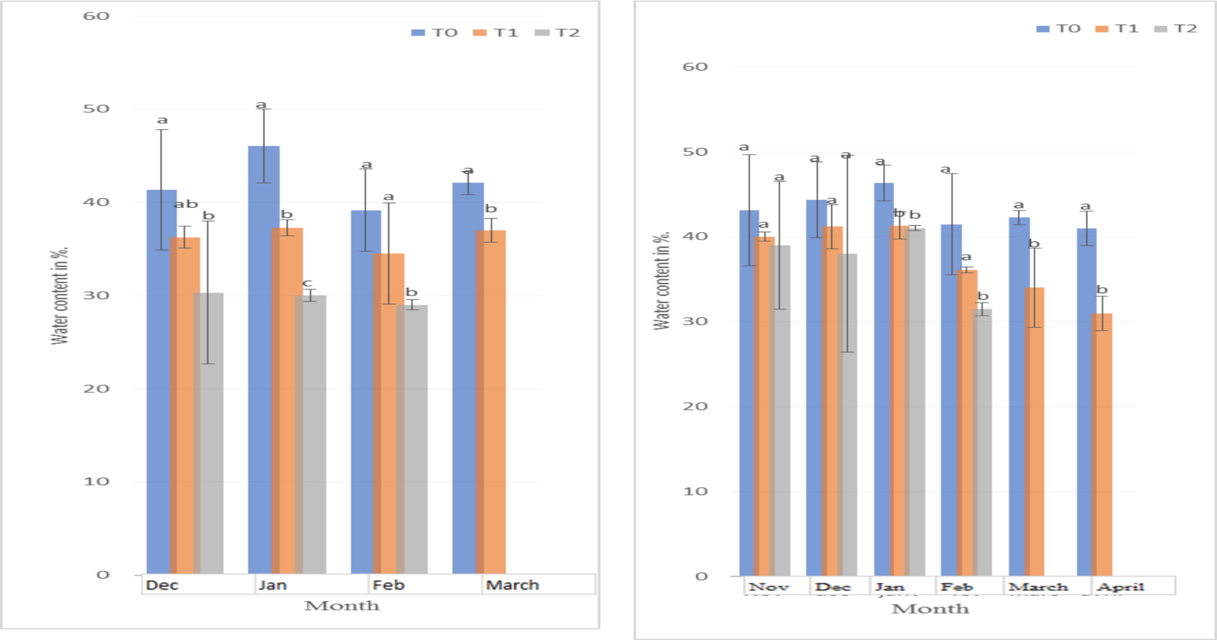
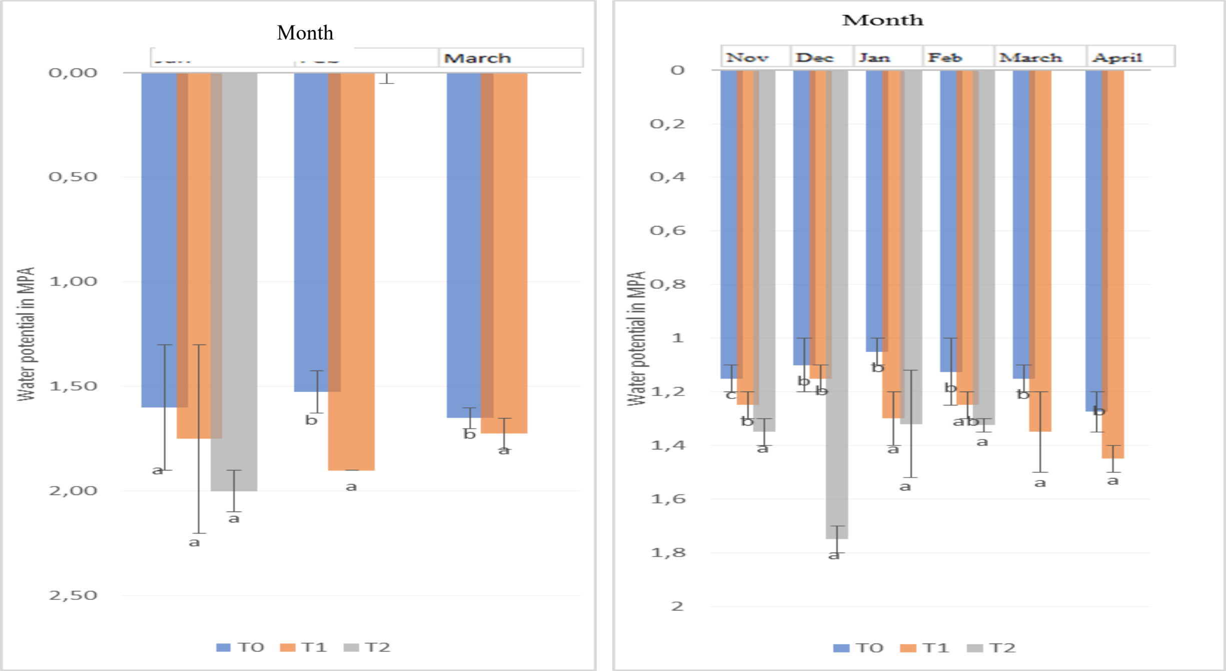


Figure. 7, 8 : variation of the relative water content according to the different water treatments (100, 60 and 20% ET0) of juvenile adult sage.

Adult Stage.

From the results in Figure 10 which illustrates the effect of water stress on plant water potential of adult sage, it can be seen that the sage kept the same trend as that of the Juvenile Stage where the low values were observed for the moderate and severe treatment during January (24%) and (26%) respectively. The single criterion analysis of variance confirmed that the recorded difference was significant for the applied treatments (p ≤0.05).



Figures 9, 10: Variation of leaf water potential according to the different water treatments (100, 60 and 20% ET0) of juvenile adult sage.

3.2.3. The quantum yield of PSII (ΦPSII).

- ***Juvenile stage.***

Figure 11 represents the effect of different levels of water stress on the PSII quantum yield of young sage seedlings. As water stress becomes severe, ΦPSII decreases. The largest decrease in chlorophyll fluorescence compared to the control is 11% was recorded in February for the moderate treatment and 20% in December for the severe treatment. However, these variations were not statistically significant between the different water regime treatments ($p \geq 0.05$).

- ***Adult Stage.***

According to the results in Figure 12, which illustrate the effect of water stress on chlorophyll fluorescence, it can be seen that the sage kept the same trend as that of the Juvenile Stage. The results clearly show a strong decrease for the severe treatment during February (25%). The latter finding is confirmed by ANOVAI analysis ($p \leq 0.05$).

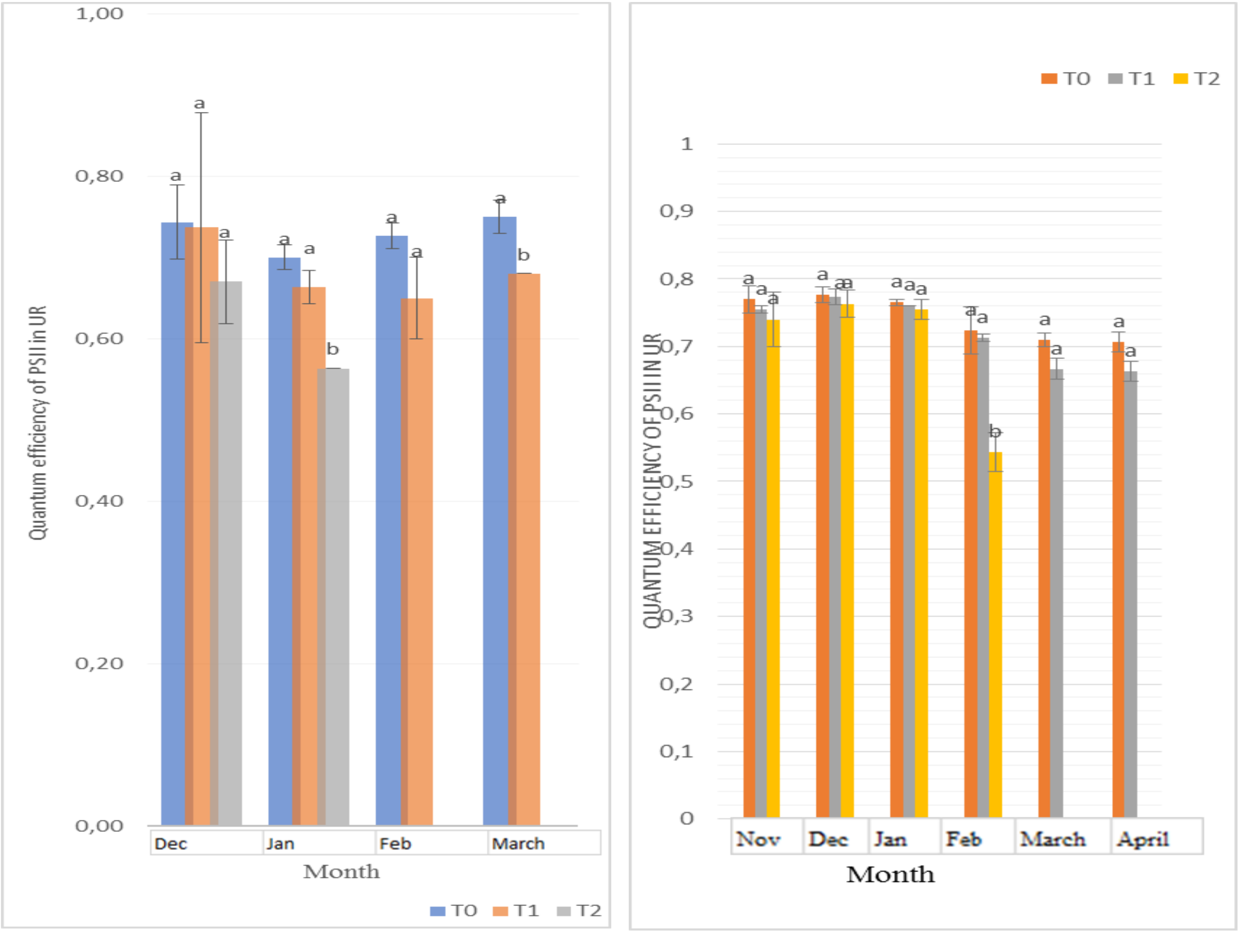


Figure. 11, 12: Effect of different levels of water stress (100; 60 and 20% ET0). On the quantum yield of PSII of juvenile and adult sage.

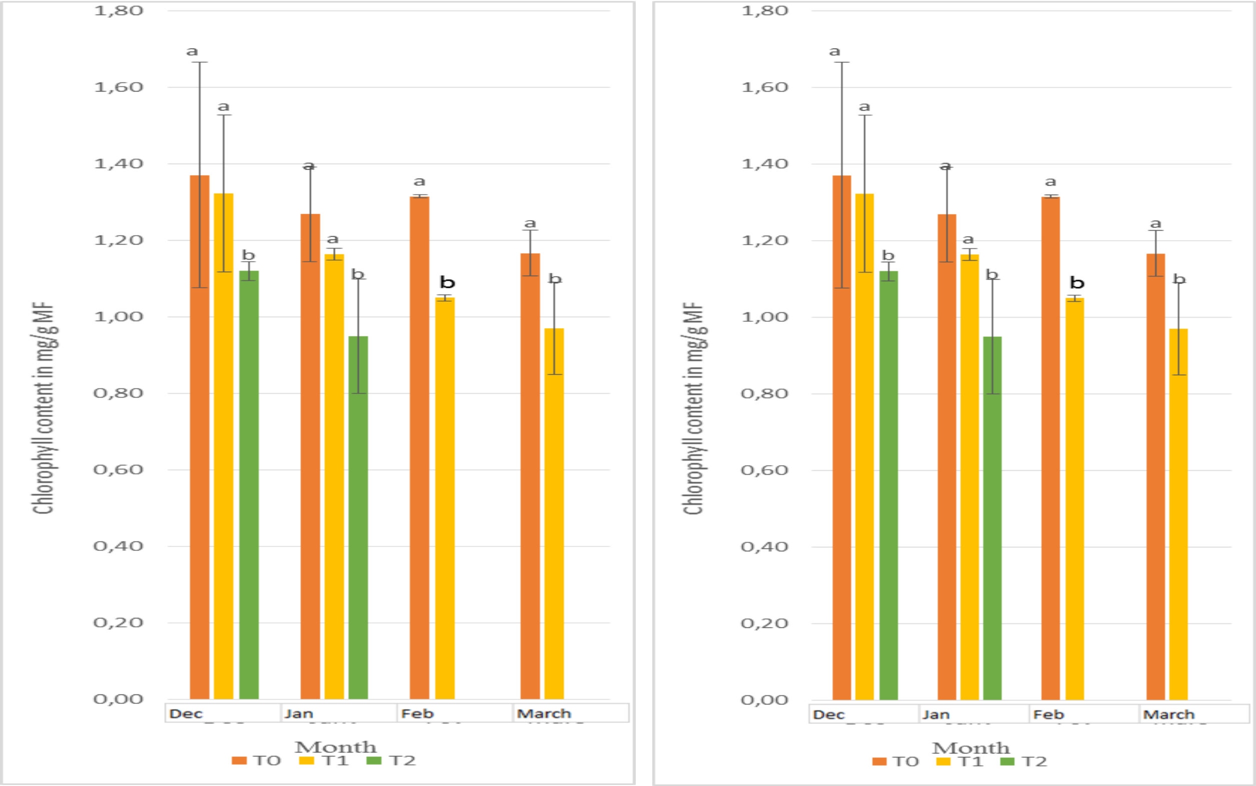
3.2.4. Total chlorophyll content (a+b)

- ***Juvenile stage.***

Figure 13 shows, on the one hand, a variation of the total chlorophyll content (a+b) of all treatments between December and March with a peak in December corresponding to 1.37, 1.32 and 0.93 mg/g MF respectively for the 100%, 60% and 20% ET0 treatments. On the other hand, the total chlorophyll content (a+b) decreases correlatively with the increase of the water stress degree. Under severe stress conditions (20% ET0), a more or less pronounced decrease in chlorophyll content was observed compared to the control. This decrease becomes very important in March when the chlorophyll content dropped from 1.16 to 0.86 mg/g MF, a reduction of 25% compared to the control. These variations are statistically significant between the different water regime treatments during the trial. Moreover, the results also show a correlation (R^2 =0.91) between the decrease in leaf area and the decrease in chlorophyll content.

- ***Adult stage.***

According to the results of Figure 14, which illustrates the effect of water stress on the chlorophyll content of adult sage plants, it can be seen that as water stress increases, the total chlorophyll content (a+b) decreases. In fact, the chlorophyll content drops by 20% when switching from the control to the moderate treatment (60% ET0) during the month of March, and by 21% when applying the severe stress (20% ET0) in February.



Figures 13, 14: Effect of different levels of water stress (100; 60 and 20% ET0) on the chlorophyll content of juvenile and adult sage.

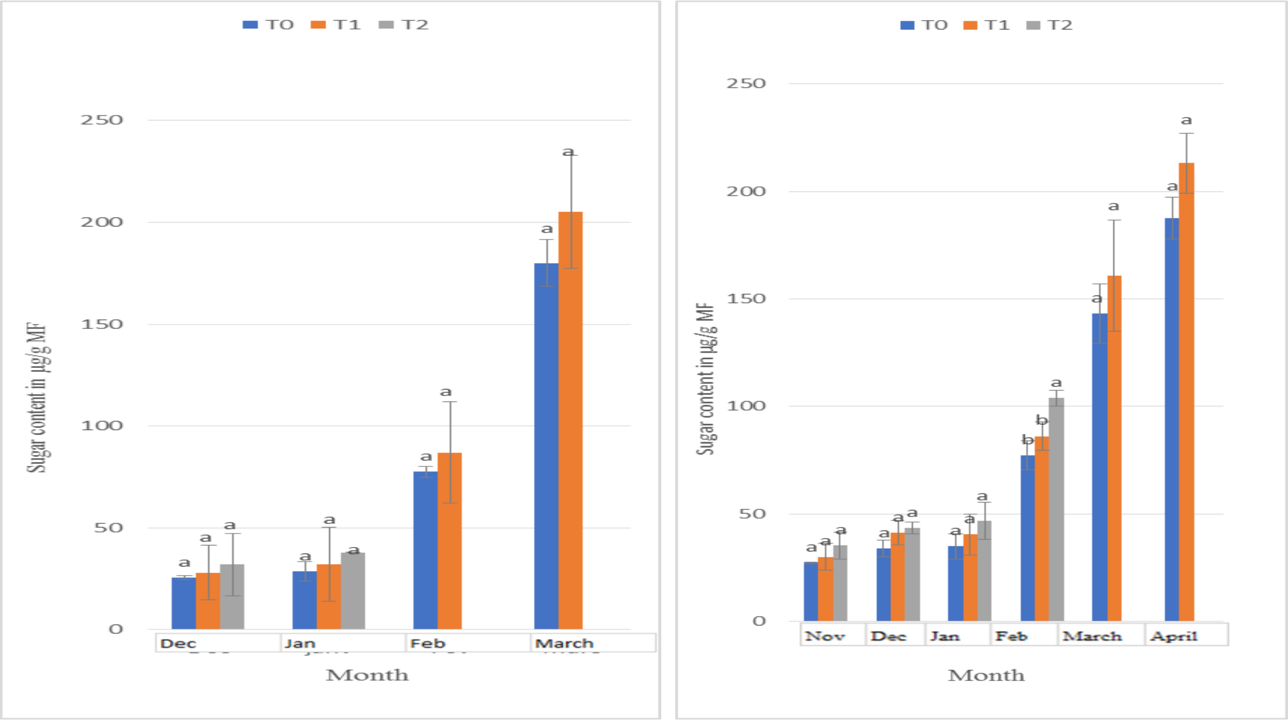
3.2.5 Sugar content.

- ***Juvenile stage***

Compared to plants watered at 100% ET0, water-restricted sage plants produced more sugars in their foliage (Figure15). A severe water deficit (60% ET0) further amplified the accumulation of soluble sugars in leaf tissue especially in the last months of the trial. The analysis of variance did not reveal any significant difference.

- ***Adult stage***

From the results of Figure 16, which shows the effect of water stress on adult sage plants, it can be seen that the sage was able to synthesize more sugar compared to the juvenile stage where the highest percentages were recorded during the month of February in the moderate and severe treatment 77.09 ; 86.13 and 103.81µg/g MF increases of 13 and 34% compared to the control.



Figures 15, 16: Effect of different levels of water stress (100; 60 and 20% ET0) on soluble sugar content in juvenile and adult sage.

3.2.6 Proline content.

- ***Juvenile stage.***

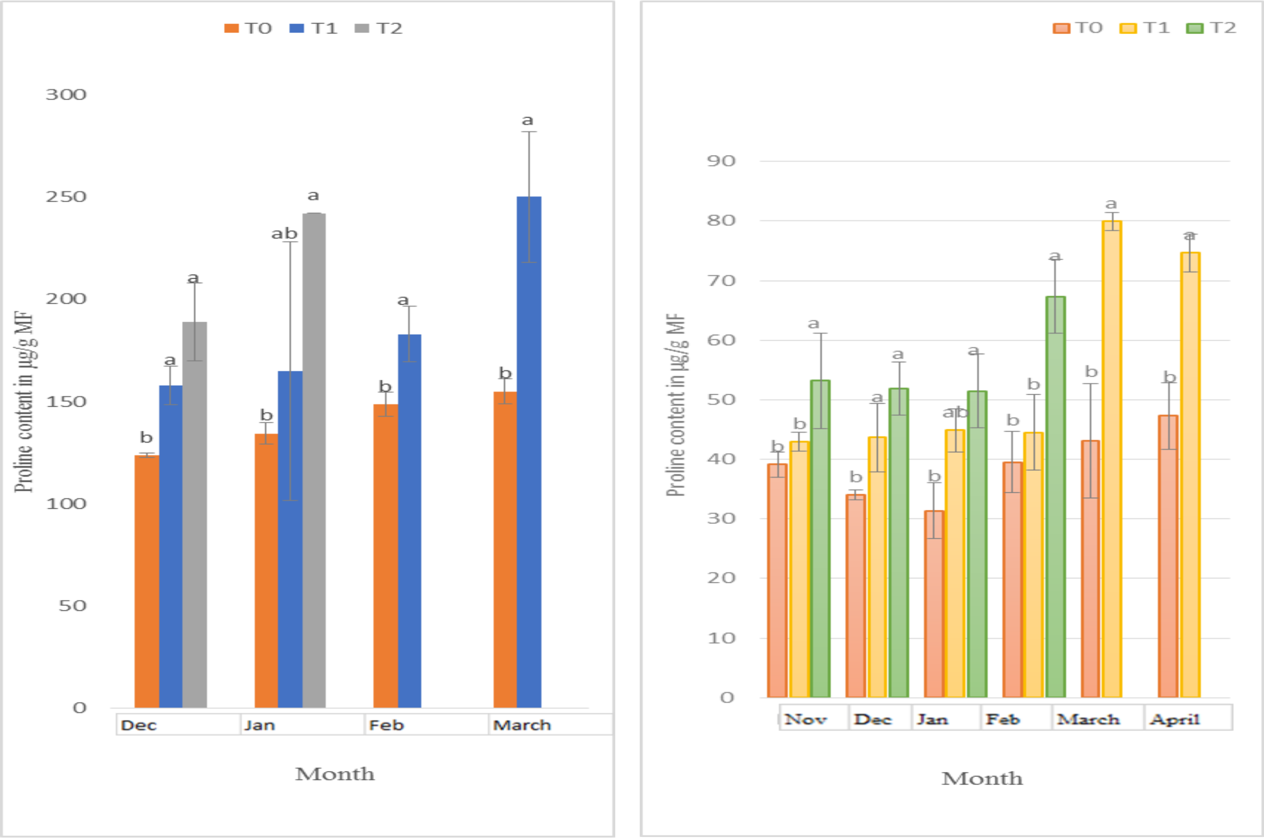
Compared to plants watered at 100% ET0, water-restricted plants produced more proline in their foliage (Figure 17). Moderate and severe water deficit (60 and 20% ET0) further amplified proline accumulation in leaf tissue of juvenile sage, reaching almost 2 times that of the control in March for moderate stress. These results reveal a highly significant difference in the applied treatments ($p \leq 0.01$) compared to the control.

- ***Adult stage***

The analysis of the results shows that the sage kept the same growing style as that of the Juvenile Stage where the largest increases were observed respectively for the severe treatment during the month of February 71% followed by plant dieback. The single criterion analysis of variance showed that these variations are statistically significant between the different treatments of the water regime ($p \leq 0.05$) (Figure 18).

3. Discussion

In this study, the sage was subjected to two situations of water stress (moderate stress imposed by an irrigation of 60% ET0 and severe stress by an irrigation of 20% ET0), was evaluated by measuring the changes recorded in some of their morphophysiological and biochemical parameters. All the seedlings experienced a reduction in their morphological development capacities, resulting in a reduction of their leaf surfaces, their growth in height and even complete exhaustion of the plants in the case of the severe treatments towards the end of the experiment. These plants also experienced a change in the ratio between their above-ground and below-ground biomass, depending on the intensity of the water stress and the age. These results seem to converge with previous work in *Melissa officinalis* (L.) (Ozturk *et al.* (2004), *Matricaria recutita* (L.) Baghalian *et al.* (2011), *Satura hortensis* (L.) Baher *et al.* (2002), lavender and Greek sage chrysargyris *et al*. (2016).



Figures 17, 18: effect of the different levels of water stress (100, 60 and 20% ET0) on the leaf proline content of juvenile adult sage

We also found that the ratio of root biomass to aboveground biomass indicates a shift to the root part relative to the aboveground part at the juvenile stage. This suggests the hypothesis that our seedlings chose the belowground growth pathway at the juvenile age. The plants thus adopted a balance approach between aerial and root biomass which is considered by several authors as a criterion of drought resistance bakht *et al.* (2011); Yoshida *et al.* (2002) to optimize their resistance to water stress and to allow a better water availability which is in agreement with the works already done on some lamiaceae (sage) Bettaieb *et al.* (2009).

However, the reduction of BR/BA ratios, recorded in adult plants, can be explained by the fact that the underground part of the stressed plants, having benefited from the allocation of root biomass during the juvenile period, became able to offer a more sustained production of aerial biomass than before. These results add to those reported by Monroy-Ata and Mc Millin (1995) who mentioned that lack of water would stimulate root biomass in order to maintain a maximum rate of above-ground growth, similar results have been reported in *Casuarina glauca* Alouchi *et al.* (2003), *Pinus ponderosa* Mc Millin .(1995) and *Quercus robur* and *Fagus sylvatica* Van Hees, (1997). We noted that the relative water content underwent the same trend of reductions in either moderate or severe mode by recording significant maximum decreases in juvenile and adult age, accompanied by a reduction in water potentials. According to Girousse *et al.* (1996), a decrease in leaf water potential from -0.4 to -2 MPA, is the cause of a significant increase in amino acid concentration in alfalfa (*Medicago sativa*).

Water stress caused losses in chlorophyll. This decrease in chlorophyll can be explained by the excess synthesis of enzymes such as chlorophyllase, responsible for the degradation of chlorophyll that can damage the photosynthetic apparatus Levent Tun *et al.* (2008) and stimulates the increase of osmoticums (proline) suggesting the presence of a competition between the two compounds on their common precursor Tahri *et al*. (1998).

The biochemical evaluation through the determination of sugars and proline allows us to note that the application of moderate or severe water stress caused maximum increases in the content of soluble sugars and proline. This increase is considered to be a distress signal emitted by plants to allow protection of membranes and enzyme systems especially at the juvenile age under severe stress, and which could also play a role in the regulation of cytoplasmic pH and in the constitution of a nitrogen reserve for the cell Tahri *et al.* (1998) . Several studies have shown that proline content increases during water stress, and its accumulation is associated with improved drought tolerance Seki et al.(2007) ; Zhang *et al .(*2010). The recent study of Mohammadi-Cheraghabadi *et al.* 202,) indicated that the water stress decreased the Essential oil Yield oscilated around at 75% available soil water depletion (ASWD).

Conclusion

In this study, the performances from a morphophysiological and biochemical point of view were discussed in *Salvia officinalis* and which showed that this species is relatively tolerant to water stress at moderate doses in the adult stage and can be recommended for cultivation in areas of the Mediterranean basin.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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(2023) ; http://www.jmaterenvironsci.com