Effects of water stress on the morphophysiological and biochemical parameters of rosemary under a glass greenhouse.

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Abstract:

Drought is a limiting factor affecting agricultural production globally, particularly in arid and semi-arid regions. It is estimated that more than 800 million hectares of land worldwide are affected by abiotic stress. Morocco, through its floristic diversity, is required to optimize the production and multiplication of its plant resources with a view to sustainability for future generations.

As part of the study of the effect of water stress on the physiological, morphological and biochemical behaviors of the plant, we were interested in Rosmarinus officinalis given its medicinal, aromatic and socio-economic interests in order to evaluate its tolerance to water stress at juvenile and adult stages by applying three water treatments: 100, 60 and 20% ET0. Rosemary's tolerance to water stress was well evaluated in this study and showed that this species is relatively tolerant to water stress at moderate degrees.

Key words: water stress, morphological, physiological, biochemical parameters, water treatments 100, 60 and 20% ET0, juvenile and adult stages.

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Résumé :

La sècheresse est un facteur limitant affectant la production agricole à l'échelle mondiale, en particulier dans les régions arides et semi-arides. Il est estimé que plus de 800 millions d'hectares de terres dans le monde sont touchés par le stress abiotique. Le Maroc par sa diversité floristique est prié d'optimiser la production et la multiplication de ses ressources végétale en vue d'une pérennité pour les générations du futur.

Dans le cadre de l'étude de l'effet du stress hydrique sur les comportements physiologique, morphologique et biochimique de la plante, nous nous sommes intéressés à *Rosmarinus officinalis* vu ses intérêts médicinaux, aromatiques et socio-économiques afin d'évaluer sa tolérance aux stress hydrique aux stades juvénile et adulte en appliquant trois traitements hydriques : 100, 60 et 20 % ET0.

La tolérance du Romarin au stress hydrique a été bien évaluée dans cette étude et a montré que cette espèce est relativement tolérante au stress hydrique à des degrés modérées.

Mots clés : stress hydrique, paramètres morphologiques, physiologiques, biochimiques, traitements hydriques 100,60 et 20% ET0, stades juvénile et adulte.

INTRODUCTION

Aromatic and medicinal plants are generally associated with a high antioxidant potential, hence their multiple benefits for human health. The business of dried plant extracts drives a significant portion of the economies of developing countries (Chetouani et al., 2023).

In recent years, the global climate has been negatively affected by global warming. Consequently, drought (water stress) and scarcity of water resources are becoming limiting factors affecting agricultural production and essential oil extracts globally, particularly in arid and semi-arid regions, where rainfall is irregular due to climate change, which will become more frequent and persistent in the future (Guillaume, 2003). The combination of summer droughts and erratic precipitation makes water one of the scariest resources (Joffre et al, 1999 ; Iglesias et al, 2006). Thus, Thuiller et al (2005) classify the Mediterranean region as particularly sensitive to drought, which allows the development of a flora rich in medicinal and aromatic plants adapted to the climate such as rosemary, which requires us as a scientific community an interest particularly to extract the maximum economic benefit without disturbing the ecological and floristic balance knowing that the market for plant extracts mobilizes significant capital on a global scale (Siqueira Leite et al., 2018; Parham et al. al.,2020; Sabbahi et al.,2020).

In this study, we will focus on the behavior of rosemary at two stages (juvenile and adult) depending on the intensity of water stress in a glass greenhouse to evaluate its tolerance to lack of water at the two different stages, to understand the behavior and how rosemary will react to water stress for the implementation of cultural practices adapted to optimize the production of rosemary biomass in the context of eastern Morocco.

1. MATERIALS AND METHODS

1.1 Experimental site.

The experiment was conducted under a glass greenhouse at the experimental station of the Faculty of Science of Oujda at an altitude of 661 m, latitude 34 ° 39 '07' 'North and longitude 01 ° 53' 01 'West (GPS BackTrack Bushnell). A Min-Max thermometer was used to determine the minimum and maximum daily temperature inside the greenhouse, which showed an average temperature of 24.5±0.2 during the test periods.

1.2. Plant material.

The 10 cm cuttings were taken in July 2015 to prepare them for the stress experiments at the juvenile stage, which will begin in December. For the plants at the adult stage and under the same experimental conditions, the cuttings taken in July will be prepared for the trial in November and which all come from rosemary mother plants planted at the experimental station of the Faculty of Sciences of Oujda.

The major selection criteria for this plant are essentially linked to the valorization of the biomass and the content of essential oils of the plant which is distributed in arid and semi-arid zones to optimize production and exploitation without compromising ecological balance (Barkaoui et al.,2022).

1.3. Conduct of the trial.

Three water treatments T0, T1 and T2 corresponding respectively to 100, 60, and 20% ET0 were used in this trial. All the plants in the trial were watered with 100% of the ET0 (reference evapotranspiration) calculated with reference to the values of the city of Oujda (MARA, 1978).

ET0 = reference evaporation.

The trials were installed in a glass greenhouse of dimensions 3 x 5 x 2.8 m (Temperature 24.5 ±0.2°C) at the experimental station, during the whole trial period from December 1st, 2015 to 30th March 2016 (4 months) for juvenile plants and from 1st November 2016 to 30th April 2017 (6 months) for adult plants.

Juvenile plants are transplanted individually into buckets, filled with a peat/sand mixture (2V/V).

Characteristics of the substrate:

PH: 6.0 ; salinity: 1.5g/l ; N (cacl2): 300mg/l ; P2O5: 150mg/l ; K2O: 370mg/l (Fixed values Gütegemeinschaft substrate für pflanzenbau e.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 three samples ± standard deviation. (Source: Oriental Center of Water Sciences and Technologies of the Faculty of Sciences of Oujda).

Table I: Chemical composition 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 apical height (in cm).

The growth in length of the aerial part is evaluated, each month using a graduated tape in centimeters (cm) from the base of the collar to the top of the shoot.

1.5.2. Leaf area (cm²).

Take leaves from the base for each plant and the area is estimated directly by using AUTOCAD 2010 software by carefully digitizing the edges of the leaf.

Take the leaves from the base for each sample

Spread each sheet on an A4 paper bearing the name of the sample.

Scan the sheets with a flatbed scanner, as an image (JPG)

Determine leaf area using AUTOCAD 2010 software

The leaf area is determined each month by taking three leaves from each treatment (one leaf/repetition/treatment). The number of leaves to estimate the total leaf area multiplied by the unit area. The leaf area is determined each month.

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 (LY et al., 2014). Cumulative aboveground and root biomass expressed in grams were determined by weighing using a precision scale type AND GF300.

1.6. Physiological and biochemical parameters.

1.6.1. Relative water content (RWC %).

According to Shonfeld et al (1988) in Nouri (2002), the relative water content constitutes a reliable parameter to evaluate the tolerance to drought and can have a direct physiological meaning on the hydric state of the plant (Ritchie et al, 1990). Performed once a month the relative water content of the leaf was determined by the method described by Barrs (1968). The relative water content is calculated by the formula of Clark and Mac-Caig (1982) in Mouellef (2010) :

RWC = (PF-Ps) / (PT-Ps) *100

RWC : Relative water content (%), PF : Fresh weight (g),

PT : Weight at full turgor (g), PS : Dry weight (g).

1.6.2. Baseline leaf water pressure.

Leaf water potential measurements are made using the Scholander pressure chamber (Scholander et al. 1965) with a compressed nitrogen source, a flow regulator and an electronic manometer with an accuracy of 0.025 MPA. The progressive increase in pressure causes the first drop to exit, expressed in MPA, and corresponds to the opposite of the water potential of the leaf. Measurements were made with three replicates per treatment.

1.6.3. Measurement of PSII quantum yield (ΦPSII).

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

- F0 as being a fluorescence in the initial state : Intensity of the fluorescence when all the centers
PSII reaction rooms are open.
- Fm or maximum fluorescence : Intensity of fluorescence when all the reaction centers of the PSII are closed.
- Fv/Fm : This is the ratio of variable fluorescence (Fm-F0) and maximum fluorescence.

It reflects the efficiency of the PSII to use light for photochemical conversion. Its value is about 0.8 in a healthy plant and decreases in stressful cases.

The leaf of each seedling is directly attached to the head of the FMS with a clip. The chlorophyll fluorescence of the leaves is measured at morning room temperature.

1.6.4. Determination of Chlorophyll.

Chlorophyll is extracted according to the procedure described by Tran et al (1995). It consists of 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 matter 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 method adopted for the determination of this amino acid is that 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 $\lambda = 528$ nm using a typical spectrophotometer (RAYLEIGH VIS-7220G). A standard range was previously established and the contents are expressed as µg of proline / g of MF.

1.6.6. Determination of soluble sugars.

The determination was carried out by referring to the method of Yemn and Willis (1954), the principle of the reaction is based on the condensation of the products of degradation of neutral oses by sulfuric acid. The latter is very concentrated and transforms the oses into furfural derivatives, which give a blue-green coloration with anthrone. The technique consists of soaking 100mg of fresh leaf material for 24 hours in 5 ml of 80% ethanol, then diluting the extract obtained 10 times with 80% ethanol then taking 2 ml of the solution obtained to which 4 ml of anthrone (0.2%).

The whole is kept in an ice bath and finally place the tubes in a water bath at 92°C for 8 minutes.

After cooling for 30mn in the dark, read the DO at λ =530nm with a spectrophotometer (RAYLEIGH VIS-7220G). The soluble sugar content of the leaves (µg/gMF) was calculated with reference to a standard glucose range from a stock solution.

1.7. Experimental setup for water stress.

The trial consists of 3 blocks with a total of 45 plants and each stage (juvenile and adult). 5 plants/treatment * 3 treatments * 3 replications). Blocks indicate replicates and sub-blocks represent treatments.

1.8. Statistical analysis

The results obtained were subjected to a one-way analysis of variance (ANOVA). 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.

2. RESULTS:

2.1. Morphological parameters.

2.1.1. The height of the plant.

- **Juvenile stage.**

Figure 1a represents the variation in plant height growth of rosemary with increasing water stress intensity. The highest values were recorded for the control treatment (100% ET0), while the lowest values were recorded for the 60% and 20% ET0 treatments during February with reduction rates in the order of 17 and 28%, respectively, compared to the control, with 17% discount for T1 treatment in March and total depletion for the severe treatment. Single-criterion analysis of variance showed that the differences observed during the first months of the experiment were not significant ($p \geq 0.05$); whereas the differences recorded in February and March were significant $p \leq 0.05$.

- **Adult stage**:

From the results in Figure 1b that illustrate the variation in stem height of rosemary at the adult stage as a function of water treatments, it can be said that stem height growth was not influenced by the application of water stress during the first months of the trial except for December where reductions were recorded for moderate and severe treatment where analysis of variance did not reveal a statistically significant difference ($p \geq 0.05$). However, during the last two months (March-April) the application of severe stress (20%) induced total plant death.

2.1.2. Leaf surface

- **Juvenile stage**

The results of leaf area variation with different water treatments of rosemary at the juvenile stage are shown in Figure 2a. The results showed a decrease in leaf area with increasing water stress intensity. Indeed, the most important decrease was recorded in February with respective reductions of 24 and 35% for the moderate and severe treatments. The one-way analysis of variance confirmed that the differences recorded between the treatments studied were significant $p \leq 0.05$.

- **Adult stage.**

According to the results of Figure 2b which illustrates the effect of water stress on the leaf area of rosemary at the adult stage, it can be seen that the rosemary kept the same trend as that of the juvenile stage where the most striking reductions were observed in January (16%) followed by a total exhaustion of the plants during March and April for the severe treatment.

2.1.3. Ratio between root and aerial biomass Br/Ba at the juvenile and adult stages

The results of the variation of the root biomass to aboveground biomass ratio with different water treatments are shown in Figure 3a. The results showed that the response of the plant to the applied stress changed according to the age of the plant. Indeed, the juvenile stage externalized a positive variation of the Br/Ba ratio with increases of 7 and 5% respectively for the moderate and severe treatment compared to the control. However, the adult stage showed reductions compared to the control with reductions of 6 and 13%. Single-criterion analysis of variance showed that the recorded differences were not significant between the different treatments ($p \geq 0.05$).

2.2. Physiological and biochemical parameters.

2.2.1. The relative water content (RWC).

- **Juvenile stage.**

The results of the variation of the relative water content (RWC) according to the different water treatments are shown in Figure 4a. The results show that the different levels of water stress applied induce a decrease in the RWC of rosemary at the juvenile stage. Indeed, the most significant decrease in the relative water content was recorded for the moderate treatment of 11% in March and 21% in February for the severe treatment compared to the control.

- **Adult stage**.

According to the results of Figure 4b, which illustrate the effect of water, and stress on the RWC of rosemary plants at the adult stage. Indeed, it can be seen that the rosemary kept the same trend as that of the juvenile stage where the most marked decreases were observed during April with a reduction that exceeds 43% for the moderate treatment, accompanied by a total destruction of the plants for the severe treatment.

The analysis of variance with only one criterion confirmed that the differences recorded between the treatments during March and April are very significant (p .0.05).

2.2.2. Leaf water potential.

- **Juvenile stage**

The results of the variation in leaf water potential according to the different water treatments are shown in Figure 5a. They showed a decrease in water potential as a function of the increase in stress intensity. Indeed, the lowest values were observed in March where the moderate treatment marked a reduction of 11% accompanied by a total destruction of the plant for the severe treatment. The analysis of variance did not reveal a statistically significant difference between the different treatments.

- **Adult stage**

The results in Figure 5b reflect the effect of water stress on the water potential of adult rosemary plants. It can be seen that rosemary kept the same trend as that of the Juvenile stage where the most flashing decreases were observed for the moderate treatment during March and April.

The ANOVA 1 showed a significant difference between the treatments during March ($p \leq 0.05$).

2.2.3. Chlorophyll fluorescence.

- **Juvenile stage.**

Figure 6a represents the effect of different levels of water stress on the quantum yield of PSII of young rosemary seedlings. As the water stress becomes severe, the ΦPSII of young seedlings tends to decrease. Indeed, the moderate treatment recorded a value of 0.72 during February and March, which is 4% and 7% reductions, respectively, compared to the control. However, the decrease found between the moderate treatment and the control was not statistically significant during the last two months of the trial ($p \geq 0.05$).

- **Adult stage.**

Figure 6b shows the effect of water stress on the quantum yield of PSII of rosemary plants in the adult stage. From the results, it can be noticed that rosemary kept the same trend as that of the juvenile stage where the most striking decreases were observed for the moderate treatment during March and April, respectively. For the severe treatment, a significant reduction was recorded in February followed by a total destruction of the plant.

2.2.4. Effect on total chlorophyll content in rosemary

- **Juvenile stage.**

The results of the effect of different levels of water stress on total chlorophyll content are shown in Figure 7a. The latter decreases correlatively with the increase in the degree of water stress applied in juvenile rosemary, especially during the last two months of the trial. The moderate treatment showed a slight decrease in TCT of 20% during March while the severe treatment showed a reduction of 18% in February with a total exhaustion of the plant in March. These results are confirmed by statistical analysis, which showed significant differences except for December $p \leq 0.05$.

- **Adult stage.**

Figure 7b illustrates the effect of water stress on the chlorophyll content of adult rosemary plants, it can be seen that the evolution of chlorophyll followed the same pattern as that of the juvenile stage where the most striking decreases were observed for the moderate treatment during April. The single criterion analysis of variance confirmed that the recorded difference is significant for the applied treatments ($p \leq 0.05$).

2.2.5. Sugars content.

- **Juvenile stage.**

The effect of different levels of water stress on the leaf sugar content of young rosemary seedlings is shown in Figure 8a. As water stress becomes more severe, the leaf sugar content increases to reach maximum values at 20% ET0. Indeed, the largest increase in leaf sugar content was recorded during March for the moderate treatment which was 14% and 56% for the severe treatment in January. Compared to the control. However, this observed difference was not statistically significant (p≥0.05).

- **Adult stage.**

According to the results in Figure 8b which illustrates the effect of water stress on adult rosemary plants, it can be seen that the maximum values of sugar content were recorded in the severe treatment where the highest increase was marked during February with +118% compared to the control. While the moderate treatment showed a statistically significant increase in sugar content only during the first months of the trial.

2.2.6. Proline content.

- **Juvenile stage**

The results of the effect of different levels of water stress on proline content are shown in Figure 9a. From the results obtained, it can be seen that there is a positive correlation between the proline content and the degree of water stress applied. The greatest increase in proline content was recorded during January in the case of the severe treatment with 81% compared to the control, followed by an estimated increase of 32% in March for the moderate treatment. These results are verified by the single-criterion analysis of variance, which confirmed that the differences recorded between treatments are highly significant (p≤0.01).

- **Adult stage.**

The results in Figure 9b show the effect of water stress on the proline content of adult rosemary plants. It can be seen that rosemary kept the same pattern as that of the juvenile stage where the largest increases were observed in the moderate treatment during March (109%) and April (124%). The one-way analysis of variance showed that these variations were statistically significant between the different treatments applied (p≤0.05).

3. DISCUSSION.

In plants, and depending on the genotype and the adaptive strategy of each species, the effect of water stress can be translated by morphological modifications aiming at increasing water absorption, decreasing transpiration and reducing competition between organs for assimilates, modifications thus affecting both the aerial and the subterranean part of the plant (Bajji, 1999). In this study, rosemary (*Rosmarinus officinalis*) 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) and their capacity of adaptation, was evaluated by measuring the modifications occurred in some of their morphological, physiological and biochemical parameters. All the rosemary plants experienced a reduction in their morphological development capacities, resulting in a reduction of their leaf surfaces, and their growth in height up to the complete desiccation of the plants for the severe treatments towards the end of the trial. These plants also experienced a change in the ratio between their aboveground and belowground biomass, depending on the intensity of the water stress and depending on the age. We also noticed that the ratio of root biomass to aboveground biomass indicates a shift from aboveground to root biomass at the juvenile stage, suggesting that our plants favored the root development pathway at the juvenile age. Thus, the plants adopted a strategy of balancing 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 for better water availability and more precisely for their survival despite plant production.

The results indicate that the lack of water induced, at the same time, a shrinkage of the leaf surface and a reduction of the growth in height especially at the juvenile age and this, as well, in case of moderate and severe stress. These results seem to confirm some results obtained by previous works performed 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)).

The decrease in growth parameters could, therefore, result from the preferential allocation of plants at juvenile age for the production of root biomass despite the development of the aerial part, which corroborates with work already carried out on certain Lamiaceae (sage) (Bettaieb et al, 2009). However, a drop in BR/BA ratios, observed in adult plants, may be explained by the fact that the root system of stressed plants, having benefited from the allocation of root biomass during the juvenile period, became capable of supporting a more sustained production of aboveground biomass than before.

These results are consistent with those reported by Monroy-Ata and Mc Millin 1995 who suggested that lack of water would modulate root biomass to maintain a maximum rate of aerial 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 found that the relative water content underwent the same trend of reductions in either moderate or severe mode recording significant maximum reductions in juvenile and adult age, accompanied by a drastic decrease in water potentials, which will undoubtedly stimulate the synthesis of osmoticums to save a vital minimum of water resources. 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 chlorophyll losses through stomatal closure, which led to a decrease in CO2 uptake and thus a decrease in net photosynthetic uptake, which corroborates the work of Tahri (1998) on wheat. This decrease in chlorophyll may also be due to 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 stimulate the increase of osmoticums (proline) which suggests the presence of competition between the two compounds on their common precursor (Tahri et al, 1998). 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 soluble sugar content. These increases may be of considerable importance, as accumulated solutes are involved in osmotic adjustment, which is an important mechanism in drought tolerance. Similarly, proline marked maximum increases in adult and juvenile rosemary under moderate or severe stress respectively, which represents a distress signal emitted by the plants to synthesize osmoregulator to allow protection of membranes and enzyme systems, especially in young organs under severe stress.

The accumulation of proline in important quantity is a response to the lack of water resources following the decrease of the root biomass recorded and could play a role as osmoticum to regulate the cytoplasmic pH or to constitute a nitrogen reserve used by the cell (Tahri et al, 1998). It appears that proline protects protein structure against denaturation caused by water stress, and stabilizes cell membranes through interaction with phospholipids and its accumulation is a common metabolic response of higher plants to water deficit (Rhodes et al, 1999). 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) to help maintain cell turgor, which may allow cell enlargement and plant growth during water stress, which supports our results found.

CONCLUSION:

Besides morphophysiological reductions, proline and carbohydrate concentrations in rosemary increased significantly in response to water stress by activating various metabolic processes, which has been demonstrated in many species under different stress situations (osmotic, water, heat) (Hossain et al, 2013e) and that the Br/Ba ratio has shown its reliability and remains a good indicator in abiotic stress studies.

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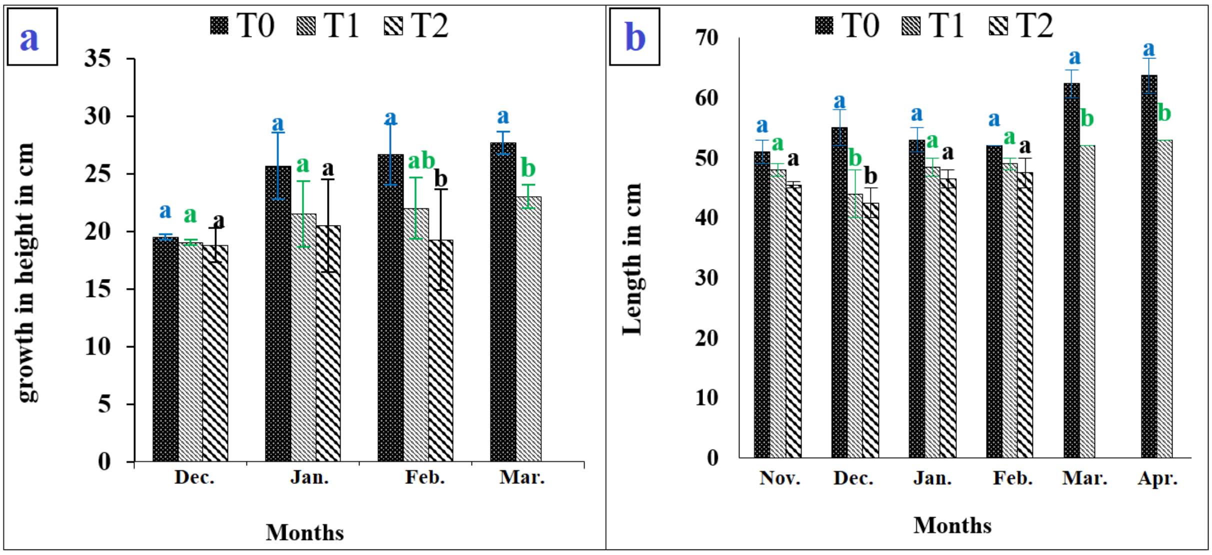

Figure 1: Effect of different levels of water stress on the average height of the stems of juvenile (a), and adult rosemary (b).

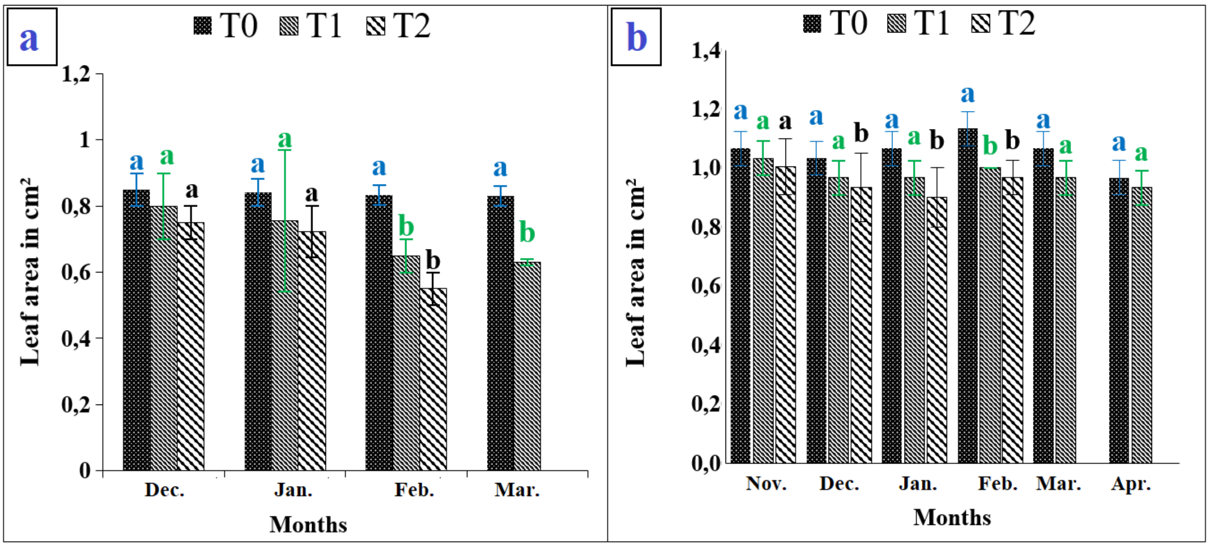

Figure 2: Effect of different levels of water stress on the average leaf area of juvenile (a) and adult rosemary (b).

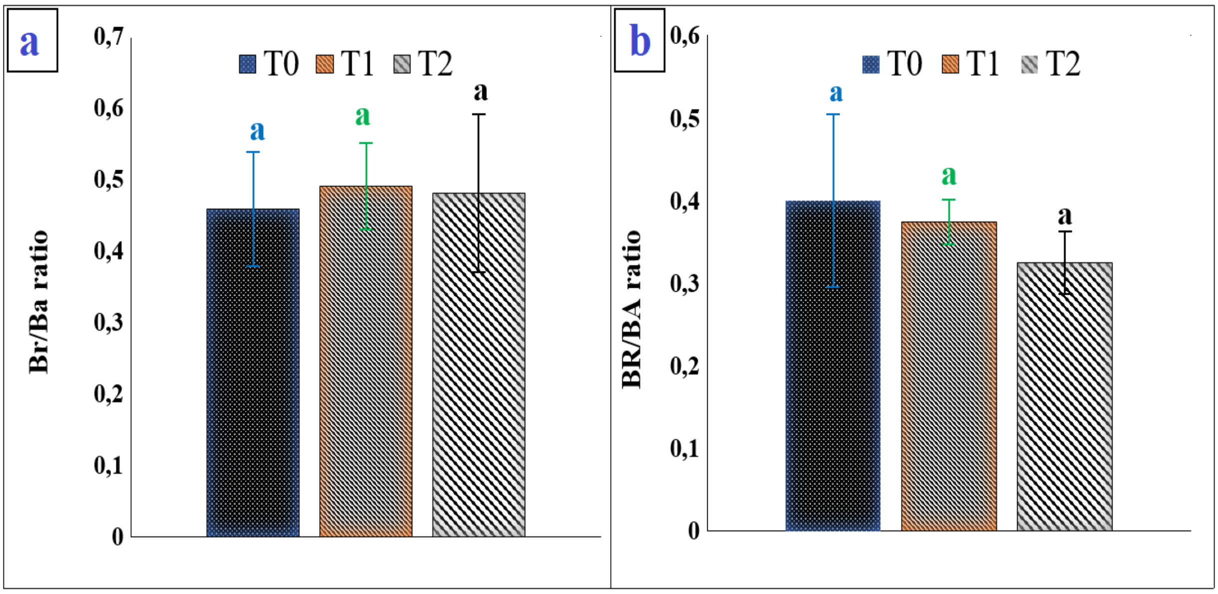

Figure 3: Effect of different levels of water stress on the Br/Ba ratio of juvenile (a) and adult rosemary (b).

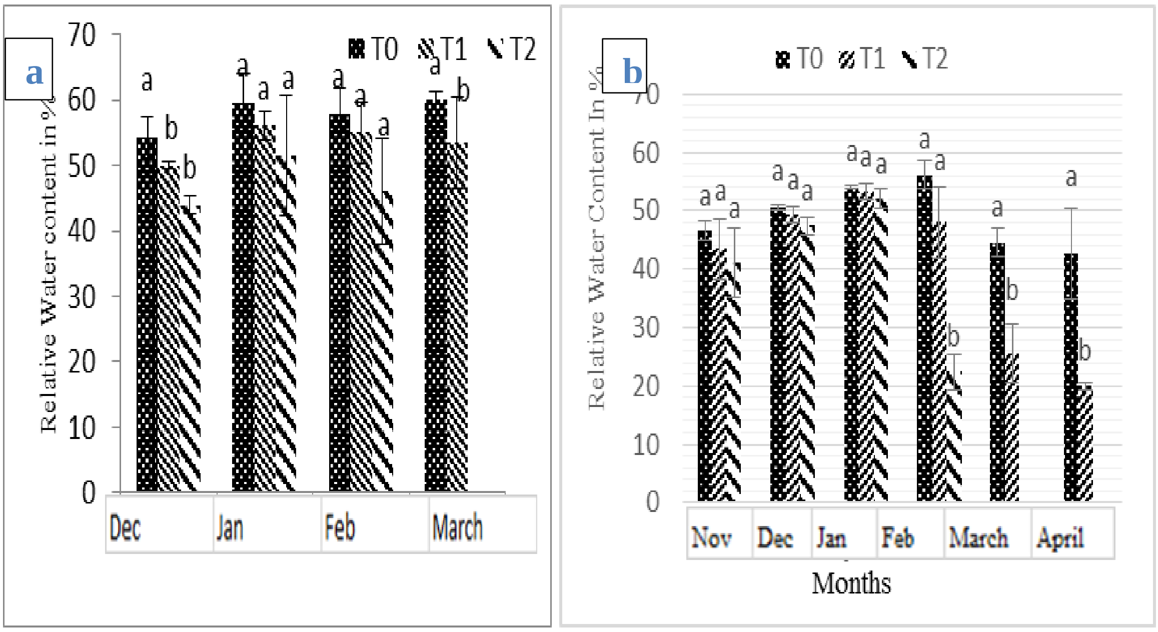

Figure 4: Effect of different levels of water stress on the RWC of juvenile (a) and adult rosemary (b).

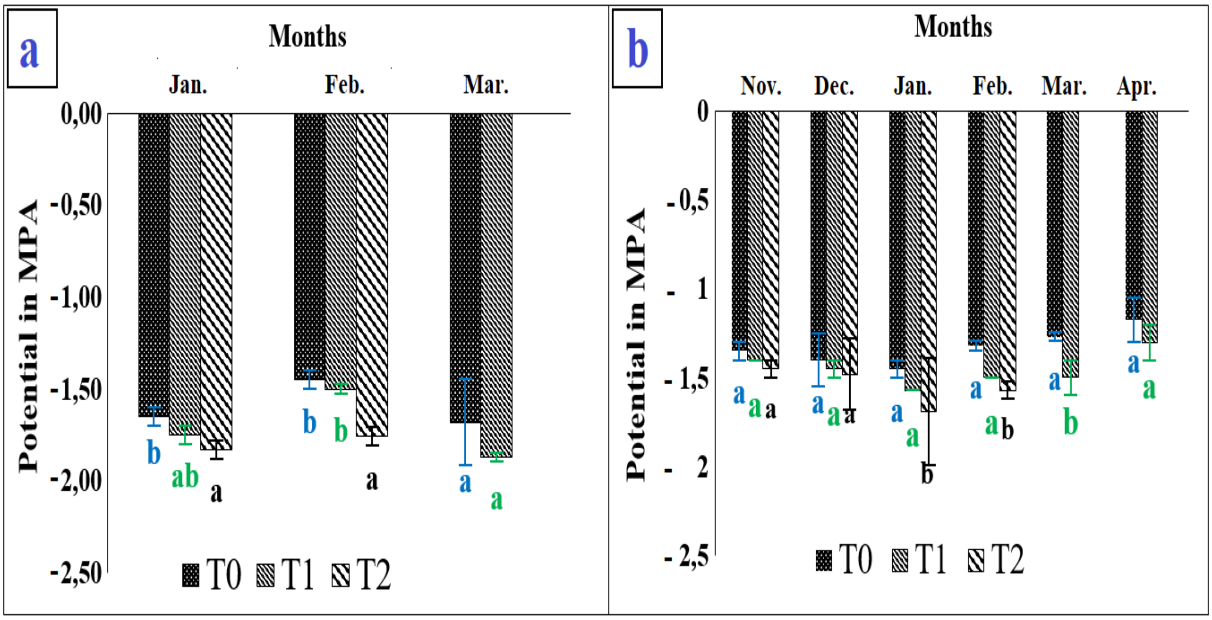

Figure 5: Effect of different levels of water stress on water potential of juvenile (a) and adult rosemary (b).

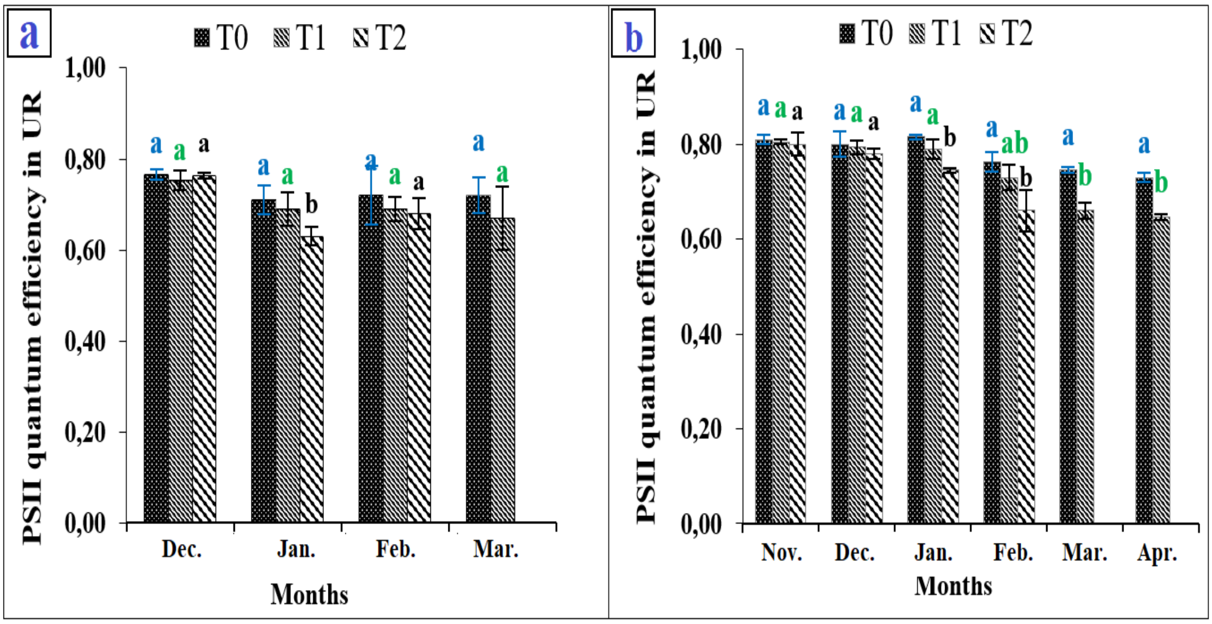

Figure 6: Effect of different levels of water stress (100, 60 and 20% ET0) on the quantum yield of PSII from juvenile and adult rosemary.

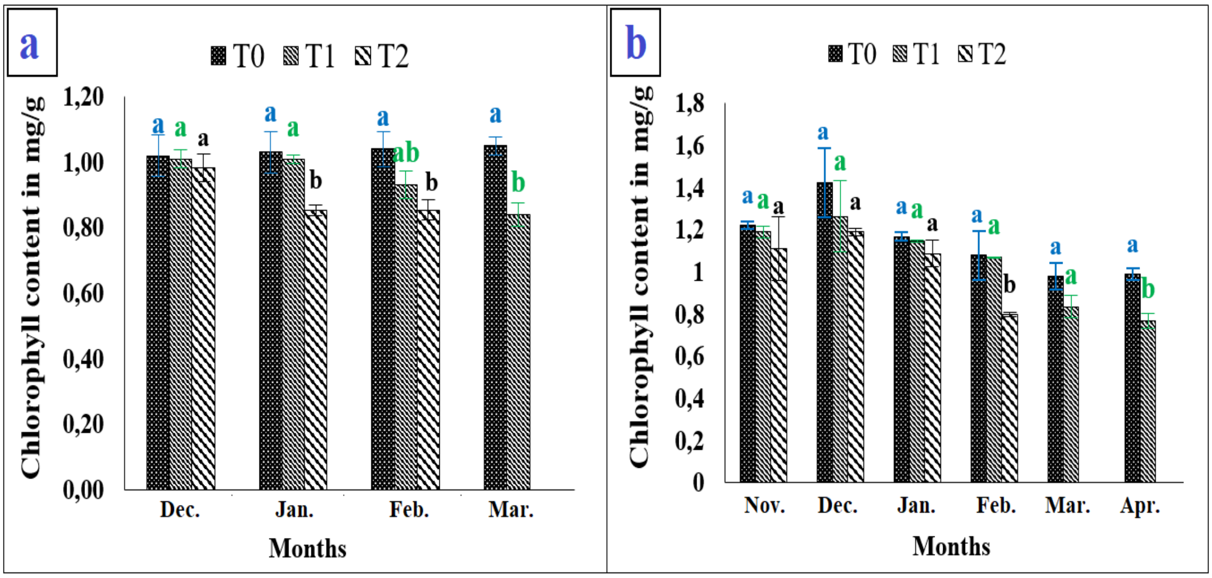

Figure 7: Effect of different levels of water stress on chlorophyll content of juvenile (a) and adult rosemary (b).

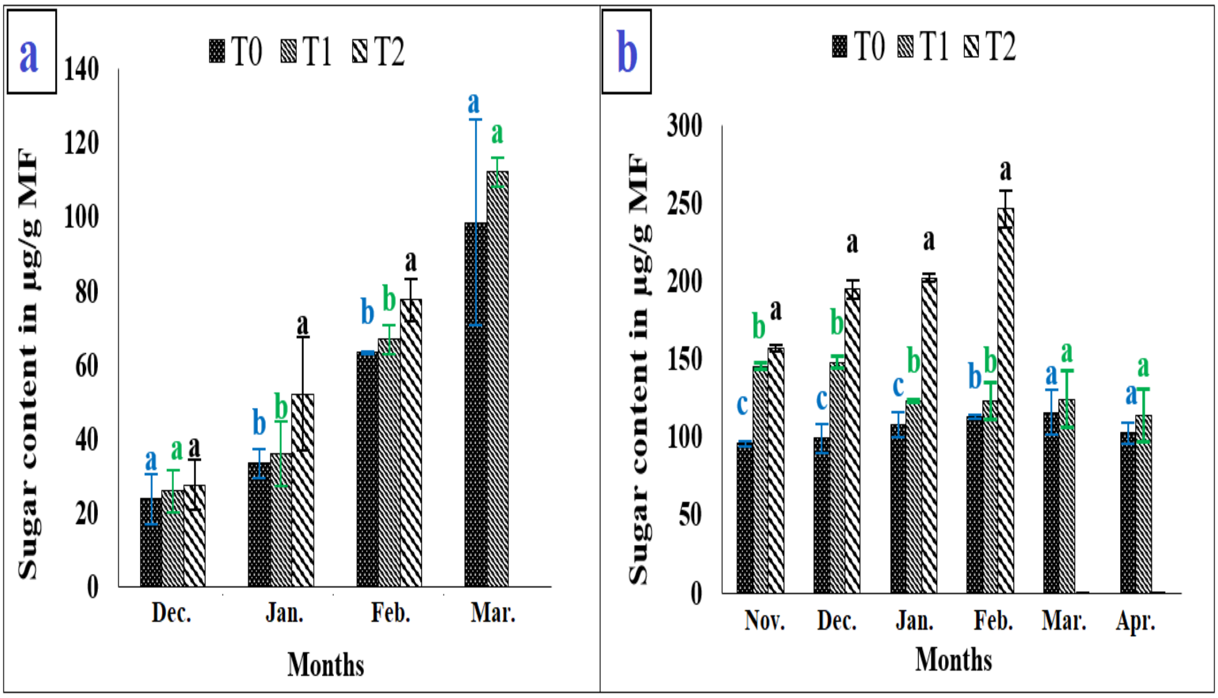

Figure 8: Effect of different water stress levels on sugar content of juvenile (a) and adult rosemary (b).

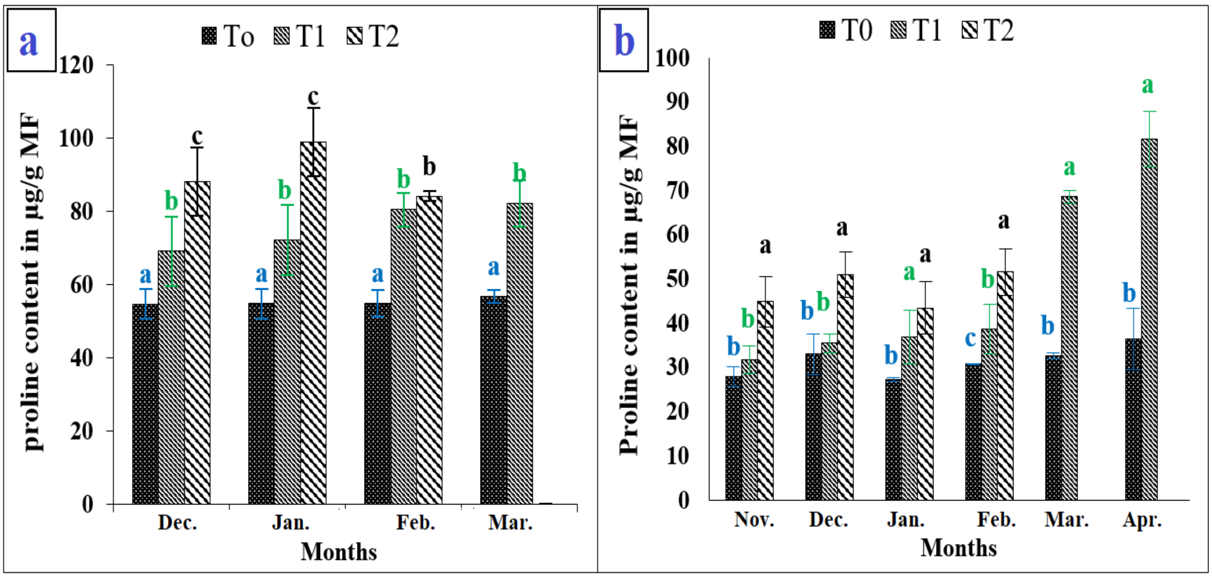

Figure 9: Effect of different levels of water stress on the proline content of juvenile (a) and adult rosemary (b).

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