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The Effect of Water Deficit on Growth and Some Physiological Responses of Yew (*Taxus baccata* L.), as an Ornamental Tree

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Abstract

Drought is the most important environmental stress, which is usually defined as an external factor that has adverse effects in plants. The endangered yew species is very important for human societies for medicinal and ornamental reasons. The roots and leaves of this plant produce biochemical compounds such as flavonoids, alkaloids and tannins that act as anti-inflammatory, anti-cancer, anti-bacterial and anti-viral. The present research investigated the effect of different levels of drought stress (100, 75, 50 and 25 % of field capacity) on the growth and biochemical characteristics of the yew plant in a greenhouse environment in three replications in the form of a completely randomized design. In this research, which used 3-year-old seedlings, the fresh-to-dry weight ratio of shoots and roots, chlorophyll a and b and the ratio of these two, the relative content of leaf water, the activity of superoxide dismutase and catalase enzymes, and the percentage and yield of essential oil were evaluated. The results showed that the growth traits and photosynthetic pigments and the relative water content of the leaves decreased with the increase in the severity of drought stress. Of course, the activity of catalase and superoxide dismutase enzymes increased with the intensity of drought stress. The highest percentage of essential oil was obtained in semi-severe drought stress conditions (50 % FC). With the increase of drought stress up to 25 % FC, the percentage of essential oil decreased. Most of the growth and physiology parameters of yew plant at 75% FC compared to 100 % FC were not significantly different, so it seems that this plant shows good tolerance in the condition of 75 % FC and has an acceptable yield. Therefore, it is recommended to cultivate this valuable plant in favorable environmental conditions with sufficient water and/or with only in mild stress conditions.

Keywords: Antioxidant enzymes, Common yew, Dehydration stress, Essential oil.

INTRODUCTION

Drought is the most important environmental stress, usually defined as an external factor that leaves adverse effects. In most cases, stress is measured about growth (biomass accumulation) or primary assimilation processes (CO_2 and mineral absorption) related to overall growth (Pamungkas *et al*., 2022). Drought does not immediately create its effect, as plants use protective mechanisms to delay or halt chemical and thermodynamic disturbances within the cell (Shukla *et al*., 2018). Plants use various strategies to shield their tissues from the harmful effects of reactive oxygen species (ROS) and mitigate the potentially risky outcomes of water scarcity. In plants, innate antioxidant systems consist of such as superoxide dismutase and catalase as well as non-enzymatic antioxidants like certain primary metabolites (proline) and secondary metabolites (phenolic compounds, terpenoids, and alkaloids). These antioxidants aid plants retain their proteins and membranes, neutralize ROS, and maintain cellular redox potential (Soares *et al*., 2019).

The yew (*Taxus baccata* L.) is one of the species that is endangered due to extinction. This plant is highly important for human societies due to its medicinal and ornamental reasons. The roots and leaves of this plant produce chemical compounds like flavonoids, alkaloids, and tannins, which serve as anti-inflammatory, anti-cancer, antibacterial, and antiviral agents. The yew plant, with its attractive shape and colorful flowers, along with leaves that change colors in different seasons, can enhance gardens, parks, and green spaces, adding to the beauty and diversity of nature (Alavi *et al*., 2019; Shahmohammadi *et al*., 2023).

Investigating the effect of different soil moisture regimes on morphological, physiological and phytochemical responses in previous researches (Minaei *et al*., 2019; Gholizadeh *et al*., 2020; Arpiwi *et al*., 2023) showed that the fresh and dry weight of the plant decreased and the content of antioxidant enzymes increased. The highest percentage of essential oil and the highest yield of essential oil were obtained in mild treatment. Reduction of leaf water capacity is the primary result of drought in plants, thus reducing photosynthetic activity by stomatal closure, membrane damage, and accumulation of reactive oxygen species (ROS) (Shukla *et al*., 2018). According to previous studies, different levels of drought stress increased the antioxidant and phenolic constituents in olive (Gholami *et al*., 2022) and sweet cherry (Hojjati *et al*., 2023), essential oil content in lemon verbena (Hosseini *et al*., 2021), *Thymus daenensis* and *Thymus vulgaris* (Askary *et al*., 2018).

The main aim of the present study is to investigate the impact of drought stress on yew growth and biochemical characteristics. It also examines the potential for cultivating this ornamental-medicinal tree under different water scarcity stress conditions.

MATERIALS AND METHODS

Experimental design and treatments

The present study was conducted in the spring of 2022 in a greenhouse located in the Vardavard area of Tehran. The greenhouse had a photoperiod of 16 hours of light and 8 h of darkness, with a relative humidity of 65 to 75 percent, maximum temperature of 29 ºC, and minimum temperature of 15 ºC.

The experiment was based on a completely randomized design with three replications under four levels of drought stress treatments (control-100%, 75%, 50%, and 25% of FC). The drought stress was imposed based on the soil field capacity. The relative soil moisture at the field capacity and wilting point was determined using a pressure plate apparatus. Subsequently, the amount of water required based on the moisture treatments at 100%, 75%, 50%, and 25% of the soil FC was applied. Table 1 presents the physical and chemical characteristics of the pot soils.

Table 1. Physical and chemical characteristics of potting soil.

Parameter	The value
Total nitrogen (%)	0.16
Absorbable phosphorus (mg/kg)	14.4
Absorbable potassium (mg/kg)	262
Organic carbon (%)	0.59
Loam (%)	47
Clay (%)	27
Sand (%)	26
Electrical conductivity (m.mh)	1.2
pH	7.1
Class	Clay loam

The ratio of fresh weight to dry weight of aerial parts and roots

Initially, the fresh weight of aerial parts and roots after harvesting was measured using a digital scale with an accuracy of 0.1 g. Then, the samples were dried in an oven at 72 ºC for 24 h, and their dry weight was determined using a digital scale with an accuracy of 0.1 gr. The ratio of fresh weight to dry weight was then calculated (Inbar *et al*., 1994).

Chlorophyll content of leaves

Amount of 0.1 g of fresh needle leaves were completely ground in a mortar with 3 ml of 80% acetone until the final volume of the extract reached 15 ml. The extract was then centrifuged at a speed of 5000 × g for 10 min. A spectrophotometer (Shimadzu UV-160) was used to measure the absorption levels of the samples. The chlorophyll a (Eq. 1) and chlorophyll b (Eq. 2) content were calculated, followed by the calculation of the ratio of chlorophyll a to chlorophyll b (Arnon, 1967).

Equation 1: $[(12.7\times A663) - (2.69\times A645)] \times V / 1000 \times W$ = mg of chlorophyll a per g of fresh leaf
Equation 2: $[(22.9\times A645) - (4.69\times A663)] \times V / 1000 \times W$ = mg of chlorophyll b per g of fresh leaf

In the above equations, A represents the absorption at the specified wavelength, V is the final volume of 80% acetone in ml, and W is the weight of the fresh leaf in g.

Relative water content (RWC)

To measure this parameter, the youngest developed leaf of the plant was separated and quickly weighed with an accurate laboratory scale LiBROR AEL model 40SM with an accuracy of 0.0001. Then, the leaves of each treatment were immersed separately in a closed test tube containing distilled water for 4 to 5 hours, and after this period, the leaves were removed from the test tube and dried using filter paper, and weighed again. Until their weight is obtained in the state of complete turgor. To calculate the dry weight, the leaves were placed in a 70 °C oven for 48 hours and then weighed. Relative leaf water content was calculated from the following equation, where FW, leaf wet weight; DW is the dry weight of the leaf and TW is the saturated weight of the leaf (Ritchie *et al*., 1990).

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

Superoxide dismutase activity (SOD)

The activity of SOD enzyme was measured on the samples of fresh needle leaves of the plant. The activity of this enzyme was determined by its ability to inhibit the photochemical reduction reaction of nitrobuterazolium (NBT). The reaction mixture contains 50 mM phosphate buffer (pH=7), 13 mM methionine, 0.1 mM Na-EDTA, 75 µmol nitrobuterazolium (NBT), 75 µmol riboflavin, and 100 µL of extract. 3 ml of the above solution without enzyme extract was poured into two test tubes, one was placed in the device away from light and the other was placed in the presence of fluorescent light as a control. Every two min., the absorbance of the solution was read in the photometric mode and the wavelength of 560 nm with a spectrophotometer (Shimadzu UV-160). The activity of this enzyme was calculated in terms of enzyme units per milligram of protein (Giannopolitis and Ries, 1977).

Catalase enzyme activity

In order to extract enzymes, the sample of fresh needle leaves of the plant was crushed in a Chinese mortar with liquid nitrogen and turned into powder. Then, 0.5 ml of sodium phosphate buffer with pH equal to 6 was added to it and centrifuged at 13000 rpm. In order to measure the total protein and enzyme activity of the samples, the supernatant was taken from the solution. A spectrophotometer (Shimadzu UV-160) was used to measure catalase enzyme activity at a wavelength of 240 nm for 30 seconds. 20 mM sodium phosphate buffer with pH equal to 7 and 20 microliters of 30% hydrogen peroxide (H_2O_2) were used as electron acceptors. The amount of catalase activity was expressed in terms of enzyme units per milligram of protein (Luck, 1965).

The percentage and yield of essential oil

In order to measure the dry weight of the aerial parts of the plant (the upper branches which include the needles and part of the sub-stems of the plant) and also to prepare the sample for the preparation of essential oil, the plants after harvesting for two weeks at normal temperature (control), dried in the laboratory environment. In order to calculate the percentage of essential oil, 100 g of the dry matter of the plant was transferred to an essential oil extraction device (Clonger) and the process of extracting the essential oil contained in the plant was carried out for 4 h. Essential oil yield was obtained from the product of essential oil percentage in dry weight (Ghasemi Dehkordi and Taleb, 2018).

Statistical analysis

The obtained data were analyzed in the form of a completely random design, and Duncan's test and SAS 9.4 software were used to compare the mean.

RESULTS AND DISCUSSION

Drought stress was significant on wet-to-dry weight ratio of shoot and chlorophyll b at five percent level and on chlorophyll a at one percent level (Table 2).

Table 2. Results of variance analysis of drought stress treatment on growth characteristics of yew.

S.o.V	df	MS				
		fresh shoot / dry shoot	fresh root / dry root	Chlorophyll a	Chlorophyll b	Chlorophyll a / b
Drought stress	3	0.22^{*}	0.13^{ns}	0.13^{**}	0.01^{*}	0.07^{ns}
Error	8	0.07	0.08	0.002	0.002	0.23
CV (%)	-	5.99	7.03	5.42	17.67	12.05

*, ** and ^{ns}: significant at P < 0.05, P < 0.01 and insignificant based on the Duncan's test, respectively.

Based result, with escalating drought stress, the ratio of fresh weight to dry weight of aboveground parts rose. As plant-available water decreased (under severe drought stress (25% FC)), this ratio hit its lowest numerical value (Table 3), suggesting a drop in tissue water content and a rise in the dry weight ratio of aboveground parts.

Table 3. The comparison results of the average effect of drought stress on the fresh-to-dry weight ratio of aerial parts and photosynthetic pigment in yew.

Drought stress	Fresh shoot / dry shoot	Chlorophyll a	Chlorophyll b
		(mg/g F.W.)	
100 F.C	4.38^{ab}	1.05^{a}	0.28^{a}
75 F.C	4.37^{ab}	1.03^{a}	0.25^{ab}
50 F.C	4.57^{a}	0.77^{b}	0.20^{bc}
25 F.C	3.94^{b}	0.62^{c}	0.15^{c}

*In each column, means with similar letter(s) are not significantly different (P < 0.05) using the Duncan's test.

Drought stress can significantly impact plant weight through various pathways. Under drought conditions, root, stem, and cell division growth decreases, leading to reduced plant weight due to decreased new tissue production. Drought stress can also alter cell structure, such as cell hardening and shape changes in dry conditions. These structural changes can decrease cell volume and plant weight. (Billah *et al*., 2021).

Photosynthetic pigments

Based on the results of increasing drought stress intensity, the levels of photosynthetic pigments (chlorophyll a and b) decreased. The lowest levels of chlorophyll a and b were linked to the 25% FC drought stress treatment (Table 3). In dry conditions, plants experience water deficiency, leading to reduced water uptake dependent on photosynthesis in the leaves. This reduction in water uptake against evaporation decreases chlorophyll production (Shahmohammadi *et al*., 2023). Under drought stress conditions, free radicals such as hydroxyl radicals (OH-) increase due to improper use of electron transfer in the photosystem and can degrade chlorophyll. This action leads to a reduction in chlorophyll in the plant (Jacomassi *et al*., 2022). Reduced plant growth during drought stress initially occurs with a decrease in the rate of absorption surface growth and subsequent inhibition of photosynthesis. The reduction in the rate of photosynthesis during drought stress can be attributed to stomatal factors (Chowdhury *et al*., 2017; Denaxa *et al*., 2020).

Drought stress resulted in significant differences in relative leaf water content, SOD, and catalase at the 1% level, and in essential oil yield at the 5% level (Table 4).

Table 4. Results of variance analysis of drought stress treatment on biochemical characteristics and essential oil of yew.

S.o.V	df	Relative leaf water content	Superoxide dismutase	Catalase	Essential oil percent	Essential oil yield
Drought stress	3	303.33^{**}	15.05^{**}	0.09^{**}	0005^{*}	4408.43^{ns}
Error	8	7.83	0.43	0.01	0.001	3291.93
CV (%)	-	3.62	14.48	17.19	17.62	28.55

*, ** and ^{ns}: significant at P < 0.05, P < 0.01 and insignificant based on the Duncan's test, respectively.

The relative leaf water content decreased with increasing drought stress intensity in yew. The highest numerical value of relative leaf water content was associated with 100% FC, which statistically grouped with 75% FC (Table 5).

Table 5. Comparison results of the average effect of drought stress on relative leaf water content, enzyme activity and essential oil yield in yew.

Drought stress	Relative leaf water content (%)	Superoxide dismutase	Catalase	Essential oil percent
		(mg/g F.W)		
100 FC	86.67[a]	2.67[c]	0.29[b]	0.15[c]
75 FC	83.67[a]	3.03[c]	0.33[b]	0.19[ab]
50 FC	74.67[b]	4.87[b]	0.54[a]	0.24[a]
25 FC	64.33[c]	7.57[a]	0.65[a]	0.17[b]

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.

Reduction in relative leaf water content is the first effect of drought stress on plants. Decreased leaf water potential due to low relative leaf water content leads to stomatal closure (El Sabagh *et al*., 2019). The reduction in water level causes plant cells to experience decreased turgor pressure, leading to cellular damage, wilting, and reduced plant growth. High relative water content in plants helps neutralize oxygen species and osmotic stress resulting from drought, potentially aiding in higher yields (Gurumurthy *et al*., 2019).

Catalase and superoxide dismutase

In this study, the activity of antioxidant enzymes (superoxide dismutase and catalase) in yew increased under drought conditions (Table 5). Increased enzyme activity, due to their role in removing oxygen from the environment, helps reduce light respiration and the compensation point of carbon dioxide. Additionally, they remove hydrogen peroxide from the environment and compensate for the lack of oxygen resulting from the inhibitory reaction (Kapoor *et al*., 2020). The rate of reactive oxygen species (ROS) production significantly increases during stress. Many biological processes are affected by reactive oxygen molecules, one of which is the function of antioxidant enzymes. In response to this oxidative damage, plants evolve tolerance mechanisms (increasing the activity of antioxidant enzymes such as catalase and superoxide dismutase) and develop resistance to non-biological stress (Mahmud *et al*., 2023).

Essential oil yield

Increasing stress intensity up to 50% FC led to an increase in the percentage of essential oil in yew, while at 25% FC, the percentage of essential oil decreased (Table 5). Glandular trichomes responsible for essential oil production change under stress and fertilization conditions, leading to changes in the amount of essential oil (Oguz *et al*., 2022). Medicinal plants exhibit different responses in essential oil production depending on the type and magnitude of stress. Drought stress at 60% FC led to an increase in the amount of essential oil, while at 40% FC, it led to a decrease in the essential oil of marigold (Bidabadi *et al*., 2020). Consistent with the current research, an increase in the percentage of essential oil under moderate stress and a decrease under severe stress conditions were reported in coriander plants (Amiripour *et al*., 2021).

CONCLUSION

Significant differences were observed in the growth, photosynthetic pigments, and biochemical traits of yew (*Taxus baccata* L.) plants under drought stress. Increased drought stress resulted in decreased growth, chlorophyll a and b levels, and leaf relative water content, while the catalase and superoxide dismutase enzyme activities increased under such stress. In response to drought stress, the yield of essential oil increased up to 50% FC, while under severe drought stress (25% FC), essential oil synthesis decreased, possibly due to the plant's energy being directed towards growth and biochemical activities for adaptation to adverse conditions, thereby reducing the production of compounds such as essential oil. It seems that the yew plant exhibits good tolerance under the condition of 75% FC and has an adequate yield because the majority of its growth and physiological characteristics at 75% FC compared to 100% FC were not significantly different. As a result, it is recommended to cultivate this valuable plant in an ideal habitat with sufficient water and/or under mild stress.

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