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Effects of Three Different Water Levels on Growth and Physiological Parameters of *Euonymus japonicus* Thunb., Shrub

[1]*Ali B. Abu Shehab,* [1]*Jamal S. Sawwan and* [2]*Taleb Rateb Abu-Zahra*

[1]Horticulture and Crop Science Department,
School of Agriculture, University of Jordan, Amman, Jordan
[2]Department of Plant Production and Protection,
Faculty of Agricultural Technology, Al-Balqa' Applied University, As Salt, Jordan

Abstract: In Jordan, water shortage due to limited water resources and increased population growth cause a major challenge to agriculture sector. The decreasing water supply available for agriculture forces us to search for plants with low water requirements. Thereby, drought tolerant plants are considered an important method to conserve water use. Physiological and morphological parameters have been developed as tolerance mechanisms by plants during water shortage period. Xeriscape refers to the origination of an attractive landscape while reducing water use. Thus, this experiment was conducted to evaluate the effects of three different water levels on growth and physiological parameters of *Euonymus japonicus* Thunb. The soil moisture contents were maintained at three levels expressed as a fraction of the container soil water capacity; 75-100% (control, non-stressed), 50-75% (moderate water deficit) and 25-50% (severe water deficit) throughout the duration of experiment which lasted for six months. Results obtained showed that water stress conditions reduced plant vegetative growth (plant height, leaf area, shoot and root dry weight and root/shoot ratio), but plant width was not affected. On the other hand, stress conditions lowered the leaves chlorophyll content and reduced the RWC, but increased the proline content, while WUE was not affected.

Key words: *Euonymus japonicus* · Chlorophyll · Proline · Stress: RWC · WUE

INTRODUCTION

In Jordan, irrigation water uses 71% of the water demand and 64% of water supply in Jordan [1]. Moreover, In the future water shortages will be periodic due to water limitation and increase population growth [2]. Abiotic stresses are the main cause of decreased crops production worldwide. Major stresses such as drought, extreme temperatures and high soil salinity might cause 50% losses or more of the yield of major crops. Savings of 50-60% of home water consumed for irrigation during the summer season through using of xeriscape is considered one of essential techniques that also promote water conservation in residential landscapes [3].

Maintenance of xeriscape is not different from traditional landscape, which includes irrigation, irrigation system maintenance, fertilization, pest and disease control, pruning and weed control [4].

Appropriate plant selection, especially drought tolerant plants, is one of the important principles in a xeriscape-type landscape. Ornamental plants in urban landscapes are subjected continuously to water shortage, therefore it's important to select drought tolerant plants that have not lost their aesthetic value [5].

Proline accumulation is a sign of injury and not as a tolerance mechanism of plant under water deficit. In accordance, great arguments were found about the protective role of proline under water deficit [6]. Proline accumulation can be used as a metabolic marker for environmental stresses in plant tissue especially under drought stress conditions [7].

Unfortunately, until now few studies have dealt with the effect of drought on ornamental landscape plants. Therefore, currently studies on the effect of drought on plants have taken priorities especially under increasing aridity due to climatic changes.

Corresponding Author: Taleb Rateb Abu-Zahra, Department of Plant Production and Protection, Faculty of Agricultural Technology, Al-Balqa' Applied University, As Salt, Jordan.

Euonymus japonicus Thunb. is a member of Celastraceae family, native to China and Japan. It is an evergreen shrub with opposite, simple, leathery and dark green leaves. Medium to fast growth rate and can tolerate poor soil and drought. Numerous cultivars are widely grown and used as hedges, spreading shrubs or trained as miniature trees [8].

The aim of study is to evaluate the effect of induced water deficit on growth and physiological parameters of *Euonymus japonicus* Thunb.

MATERIALS AND METHODS

Plant Material and Growing Conditions: A popular ornamental landscape shrub (*Euonymus japonicus* Thunb Ait.) in Jordan, were selected to study the effect of induced water deficit on their growth and physiological parameters. The experiment was conducted in the greenhouse of the Faculty of Agriculture at the University of Jordan, Amman, Jordan during the period from April to October 2023. Daily reading at noontime of both temperature and humidity were recorded using electronic digital thermometer/hygrometer (Model WSD-2A, China) placed in the middle of the growing bench in the greenhouse.

The average maximum greenhouse air temperature was 30°C for the period of study and average relative humidity of the air was around 30%.

Uniform size and healthy plants of the above shrub were obtained from a local commercial nursery in early March 2023. Shrubs growing as a single shoot were selected for the uniformity of size.

The shrub was transplanted into standard 9 L plastic pots with 26 cm diameter and 24 cm depth. Eight kilograms of oven dried homogenized soil were used per pot.

Soil Characteristics: The soil was collected from the Jubeiha agricultural research station at the University of Jordan, Amman, Jordan. Prior to the start of experiment, the soil was homogenized by manual mixing four times using shovel, which ensured thoroughly tumbling and mixing of the soil. Then the soil was sterilized by heating in an oven at 75°C for three days.

Physical soil analysis revealed that the soil texture is clay and composed of 17.8%, 27.9% and 54.3% of sand, silt and clay, respectively. The soil pH was measured by paste extract and found to be 7.9 [9].

Soil Water Contents: A soil sample was taken from the homogenized air-dried soil and was used to determine gravimetric soil moisture using pressure plate [10]. Gravimetric soil moisture at both field capacity and wilting point were 37.8 and 21.04%, respectively.

Water Supply Treatments: All the plants were initially maintained under non-stress conditions for four weeks from transplanting by regular irrigation to the upper limit of the watering regime (75-100%) of container capacity by weighing the pots every day at 10:00 AM and once water content reach its lower limit 75% of field capacity the pots were re-watered to bring it back to the 100% field capacity by adding the amounts of water that equal to the loss in weight from the pot for 4 weeks prior to the start of the experiment as a recovery period to remove any previous stresses [11].

Growth (Plant height, width and average leaf surface area) and physiological (Chlorophyll content, relative water content and proline content) measurements were taken before the beginning of the experiment on day 0 (last day plants were watered to the upper limit of the watering regime 75-100% once they reach its lower limit of container capacity) for initial measurements (pre-drought) before starting the different water deficit treatments [12].

One healthy, unshaded and third lateral fully expanded leaf from each plant was collected on day 0 for initial physiological measurements.

Beginning on May 1, 2023, the planted pots were exposed to three levels of watering regimes established as a fraction of the soil field capacity, namely:

- 75-100% of container capacity (control, non-stressed),
- 50- 75% of container capacity, (moderate water deficit)
- And 25- 50% of container capacity (severe water deficit).

All pots corresponding to the assigned water regimes were irrigated to the upper limit of the regime once they reach its lower limit. Thus, the control pots were irrigated only once they reached 75% of the F.C. to bring it to 100% F.C. The other two watering regimes were treated similarly.

The water consumption in the pots was monitored gravimetrically by weighing individual pot daily at 10:00h AM to maintain and restore the moisture level with a balance (capacity 20 kg, Dial Spring Scale, model SPR) throughout the experimental period. The plant weight was neglected. The loss in water was replenished when it reached the lower limit of the watering regime and recorded.

Different levels of watering regimes were maintained during the entire experiment which lasted for six months.

Data Collection: Both growth parameter data and physiological data were collected at 0, 2, 4, 6 months from the start of the experiment.

At the end of every 2-months interval, the growth parameters (Plant height, width and average leaf surface area) were measured and plant materials were sampled for physiological analysis (Chlorophyll content, relative water content and proline content).

Leaf samples were collected at mid-day (11:00 h to 13:00 h) to meet the period when water stress was assumed to be greatest.

Shrubs infected with insect mealy bugs during the experiment period were treated with a systemic insecticide (Sweeper 20%, Wylson.chem, China).

Vegetative Growth Parameters: All vegetative growth parameters were estimated according to procedures outlined [13].

Plant Height and Width (Diameter at the Widest Point): Were recorded in (cm) at the beginning of the experiment and at the end of every 2 months. Plant height was measured with the aid of a meter rule from the base of the stem at the soil surface to the terminal bud of the main stem. Plant width was also measured by a meter at widest point on the growing shrubs to determine canopy cover.

Average Leaf Surface Area (cm^2): Was measured nondestructively during the experiment with a leaf area meter (Area Meter AM 300, UK). Five mature leaves from each plant were randomly selected and drawn on a green carton while attached to the plant. Then the drawn leaves on a green carton were cut and spread against a white background and covered with a sheet of firm, transparent plastic before passing the hand scanner over it to determine the leaf area (LA). The average leaf area was measured by dividing the total leaf area by the total number of leaves.

Total Plant Dry Weight: At the end of the experiment the soil was gently washed from roots and the plants were divided into shoots and roots. These were oven dried at 70°C until they reach a constant weight to measure the respective dry weights. Root to shoot ratios were calculated by dividing root dry weight by shoot dry weight [14].

Physiological Measurements

Chlorophyll Content: The third youngest fully expanded and exposed leaf from the apex was collected between 11:00 h and 13:00 h. The detached leaf from the plants was immediately enclosed in a paper bag and then taken back to the laboratory for analysis.

The fresh weight (FW) of each leaf sample was weighed then the leaf was finely cut and placed in a 30 ml plastic vial along with 15 ml of 80% acetone (Tedia, United States) and blended using a homogenizer (Ultra-Turrax T25, Germany). Extraction in 80% acetone (80 ml of acetone made up to 100 ml with 20 ml of distilled water) was done as quickly as possible at room temperature.

The homogenate comprising chlorophylls (both chlorophyll a and b) were filtrated by filter paper (Ederol No. 1, Germany). Then 2 ml of the leaf extract (supernatant) was transferred with a micropipette (BioTina, Germany) into 3 ml path length of spectrophotometer cuvette (Greiner, Germany) and absorbance was read against 80% acetone blank in a spectrophotometer (Thermo fisher scientific, USA) at 645 nm (for chlorophyll b) and 663 nm (for chlorophyll a).

Then the chlorophylls were quantified based on the Arnon equations as follows:

Total chlorophyll (μg/ml) = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll a (μg/ml) = 12.7 (A_{663}) - 2.69 (A_{645})

Chlorophyll b (μg/ml) = 22.9 (A_{645}) - 4.68 (A_{663})

were,

A = Absorbance at specific wavelength (A_{645} is the solution absorbance at 645 and A_{663} is the absorption at the 663).

Chlorophyll a/b ratios were calculated by dividing chlorophyll a content by chlorophyll b content.

Chlorophyll contents as μg g^{-1} leaf fresh weight were calculated as following:

[(μg/ml) ×V]/ W

were,

μg/ml = Micro gram of chlorophyll per each ml.

V = Final volume (ml) of chlorophyll extracted in acetone 80%.

W = Fresh weight (gm) of leaf sample. According to procedures outlined [15].

Relative Water Content of the Leaves (RWC): It was measured using the third youngest fully expanded and exposed leaf from the apex collected from each plant at each sampling date with four replicates for each treatment. The detached leaf was immediately sealed in a paper bag and then taken back to the laboratory for determination. These measurements were carried out between 11:00 h and 13:00 h.

RWC was determined according to procedures outlined [16]. In which, leaf for RWC was weighted immediately to obtain a fresh weight (FW) then was floated in distilled water inside a closed Petri dish and lasted for 2 h under dark condition in the laboratory (temperature about 21°C), the leaf was weighted again after gently wiping the water from leaf surface with tissue paper to obtain the turgid weight (TW). Leaf then was dried in the pre heated oven at 70°C for 24 h and their dry weights was measured (DW). The RWC was calculated using the formula outlined [17]:

$$RWC = \frac{FW - DW}{TW - DW} \times 100\%$$

Proline Content: Third fully expanded leaves were collected between 11:00 h and 13:00 h and immediately enclosed in a paper bag and then taken back to laboratory for analysis.

Proline accumulation in fresh leaves was determined spectrophotometrically according to the method outlined [18]. The proline estimation was based on the formation of brick red color by acidic ninhydrin reagent that dissolved in toluene.

Reagents: Sulfosalicylic acid solution: three grams of sulfosalicylic acid (Sigma, United States) was dissolved in 100 ml of distilled water.

Ninhydrin Reagent: Was prepared by stir until dissolved 2.5 g ninhydrin (Sigma, India) per 100 ml of a solution containing glacial acetic acid (Sigma, Germany), distilled water and phosphoric acid (Carbon group, Ireland) at a ratio of 6:3:1.

Procedure: The fresh weight (FW) of each leaf sample was weighed then the leaf was finely cut and placed in a 30 ml plastic vial along with 10 ml of a 3% (w/v) aqueous sulfosalicylic acid solution and blended using a homogenizer (Ultra-Turrax T25, Germany). The homogenate was filtered by filter paper (Ederol No. 1, Germany) and clear filtrates were then used in the assay. Then 2 ml of the clear filtrate was transferred with a micropipette (BioTina, Germany) into screw cap tube (15 ml Centrifuge tubes, JET BIOFIL) and mixed with equal volumes of glacial acetic acid and ninhydrin reagent and then the closed test tube with the reaction mixture was incubated for 1 h at 100°C boiling water bath. Brick red colors were developed. The reaction was stopped by placing the test tubes in a water bath at room temperature (21°C) for 5 minutes to cool reaction mixtures. The reaction mixture was extracted with 4 ml toluene (Tedia, United States) that was mixed vigorously for 15-20 seconds. Then 2 ml of toluene layer containing chromophore phase was separated from the aqueous phase by micropipette and transferred into 3 ml path length of spectrophotometer cuvettes (Greiner, Germany). Readings were taken immediately at a wavelength of 520 nm using toluene as a blank in a spectrophotometer (Thermo fisher scientific, USA).

Proline standard curve was prepared by using L-proline (Sigma, United States) from a range (10-100µg ml^{-1}). Stock solution of 10 mg/ml L-proline was prepared in distilled water. In Eppendorf tubes 2 ml of dilution from 10 to100 µg/ml was prepared from the stock solution in distilled water and vortex thoroughly. Then 2 ml of all diluted proline were transferred separately into screw cap tubes and mixed with equal volumes of glacial acetic acid and ninhydrin reagent and then the steps was completed as described previously. A standard curve of proline concentration versus absorbance was made.

Proline concentration (µg /ml) of different samples were estimated by referring to standard curve using standard equation ($y= 5.4657x + 4.6324$, $R^2 =0.996$).

Finally, the proline concentration was calculated on a fresh weight basis and expressed as µmol proline g^{-1} FW by using the following formula:

[(µg proline/ml × ml toluene) / 115.5 µg/ µmole] / [(g sample)/5]

were,

µg proline/ml = Concentration of proline in samples determined by referring to standard curve.

ml toluene = Amount of toluene used for each sample.

115.5 = Molecular weight of proline.

g sample = Fresh weight of leaf sample.

Water Consumption and Water Use Efficiency: The amount of water added to each pot after weighing to bring back to 100%, 75% and 50%, respectively, of container capacity was summated individually for each pot during the treatment period and used in calculating the water use efficiency (WUE) as ratio of the total dry matter to the total water applied during the study period [19].

Before applying the treatments at planting time, four additional plants were partitioned into shoots (shoots were removed to the crown) and roots and then shoot dried at 70°C until a constant mass was obtained for initial shoot dry weight values on day 0 before starting the different water deficit treatments.

And also, at the end of the experiment, four plants per treatment were harvested and separated into shoots and roots to determine dry biomass at the end of the experiment. These were oven dried at 70°C until they reach a constant weight to measure the respective dry weights.

Shoot biomass gain was calculated as the difference between final shoot dry weight and initial shoot dry weight.

Cumulative water added during the experiment period was determined gravimetrically. Each individual pot was weighed daily at 10:00h AM in the morning to maintain and restore the moisture level. The total water added was reported in liters.

Water use efficiency was expressed as kilogram dry matter/ cubic meter (m^3) water according to Still and Davies [20] and calculated as following:

$$WUE = (DW\ final - DW\ initial)\ /TWA$$

were,

WUE	=	Water use efficiency.
DW final	=	Dry weight of shoot 6 months after water stress induction.
DW initial	=	Dry weight of shoot 0 day.
TWA	=	Total water added during the same experimental period.

Statistical Analysis: The experiment was arranged as a completely randomized design (CRD) with three water regimes and four replications per treatment (12 plants in total). The experimental unit is a single plant per pot.

Since water deficit stress was induced for 6 months and measurements were taken on 0 day and after 2, 4, 6 months, the measurements were effectively repeated measures and hence repeated measures analysis was used.

Data were analyzed using the Mixed Model Procedure. Means were separated using the Fisher protected LSD pairwise mean comparisons at probability level P=0.05.

All analyses were performed using the statistical analysis system (SAS 2002, version 9.0; SAS institute, Inc., Cary, North Carolina, USA) [21].

RESULTS AND DISCUSSION

In this experiment, mortality of euonymus shrubs was 25%, after two months of induced sever water deficit treatments (data not shown).

Crops yield losses worldwide from drought exceeding the cumulative loss from all other stresses [22]. Furthermore, Water deficit affect plant growth through affecting photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters. Understanding morphological, physiological and anatomical changes that occurred in the plant during water deficit could be used to select or create new varieties with high productivity under water shortage period. On the other hand, understanding the response of plants to water deficit is essential for making tolerant stress crops [23].

Growth Parameters:

Plant Height: Under non-stress conditions, plants exhibited the greatest height, whereas severe stress treatments resulted in the most pronounced reduction in plant height (Table 1). This overall decrease in plant height increments aligns with old findings [24], who observed similar reductions under-water stress. Water stress significantly impacts plant height, as reported by Petropoulos *et al.* [25]. Khan *et al.* [26] also noted a decrease in plant stem height with increasing water stress across various watering regimes.

Plant Width: The results indicated that plant width was unaffected by the different water treatments (Table 1), showing no significant differences across the water regimes. These findings contrast ancient results [25], who reported a reduction in plant width of citrus seedlings under water stress conditions.

Average Leaf Area (ALA): Throughout the experiment, shrubs exposed to moderate and severe water stress exhibited the smallest average leaf area compared to those in the control treatment (Table 1). Moderate water deficit treatment led to a 36.46% decrease in ALA, while severe water deficit conditions resulted in a 39.97% reduction in ALA compared to the control shrubs.

These findings align with those of Wullschleger *et al.* [27], who observed reduced leaf area in Populus and Amaranth plants under water deficit conditions. High leaf areas under favorable conditions enhance photosynthesis and growth rates. However, during water stress, plants often reduce biomass allocation to leaf area to minimize

Table 1: Effect of three water regimes on growth parameters of Euonymus shrubs:

Treatments	Plant height (cm)	Width increment (cm)	Average Leaf area (cm^2)
Control (75-100%)	15.75 a	6.2 a	7.13 a
Moderate water deficit (50-75%)	11.83 b	4.8 a	4.53 b
Severe water deficit (25-50%)	10.20 b	7.1 a	4.28 b

Means within each column having different letters are significantly different according to LSD at 5 % level.

Table 2: Effect of three water regimes on total dry weight and root/shoot ratio of Euonymus shrubs:

Treatments	Shoot dry weight (gm)	Root dry weight (gm)	Root/shoot ratio
Control (75-100%)	14.82 a	10.1 a	0.7 a
Moderate water deficit (50-75%)	11.50 a	5.8 b	0.5 b
Severe water deficit (25-50%)	8.87 a	3.2 b	0.36 b

Means within each column having different letters are significantly different according to LSD at 5 % level

water loss [28]. Basal and Unay [29] reported that reduced leaf number and size under water deficit conditions correspondingly diminished photosynthesis and growth rates.

Shoot Dry Weight: The reduction in shoot dry weight across different water regimes was not significant (Table 2). Under moderate water deficit conditions, Euonymus shrubs experienced a 22.40% decrease in shoot dry weight, while severe water deficit conditions led to a 40.15% reduction compared to the control shrubs.

Wu and Bao [30] explained that changes in plant structure, dry matter accumulation, stomatal conductance and osmotic potential in response to drought are due to adaptive morphological and physiological mechanisms.

Root Dry Weight: Under non-stress conditions, Euonymus shrubs exhibited the highest root dry weight, with a mean value of 10.1 g (Table 2). However, severe water stress treatments significantly reduced root dry weight to 3.2 g.

Moderate water deficit reduced root dry weight by 42.57%, while severe water deficit treatments led to a 68.31% decrease compared to the control shrubs.

Previous studies have noted that stomatal closure is one of the earliest responses of plants to water deficit [31, 32]. In addition, Yordanov *et al.* [33] indicated that stomatal conductance is more closely related to soil moisture than to leaf water status.

Root per Shoot Ratio: The impact of water stress on the root/shoot ratio was significant in Euonymus shrubs. Under non-stress conditions, Euonymus shrubs exhibited the highest root/shoot ratio, with a mean value of 0.70, compared to other water deficit treatments (Table 2).

Abdul Jaleel *et al.* [23] reported a decrease in biomass across all sunflower genotypes under water deficit conditions. Water availability influences photosynthesis, dry matter production and its distribution [28]. Similarly, [34] found that water stress reduced the root area for water and nutrient absorption, which in turn affected overall plant growth.

Physiological Measurements

Chlorophyll Content: Total chlorophyll content was significantly reduced in shrubs subjected to moderate and severe water stress compared to control treatments throughout the experiment (Table 3). Specifically, moderate water deficit decreased total chlorophyll by 21.66%, while severe water deficit reduced it by 36.38% compared to the control.

These findings are consistent with previous research. Many studies have documented reductions in chlorophyll content due to drought stress, with varying degrees of impact depending on the duration and severity of the stress Zhang and Kirkham [35]. Kirnak *et al.* [36] observed a 55% reduction in total chlorophyll content under water deficit conditions and Kiani *et al.* [37] reported significant decreases in chlorophyll content in sunflower plants under severe water stress.

Chlorophyll a and b: Chlorophyll content significantly decreased under both moderate and severe water deficit conditions (Table 3), with reductions of 20.65% under moderate stress and 65.22% under severe stress. In contrast, the changes in chlorophyll b content were less pronounced. Severe water deficit reduced chlorophyll b by 40% compared to control treatments.

These findings align with those of Manivannan *et al.* [38], who reported decreases in chlorophyll a, b and total chlorophyll content in various sunflower species under water deficit conditions. The reduction in chlorophyll content is linked to the inhibition of photosynthesis under water deficit conditions [39]. Similarly, Anjum *et al.* [40] attributed decreased photosynthesis under limited water availability primarily to the loss of chlorophyll content.

Table 3: Effect of three water regimes on chlorophyll content of Euonymus shrubs

Treatments	Chlorophyll content (μg g^{-1}FW)	Chlorophyll a (μg g^{-1}FW)	Chlorophyll b (μg g^{-1} FW)
Control (75-100%)	1325 a	920 a	405 a
Moderate water deficit (50-75%)	1038 b	730 b	308 ab
Severe water deficit (25-50%)	843 b	600 b	243 b

Means within each column having different letters are significantly different according to LSD at 5 % level

Table 4: Effect of three water regimes on physiological measurements of Euonymus shrubs

Treatments	Relative water content (RWC) %	Proline content (μmol g^{-1}FW)	Water use efficiency (WUE) (Kg m^{-3})
Control (75-100%)	96.36 a	2.91 c	0.32 a
Moderate water deficit (50-75%)	90.40 b	5.95 b	0.27 a
Severe water deficit (25-50%)	84.82 c	13.66 a	0.23 a

Means within each column having different letters are significantly different according to LSD at 5 % level

Relative Water Content of Leaf (RWC): Relative water content (RWC) in the leaves of Euonymus shrubs decreased with the onset of water deficit, with the most pronounced reductions observed under severe stress conditions (Table 4). Euonymus shrubs in control treatments had high RWC. As water deficit increased, RWC in the shrubs decreased compared to control plants. Specifically, moderate water deficit treatment reduced RWC by 4.27%, while severe water deficit conditions led to an 11.97% decrease in RWC relative to the controls.

These findings are consistent with recent studies. Yang and Miao [41] reported that water deficit led to a decrease in RWC of 23.3% in *Populus cathayana* and 16% in *Populus kangdingensis*. Similarly, Munne *et al.* [42] observed reductions in RWC of 40% and 30% in *Rosmarinus officinalis* and *Melissa officinalis*, respectively, under water deficit conditions.

Proline Contents: Proline content in the leaves of *Euonymus* shrubs was monitored throughout the water deficit period. Significant increases in proline levels were observed in response to water stress (Table 4). Under severe stress conditions, Euonymus shrubs exhibited a markedly high leaf proline content compared to other water treatments. Specifically, proline content increased by 104.47% under moderate water deficit and by 369.41% under severe water deficit, relative to control shrubs.

Extended drought conditions can severely impact plants, prompting them to adopt various morphological, biochemical, physiological and developmental adaptations to mitigate damage [43]. Proline, as a compatible solute, accumulates in the cell cytoplasm without disrupting cellular metabolism or structure [44]. These findings are consistent with Upadhyay and Panda [45], who reported elevated proline levels in plants under water deficit. Similarly, Saglam *et al.* [43] observed increased proline content in *Ctenanthe setosa* under water stress. The accumulation of proline under water deficit conditions is likely due to reduced proline oxidation or enhanced biosynthesis [46].

Water Use Efficiency (WUE): Moderate water stress treatments led to a decrease in the water use efficiency (WUE) of Euonymus shrubs compared to the control, although the reduction was not statistically significant (Table, 4). In contrast, severe water stress resulted in a marked reduction in WUE for Euonymus shrubs.

These findings are inconsistent with those of Seghatoleslami *et al.* [47], who reported increased water use efficiency under water stress conditions. The decreased WUE in Euonymus shrubs under moderate and severe water deficits may be attributed to a reduced rate of CO_2 assimilation, which adversely affects WUE. This reduction in dry matter production under water stress could be due to both stomatal and non-stomatal limitations, which are key factors contributing to decreased photosynthetic rates during water deficits [7, 48].

CONCLUSIONS

In Jordan, water scarcity poses a significant challenge to the development and expansion of landscaping. The demand for water is increasingly outpacing supply due to natural population growth and other factors. Therefore, efficient water management and the selection of low-water or drought-tolerant species are crucial strategies.

The Euonymus plant has demonstrated notable tolerance to water deficit conditions. It showed lower reductions in shoot-dry weight under moderate and severe water deficits, indicating its resilience.

Additionally, a positive correlation was observed between proline content and water stress, with proline levels increasing twofold and threefold under moderate and severe water deficits, respectively. Reductions in water application during moderate stress treatments contributed to water conservation but only affected the shoot dry weight of Euonymus compared to the control.

Overall, water stress conditions led to reductions in plant vegetative growth, including plant height, leaf area, shoot and root dry weight and root-to-shoot ratio, though plant width remained unaffected. Stress conditions also decreased chlorophyll content and relative water content (RWC) but increased proline content, while water use efficiency (WUE) remained unchanged.

Further research is needed to explore how water deficit impacts growth and physiological responses across different genotypes in both controlled and field environments, particularly for evaluating their suitability for xeriscaping. Additionally, studies should focus on the introduction of wild flora and shrubs, assessing their water requirements and adaptability to xeriscape settings.

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