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The Impact of Effective Microorganisms (Baikal EM1) on Some Physiologic Parameters of Young Chestnut Trees (*Castanea Sativa* Mill.)

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Abstract

In this study, the effects of the mixture composed of effective microorganisms named Baikal EM1 on the physiologic parameters of young chestnut trees (seedlings) were studied. Baikal EM1 improves the soil microflora and increases the yield, quality and safety of agricultural products. In addition, Baikal EM1 enables nutrient uptake by plants and enriches the soil in terms of vitamins, amino acids and biologically active ingredients. Within the scope of the study, the physiological characteristics of chestnut seeds and control samples, which had been treated with Baikal EM1, were observed for one year. The survival rate and the average height of the living young trees, which had been treated with Baikal EM1, were 8.5% and 34.7% higher than the control samples, respectively. Chlorophyll a (Cl a), chlorophyll b (Cl b), carotenoid, nitrogen and protein ratios determined on the leaves showed an increase compared to the control samples. An increase by 62.3% and 40%, respectively were determined in DNA and RNA ratios.

Key words: Baikal EM1, chestnut tree, chlorophyll, effective microorganisms, plant physiology, survival rate.

Efektif Mikroorganizmaların (Baykal EM1) Genç Kestane Ağaçlarının (*Castanea sativa* Mill.) Bazı Fizyolojik Parametrelerine Etkisi

Öz

Bu çalışmada Baikal EM1 isimli efektif mikro organizmaların oluşturduğu karışımın genç kestane ağaçlarının (fidanlarının) fizyolojilerine olan etkileri araştırılmıştır. Baykal EM1 toprağın mikroflorasını iyileştirmekte, tarımsal ürünlerin verimliliğini, kalitesini ve güvenliğini arttırmaktadır. Ayrıca Baikal EM1 bitkilerin besin alımını kolaylaştırır ve toprağı vitamin, amino asit ve biyolojik olarak aktif maddelerle zenginleştirmektedir. Çalışma kapsamında Baikal EM1 ile muamele edilen kestane tohumları ve kontrol örneklerinin fizyolojik özellikleri bir yıl boyunca gözlemlenmiştir. Baikal EM1 ile muamele edilen tohumların hayatta kalma oranı (survival rate) ve ortalama yüksekliği kontrol örneklerinden sırasıyla %15 ve %34.7 daha yüksektir. Yapraklar üzerinde belirlenen klorofil a (Cl a), klorofil b (Cl b), karotenoid, nitrojen ve protein oranları kontrol örneklerine göre artış göstermiştir. DNA ve RNA oranlarında sırasıyla %62.3 ve %40 artış belirlenmiştir.

Anahtar Kelimeler: Baikal EM1, kestane ağacı, klorofil, efektif mikroorganizmalar, bitki fizyolojisi, hayatta kalma oranı.

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1. Introduction

Chestnut tree, which is mostly known for its fruit, has an extensive area of usage due to its anatomical, physical and mechanical properties. Chestnut is a healthy, delicious and nutritious fruit. Chestnut, which involves minerals such as starch, fibre, proteins, potassium, magnesium, iron, phosphor, calcium and B1, B2 and C vitamins, is a substantial food. Chestnut tree is highly important because it prevents erosion and loss of water. House, furniture and dowry chest are made using the dark coloured and hard lumber of the chestnut, whilst baskets and large baskets are made from the suckers. As it resists against rotting, it is used in railway tracking, ship building and as electric pole. Its leaves and flowers are used in drugs and cosmetics industry. Anatolian chestnut starts from the Caucasus through the north and northwest of Anatolia and Europe. Besides, the whole of Spain, Albania, east and south Balkans, the whole of Italy and the Alps are within the propagation area of chestnut (Saatcioglu, 1969). In Turkey, in areas, where chemical fertilizers are not used appropriately, used excessively and in unbalanced way, environmental problems such as the pollution of soil and water resources are encountered (Yilmaz, 2005). In addition to the precautions taken within this scope, the use of natural resources and environmentally friendly utilizations will be expanded in drug and irrigation inputs and production of organic products will be encouraged. In line with this, the utilization of organic resources, which are to replace chemical fertilizers in order to prevent environmental pollution, should also be expanded in our country as it does in developed countries (DPT, 2000). EM-technology (EM- effective microorganisms) discovered by Japanese scientist Higa T. is known for big successes (Higa, 1994 a, 1994b). Microbiological preparation (fertilizer) called Baikal EM1 lies at the basis of EM-technology in Russia. The Baikal EM1 is distinguished due to the fact that it is ecologically pure, easy to use and highly effective (Shablin, 2004).

Today biotechnology solves many problems in the field of agriculture and forestry. And one of the solutions is the EM - technology (Effective Microorganisms Technology) which has been widely used in organic agriculture recently. Microbiologic fertilizer called Baikal EM1 lies behind the EM - technology of Russia. Baikal EM1 production in Russia began in 1998. It is a liquid with the pleasant silage smell, packaged in plastic bottles in capacity of 0.5 and 1.0 litre with 1 year of storage period. Baikal EM1 improves the microflora of soil, increases productivity of agricultural crops and raises their quality and safety. Baikal EM1 is an aqueous solution containing photosynthetic bacteria (Rhodopseudomonas palustris, Rhodobacter sphaeroides, Rodobacter capsularus), lactic acid bacteria (Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus salivarius, Lactobacillus delbrueckii), yeast and products of their activities. Baikal EM 1 improves the microflora of soil, increases the yield of agricultural crops and raises their quality and safety. Baikal EM1 activities enables nutrient uptake by plants and enrich the soil in terms of vitamins, amino acids and biologically active substances. Soaking of seeds in Baikal EM1 and spraying of plants during the vegetation period provides resistance against diseases, pests and unfavourable weather conditions (Shablin 2004). Baikal EM1 has no mutagenic, teratogenic, carcinogenic, allergenic or pyrogenic effects. Microorganisms of a fertilizer transform the elements of a plant nutrition in an assimilating manner, enrich the soil and compost it with vitamins, amino acids and biologically active substances. The soil becomes friable and well structured (Blinov 2008). The microbiological substances, Baikal EM1 lays on the basis of the Russian EM-technology. It is a unique complex of various microorganisms existing in the nature and is contained in lactic products, cheese and wine. Groups of lactic acid, nitrogen fixing bacteria, photosynthetic bacteria and yeast are included in its structure. The lactic acid bacteria (these are beneficial organisms widely found in fermented foods and in the gastrointestinal tract of healthy humans and animals) are Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus salivarius and Lactobacillus delbrueckii. The phototrophic purple non-sulphur bacteria, aka PNSB (these are widely found in ponds, soil, on plant leaves, ice, snow and icicles) are Rhodopseudomonas palustris, Rhodobacter sphaeroides and Rhodobacter capsulatus. The yeast is Saccharomyces cerevisiae (these are beneficial organisms widely found in fermented foods and the GI (gastrointestinal) tract of healthy humans and animals). These substances possess a wide range of action, which favourably distinguishes them from other microbiological substances contained, as a rule, one to two species of useful microorganisms.

The purpose of this study is to introduce Baikal EM1, which is composed of microorganisms, and to examine its impacts on some physiologic processes in chestnut trees.

2. Material and Methods

This study was carried out in the Gokcebey Plantation of Zonguldak Regional Directorate of Forestry (41°18′29″N 32°08′30″E) and in the laboratories of Bartin Faculty of Forestry. The growing of the young chestnut trees was made in the plantation, whilst the physiologic analyses were carried out in the laboratories. Chestnut seeds were bathed in Baikal EM1 for 12 hours before they were planted. The ratio of Baikal EM1 was 1:100 (10 ml: 900 ml pure water). The seeds in the control variable were bathed in 1 litre of pure water. On

October 26 (2007), the seeds were planted on the seedbeds prepared. The width of the seedbeds was 120 cm, whilst the height was 20 cm and length was 300 cm. Soil characteristics were identified through physical and chemical soil analyses. Physical analyses; sand - 70.6 %; clay - 12.5 % and dust - 16.9 %. Chemical analyses; pH – 8.0; total CaCO3 - 4.18 % and organic material – 5.05 %.

In the following year (2008), herbal analyses were made on the chestnut leaves on the 15th of July, August and September. The determination of the chlorophyll on the plant leaves was made using photoelectric chlorometer (FEK-M) method. Determination of total nitrogen and protein was made using Kjeldahl method (Bremer 1965). The principle of Kjeldahl method is standardized in all countries. Turkova method was used in determining nucleic acids (DNA, RNA) (Turkova, 1965).

3. Results and Discussion

At the end of the 1 year long vegetation season, the number of young trees in all of the seedbeds in the area was counted and the rate of young tree survivals were determined and presented in Table 1.

Table 1. Rates of young tree survivals.

Variants	Number of planted trees	Number of surviving trees	Rate of Survival
Control	150	130	86
Baikal EM1	150	148	98.9

p = 0.001 significance level.

It was seen that 98.9 % of the young trees processed with Baikal EM1 survived at the end of the vegetation period. On the other hand, it was seen that 86 % of control variables survived. In addition to that, the means, minimum and maximum heights of young trees, standard deviations and standard error values of the 1+0 aged young trees at the end of the vegetation season are given in Table 2. At the same date, the height of the living young trees was measured. The means, minimum and maximum values, standard deviations and standard error values are presented in Table 2.

Table 2. Heights of the young trees.

Variants	Number of trees	The average height (cm)	Standard Deviation	Standard Error	Minimum Height (cm)	Maximum Height (cm)
Control	130	28.5	5.7	0.5	16.7	47.1
Baikal EM1	148	38.4	5.5	0.5	28.8	53.5

p = 0.001 significance level.

When Table 2 is examined, it is seen that average young tree height in control variables is 28.5 cm, minimum young tree height is 16.7 cm and maximum young tree height is 47.1 cm whilst the average height of the young trees treated with Baikal EM1 is 38.4 cm, the minimum height of the young trees treated with Baikal EM1 is 28.8 cm and the maximum height of the young trees treated with Baikal EM1 is 53.5 cm.

The role of pigments in plant processes is indispensable, whereas the synthesis of photosynthetic pigments is genetically controlled, but it also depends on environmental factors. It is known that the pigments presented in thylakoid membranes consist largely of two kinds of green chlorophylls which are chlorophyll a and chlorophyll b. Also present are yellow-to-orange pigments classified as carotenoids. However, photosystem II (PS II) contains chlorophyll a, β carotene (connected to two major proteins), and a small amount of chlorophyll b. These green bands represent light-harvesting complexes of pigments and protein, one of which functions with photosystem I (PS I) and the other one functions mainly with PS II. Their functions are to absorb light energy and transfer it to the proper photosystem where it eventually reaches P700 or P680. As it is known, chlorophyll is an organic material which has macromolecules and which gives the plants and in particular, its leaves the green colour. Chlorophyll absorbs light and shows fluorescence and have catalyser characteristics. In fact, the essential role of chlorophyll in photosynthesis is related to this fluorescence characteristic. There are red-orange materials in the leaves of the plants which are covered with green-coloured chlorophyll. These are named as carotenoids and they work in cooperation with chlorophyll in high plants, algue and as β-carotene in photosynthesis (Libbert, 1974). During the vegetation period (in July, August and September) chlorophyll a (Cl a), chlorophyll b (Cl b) and carotenoid analyses were carried out on chestnut leaves. The results of these analyses are given in the Tables 3 and 4 below.

Table 3. The amount of chlorophylls (a.b) and Cl a+Cl b in chestnut leaves (mg/g dry material).

Variants	July			August			September		
	Cl a	Cl b	Cl a+b	Cl a	Cl b	Cl a+b	Cl a	Cl b	Cl a+b
Control	5.75	2.0	7.75	5.77	2.12	7.89	5.77	2.13	7.90
Baikal EM1	5.98	2.15	8.13	6.04	2.18	8.22	6.05	2.18	8.23

p = 0.001 significance level.

When Table 3 is examined, the results of the analyses carried out indicates that the highest amount of chlorophyll (C1 a and C1 b) in each month and in average is in young trees treated with Baikal EM1. When the amount of chlorophylls is compared in terms of months, it is seen that the highest value is in August and September in all processes. Carotenoids have two important roles in photosynthesis. First of all, carotenoids prevent the breakdown of chlorophylls against light and oxygen. Secondly, it is about the role they have in light absorption. Within the photosynthetic system, carotenoids contribute to photosynthesis by absorbing light energy at certain wave lengths and transferring it to chlorophyll (Seely, 1966). The results of the analyses indicate that the highest amount of carotenoid in each month and in average is in young trees treated with Baikal EM1. On the other hand, the lowest carotenoid amount is found in control variables. When the amount of carotenoid is compared in terms of months, it is seen that the highest value is in August and September in all processes. This research has clearly demonstrated the positive effect of the Baikal EM1 on the synthesis of chlorophylls, carotenoids and photosynthesis processes. It is a very important element for plants that nitrogen gets into the structure of proteins to a large extent. It also combines with carbon compounds in the plants, and thus forms different molecules and gets into the compounds of vitamins which work as co-enzymes in different respiration enzymes as well as nucleic acids. In addition to having a dominant role in the structure and functionality of the cell, proteins also carries genetic information as molecular intermediates. The high molecular polymers of amino acids, which are the basis of living, are called proteins and these are defined as the main compounds of protoplasm (Rubin 1976). Table 5 shows the amount of average nitrogen and protein found in chestnut leaves in each month (in dry weight).

Table 5. Average nitrogen and protein amounts in chestnut leaves (%).

Variants	Nitrogen			Protein		
	July	August	September	July	August	September
Control	0.71	0.69	0.65	4.44	4.31	4.06
Baikal-EM1	1.26	1.23	1.23	7.87	7.69	7.69

p= 0.001 significance level.

When Table 5 is examined, it is seen that the highest nitrogen and protein values are found in Baikal EM1 variables. In terms of months, it is seen that the highest value is in July. As it is known, nucleic acids (DNA and RNA) are the most important chemical compounds of cell nucleus. In the DNA of each living thing, there is a unique nucleotide sequence and all information is coded in this nucleotide sequence. This enables the development, life and transfer of characteristics of the species (Knorre 1998). In Table 6, DNA and RNA values on chestnut leaves have shown.

Table 6. DNA and RNA values on chestnut leaves (in July) (mg/g in fresh material).

Variants	Average DNA	Average RNA
Control	19. 67	128. 82
Baikal EM1	31. 94	180. 35

According to the results in Table 6, DNA and RNA amounts are higher in Baikal EM1 variable compared to control variable. In addition, the highest values belong to RNA.

4. Conclusion

In this study, the effects of the effective microorganism Baikal EM1 on the physiological properties of young chestnut seedlings were investigated. Baikal EM1 performed well compared to control samples. Results of experiments carried out with chestnut showed that the drug Baikal EM1 stimulated the processes of growth and development which was to confirm the maintenance of photosynthetic pigments, nitrogen, proteins, DNA and RNA in leaves. Contents of those essential for plant body metabolites of chestnut leaves is increased by treatment with this drug which may be related to optimizing the functional state of the cell organelles.

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