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Effect of different concentrations of effective microorganisms (Baikal EM1) on the root collar diameter and height growth in the seedlings of Anatolian black pine [*Pinus nigra* Arnold. subsp. *pallasiana* Lamb. (Holmboe)]

Atilla Atik
Department of Landscape Architecture, Inonu University, 44280, Malatya, Turkey.
e-mail: atikatilla@hotmail.com

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Abstract

In the present study, effect of Baikal EM1, a product of EM technology, on the root collar diameter and height growth of 1+0 aged seedlings of Anatolian black pine [*Pinus nigra* Arnold. subsp. *pallasiana* Lamb. (Holmboe)] growing in Kozcağız Temporary Nursery was investigated. Seedlings were treated with three different concentrations of Baikal EM1 solution from seed dibbling. Related morphologic parameters were measured in four treatment types at the end of vegetation season. Statistical comparison of morphologic data for treatments was performed using ANOVA test while the treatments were categorised using Duncan test. Compared to control, mean root collar diameter growth was found to be 20% higher in seedlings in T1 treatment type (Baikal EM1 concentration of 1:1000) and nearly 31% more in T2 and T3 treatment type while height growth was 22% more in T1 treatment and 34% in T2 and T3 treatment. Difference between treatment groups for root collar diameter and height growth was found to be statistically significant in from the results of ANOVA test ($p<0.05$). According to the results of Duncan test, T2 and T3 treatment categories where Baikal EM1 was used in the concentration rates of 1:500 and 1:100 respectively, took place in the same group considering both morphological values. It was suggested form the result of the study that Baikal EM1 should be used in the concentration of 1:500 for the 1+0 aged seedlings of Anatolian black pine originating from Kumluca and growing under the conditions of Kozcağız Temporary Nursery. Findings of the study were in convenience with literature and Baikal EM1, a product of EM technology, was observed to contribute positively to the root collar diameter growth of the seedlings of Anatolian black pine. It is thought that Baikal EM1 can be used to obtain quality black pine seedlings in nurseries by developing root collar and plant height. It was also suggested that effect of Baikal EM1 on the growth of seedlings in different growing mediums of nurseries should be investigated by taking different species including forest and fruit trees at various ages.

Key words: Effective microorganisms, seedling height, root collar diameter, ANOVA, nursery, Baikal EM1.

Introduction

It was determined from recent inventory studies that 21.5 million ha of forest land covers 28% of Turkey's surface area, 11.2 million ha (52%) of which is productive forest and the remaining 10.3 million ha (48%) including degraded coppice is low or non-productive no yield at all or low yield. Conifer forests cover nearly 61% (13.2 million ha) of Turkey's total forest area [1].

Anatolian black pine [*Pinus nigra* Arnold. subsp. *pallasiana* Lamb. (Holmboe)] is one of the commonest and economically important native conifers in Turkey [2] and has the second largest expansion area (4.2 million ha) after red pine (*Pinus brutia* Ten.) among coniferous species [3]. Black pine may grow naturally in seven geographical regions of Turkey except Eastern Anatolia [4]. Anatolian black pine constitutes pure or mixed forests in the mountainous parts of Turkey's coastal regions, even goes into steppe. It constitutes forest stands which are pure or mixed with oriental beech (*Fagus orientalis* Lipsky.), oak (*Quercus* sp.), red pine (*Pinus brutia* Ten.), Lebanon cedar (*Cedrus libani* A. Rich.), Scots pine (*Pinus sylvestris* L.) and juniper (*Juniperus* sp.) species on the inward slopes of North Anatolian mountains, on the northern slopes of the Western and southern Anatolia. It surrounds the coastal areas of Middle and Western Black Sea, Marmara, Aegean and Mediterranean Regions and goes into Central Anatolian steppe. Its naturally vertical distribution is between 700 and 2100 m [2, 5-9].

Black pine is the only species to be planted in each of seven geographic regions of Turkey [4]. In order to meet the need for seedlings, 112 million black pine seedlings were grown in 2012 in the nurseries. Black pine is in the first row for seedling propagation in the rate of 22% in Turkey [1].

Generally, afforestation value of seedlings can be measured by considering the quality of their seedlings [10]. However, one of the most important problems of nurseries in Turkey is the low fertility of their soils. Poor management for a long time and deficiency of organic and artificial fertilizer caused reduced productivity of nurseries by decreasing the quality of produced seedlings [11].

As in other agricultural activities, use of chemical fertilisers in nurseries will deter natural reserves both directly and indirectly, therefore, there is a need for biological and natural materials in order to increase the fertility of nurseries and produce quality seedlings.

An important component of organic agriculture is effective microorganisms (EM) technology and it is recognized as such in

many countries. In 1988 in Japan (Higa Teruo) and 1997 in Russia (Shablin), the preparation of effective microorganisms was achieved using EM86 technology [12, 13]. In Russia, this preparation was given the name Baikal EM1. Effective microorganisms make amino acids useful to plants, and organic acids, polysaccharides and vitamins strengthen their immune systems. Baikal EM1 consists of a water solution which contains compounds that promote nitrogen fixation and photosynthesis, along with lactic bacteria, yeast and other components that these microorganisms need to live [13].

Several studies have so far been conducted related to the positive effects of effective microorganisms on product yield in agricultural field. Extraordinary effects of these microorganisms have been revealed by Chagas *et al.* [14] in Brazil on papaya, turf grass in Netherlands and Austria [15, 16], apple in Japan [17]. These effects may be attributable to several related factors among which are the redundancy of nutrient elements freed by the separation of organic materials when treated with EM solution [18] and high frequency of photosynthetic [19] and protein activities [20]. It is also stated that EM use can help increase the plant resistance to water stress [21] and carbon mineralisation [15], improve soil properties [22] and aerate plant roots better [23].

In the present study, effect of Baikal EM1 on seedling height and root collar diameter, which are significant morphological parameters in determining seedling quality, in 1+0 aged black pine seedlings growing in Bartın-Kozcağız Temporary Nursery was investigated.

Materials and Methods

Study area: Anatolian black pine [*Pinus nigra* Arnold. subsp. *pallasiana* Lamb. (Holmboe)] seedlings growing in the temporary nursery in Kozcağız and originating Kumluca. Kozcağız Temporary Forest Nursery is on Kozcağız town centre (41° 28' 33" N and 32° 20' 41"E) in Western Black Sea Region of Turkey. The nursery is 20 km far from the Bartın city centre. The elevation of the region is 75 m and its general aspect is in West course.

According to the data obtained from Bartın Meteorology Station which is at 33 m altitude, mean annual, minimum and maximum temperatures are 12.6°C, 0.3°C and 18.8°C, respectively annual rainfall is 1035 mm [24]. Length of vegetation period in the area is totally seven months from April (11.1°C) to October (13.6°C). Total rainfall in vegetation period is 527 mm (Table 1). According to Thornthwaite method, the area has a climate that is indicated by B_2B_1'rb4' symbols which means a location showing close features to oceanic climate that is meso thermal and has little or no water shortage (Fig. 1).

The land on which the nursery was established is plain and its soil consists of a medium heavy texture such as sandy clay with pale brown to dark colour. Total sand, clay and dust contents of the nursery soil are 70.6%, 12.5% and 16.9%, respectively. The reaction of soil (pH) is 8.0 and it is semi-alkali. Lime rate in the soil is 4.18, not rich in lime. Its organic substance rate is 5.05% (Table 2).

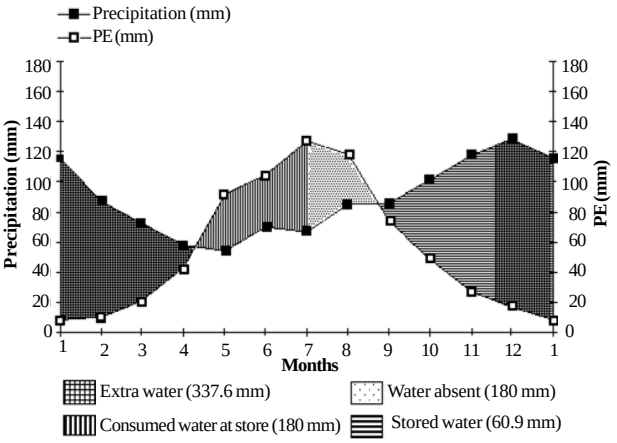

Figure 1. Water balance graphic of study area.

Seed sowing and preparation process: Facial sterilisation was applied to seeds before sowing by soaking them in a solution containing 40% sodium hypochlorite for 30 min and rinsing in distilled water to remove remaining sodium hypochlorite.

Seeds were then incubated in three different aqueous solutions of Baikal EM1 for 18 h in the dark at 22°C, which are 5 ml in 5 l of water, 1:1000; 10 ml in 5 l of water, 1:500; 50 ml in 5 l of water, 1:100) in. Seeds in control group were incubated in distilled water for the same period of time and planted in seedbed.

Seeds were sown at a 6 cm distance and in the depth of 1 cm on parallel lines 15 cm far from each other in seedbed in the middle of March. Seeds were then covered with a layer of material composed of sawdust and silt and pressed using a cylinder.

Study was designed according to randomized block experiment design with three repetitions for each of four treatments thus constituting 12 blocks (seed beds). The number of seeds to sow was determined considering the desired number of seedlings to be obtained. It was planned to measure the changes in root collar diameter and height of 1+0 aged seedlings over 100 seedlings in order to determine the size of effect caused by treatments on the development of two parameters at the end of vegetation season. From this point of view, the number of seeds to sow increased in the rate of 50% by considering the seed loses (including those not capable of germination or dying after germination) and 50 seeds were added for each repetition while determining the number of seeds to be 150 for each treatments and sowing 600 seeds in 12 blocks.

After the completion of germination in all seed beds, Baikal EM1 solution prepared in the concentrations given above was applied to the seedlings in the middle of months from April to October. During the same period control blocks were irrigated with distilled water equal to the volume of solution.

Data collection and analyses: Height and root collar diameter of randomly selected from each treatment type 100 black pine seedlings at the age of 1+0 were measured at the end of the vegetation season in nursery.

Table 1. Mean climatologic values from Bartın Meteorology Station.

Climatic parameters	Months												Annual Mean
	1	2	3	4	5	6	7	8	9	10	11	12	
Mean Temp. (°C)	4.1	4.7	7.0	11.1	15.5	19.7	22.0	21.6	17.6	13.6	8.9	5.7	12.6
Mean Max. Temp (°C)	9.1	10.2	13.	17.7	22.0	25.9	28.1	28.1	24.8	20.3	15.4	10.9	18.8
Mean Min. Temp (°C)	0.3	0.5	2.4	6.0	9.7	13.3	15.6	15.5	12.0	8.8	4.3	1.8	0.3
Rainfall (mm)	105.0	82.5	72.6	57.9	52.1	73.4	62.9	77.5	88.4	114.8	116.8	131.1	1035

Table 2. Soil structure of nursery.

Sand (%)	Clay (%)	Dust (%)	Soil type	pH	Total $CaCO_3$ (%)	Organic substance (%)
70.6	12.5	16.9	Sandy clay	8.0	4.18	5.05

The measurement of seedling height from root collar level to the connection point of terminal bud with the bole at 1 mm sensitivity, and root collar diameters were measured at 0.01 mm sensitivity.

Descriptive statistics were applied for the values of obtained height and root collar diameter data and Kolmogorov-Smirnov test were used to determine whether a set of observations is from a homogenous distribution. Then analysis of variance (ANOVA) test was applied for the evaluation of height and root collar diameter data of black pine seedlings at various different aqueous solution of Baikal EM1.

In the case of significant difference as the result of variance analysis, related categorisation was performed considering Duncan test. Data were transferred to computer and analysed statistically using MS Office and SPSS 18.0 software package.

Results

According to treatment type the results of one sample Kolmogorov-Smirnov test and measurement root collar diameter and height growth of black pine at 1+0 age seedlings were given in Table 3.

Root collar diameter and height growth of seedlings in all treatment types constituted using three different Baikal EM1 concentrations are higher than controls. When treatment types are compared with each other, the highest height and root collar diameter growth was seen in T3 followed by T2 and T1 (Table 3, Figs 2 and 3).

Mean differences of root collar diameter and seedling height in T3 compared to control are 1.06 mm and 3.0 cm, respectively.

According to the results of one sample Kolmogorov-Smirnov test, values of root collar and seedling height are convenient to normal distribution (Table 3).

Values of root collar diameter and seedling height were subjected to logarithmic transformation in order to achieve variance homogeneity between treatment groups ($\log_{10}$). Results of one direction variance analysis (ANOVA), related to the effect of the treatments on diameter and height growth of seedlings, are presented in Table 4. It can be seen from Table 4 that the difference in root collar and height between treatment types is statistically significant ($p<0.05$). Results of Duncan test showing the distribution of differences are given in Tables 5 and 6.

Black pine seedlings were classified into three groups according to the results of Duncan test considering treatment types for root collar diameter and height growth. Seedlings in control for both parameters constituted Group 1, those in T1 treatment type were Group 2 while seedlings in T2 and T3 treatment types were Group 3 (Tables 5 and 6).

Discussion

Mean number of black pine seedlings produced yearly in Turkey is nearly 110 million [1]. Success in afforestation depends greatly on several factors on which practitioners can have limited or no effects such as climatic, edaphic and physiological. However, seedling quality is a factor which can be affected by authorities of the subject. High quality seedlings have to be used in plantation works which are both expensive and time consuming [10, 25].

Parameters considered in the determination of seedling quality are some morphological features such as root collar diameter, height, robust index, stratum, seedling dry weight, root percentage and physiological features such as plant water capacity, root regeneration potential. However, morphological parameters are used more in the studies related to seedling quality categorisation both in Turkey and the world since it is practical and has high applicability [10, 25-28]. Standards of TSI (Turkish Standards Institution) based on morphological parameters are used for the classification of seedlings produced in forest nurseries in Turkey.

It was determined from the findings of the present study that Baikal EM1 has positive effect on the development of root collar diameter and height of 1+0 aged black pine seedlings growing in Kozcağız Temporary Forest Nursery.

Mean root collar diameter and height of seedlings increased in the rates of 20% and 22%, respectively, in T1 treatment type where Baikal EM1 was applied in the concentration of 1:1000 while 31% and 34% in T2 and T3 treatments where concentrations were 1:500 and 1:100 respectively when compared to control. Mentioned seedling parameters increased when the concentration of Baikal EM1 increased from 1:1000 to 1:500 while no significant increase was seen when the concentration increased from 1:500 to 1:100. This condition is supported by the results of Duncan test which show that T2 and T3 treatment types are in the same group.

Effective microorganisms are found to play roles in the increase of seed germination rate, development of root system, stimulation of photosynthesis, protein and chlorophyll formation and acceleration of plant growth and development [20].

It was reported in another study, using Baikal EM1 (concentration of 1:100) and 1+0 aged oriental spruce seedlings that mean height

Table 3. The results of one sample Kolmogorov-Smirnov test and measurement of root collar diameter and height growth of seedlings.

Compared Feature	Treatment type [a]	N	μ	X_{min}	X_{max}	σ	One-sample Kolmogorov-Smirnov test				
							Most extreme differences				
							Absolute	Positive	Negative	Z	Asymp. Sig.
Root collar diameter (mm)	Control	100	3.35	2.99	3.90	0.20	0.078	0.078	-0.041	0.777	0.582 [b]
	T1	100	4.02	3.24	4.82	0.29	0.083	0.051	-.0.083	0.825	0.504 [b]
	T2	100	4.40	3.34	5.24	0.37	0.073	0.073	-0.071	0.729	0.663 [b]
	T3	100	4.41	3.40	5.18	0.34	0.070	0.062	-.0.070	0.696	0.718 [b]
Height (cm)	Control	100	8.5	7.6	9.4	0.47	0.105	0.082	-.0.105	1.050	0.220 [b]
	T1	100	10.4	8.4	12.0	0.72	0.098	0.075	-.0.098	0.982	0.290 [b]
	T2	100	11.4	8.9	13.1	0.89	0.111	0.074	-0.111	1.113	0.167 [b]
	T3	100	11.5	9.0	13.2	0.84	0.070	0.051	-.0.070	0.696	0.718 [b]

[a]: Control-Treatment with distilled water; T1-5 ml in 5 l of water, that is, 1:1000; T2-10 ml in 5 l of water, that is, 1:500; T3-50 ml in 5 l of water, that is, 1:100.
[b]: Asymp. Sig.>0.05, test distribution is normal.

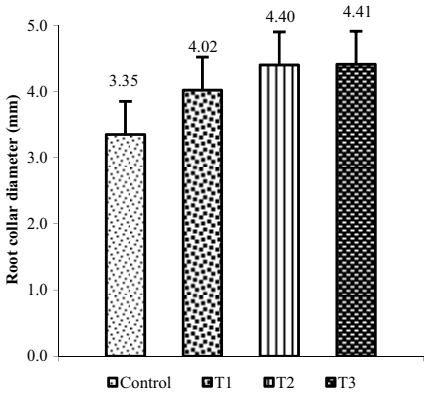

Figure 2. Effect of different concentrations of Baikal EM1 on seedling root collar growth.

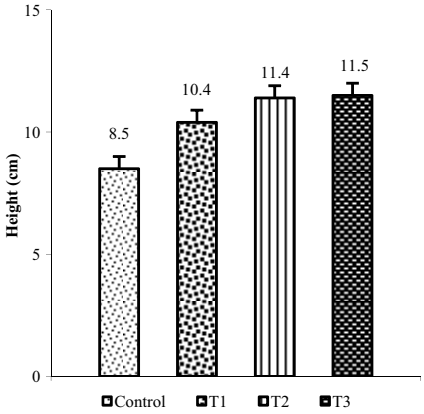

Figure 3. Effect of different concentrations of Baikal EM1 on seedling height growth.

Table 4. The results of ANOVA for root collar diameter and height.

Compared Feature		Sum of Squares	df	Mean Square	F	P
Root collar diameter (mm)	Between Groups	0.946	3	0.315	336.052	0.000 [a]
	Within Groups	0.372	396	0.001		
	Total	1.318	399			
Height (cm)	Between Groups	0.929	3	0.310	293.567	0.000 [a]
	Within Groups	0.418	396	0.001		
	Total	1.346	399			

[a]: P<0.05, 5% significant to confidence level.

Table 5. Result of Duncan test for root collar diameter.

Treatment	N	Subset for alpha=0.05		
		1	2	3
Control	100	3.35		
T1	100		4.02	
T2	100			4.40
T3	100			4.41

Table 6. Result of Duncan test for height.

Treatment	N	Subset for alpha=0.05		
		1	2	3
Control	100	8.5		
T1	100		10.4	
T2	100			11.4
T3	100			11.5

growth and root collar diameter increased in the rates of 29% and 24%, respectively. It was observed when the values of nitrogen, protein and nucleic acid were evaluated in the same seedlings in vegetation season that mean rates of nitrogen and protein, RNA and DNA in Baikal EM1 treated seedlings were 59%, 45% and 36% higher compared to control [29, 30].

It was stated by Bruggenwert that EM increased in C, N and P component of soil in rangelands in the rates of 20%, 30% and 60%, respectively [31].

It was reported that EM increased the rate of organic matter from 12.9% to 32.0% in rangelands areas and pH rate from 5.4 to 5.7 [32].

Findings of the present study was found to be in convenience with those of literature and Baikal EM1, a product of EM technology, was determined to increase the root collar diameter and height growth of Anatolian black pine at a statistically significant level ($p<0.05$).

Conclusions

Black pine is the second most prevalent forest tree species among conifers in Turkey after red pine. In addition, this species is one of the most important tree species in the country since it is planted extensively in each of seven geographical regions of Turkey for the economic value of its wood.

In the present study, effect of Baikal EM1 in three different concentrations on root collar diameter and height growth of 1+0 aged seedlings of black pine. It was determined that Baikal EM1 contributed positively to seedling root collar diameter and height, which are significant parameters for seedling quality categorisation. Increase in these parameters was seen in the same rate in T2 and T3 treatments using the concentrations of 1:500 and 1:100, respectively. From the results of the study it was found that optimum benefit may be obtained by using 1:500 concentration of Baikal EM1 under the conditions of Kozcağız Temporary Forest Nursery.

From the study it was also determined that the use of Baikal EM1 in black pine seedling could contribute positively to the production of quality seedlings by causing larger root collar diameters and height.

It was suggested that effect of Baikal EM1 on the development of seedlings from different species and at various ages in nurseries with different edaphic, climatic, physiographic and biologic conditions using different production techniques for different aims.

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