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The influence of effective microorganisms on physiological characteristics of containerized taurus cedar (*Cedrus libani* A. Rich.) seedlings

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SILVICULTURE

ABSTRACT

Background: In this study, the impact of Effective Microorganism (EM) applications on some physiological characteristics of 2+0 aged, containerized Taurus cedar (*Cedrus libani* A.Rich.) seedlings were investigated. EM-1, EM-A, EM-5, and EM-Gold were used as effective microorganism varieties. EM application was repeated twice at two different times. The first application was carried out in April-May 2017 at the beginning of the vegetation period, and the second application was carried out in June-July, when the growth of seedlings was the most active period, according to a randomized plot design with three replications.

Results: Physiological measurements on seedlings were carried out at the end of the second vegetation period. According to the results of the study, it was determined that the EM type, dose and application time factors had a significant effect on physiological characteristics such as Chlorophyll a, Chlorophyll b, Total Chlorophyll, Photosynthesis Rate, Transpiration and Relative Humidity. EM-A had a positive effect on photosynthesis rate and EM-5 had a positive effect on all other measured physiological parameters. High dose of EMs had the most positive effect on relative humidity, while medium dose of EMs had the most positive effect on chlorophyll values, transpiration rate and photosynthesis rate. In addition, it was determined that EM application at the beginning of the vegetation period had an increasing effect on all measured physiological parameter values.

Conclusion: According to the results of the study, it was concluded that EMs have a positive effect on the physiological quality characteristics of Taurus cedar seedlings and can contribute to the production of seedlings with higher adaptability in afforestation.

Keywords: Effective microorganism, *Cedrus libani*, Taurus Cedar,seedling quality, physiology.

HIGHLIGHTS

EM application positively affects photosynthetic pigments and increases the rate of photosynthesis, coinciding with the period when the plant awakens, and physiological processes accelerate.
EMs effected on chlorophyll a, chlorophyll b, total chlorophyll, relative humidity (%) and transpiration rate, significantly.
EMs play a critical role in improving physiological characteristics that significantly affect seedling quality. So, it should be used for the seedling propogation, effectively.
EM treatments may also be used in forestry to cultivate high quality seedlings and achieve high quality plantation as well as serve as a tool to increase the performance of forestation.

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INTRODUCTION

Cedrus libani A. Rich., Taurus cedar, a forest tree that establishes pure and mixed stands in the Taurus orogenic belt in Turkey, makes its wide, vertical amplitude natural expansion happen from the sea level up to 1,400-2,200 m altitude in the mountains of Syria and Lebanon outside Turkey (Quézel and Médaıl, 2003; Ayan et al., 2018). Taurus cedar is a very tolerant variety to harsh winter conditions and high temperatures in summer. Its high tolerance has made the cedar tree a frequently preferred variety in afforestation (Ata, 1995). Apart from the natural distribution areas of the Taurus cedar, the existence of successful plantations within the borders of 25 different provinces in five different geographical regions of Turkey confirms that it is a variety with high plasticity (Ayan et al., 2017).

The use of containerized seedlings (Ayan, 2007), different growing medium substrates (Ayan, 1999, 2001, 2002a, 2002b; Ayan and Tüfekçioğlu, 2006; Ayan and Tilki, 2007), slow working fertilizers (Ayan, 1998), different growing processes – "greenhouse-open area-shaded area" (Ayan et al., 2000), many cultural processes based on the seedling development stages determined according to the ecological conditions of the nursery (Yer and Ayan, 2011) have been reported in various studies to have an effect on the quality of seedlings to be used in afforestation. In particular, soil fertility is effective on the quality of seedlings to be used in afforestation areas. However, one of the most important problems of nurseries in Turkey is the low fertility of nursery soils (Atik, 2013). Long-term mismanagement on nurseries, lack of organic matter in nursery lands and malpractices in the use of artificial fertilizers reduce the quality of the seedlings and cause a decrease in the productivity (Yılmaz, 1988). Furthermore, the use of chemical fertilizers in forest nurseries, as in agricultural areas, directly or indirectly affects the natural structure of the soil. Therefore, the need for biological and natural materials has gained more importance in recent years to increase the current yield of nurseries and to produce quality seedlings.

Some outcomes have been expressed those chemical fertilizers used in forest nurseries and agricultural production give positive results in meeting plant nutrients (Özdemir, 1989; Kulaç, 2016). However, Effective Microorganism (EM), i.e., effective, active, or beneficial microorganism technology has started to develop in recent years due to the harmful effects of chemical fertilizers or pesticides on human health and nature -it was established by the Japanese philosopher and naturalist Mokichi Okada in the 1930s, considering the long-term harm of chemicals.

EM consists of mixed cultures of natural and beneficial microorganisms that are widely used in soil and is applied to plants as inoculants to increase soil quality, plant growth and yield (Higa and Parr, 1994; Iwaishi, 1994; Iwahori and Nakagawara, 1996). EMs, which are mostly used in agriculture, have become an important part of natural agriculture (Calıskan, 2018). Although EM consists of numerous microbial varieties, the predominant populations are lactic acid bacteria, yeasts, actinomics, and photosynthetic bacteria.

It is known that EM preparations have positive effects on the improvement of soil production properties (Higa and Wididana, 1991; Valarini et al., 2003) and that EMs accelerate the mineralization of organic matter in the soil (Daly and Stewart, 1999). EMs are explained to be natural products that have positive effects on increasing beneficial soil microflora, improving vegetative growth of plants, and increasing resistance to insect diseases and pests (Daly and Stewart, 1999). Although the positive effects of EM on plant growth, yield and quality have been suggested by many studies, there are still many questions as to which EM cultures or which combinations of these cultures are most effective for alleviating certain chemical, physical and microbiological problems in existing soils (Calıskan, 2018).

Many studies have been conducted on the positive effects of EM on crop yield in agricultural areas (Chaudhry et al., 2005; Piskier, 2006; Javaid, 2006). On the extraordinary effects of these microorganisms, some studies have been conducted on chamomile (*Chamomilla spp*. or *Chamomilla sp*.) on grass in the Netherlands and Austria (Daly and Stewart, 1999; Fujita, 2000) and on apples in Japan (Fujita, 2000). Although EM is widely used especially in agriculture, the information about the effectiveness of EMs in forestry is quite limited. Atik (2013) has studied the effect of EM on seedling height and root collar diameter in *Pinus nigra* J.F.Arnold.

In this research, it was aimed to evaluate the effect of EM application on the physiological quality characteristics of Taurus cedar seedlings, which is one of the most preferred species in afforestation investigations in Turkey and has high plasticity, showing high adaptability in different environments both within and outside its natural range.

MATERIAL AND METHODS

Material

The study, which was conducted on 2+0 aged, polyethylene-containerized (Dimensions: 11 x 25 cm) Taurus cedar seedlings originating from Eğirdir, Isparta, was carried out in Daday State Forest Nursery affiliated to Kastamonu Regional Directorate of Forestry. Brief information about the nursery where the study was carried out is given in Table 1.

Table 1 General information of Daday State Forest Nursery

FEATURES	VALUES
Latitude	41° 22' 16"
Longitude	33° 46' 38"
Exposure	South
Elevation from Sea Level	800 mt
Annual Average Temperature	9,9 °C
Annual Maximum Temperature	19,9 °C
Annual Minimum Temperature	--0,3 °C
Annual Precipitation	611 mm
Annual Average Relative Humidity	% 50
Vegetation Period	May-October

A mortar mixture consisting of 60% mineral soil, 5% fine sand, 20% humus and 15% burnt barn manure was used for seedlings grown in polyethylene containers.

Method

Preparation of Effective Microorganisms

Routine nursery cultural processes were applied to the seedlings, which were considered as research objects in the nursery, and irrigation was carried out once a week based on observation with a sprinkler system. The research was established according to the "Random Plots Trial Design" in open area conditions.

Four different EM products, EM-1, EM-5, EM-A, and EM-Gold, were used in the study. Solutions of 30%, 60%, 90% from products EM-1, EM-A, and 10%, 20% and 30% from EM-5, EM-Gold products (adjusted as low, medium and high dose) were prepared in 1.5 litre containers. They were applied to the seedlings as a total of 12 different treatments of three different concentrations of four different products (Table 2).

EM application was carried out by directly pulverizing to the mortar on the surface of the container and the above-ground parts of the 2+0 aged, containerized seedlings, which continue their development in the containers; in April before they enter the vegetation period, and in the first week of June, when the growth of the seedlings is most active (rapid development stage). The trial was set up with three replications of each treatment, with each plot represented by 10 seedlings. Four different types of EM were applied in three different doses at two different application times (before and during the vegetation period).

For the first trial, EM solutions were treated by spraying on each plot in the first week of April 2017 before entering the vegetation period. The second application was repeated one month after this procedure, that is, in the first week of May. For the second trial, each plot was treated with EM solution by spraying in the first week of June 2017 during the vegetation period. The second application was repeated one month after this procedure in the first week of July.

Seedling Physiological Features

The needles were taken and chlorophyll a, chlorophyll b, total chlorophyll, photosynthesis rate and relative humidity (NNI%) were determined after EM applications, at the end of January, when the seedlings were in true dormancy at the end of the vegetation period. Measurements of the amount of cumulative transpiration (S) were carried out after the pluck.

Determination of chlorophyll analysis: For the measurement of chlorophyll amounts, needle leaves were taken from the terminal shoot of the seedling, crushed in a pestle and treated with ethanol (1 g pure chlorophyll, 25 ml ethanol) to form a solution. Using the FEK-M method, the amount of chlorophyll in the solution was determined with the help of photoelectrocolorimeter (Dmitriyeva and Kefeli, 1991). By means of this method, the amount of chlorophyll in the solution was determined by how much of the applied radiation was absorbed by the solution, that is, its optical frequency (density) (Dutton et al., 1943; Atik, 2008; Aydınoğlu, 2014).

Table 2 Application levels of EM types, contents and doses.

EM Type	Explanation of EM Contents	Dose (%)		
		Low	Medium	High
EM-1	It is used effectively in agriculture and includes all the beneficial properties of EM (EM Agrıton Turkey, 2018). It contains 96% water, 3% Molas, 1% lactic acid bacteria *Lactobacillus casei* (Orla-Jensen 1916) Hansen & Lessel 1971.	30	60	90
EM-A	It includes enzymes, antioxidant substances, organic acids, bioactive substances, minerals, natural hormones and other beneficial substances that increase the resistance of the plant against diseases and pests, low temperatures and frosts (EM Agrıton Turkey, 2018). It contains lactic acid bacteria (*Lactobacillus fermentum* Beijerinck 1901), L. plantarum (Orla-Jensen 1919), *L. rhamnosus* (Hansen 1968), *L. casei* (Orla-Jensen 1916) Hansen & Lessel 1971, L. delbrueckii Weiss et al., 1984), yeasts (*Saccharomyces cerevisiae* Meyen ex EC Hansen), *photosynthetic bacteria* (*Rhodopseudomonas palustris* (Molisch 1907) van Niel 1944), heterotrophic bacteria Bacillus subtilis Ehrenberg, 1835 and beneficial organisms that can survive under pH 3.5 in EM-A (EM-A, 2021).	30	60	90
EM-5	It is an important group of effective microorganisms that increase the resistance against diseases and pests, reduce the need for chemical pesticides when applied continuously, and are used after germination, before the emergence of diseases and pests (EM Agrıton Turkey, 2018). It contains lactic acid bacteria (Lactobacillus delbrueckii Weiss et al., 1984, L. plantarum (Orla-Jensen 1919), L. rhamnosus (Hansen 1968), L. casei (Orla-Jensen 1916) Hansen & Lessel 1971), yeasts (Saccharomyces cerevisiae Meyen ex EC Hansen), phototrophic bacteria (Rhodopseudomonas palustris (Molisch 1907) van Niel 1944) (PTTAVM.com, 2020).	10	20	30
EM-Gold	It contains antioxidant substances and various vitamins produced for humans (EM Agrıton Turkey, 2018). It is a special product based on sugar cane molasses and yeast extract fermented by microorganisms in a mixture of water, coral lime and nigari (PRODUKTE.DE, 2021).	10	20	30

The determination of Photosynthesis rate: Photosynthesis rate was analysed with the LICOR-6200 portable photosynthesis measuring device. Photosynthesis rate was measured in the exposed grown needle leaves of 5 sample seedlings taken from each plot. Measurements were recorded between 9:00-12:00. The results obtained are expressed as μmol m^{-2} s^{-1}.

Relative humidity percentage (RH%): Needle leaves were cut in certain sizes (to cover an area of 1 cm^2). They were weighed with the help of precision scales immediately after being cut and their wet weights (WW) were measured. Then, these needle leaves were kept in pure water until they became turgescent (approximately 4-5 hours). Turgorized needle leaves were weighed again with a precision scale and their turgor weights (TA) were recorded. Needle leaves in turgorized state were taken into an oven at a temperature of 102 ± 3 °C, and left for 24 hours to become oven-dry, and then the oven-dry weights were recorded (KA). The relative humidity of the needles taken was determined with the help of the following formula (Dhanda and Sethi, 1998).

$$NNI (\%) = [(YA-KA) / (TA-KA)] \times 100 \quad [1]$$

Cumulative transpiration (S): 5 seedlings belonging to each application were removed from the containers, then washed and cleaned, and brought to the laboratory. A sensitive cleaning process was carried out again without damaging the stem and roots of the seedlings. After this process, the seedlings were cut from the NWF level and the stems of the seedlings were placed in distilled water at +4 °C until they were fully saturated (approximately 24 hours). The stems of the seedlings that became fully saturated (FS) were purified from the water with the help of a paper towel. The fully saturated seedlings, whose weights were measured, were immediately taken into the air-conditioning cabinet at 25 °C ambient temperature, 60-65% relative humidity and 4000-4500 lux light intensity. Since the moisture content of the seedlings would decrease rapidly after being placed in the cabinet, weight measurements were continued at 15-minute intervals, and the measurement intervals were extended to 30, 45, and 60 minutes since moisture loss would slow down further in the future.

Finally, the seedlings, which were measured at 420 minutes intervals, were taken from the air-conditioning cabinet and their weight (DDA) was measured, and the water pressure values were measured with the help of the Scholander device. The seedlings were placed in an oven that was kept ready at 104 °C, and their oven-dried weight (KA) was measured after the seedlings which were dried for 24 hours were taken out of the oven. Cumulative Transpiration values were calculated with the help of the following formula (Dirik, 1994).

$$S=(TDH-DDA)/KA \times 100 \text{ gr } (H_2O/100 \text{ gr dry weight}) \quad [2]$$

Statistical Evaluations

Multiple variance analysis was applied to reveal the individual and interactive effects of EM type, EM dose, and application time factors on all measured and calculated physiological characteristics, and Duncan's multiple test was applied with the help of SPSS package program for process comparison according to variables. The data determined as a percentage and counted before the variance analysis were subjected to variance analysis after the necessary transformations were applied.

RESULTS AND DISCUSSION

The EM type, dose and application time factors applied to the seedlings created a significant difference on the individually measured variables chlorophyll a, chlorophyll b, total chlorophyll, photosynthesis rate, relative humidity, and cumulative transpiration while the effects of the double and triple interactions of the factors on the physiological characteristics of the seedlings were found to be statistically insignificant (Table 3).

It was determined that EM 5 variety caused the highest values on chlorophyll a, chlorophyll b, total chlorophyll, cumulative transpiration and relative humidity, while EM-A had the highest effect on photosynthesis rate. While all applied EM doses caused higher values in all physiological variables compared to the Control treatment, medium level EM application between three doses was more effective than low and high dose applications. The highest value was obtained with high-dose EM application only on the relative humidity variable. In terms of the application time of EMs, it was determined that the application during the April-May period, which is the rapid development phase of the seedlings and before the vegetation starts, has an effect on increasing the physiological characteristics of all seedlings measured compared to the application in the June-July period (Table 4).

Various studies have been carried out on the seedling quality and afforestation performance of Taurus cedar, which is the most commonly used coniferous variety in afforestation together with Anatolian black pine (*Pinus nigra* J.F.Arnold subsp. pallasiana (Lamb.) Holmboe) in Turkey. Semerci (2005), in his study on Taurus cedar seedlings, emphasized that the physiological characteristics have a strong effect on the survival potential of the seedlings. Sarı and Deligoz (2019), in their study on the effect of the physiological state of Taurus cedar seedlings on the time of pluck, stated that appropriate seedling pluck should be done not only on the basis of morphological characteristics, but also on physiological parameters. Mercan (2010) indicated that in the studies conducted in Mediterranean countries such as Spain, the success of both seedling holding and seedling growth is higher in arid afforestation areas of seedlings that are well fed in the nursery environment. Ritchie and Shula (1984) stated that morphological parameters and physiological parameters show parallelism.

Pigments such as chlorophyll, anthocyanins and carotenoids in plants perform very important functions. Especially chlorophyll has an important function in photosynthesis, which is necessary for the growth and development of the plant (Schaefer and Wilkinson, 2004; Gould and Lee, 2002; Gouldet al., 2002). Photosystem I (PSI-P700) and Photosystem II (PSII-P680) are photoreceptors that contain chlorophyll *a* and chlorophyll *b* and have an important function in photosynthesis (Poethig, 2013). In his study of the effects of drought on photosynthesis and Photosystem II heat tolerance in *Cedrus libani* and *C. atlantica* (Endl.) Manetti ex Carrière seedlings, Epron (1997) stated that *C. libani* exhibited a significantly

Table 3 The results of variance analysis of the physiological characteristics of the seedlings.

Measurement	Application Time		EM dose		EM Type		App. Time x EM Dose		App. Time x EM Type		EM Dose x EM Type		App. Time x EM Dose x EM Type	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Chlorophyll *a*	1099.6	0.00**	261.98	0.00**	673.76	0.00**	0.875	0.42	4.032	0.09	3.154	0.07	0.166	0.988
Chlorophyll *b*	419.2	0.00**	89.98	0.00**	202.49	0.00**	0.502	0.607	1.43	0.236	2.476	0.28	0.021	0.96
Total Chlorophyll	196.6	0.00**	476.9	0.00**	476.9	0.00**	0.855	0.423	3.05	0.32	2.94	0.11	0.03	1.00
Photosynthesis rate	353.8	0.00**	206.6	0.00**	2526.5	0.00**	0.139	0.870	0.77	0.513	7.20	0.06	0.14	0.95
Relative humidity	165.2	0.00**	110.7	0.00**	47.19	0.00**	0.19	0.828	0.083	0.97	1.08	0.38	0.22	0.94
Cumulative trn.	1049.8	0.00**	664.9	0.00**	554.9	0.00**	66.16	0.06	120.5	0.07	2.08	0.177	4.58	0.67

higher heat tolerance than *C. atlantica*. As pointed out by Havaux (1993), even a small difference in heat tolerance has important consequences in the plant's struggle for existence. Epron (1997) stated in his study that the combination of high light and high temperature stresses greatly changed the PSII photochemistry in cedar needles. This is common because high temperatures increase photoinhibition of photosynthesis in many varieties (Ludlow, 1987; Al-Khatib and Paulsen, 1989; Gamon and Pearcy, 1990). In his study on the effects of drought preconditioning on the heat resistance of Photosystem II and the sensitivity of photosynthesis to heat stress in cedar seedlings (*Cedrus brevifolia* (Roxb.) G. Don, *C. libani*, *C. atlantica*), Ladjal et al. (2000) indicated that differences in heat tolerance of PSII reflect differences in the sensitivity of photosynthesis to heat stress. Due to the net CO_2 assimilation rate is inhibited more by heat stress in *C. atlantica* than in *C. libani*, well-watered seedlings are more susceptible than drought-preconditioned seedlings. PSII activity is one of the most heat sensitive functions in plants and loss of PSII activity occurs in a very narrow temperature range. This behavior is likely to contribute to Mediterranean cedars' ability to tolerate the adverse hot and dry seasons that characterize their natural habitats during the summer months. In addition, Ozel (2016) stated that air pollution negatively affects the total chlorophyll content of cedar seedlings in his study on the effects of pollutant particles from Bartın Cement Factory on the development of Taurus cedar seeds and leaves in the planting area around the factory. This shows that not only temperature and drought but also polluting factors affect the physiological development of cedar seeds and seedlings. In the findings obtained in the study, it was determined that EM applications had a positive effect on chlorophyll a and chlorophyll b, which have important functions in photosynthesis. Especially, EM-5 variety gave statistically significant results in the amount of chlorophyll a and chlorophyll b compared to the Control treatment and other EM varieties. It was determined that medium (20%) dose was sufficient as an application dose. The positive effect of EM application on chlorophyll a and chlorophyll b will have a positive effect on PSII, which is important in drought, and may increase the field performance of seedlings for Taurus cedar.

Turkey is in a geography dominated by the Mediterranean climate, where climate change is the most risky, and as a result, summer drought emerges as an

Table 4 Duncan test results for physiological measurements according to EM type, dose and application time.

EM Type	Chlorophyll a (mcg/ml)	Chlorophyll b (mcg/ml)	Total Chlorophyll (mcg/ml)	Rate of Photosynthesis	Relative Humidity (RHI %)	Cumulative Transpiration (H2O/100 g)
	Mean and Std. Error (X+Sx)					
Control	1.94±0.03 e	1,80±0,04 e	3,74±0,07 e	670,50±3,45 e	38,06±0,8 e	83,68±0,9 e
EM-1	2.66±0.04 b	2,45±0,04 b	5,11±0,09 b	777,23±3,9 b	46,65±0,6 b	106,05±1,6 b
EM-5	2.90±0.04 a	2,67±0,04 a	5,57±0,09 a	722,07±3,84 c	47,96±0,6 a	115,34±1,8 a
EM-A	2.56±0.04 c	2,36±0,04 c	4,93±0,08 c	838,83±3,02 a	43,80±0,6 d	105,04±1 c
EM-GOLD	2.27±0.04 d	2,13±0,04 d	4,39±0,07 d	709,73±2,5 d	44,22±0,54 c	99,04±15 d
EM Dose	**Chlorophyll a (mcg/ml)**	**Chlorophyll b (mcg/ml)**	**Total Chlorophyll (mcg/ml)**	**Rate of Photosynthesis**	**Relative Humidity (RHI %)**	**Cumulative Transpiration (H_2O/100 g)**
Control	1.94±0.03 d	1.80±0.04 d	3.73±0.08 d	670.50±3.5 d	38.06±0.8 d	83.68±1.2 d
Low	2.60±0.04 b	2.41±0.04 b	5.01±0.09 b	756.80±8.29 b	43.11±0.5 c	105.52±1.6 b
Medium	2.74±0.04 a	2.52±0.04 a	5.26±0.09 a	778.47±9.1 a	45.49±0.5 b	113.13±1.2 a
High	2.46±0.05 c	2.26±0.04 c	4.72±0.09 c	750.62±7.9 c	48.37±0.44 a	100.44±0.9 c
Application Time	**Chlorophyll a (mcg/ml)**	**Chlorophyll b (mcg/ml)**	**Total Chlorophyll (mcg/ml)**	**Rate of Photosynthesis**	**Relative Humidity (RHI %)**	**Cumulative Transpiration (H_2O/100 g)**
April- May	2.73±0.04 a	2.52±0.04 a	5.26±0.07 a	766.58±7.19 a	46.98±0.46 a	109.69±1.47 a
June- July	2.36±0.03 b	2.18±0.03 b	4.55±0.06 b	743.27±6.9 b	43.16±0.44 b	99.55±1.06 b

important abiotic factor affecting forest trees (Dirik, 1994). The rate of photosynthesis is determined by measuring the amount of CO_2 used or the amount of O_2 released during photosynthesis. The rate of photosynthesis decreases as a result of water deficiency in plants. Linares et al. (2011) stated that drought stress caused by heat in Morocco has a limiting effect on the development of older individuals of *Cedrus atlantica*, and that the decrease in precipitation has a great effect on the radial growth of young trees rather than old trees. Photosynthesis, one of the most important physiological processes, meets 90% of the plant dry matter. In addition, the rate of photosynthesis and transpiration reflects and quantifies the effects of environmental stress (Ewers et al., 2008). Tree transpiration rate is largely controlled by biological factors such as tree size and leaf area index and by environmental factors such as radiation, vapor pressure deficit (VPD), wind speed and soil moisture availability (Ewers et al., 2008; Hernández-Santana et al., 2008; Komatsu et al., 2006; Tang et al., 2006; Tognetti et al., 2009). These biophysical factors affect transpiration on a temporal and spatial scale. Plant water use and transpiration rates may increase with precipitation (Mitchell et al., 2009) and may decrease during periods of low soil water availability (Gazal et al., 2006; Luis et al., 2005). Studies show that stand transpiration increases in years with high rainfall (Macfarlane et al., 2010; Zeppel et al., 2008). Low soil water availability can increase the hydraulic resistance between the soil and the root system, prevent the movement of water from the soil to the plant leaves, and reduce transpiration rate by triggering stomatal closure (Meinzer et al., 1993; Sala and Tenhunen, 1996; Tognetti et al., 2009). Under these conditions, plants adopt protective water use strategies (Tognetti et al., 2009) and take advantage of water reserves in deeper soil layers (David et al., 2004; Hernández-Santana et al., 2008; Thomas et al., 2006). Deligöz et al. (2016) tried to reveal the responses of Cedrus libani seedlings to recurrent drought cycles by examining the morpho-physiological parameters of the seedlings. In their study, they found that drought stress caused a significant decrease in growth parameters such as height, dry weight and root collar diameter, but severe drought stress increased the root/shoot ratio. *C. libani* seedlings showed less growth, higher root/shoot ratio and proline accumulation as an adaptation mechanism to repeated drought stress. Under moderate water stress, carbohydrates obtained by photosynthesis are accumulated by plants and can be divided into differentiation processes (e.g., secondary metabolism) rather than growth (Ayres, 1993). The positive effects of EM applications on chlorophyll *a*, chlorophyll *b* and photosynthesis rate will have a positive effect on primary and secondary metabolism and photosynthesis. Thus plant secondary metabolites are involved in the biological processes of plants, which generally do not have primary functions in the maintenance of life processes in plants but deal with environmental stress in terms of adaptation and defence (Lavola and Julkunen-Tiitto, 1994; David et al., 2004; Ladjal et al., 2007; Ramakrishna and Ravishankar, 2011).

There is a strong and positive relationship between the resistance of plants to biotic and abiotic stress factors and the biochemical and physiological plant processes that form the basis of plant metabolism such as photosynthesis, respiration and transpiration (Atik and Aslan, 2015). Ladjal et al. (2007) conducted a study on the effects of soil and air drought on growth, plant water status and leaf gas exchange in three Mediterranean cedar species, *Cedrus atlantica, C. brevifolia* and *C. libani*, and they subjected the three- and four-year-old seedlings to two different irrigation regimes. Evaluating the data obtained, they found a close functional link between height growth in cedars and water availability during the growing season. Drought, which is an important problem for the Mediterranean climate, affects the plant-water balance and has negative consequences in many physiological processes such as closure of stomata, inhibition of gas exchange with the plant's environment, reduction of transpiration and inhibition of photosynthesis. It also negatively affects photosynthesis, which is of vital importance for plant growth and development. This leads to premature aging and growth inhibition in plants (Siddique et al., 2016). The rate of photosynthesis decreases, and metabolic deterioration occurs severely with the closure of stomata (Walawwe, 2014). Bayar and Deligöz (2019) investigated the changes in water relations and biochemical properties of the 22-year-old Taurus cedar and Anatolian black pine in the afforestation area during the summer drought period. In the data obtained, it was determined that the osmotic potential at the wilting point and fully saturated state of *Cedrus libani* decreased towards September. They found that Taurus cedar has a lower osmotic potential at the wilting point than Anatolian black pine in September. In the study of Semerci (2001) the osmotic potential values in full turgor state of Taurus cedar were at the level of -1.80 MPa, -2.32 MPa and -2.39 MPa in June, July and August, respectively; the water potential values at the wilting point of the same periods were -2.90 MPa, -3.30 MPa and -3.50 MPa, respectively. Bayar and Deligöz (2019) determined the proportional water content at the wilting point of Taurus cedar as 85% and 87% in July and August, respectively. Dirik (2000) states that, depending on the type of tree studied, the osmotic potential at the wilting point decreases during dry periods, and while the resistance to drought increases. The results obtained from the study of Bayar and Deligöz (2019) show that *C. libani* is a more drought tolerant variety than *Pinus nigra* due to its lower osmotic potential at the wilting point. The lack of water in the plant due to drought stresses the plant and also reduces the rate of photosynthesis. In order to mitigate this phenomenon, plants synthesize certain compounds from the soil and minimize water loss. These structures, which consist of different groups such as amino acids, organic acids and carbohydrates, provide the continuity of photosynthesis by increasing the conductivity of the stomata as they balance the leaf water pressure. Thus, it also contributes to plant growth (Örs, 2015). EM-5 variety applications will also positively affect the growth of seedlings by increasing transpiration and relative humidity, positively affecting transpiration and water intake, and positively affecting photosynthesis, that is, CO_2 fixation.

Among all EM varieties, especially EM-A caused an increase in the rate of photosynthesis and positively affected the growth of seedlings. The time of EM application, especially before and at the beginning of the vegetation period, increases the success of EM application. This is thought to be related to the fact that EM application positively affects photosynthetic pigments and increases the rate of photosynthesis, coinciding with the period when the plant awakens, and physiological processes accelerate.

It is thought that amino acids produced by photosynthesis bacteria in the structure of EMs applied to Taurus cedar seedlings ensure the continuity of photosynthesis and promote plant growth by maintaining the plant turgor balance. EM consists of mixed cultures of beneficial, naturally occurring microorganisms such as photosynthetic bacteria *(Rhodopseudomonas palustris, Rhodobacter sphaeroides),* lactobacilli (*Lactobacillus plantarum, L. casei* and *Streptococcus lactis*), yeasts (*Saccharomyces sppces*) and actinomycetes. Condor et al. (2007) defined these microorganisms as follows: Phototrophic bacteria are self-supporting microorganisms. They synthesize amino acids, nucleic acids, bioactive substances and sugars using root secretions in the soil, organic matter, sunlight and geothermal heat as energy sources. Unlike plants, they use energy from the infrared band of solar radiation (700-1,200 nm) to produce organic matter, thereby increasing the efficiency of plant growth. Metabolites produced in this way can be directly absorbed by plants or serve as substrates for other bacteria, thereby increasing the biodiversity of soil microflora. Photosynthetic bacteria, which are the main components of EM, are known to work synergistically with other microorganisms to support the nutritional requirements of plants and reduce the incidence of pathogenic microorganisms (Condor et al., 2007). Subadiyasa (1997) states that EMs can interact with the soil-plant ecosystem by suppressing plant pathogens and other disease agents, dissolving minerals, conserving energy, maintaining the microbial and ecological balance of the soil, increasing photosynthetic productivity and fixing biological nitrogen.

CONCLUSIONS

Effective microorganisms play a critical role in improving physiological characteristics that significantly affect seedling quality. Furthermore, they can support the development of plants in conditions of increased stress due to global climate change in recent years. In this study, it was determined that EMs had a positive effect on physiological variables such as chlorophyll *a*, chlorophyll *b*, total chlorophyll, photosynthesis rate, relative humidity and transpiration rate. In addition, this study sheds light on the use of different kinds of EMs in different forest tree seedlings.

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