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Effect of Biochar on Physiological Characteristics of European Yew (*Taxus baccata*) Seedling in Different Light Intensities

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ABSTRACT

Aim: This investigation aims to study the effect of biochar on gas exchange, water relations, and photosynthetic pigments of European yew (*Taxus baccata*) seedling in different light intensities (LI).

Materials and Methods: Two-year potted seedlings of European yew are placed in forest stands with canopies of closed (15% LI), semi-closed (45% LI), and open (75% LI). In late June 2018, biochar was added to potted soils at the levels of zero, 10, 20, and 30 $g.kg^{-1}$, and until the end of November, every 3 (or 4) days, 100 mL water was given to each pot.

Findings: Seedling survival at different levels of treatments was 100%. Regardless of biochar, the highest photosynthesis, stomatal conductance, intercellular CO_2 concentration, relative water content, chlorophyll (chl) contents in the closed canopy, and the highest leaf temperature in the open canopy was observed. With increasing biochar concentration, chl b and total chl increased (28-86%, respectively) in the open canopy and decreased (28 and 28%, respectively) in the closed canopy. Neither biochar nor LI significantly affected electrolyte leakage and carotenoid content.

Conclusion: Although yew seedlings maintained their vegetative quality and health in different LI, and the measured variables responded differently to light-biochar combination, it may be best to grow yew in the shade together with biochar. Since the effect of biochar becomes more tangible over time, further research in the following years can lead to more accurate findings affected by the combined light-biochar treatment.

Keywords: Carotenoid, Electrolyte leakage, Relative water content, Stomatal conductance, Total chlorophyll.

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Introduction

Biochar is a stable carbon-rich material produced by burning organic matter in the absence of oxygen or oxygen at temperatures below 700 °C, a process called pyrolysis. A high concentration of biochar increases cation exchange capacity (CEC) and nutrient uptake, which effectively controls pollution [1]. The amount of water absorbed in the soil depends on the specific level of biochar so that by adding biochar in clay soils, water accumulation is prevented and soil aeration is increased, and in sandy soils, water holding capacity is increased, and water loss is prohibited [2]. The difference between biochar and charcoal or activated carbon and other carbon materials is that biochar is often produced for soil use and has a large porosity and contact surface due to the preservation of the biomass structure. Biochar is more effective than activated carbon in increasing the uptake of polycyclic aromatic hydrocarbons and improving the dry weight of roots and stems and increasing plant growth, biomass, and nutrient uptake under drought stress [3]. The microscopic structure of biochar is one of the main determining factors in the ventilation and improvement of soil conditions. The contact surface of raw organic materials (after the pyrolysis process) multiplies after conversion to biochar [4]. Improving crop growth in biochar-amendment soils also leads to improved soil exchange capacity and increased soil water and plant available moisture [5].

In recent years, several studies have been conducted on the effect of biochar, especially on the physiological characteristics of forest tree seedlings. For example, in an investigation carried out on two years seedlings of *Pyrus ussariensis* under drought stress, biochar prevented the decrease of soil efficiency moisture and significantly enhanced chlorophyll fluorescence [6]. In another study on *Salix alba* seedling, the combination of bacterial (*Pseudomonas fluorescens*) with biochar increased the dry matter of leaf, stem, root, and the whole plant compared to the control (no bacteria-no biochar) and improved the efficiency of the plant in removing heavy metals [7]. It was also confirmed that soil drought significantly reduced photosynthesis, transpiration, and stomatal conductance of *Quercus castaneifolia* seedlings. However, the addition of biochar prevented a reduction in the amount of these variables (as well as nutrient incorporation) [8] and the number of photosynthetic pigments and leaf growth indices [9].

Radiation is a main source of energy in ecosystems and varies with season, climate, and longitude, and seedlings of forest species show different responses to light/shade, depending on their light nature. For example, increased shoot growth of *Castanea sativa* seedling has occurred in the shade [10] but that of *Fagus sylvatica* seedling in light [11]. However, in *Fagus orientalis* seedlings of the Hyrcanian forests (northern Iran) [12], as well as silver cypress (*Cupressus arizonica*) [13], shade improved survival and shoot growth. European yew (*Taxus baccata* L.) belongs to Taxaceae, like *Cupressus sempervirens* var. *horzontalis*, *Juniperus exelsa*, and *Thuya orientalis* is one of the four conifers native to the Hyrcanian forests of Iran. It is a native and ancient tree species growing in the Hyrcanian forests of Iran that have been considered in recent years because of being on the red lists of threatened species. Yew is a shade and drought-tolerant sub-montane species, but in the Sangdeh region, the individuals of this evergreen tree occur up to 2800 m a.s.l. [14]. Soils of yew habitats are humic and brown, calcareous, rich in organic matter and calcium carbonate, the pH varies between 6.5 and 7.5, and the texture is moderate to heavy and often gravelly [15]. Yew is expanding in parks, urban green spaces, yards, and villas due to its beauty and deco-

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rative values. In recent years, in the north of the country, the seedling production of yew has been started in mountain forest nurseries by the public sector. In the lowland areas, the private sector has implemented to propagate through cutting and seed in the shade or whole light so that municipalities and applicants demand the transplanted seedlings. Slow growth is a negative factor in the production of yew in nurseries and greenhouses, but soil amendments, including biochar, may play an important role in improving its growth and physiological threats. This study determines the effect of biochar on some physiological properties (gas exchanges, water relations, and photosynthetic pigments) of potted seedlings of yew in stands with different light intensities. We hypothesized that biochar could improve some physiological variables in yew seedlings under shade conditions.

Materials and Methods

In late June 2018, two-year potted seedlings of European yew were transferred to three forest stands (dominated by a mixture of *Quercus castaneifolia*, *Populus caspica*, and *Ulmus carpinifolia*) in the experimental forest of Tarbiat Modares University, northern Iran (36°17´58 ´´ N, 52°04 ´24 ´´ E). The stands included closed-canopy [15% light intensity (LI)], semi-closed canopy (45% LI) and open canopy (75% LI). Then biochar produced from hornbeam wood was added into the soil of the pots (1.5 kg) at four levels of zero, 10, 20, and 30 g kg^{-1}. The experiment was performed as a factorial (LI × biochar) in the form of randomized complete blocks in 3 replications so that 96 seedlings were placed in each radiation level. After determining the soil field capacity [16], 100 mL of water was given to each pot every 3 (or 4) days until the end of November. It is necessary to say that on fully sunny days, in each crown canopy, the accurate measurement of relative light intensity was performed with a Lux-Meter (LI-250A, USA). The average measurement of days was recorded as the relative light intensity for each canopy. Properties of biochar and potting soil were determined in the laboratory (Table 1).

Gas exchanges Measurements

Net photosynthesis rate (A), transpiration rate (E), stomatal conductance (Gs), intracellular CO_2 concentration (Ci), and leaf temperature (LT) were measured using a portable gas exchange device (LiCor Inc., Lincoln, USA). For this purpose, four seedlings were selected from each treatment combination, and from each seedling, four fully developed and healthy leaves were selected from the upper part of the seedlings [17]. Measurements were taken between 9 am, and 12 am on a sunny day with a light intensity of 1400 $\mu molm^{-2}$ (LED, Red-Blue 6400-02B LiCor Inc) in early September.

Water relations

Three of the most developed and mature leaves were selected to measure the relative water content (RWC) of each seedling. The leaves were weighed (FW), then placed in distilled water for 24 hours in the dark to absorb as much water as needed and become swollen. The swollen leaves were weighed

Table 1) Properties of biochar and potting soil.

Medium	Clay (%)	Silt (%)	Sand (%)	Texture	N (%)	P ($mg.kg^{-1}$)	K ($mg.kg^{-1}$)	ECC© ($Cmol.kg^{-1}$)	BDθ ($g.cm^{-3}$)	pH
Soil	12	12	75	Sandy-Loam	0.02	4.6	120	9.6	1.63	7.6
Biochar	-	-	-	-	0.52	88.5	1100	-	-	7

© Exchange Cation capability
θ Bulk Density

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(SW), and after placing them in the oven (temperature 70 °C) for 48 hours, their dry weight was measured (DW). The RWC was calculated according to Eq. (1) [18].

$$RWC = [(FW-DW) / (SW-DW)] \times 100 \quad \text{Eq. (1)}$$

To measure electrolyte leakage (EL), one square centimeter sample was taken from the middle of the newly matured leaves and placed in ionized water tubes. The samples were exposed to laboratory temperature for 24 hours, and their initial electrical conductivity (EC_1) was measured by a digital EC meter. The samples were then placed in a Benmari bath (80 °C) for 12 hours, and after measuring the total electrical conductivity (EC_2), the percentage of EL was determined according to Eq. (2) [19].

$$EL = [EC_1/EC_2] \times 100 \quad \text{Eq. (2)}$$

Photosynthetic pigments Measurements

Photosynthetic pigment content [chlorophyll (chl) a, chl b, total chl, and carotenoid] was measured by sampling the top three leaves of seedlings. To do this, the first 0.1 g of frozen leaves were extracted at -80 °C with 0.1 g of calcium carbonate and 4 ml of 80% acetone in the dark. The resulting extract was centrifuged at 4000 rpm for 10 minutes at four °C. The adsorption of the supernatant was read at 470, 645, and 663 wavelengths using a spectrophotometer (Lambda 45-UV/Visible, PerkinElmer, Waltham, MA, USA). Thus, the contents of chl a, chl b, total chl, and carotenoid (in mg g^{-1} fresh weight) were determined using the following Eqs. [20].

$$\text{chl a} = 12.7\,(A663) - 2.69\,(A645) \times \frac{v}{w \times 1000} \quad \text{Eq. (3)}$$

$$\text{chl b} = 22.9\,(A645) - 4.69\,(A663)\,\frac{v}{w \times 1000} \quad \text{Eq. (4)}$$

$$\text{total chl} = \text{chl a} + \text{chl b} \quad \text{Eq. (5)}$$

$$\text{carotenoid} = (A470) - 14.4\,(A663) - 63.08\,(A645) \quad \text{Eq. (6)}$$

where A is the optical absorption of samples, v is the volume of acetone used, and w is the weight of the isolated leaf tissue (gr).

Data analysis

The Kolmogorov-Smirnov test determined the normality of the data, and the Levene test did the homogeneity of variance. Two-way ANOVA was used to detect the data significance and Tukey's-HSD test to compare the means. If the data were normal and the variance was not homogeneous, the Dannett-T3 test was used. Due to the scarcity of leaf samples, treatments of 10 g.kg^{-1} biochar and 45% LI were not included in the analysis of water relations and photosynthetic pigments. Analyses were performed using the software of SPSS ver. 26.

Findings

Photosynthesis was affected by biochar, LI, and the combined effect of the two factors (Table 2), and its highest amount occurred in 15% LI- 30 g.kg^{-1} biochar (Table 3). Both factors and their interaction affected the stomatal conductance (Table 2).

With the application of biochar, the stomatal conductance reduced in the severe shade (15% LI); conversely, it improved (15.4-30.8%) in relatively whole light (75% LI); but it did not differ in intermediate light status (45% LI) (Table 3). Leaf temperature was influenced by the two factors (Table 2). In the severe shade, biochar increased the leaf temperature, and in 75% LI, 30 g.kg^{-1} biochar showed the highest leaf temperature (Table 3).

The combined effect of two factors on transpiration was significant (Table 2), and significant changes were detected in high concentrations of biochar in 15% and 75% LI (Table 4). Intracellular CO_2 concentration did not differ significantly with increasing biochar concentration (Table 2), but it decreased with increasing LI (Table 4). Chl a did not differ by biochar (Table 2), but it decreased in high LI (75%) (Table 4).

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Table 2) Two-way ANOVA on gas exchange, water relations, and photosynthetic pigments of yew seedling.

	Biochar		Light intensity		Biochar × Light intensity	
	F	P	F	P	F	P
Photosynthesis	41.75	0.000***	172.76	0.000***	4.63	0.000***
Stomatal conductance	4.07	0.011**	158.01	0.000***	13.33	0.000***
Temperature	11.07	0.000***	110.91	0.000***	9.46	0.000***
Transpiration	1.50	0.224ns	0.92	0.404ns	2.21	0.055*
Intercellular CO_2 Concentration	0.16	0.92ns	15.11	0.000***	1.07	0.39ns
Chl a	3.50	0.064ns	7.25	0.020**	4.11	0.054 ns
EL	1.56	0.286ns	3.04	0.132ns	2.94	0.129ns
RWC	2.87	0.096ns	3.55	0.084ns	12.70	0.001***
Chl b	3.87	0.050**	28.43	0.000***	40.34	0.000***
Total Chl	1.28	0.315ns	31.30	0.000***	28.98	0.000***
Carotenoid	2.87	0.096ns	3.55	0.084ns	12.70	0.001***

Ns: non significant, *,** and *** significant at level of 5% , 1% and 0.1%, respectively.

Table 3) Comparison of mean of photosynthesis, stomatal conductance, and leaf temperature of yew seedlings under the combined effect of biochar × LI.

	Li 15%				LI 45%				LI 75%			
Biochar ($g.kg^{-1}$)	0	10	20	30	0	10	20	30	0	10	20	30
Photosynthesis ($\mu mol\ CO_2\ m^{-2}.s^{-1}$)	10b	8.8cd	9.2bc	11a	7.5ef	4ij	5.5gh	7.5fg	8.5de	3j	5hi	7.4fg
Stomatal conductance ($mmol\ H_2O\ m^{-2}.s^{-1}$)	0.30a	0.22b	0.24b	0.25b	0.18c	0.16c	0.17c	0.18c	0.13d	0.17c	0.15c	0.16c
Temperature (°C)	24.5h	26.5de	26fg	25.5g	26.5ef	27cd	26.5ef	26.5ef	27.5ab	27bc	27.2abc	27.7a

- Different letters in a row are significantly different (p<0.05).

EL did not change with biochar and LI as well as their interaction (Table 2). RWC, chl b, total chl, and carotenoid were influenced by the interaction of the two factors (Table 2). The highest content of RWC, chl b, and total chl was found in 15% LI-biochar of 30 g Kg^{-1}, and that of carotenoid did in 15% LI-biochar of 20 $g.Kg^{-1}$ (Table 5).

Discussion

In the present study, at each combination treatment of biochar-LI, the survival of seedlings was 100%, and, at different levels of treatments, no seedlings died. This suggests

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Table 4) Comparison of mean of transpiration, intercellular CO_2 concentration, and chl a of yew seedlings under the separate effect of biochar and LI.

	Biochar (g.kg^{-1})				LI (%)		
	0	10	20	30	15	45	75
Transpiration (μmol H_2O m^{-2}.s^{-1})	2a	1.8a	2a	2a	2a	1.8a	1.9a
Intercellular CO_2 Concentration (μmol m^{-2}.s^{-1})	395a	396a	396a	395a	405a	392b	388b
Chl a (mg.g^{-1})	1.5a	-®	1.5a	1.3a	1.5a	-®	1.4b

® No data

-Different letters in a row of each treatment (biochar or LI) are significantly different ($p<0.05$).

Table 5) Comparison of mean of EL, RWC, chl b, total chl and carotenoid of yew seedlings under interaction effect of biochar × LI.

	Li 15%			LI 75%		
Biochar (g.kg^{-1})	0	20	30	0	20	30
EL (%)	92a	75a	85a	86a	90a	92a
RWC (%)	63b	58cd	72a	55d	60bc	54d
Chl b (mg.g^{-1})	0.7cd	1b	1.3a	0.8b	0.7bc	0.5d
Total Chl (mg.g^{-1})	2.2c	2.5b	2.8a	2.4bc	2.3c	1.8d
Carotenoid (mg.g^{-1})	0.5c	0.65a	0.55bc	0.61ab	0.47c	0.45c

-Different letters in a row are significantly different ($p<0.05$).

that even though *Taxus* is a shade-tolerant species, the radiation produced in the open canopy (75% LI) did not reduce its survival rate. Based on research in an open canopy, *Taxus baccata* (European yew) had a higher leaf area index and leaf nitrogen than *Taxus brevifolia* (Pacific yew). This indicates that *T. baccata* is more resistant to radiation than *T. brevifolia* [21]. Of course, the yew seedlings grown in the shade (produced in nurseries or appeared in natural stands) and transferred to open areas are not able to survive; on the contrary, the gradual openings of the forest stand (or gaps occurred in natural forests) affects its establishment [22,23].
According to literature, the regeneration ability of yew in the stands with a light crown canopy is better than that in the dark one. However, while facilitating the regeneration and successful establishment of the seedlings, dense shade increases the chl content, leaf area, and specific leaf area [24]. Conversely, after two years of shade adaptation, seedlings, when transferred to the open environment, their survival rate decreases, and their foliage color and freshness change. This suggests that yew seedlings are generally sensitive to sudden changes of LI [25]. In this regard, it was found that yew seedlings achieved relatively good survival (61%) in shady conditions (25% LI), but all of them died as long as exposed to whole light (100% LI) [26]. It was also reported that yew saplings had a successful establishment following the overthrow of the upper storey of European beech and the emergence of canopy opening. This implies that, generally, the gap dynamics associated with high mortality of upper storey are the

determinants of yew natural regeneration in the closed canopy [22]. In relation to shade-tolerant species such as chestnut [10], Eastern beech [12], and semi-shady species of hornbeam [27], the evidence indicate an improvement in the quantitative and qualitative attributes of these species in the shade or gaps occurred in the small crown canopy.

In our investigation, nevertheless, the survival rate was high. However, it was not significantly influenced by biochar (as well LI). Usually, the survival rate of some shade-tolerant species is reduced by fertilizing or adding soil amendments. This may be due to the lack of microorganisms, including mycorrhizal fungi. However, soil amendments play an influential role in the growth of shade-resistant species, so nitrogen fertilization positively affects the growth of yew seedlings in stands with different LI [28].

In the present study, significant changes in transpiration were observed in high concentrations of biochar-LI of 15% and 75%, and with the application of biochar, the stomatal conductance was reduced in the shade (15% LI). Conversely, transpiration improved at high LI (75%), but it did not change at intermediate LI (45%). Intracellular CO_2 concentration did not differ with biochar addition but decreased with LI. Applying biochar in the severe shade (15%) increased leaf temperature, and the concentration of 30 g.kg^{-1} biochar in the thin stand or open canopy (75% LI) caused the highest leaf temperature. This indicates that despite the low tolerance of shade-tolerant plants to drought, the yew is a unique species among conifers that are both shade-tolerant and drought-tolerant [29].

Generally, maintaining the rate of photosynthesis under closed stomates and low transpiration leads to increased water use efficiency and RWC, and this increase is essential for the improvement of the survival and performance of plants [30]. In the present study, the RWC of yew in the dense shade (15% LI) increased with biochar addition. It was also reported that the crown canopy of forest stands (by reducing sunlight and temperature) helped maintain the soil moisture and increased the RWC of the leaves [31]. Likewise, it was confirmed that biochar significantly increased (22-50%) the amount of water available to the plant and caused changes in porosity, nitrogen, and moisture content of the soil .In the present study, no significant change in EL rate was found in different treatments. However, mesophilic conductance increased with increasing biochar concentration at each light level, and the highest was recorded in severe shade-30 g.kg^{-1} biochar. Reducing mesophilic conductivity is a physiological mechanism and prevents water loss by cells and plants.

Conclusion

It was revealed that biochar improved a few physiological variables in yew seedlings under shade conditions. In general, the shade-tolerant seedlings of yew, although in different radiation conditions, maintain high survival and their vegetative quality and health (according to the authors' observations), and their measured variables showed different responses to the light-biochar combination. However, it is recommended that the seedlings be trained in shady conditions using biochar (and or microorganisms, including mycorrhizal fungi and rhizobacteria). Of course, because the effect of biochar becomes more tangible over time [30], further research in the coming years can lead to more accurate findings affected by the combined treatment of light and biochar.

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