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***Dani SARSEKOVA[1], Sezgin AYAN[2], Talgat ABZHANOV[1], Ainur NURLABI[1]***

# PRELIMINARY RESULTS OF THE EFFECT OF ARTIFICIAL MYCORRIZATION ON THE GROWTH OF SIBERIAN SPRUCE (*Picea obovata* Ledeb.) SEEDLINGS AND SOIL PROPERTIES

## SUMMARY

In this study, we investigated the effects of artificial mycorrhiza application on soil properties and seedlings growth of Siberian spruce (*Picea obovata* Ledeb.) in the Novodolinsky nursery in Kazakhstan. Before transplanting, the roots of 3-years old seedlings were subjected to the mycorrhizal solution. The mycorrhizal strain includes mycelium of fungi of the genera *Suillus* Gray, *Boletus* Bull., *Paxillus* Fr., and *Cortinarius* (Pers.) Gray. The results showed that by applying the mycorrhizal activator, there were minor changes in the absorbed bases and soil pH. The value of phosphorus in the A horizon increased twice. The survival rate of the mycorrhizal seedlings is on average 2.4% higher than in the control. Significant differences were found in terms of the variables of the yellowing needles on seedlings, the formation of buds on young shoots, and the lignified stem of the seedlings. Particularly, in late August observation, mycorrhizal seedlings showed 14.2% more lignification. Due to the lignification, seedlings might be more resistant to stress factors such as drought, frost. These preliminary results are giving the information about adaptation to extreme ecosystems of the seedlings.

**Keywords**: Ectomycorrhizal fungi, mycotrophy, survival rate, symbiosis

## INTRODUCTION

Preservation of environmentally favourable living conditions in various regions of the world directly depends on the rational and careful use of forest resources. Forest ecosystems of Central and Northeast Kazakhstan are one of the most important components of the Earth's biosphere, contributing to the ecological balance on the entire planet. Among the several categories of mycorrhizal fungi, ectomycorrhizal fungi is one of the major groups with

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[1]Dani Sarsekova, Talgat Abzhanov, Ainur Nurlabi, S. Seifullin Kazakh AgroTech. Univ., Forest Resources and Forestry, Nur-Sultan, KAZAKHSTAN

[2]Sezgin Ayan (Corresponding author: sezginayan@gmail.com), Kastamonu University, Faculty of Forestry, Silviculture Department, Kastamonu, TURKEY

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arbuscular mycorrhizal in below-ground fungal communities in forests (Peay *et al.*, 2016). Ectomycorrhizal fungi are obligate mutualistic symbionts and hence rely entirely on carbon supply from host plants (Smith and Read, 2008). While they are abundant in root systems of herbaceous plants (Hiiesalu *et al.*, 2014), they are hosted also by diverse tree species (Liu *et al.*, 2015). Ectomycorrhizal fungi have a leading and play significant roles in forest community dynamics (Smith and Read, 2008) because they promote the dominance of the specific plant families e.g., Pinaceae, Fagaceae, Betulaceae, and Dipterocarpaceae (Tedersoo and Smith, 2013). Ectomycorrhizae are found in the upper layer, which mostly contains humus, rather than the lower layer of the soils which has the mineral-rich substances (Dogmus Lehtijarvi, 2007). The ectomycorrhizal fungi improve plant nutrition in exchange for carbohydrates (Smith and Read, 1997; Sebastiana *et al.* 2018). They have an important role in the intake of Zn, Cu, Mn, Fe, Ca, K and N, especially P, which is slow in soil intake. In addition, mycorrhizas increase the efficiency water use, as well as improve soil structure and protect the soil against erosion (Dogmus Lehtijarvi, 2007). Previous studies have been emphasized that the use of mycorrhiza seedlings in afforestation, rehabilitation and restoration works in marginal sites can significantly increase the success of the plantations (Arocena and Glowa, 2006; Qiang-Sheng and Ren-Xue, 2006; Bennett *et al.*, 2017; Kharuk *et al.*, 2019). This subject is particularly important in marginal sites that have extreme ecological conditions and under higher climate change influence, than in core populations (Barbati *et al.*, 2018). The environmental conditions on poor sites favour numerous biotic harmful factors including pathogens (Haavik *et al.*, 2015).

One of the main research and applied subjects is the study of ectomycorrhizal fungi, and their significance in forest nurseries, the development of methods for artificial inoculation of plants and ways to control the ecological potential of ectomycorrhizal associations. The importance of the mycotrophic way of feeding forest trees is expressed, mainly in improving growth. Equally important, seedlings with successful mycorrhiza formation can survive best after transplantation (Taylor *et al.*, 2016). The presence of mycorrhiza and its development is an essential indicator of their quality, since plants with trees and shrubs seedlings developed mycorrhiza better take root and grow, especially on poor soils (Jo *et al.*, 2018). This symbiosis partnership allows participating in the circulation of nutrients, optimizing plant metabolism, activating mineral nutrition, and inducing resistance to drought, salinization, heavy metals, and pathogens (Taylor *et al.*, 2016). There is a consensus that these plant-fungal associations have profound impacts on nutrient cycling and vegetation dynamics in ecosystems, particularly in temperate forests (Bennett *et al.*, 2017; Taylor *et al.*, 2016; Jo *et al.*, 2018). The study of ecology and physiology of ectomycorrhiza (EcM) is concentrated mainly in Europe, North America and Australia (Smith and Read, 1997; 2008; Read, 1999; Finlay, 2005; Polenov, 2013). On the territory of the Republic of Kazakhstan, the study of mycobiota and EcM macromycetes was carried out by Nam (1998), Abiev *et al.* (2000), and Abiev (2015). Applied aspects of the mycorrhization were investigated by Meshkov (2010), what were the first studies in Kazakhstan. His research was not focused only to obtain four types of macromycetes into the investigated culture but also to develop a technology for their scaling and application in the form of mycorrhized compost for reforestation in Zailiysky Alatau (Meshkov *et al.*, 2009a; Meshkov 2010). However, some researchers emphasize that for normal development of trees, specialized strains of macromycetes fungi forming EcM is needed. In addition, particularly coniferous seedlings are not able to achieve adequate growth if they are excluded from mycorrhizal occurrence (Kais *et al.*, 1981; Alvarez and Trappe, 1983; Valdes, 1986).

Some studies have been carried out on Siberian spruce (*Picea obovata* Ledeb.) and ectomycorrhizae for different purposes. Some of these are as follows: Seasonal growth, dynamics of morphological and anatomical structure, and sugar content in ectomycorrhizal roots of Siberian spruce were studied by Tvorozhnikova *et al.* (2009); In another research; the diversity of fungal mantles of Siberian spruce ectomycorrhiza was investigated in two natural environmental gradients (the Visim State Nature Biosphere Reserve (Middle Ural; 380-685 m a. s. l.) and "Denezhkin Kamen" Reserve (Northern Ural; 305-800 m a. s. l.). As results of this reserach; Shennon's and Simpson's diversity indices of mantles types did not depend on the site altitude. It was revealed that the transformation of ectomycorrhizal diversity occurs in different ways in natural and technogenic ecological gradients (Veselkin 2010). In another study by Veselkin (2004) stated that changes in the anatomical characters of ectomycorrhiza in Siberian fir and Siberian spruce were studied in natural forests polluted with heavy metals (Cu, Zn, Cd, Pb, As, and Fe) and sulfur dioxide. As technogenic load increased, the total radius of mycorhiza terminals and plant roots included in them increased in the organic horizon and decreased in the mineral part of the soil. However, Kharuk *et al.* (2019) emphasizes in their work climate-driven changes in boreal forest including Siberian spruce; however, little is known about its symbiosis with fungi. The aim of this research was to the investigate the effect using the artificial mycorrhiza application on soil properties and seedlings growth of Siberian spruce.

## MATERIAL AND METHODS

### Study site and climate characteristics

The research was carried out in the Novodolinsky nursery (N 49º 42′ 601″, E 72º 43′ 096″) of the Kazan State University of Karaganda region of Kazakhstan.

Karaganda is about 554 m above sea level and the climate is cold and temperate. The climate classification is warm-summer humid continental climate (Dfb) according to the Köppen-Geiger climate classes. The coldest month is averaging below 0 °C (or –3 °C). The average annual temperature in Karaganda is 2.8 °C in a year, and the average rainfall is 310 mm (Table 1) (Kottek *et al.*, 2006; Peel *et al.*, 2007; URL1, 2020).

Table 1. Climate characteristics of Karaganda region (Year: 1982–2012) (URL1, 2020)

| Climate characteristics | Month | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | January | February | March | April | May | June | July | August | September | October | November | December |
| Average temperature (°C) | -15 | -14.7 | -7.9 | 4.7 | 12.7 | 18.4 | 20.5 | 18 | 12.4 | 3.3 | -6.2 | -12.1 |
| Minimum temperature (°C) | -19.5 | -19.6 | -12.7 | -1.2 | 5.7 | 11.4 | 13.9 | 11.4 | 5.7 | -1.8 | -10.5 | -16.4 |
| Maximum temperature (°C) | -10.4 | -9.7 | -3 | 10.7 | 19.8 | 25.4 | 27.2 | 24.6 | 19.2 | 8.5 | -1.9 | -7.8 |
| Precipitation (mm) | 20 | 17 | 17 | 23 | 40 | 32 | 37 | 29 | 21 | 29 | 23 | 22 |

The climate of the nursery location is continental, in some years severe, with blizzards. The experiment was started on May 23, 2019 and observation time was 97 days. The main climate characteristics during the investigation (from July 23 to August 24, 2019) were observed. Namely, the average temperature was 21.6 ± 6.8 °C, the average daily maximum temperature was 27.3 ± 4.3 °C, and the average minimum morning temperature was 15.2 ± 3.4 °C, while the number of cloudless days was 15 days. The number of cloudy days was 16, and the average cloud cover was 3.9 points. The number of rainy days was 7 days, and the precipitation level was 217 mm. The average humidity for the observation period was 49.2 ± 14.9%. The average level of the UV index was 4.5 ± 1.0 points (Anonymous, 2019).

**Site preparation and mycorrhization**

Three-years-old Siberian spruce seedlings from in the nursey of the Karaganda Region were used as research materials. The planting time was at the end of April and the beginning of May 2019. Before planting, the seedlings were stored under snow for 1 week. As morphologically homogeneous seedlings (Average height: 36 ± 8 cm) as possible were used in the research. Visual inspection of the seedlings showed that the overall condition of the seedlings is satisfactory, and no signs of wilting and necrosis were recorded. The buds were dormant, and the roots were moistened. Site preparations were performed using the MTZ – 80 tractors, and a 4-row sewing machine for the MTZ-82.1.8.

Before planting, the seedlings were subjected to the process of soaking the root system in a mycorrhizal solution using the following method: 1.1 kg of the mycorrhizal substrate (produced by Bio-Logica) was diluted in 10 liters of water with the addition of 200 g of agroperlite (produced by Morov Agro). The substrate used is free-flowing, based on peat and living mycelium of fungi, immobilized on secondary aluminosilicates. Seedlings were exposed to the mycorrhizal solution for 25 minutes. Planting of seedlings was carried out according to the generally accepted method of "Planting seedlings of forest crops in transplant section of forest nurseries" (Meshkov *et al.*, 2009a; Meshkov, 2010). The row spacing was 80 cm, while the distance between seedlings was 60 ± 10 cm. Planting depth was 15-20 cm.

Mycorrhiza was introduced into the root system at the same time as planting. The biological product called Mycorrhizal activator (produced by Bio-Logica) was also applied. This mycorrhizal activator contains active strains of living rhizosphere microorganisms and is grown based on natural material taken from the roots of a specific coniferous culture. The mycorrhizal activator included mycelia of fungi of the genera *Suillus,* [*S. variegatus* (Sw.) Richon and Roze 1888, *S. luteus* (L.) Roussel 1796, *S. sibiricus* (Singer) Singer 1945, *S. bovinus* (L.) Roussel 1796, *S. tridentinus* (Bres.) Singer 1945, *S. salmonicolor* (Frost) Halling 1983, *S. granulatus* (L.) Roussel 1796, *S. placidus* (Bonord.) Singer 1945], *Boletus*, [*B. satanas* Lenz, 1831], *Paxillus* [*P. involutus* (Batsch) Fr., 1838] and *Cortinarius* [C. *nemorensis* (Fr.) JE Lange 1940, *C.* sp. *sensu* NCL (1960)].

**Analysis of soil samples**

A detailed morphological description of genetic horizons was carried out according to standard methods (Rozanov, 2004). Three soil samples were taken from each depth (0-20 cm and 20-40 cm) and horizon. The soil samples were taken from 10-15 cm distance to the planting rows of seedlings. The following analyzes were performed: 1) Determination of the physical properties of soils (soil density (g/cm$^3$), the solid phase density of the soil by the pycnometric method, porosity (%) according to calculations, soil moisture by thermostat-weight method), determination of the granulometric composition of soils by the method of N.A. Kachinsk (Polupan, 1981), 2) Determination of physicochemical properties by potentiometric method, the amount of absorbed bases by the trilonometric method, humus (%) by the method of Tyurin (Tyurin, 1951), carbonate content by a gasometrical method, analysis of water extract (dry residue, anion composition: $CO_3^{2-}$, $HCO_3^-$, $Cl^-$, and $SO_4^{2-}$) and cations ($Ca^{2+}$, $Mg^{2+}$, $Na^+$, and $K^+$) (absorbed base, mg/equiv per 100 g soil), mobile forms of phosphorus (mg/100 g soil) by the method of Machigin B.P (Sychev, 2000), exchange potassium (mg/100 g soil) on a flame photometer, pH, pedotransfer functions (PTF) (g/cm$^3$), 3) the samples were taken by a soil drill using the continuous column method every 10 cm to 100 cm to determine soil moisture and every 10 cm to 40 cm to determine the density, density of the solid phase of the soil and wet sieving aggregate content (%) of the soil, as well as in layers 0-20 cm and 20-40 cm to determine the amount of absorbed bases, humus (%) and nitrogen, phosphorus, and potassium (NPK) (Fly, 2004).

**Seedling morphometric and phenologic characteristics**

The following parameters of the seedlings were observed end of the vegetation period and measured by digital meters without removal from the soil:

1) Survival rate (SR-%), 2) terminal shoot length (TSL-mm), 3) lateral shoot length (LSL-mm), 4) the length of needles on the young shoot (LNYS-mm), 5) the yellowing needles on seedlings (YNS-pcs), 6) the formation of buds on young shoots (FBYS-pcs), 7) the number of seedlings with young shoots (NSYS-pcs), and 8) number of lignified stem (NLS-pcs). NLS has observed two times (Mid of July and end of August) in 2019.

The data of Siberian spruce seedling for the tested parameters were observed with three replicates. Among the measured characteristics of conifer seedlings “shoot length” and “length of needles” were observed. These indicators correlate with the degree of mycotrophy of coniferous seedlings (Ivanov *et al.*, 2014).

**Statistical analysis**

In the analysis of the obtained data, the t-test was applied for each measured parameter to compare the average values of the two data sets. The measured parameters were obtained with 3 repetitions and 30 seedlings in each repetition. For the data obtained, normal distribution compliance test checks were performed, and the necessary statistical transformations (arc-sin, logarithmic) were applied. All the analyses were performed using the SPSS program (version 11, IBM Corporation, Armonk, NY, USA).

## RESULTS

**The effects of artificial mycorrhization on soil properties**

The obtained data present that the granulometric composition, i.e., the chestnut soils of the nursery, sandy loam is formed on sand. Sand fractions of 0.3-0.7 mm in size are found throughout the soil profile. Throughout the profile, the soil is loose with a sand granulometric composition. In the upper horizon $A_1$ of 0.2-17 cm of soil in the deposit, the content of physical clay is 19.2%, in the underlying horizons it gradually decreases in the parent rock and decreases to 9.24%, which can be confirmed by the sand granulometric composition of the parent rock. A similar change in the content of physical clay along the profile occurs in the soil of the experimental plot, where in the arable horizon of 0-26 cm the value of physical clay is 15.08%.

The maximum content of the fraction of physical clay is found (17.60%) in the $B_1$ horizon 26-36 cm per due to an increase in the silt fraction during washing and during irrigation. Further in the underlying horizons there is a decrease in physical clay, where in the parent rock it decreases to a minimum of 8.92% (Table 2). The humus content of the upper horizon A in control and in the experimental plot was same (1.18%). In the lower soil horizon of the control, $B_1$ 17-37 cm, is 0.74%, and in this horizon of the experimental site, $B_1$ 26-36 cm is 0.84%. The content of phosphorus in the all-soil horizons is very low however, the value of phosphorus in the A horizon has been found to increase 2-times thanks to the mycorrhizal activator compared to control (Table 3).

Table 2. Granulometric composition of chestnut soils of the nursery

| Horizon and depth (cm) | The dimension of granule, % to dry soil | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | >3 mm | 3-1 mm | 1-0,25 mm | 0,25-0,05 mm | 0,05-0,01 mm | 0,01-0,005 mm | 0,005-0,001 mm | <0,001 mm | Σ<0,01 mm |
| Control- Grassland without mycorrhization | | | | | | | | | |
| $A_1$ 0,2-17 | 0.02 | 0.35 | 38.57 | 37.19 | 5.04 | 3.20 | 6.80 | 9.20 | 19.20 |
| $B_1$ 17-37 | 0.00 | 0.04 | 39.05 | 42.79 | 4.60 | 1.28 | 4.36 | 7.92 | 13.56 |
| $B_2$ 37-62 | 0.80 | 6.05 | 19.39 | 65.85 | 4.20 | 0.88 | 3.00 | 6.68 | 10.56 |
| $B_к$ 62-87 | 0.07 | 0.22 | 24.71 | 62.33 | 3.28 | 0.32 | 4.64 | 4.72 | 9.68 |
| $B_кC$ 87-106 | 0.10 | 1.50 | 35.64 | 50.40 | 5.96 | 0.28 | 2.64 | 5.08 | 8.00 |
| C 106-124 | 0.30 | 0.92 | 33.50 | 53.78 | 3.48 | 0.48 | 3.20 | 5.56 | 9.24 |
| Experimental plot with mycorrhization | | | | | | | | | |
| A 0-26 | 0.39 | 1.76 | 39.27 | 40.21 | 5.44 | 2.32 | 5.08 | 7.68 | 15.08 |
| $B_1$ 26-36 | 0.14 | 0.63 | 41.75 | 30.53 | 10.12 | 2.84 | 2.40 | 12.36 | 17.60 |
| $B_2$ 36-67 | 0.01 | 0.11 | 32.42 | 46.14 | 7.24 | 0.16 | 3.56 | 10.48 | 14.20 |
| $B_к$ 67-107 | 0.00 | 0.47 | 40.43 | 41.89 | 8.12 | 0.24 | 3.84 | 5.48 | 9.56 |
| C 107-120 | 0.69 | 3.32 | 43.56 | 43.64 | 3.88 | 0.76 | 3.36 | 4.80 | 8.92 |

Table 3. Physicochemical properties of chestnut soils of the nursery

| Sampling depth cm | Humus% | pH | Mobile mg/100 g soil | | Absorbed base mg/equiv per 100 g soil | | | Absorption basis in% of the amount or capacity | |
|---|---|---|---|---|---|---|---|---|---|
| | | | P | K | $Ca^{2+}$ | $Mg^{2+}$ | Total | $Ca^{2+}$ | $Mg^{2+}$ |
| Control - Grassland without mycorrhization | | | | | | | | | |
| $A_1$ 2-17 cm | 1.18 | 6.6 | 0.40 | 22.20 | 7.70 | 1.60 | 9.30 | 82.79 | 17.21 |
| $B_1$ 17-37 cm | 0.74 | 6.7 | 0.30 | 10.14 | 6.20 | 1.90 | 8.10 | 76.54 | 23.46 |
| $B_2$ 37-62 cm | 0.53 | 6.7 | 0.20 | 8.71 | 5.80 | 2.15 | 7.95 | 72.96 | 23.04 |
| Experimental plot with mycorrhization | | | | | | | | | |
| $A_{пах}$ 0-26 cm | 1.18 | 7.0 | 0.80 | 32.30 | 7.35 | 1.80 | 9.15 | 80.33 | 19.67 |
| $B_1$ 26-36 cm | 0.84 | 6.7 | 0.30 | 10.00 | 6.80 | 1.80 | 8.60 | 79.07 | 20.93 |
| $B_2$ 36-67 cm | 0.59 | 6.9 | 0.20 | 5.00 | 4.10 | 1.80 | 5.90 | 69.49 | 30.51 |

The changes in the chemical properties of chestnut soils in the nursery were investigated with application of the mycorrhizal activator. The results have shown that when this treatment was applied to the soil, there were minor changes in the amount of absorbed bases and the reaction of the soil solution (Table 4).

The exchange ability of the soils is low due to the light particle size distribution. The amount of exchangeable bases ranges from 9.30-9.40 mEq per 100 g of soil in the upper layer of 0-20 cm. When the mycorrhizal activator is introduced in the soil, a slight change in the increase in the number of exchange bases occurs. With the option of using mycorrhizal activator in a layer of 0-20 cm, the number of cations increased. Changes of this value were also noted in the lower layer of this soil, where with the use of mycorrhizal activator was 8.90 mEq/100 g, and whereas this application was not the case- the sum of cations was 8.65 mEq/100 g. This value increased, which might be explained by more increased sorption process of the soil when using mycorrhizal preparation. In the layer of 20-40 cm of soil with the use of a mycorrhizal biological product, there has been an increase in the amount of cations by 0.25 mEq/100 g compared to the version without a biological product, but these changes are related to an increase in the proportion of $Mg^{2+}$ cation in the soil adsorption complex. $Ca^{2+}$ predominates in the composition of the absorbed soil bases in all cases, which accounts for 81.18-81.91% in the upper 0–20 cm layer, where in the cases by using the mycorrhizal biological product. The $Ca^{2+}$ cation has been increased very slightly compared to control (Table 4).

Table 4**.** Changes in the chemical properties of chestnut soils

| Sampling depth (cm) | pH | Absorbed base mg-eq per 100 g of soil | | | Absorbed base in% of the amount or capacity | |
|---|---|---|---|---|---|---|
| | | $Ca^{2+}$ | $Mg^{2+}$ | Total | $Ca^{2+}$ | $Mg^{2+}$ |
| Control - Siberian spruce without mycorrhizal activator | | | | | | |
| 0-20 | 6.9 | 7.55 | 1.75 | 9.30 | 81.18 | 18.82 |
| 20-40 | 7.1 | 6.85 | 1.80 | 8.65 | 79.19 | 20.81 |
| Siberian spruce by applying the mycorrhizal activator into the soil | | | | | | |
| 0-20 | 6.7 | 7.70 | 1.70 | 9.40 | 81.91 | 18.09 |
| 20-40 | 6.9 | 7.00 | 1.90 | 8.9 | 78.65 | 21.35 |

A negligible amount of the soil density has been decreased when compared to the control. Depending on the density of the solid phase in different variants of the soils, the porosity in the upper 0-10 cm layer has changed in the range of 51,03-51,66%. In the lower layer of 10-20 cm, these values vary from 50,99-51,76% (Table 5). According to porosity, these arable soils are rated as satisfactory. In the fallow variant, the upper soil layers are dense and have a porosity of less than 50%.

According to the structural analysis for wet sieving, in all variants of the soil layer 0-40 cm the number of water-resistant aggregates is higher than 51.06%, which indicates a satisfactory degree of structurally of the soils. In all variants using the biological product of chestnut soils, a slight increase in the aggregates is greater than 0.25 mm of soils by 0.78% in the 0-20 cm layer and 2.83% in the 20-40 cm layer (Table 6).

**Table 5.** Change of physical properties of chestnut soils

| Depth of sampling cm | The density of the soil g/cm$^3$ | PTF soil g/cm$^3$ | Porosity % |
|---|---|---|---|
| Control - Siberian spruce without biological product | | | |
| 0-10 | 1.18 | 2.41 | 51.03 |
| 10-20 | 1.25 | 2.55 | 51.76 |
| Siberian spruce by applying the mycorrhizal activator into the soil | | | |
| 0-10 | 1.16 | 2.40 | 51.66 |
| 10-20 | 1.23 | 2.51 | 50.99 |

Table 6. Change in water-resistant aggregates of chestnut soils

| Depth of sampling cm | Wet sieving aggregate content, % | | | | ∑> 0.25 mm |
|---|---|---|---|---|---|
| | 1-2 mm | 0.5-1 mm | 0.25-0.5- mm | <0.25 mm | |
| Control - Siberian spruce without biological product | | | | | |
| 0-20 cm | 4.32 | 16.13 | 31.00 | 46.11 | 51.45 |
| 20-40 cm | 5.03 | 14.25 | 31.78 | 47.39 | 51.06 |
| Siberian spruce by applying the biological product into the soil | | | | | |
| 0-20 cm | 3.33 | 17.45 | 31.45 | 46.22 | 52.23 |
| 20-40 cm | 2.45 | 18.79 | 32.65 | 45.11 | 53.89 |

**The effects of artificial mycorrhization on seedling characteristics**

The results show that the survival rate of the mycorrhizal seedlings of Siberian spruce is on average 2.4% higher than the control. However, there has not been found a statistically significant difference between control and mycorrhizal seedlings as to variables of SR (%), the number of the seedlings with blooming buds (pcs), and the number of seedlings with active shoots (%). A statistically significant difference between control and mycorrhizal seedlings was determined in terms of the NSYS variable. The fact that mycorrhizal seedlings have less yellowing in needles than control seedlings can be considered as a positive effect of artificial mycorrhiza application. It can be stated that the resistance of mycorrhizal seedlings increases thanks to artificial mycorrhization. In control seedling, the number of seedlings with young shoots (pcs) is higher in both the terminal and the lateral branches (Table 7). Mycorrhizal seedlings have more buds in young shoots than control seedlings can be evaluated as a positive indicator. Some of the buds are potential shoots of the following year. This can be considered as a sign that mycorrhizal seedlings will form a stronger crown.

Table 7. Survival rate and key growth biometric indicators of Siberian spruce seedlings after mycorrhization

| Treatment | Survival rate (SR) (%) | Number of seedlings with young shoots (NSYS) (pcs) | | Terminal shoot length (TSL) (mm) | Lateral shoot length (LSL) (mm) | The length of the needles on the young shoots (LNYS) (mm) | Yellowing needles on seedlings (YNS) (pcs) | The formation of buds on young shoots (FBYS) (pcs) |
|---|---|---|---|---|---|---|---|---|
| | | Terminal | Lateral | | | | | |
| Control | 70.4 | 103 | 111 | 29.1 ± 10.15 | 21.0 ± 7.95 | 18.4 ± 5.28 | 152 | 0 |
| Mycorrhiza applied saplings | 72.8 | 83.33 | 93.33 | 29.07 ± 8.64 | 22.53 ± 6.69 | 20,8 ± 6.91 | 103.33 | 9.7 |
| *t test, P level* | 0.939 ns | 5.502 ** | 7.489 ** | 0,021 ns | 1,946 ns | 2.396 ns | 6.720 ** | 2.857 * |

*P* significance level; ns: non-significant, * $P < 0.05$: ** $P < 0.01$; *** $P < 0.001$

There was no significant difference between control and mycorrhizal seedlings as the key growth biometric indicators such as terminal shoot length, lateral shoot length, and the length of the needles on the young shoots. However, significant differences were determined in terms of the variables of the yellowing needles on seedlings, and the formation of buds on young shoots (Table 7).

In the control treatment, the number of seedlings in which yellowing of the needles is observed is 86.4% of the total number of living seedlings. In experimental replications, this parameter was significantly lower at the level of $p<0.01$ according to the control.

Table 8. The effect of mycorrhization on the process of lignification of the seedlings

| Month | The number of lignified stems (NLS) | | |
|---|---|---|---|
| | Control | Mycorrhizal seedlings | *t value, P level* |
| | % | % | |
| Mid of July | 38,8 | 51,2 | 2,939** |
| End of August | 42,9 | 57,1 | 17,391*** |

*P* significance level; ns: non-significant, * $P < 0.05$: ** $P < 0.01$; *** $P < 0.001$

The number of seedlings with lignified stem was observed twice in mid-July and at the end of August. In both periods, statistically significant differences were determined between control and mycorrhizal seedlings. Particularly, in late August observation, mycorrhizal seedlings showed 14.2% more lignification than the control treatment. This value would be reflected in the survival percentage of seedlings in the next years (Table 8).

## DISCUSSION

Meshkov *et al.* (2009b) have emphasized that priority should be given to forest rehabilitation on burned areas and cut-over lands, including the ribbon-like relict pine forests of the Irtysh region, the Kazakh upland, plain forests of Kostanai Province. In addition, Meshkov *et al.* (2009b) and Sarsekova *et al.* (2016; 2020) recommended that in many parts of Kazakhstan, in the degraded forest areas, mycorrhizas should be used as a major improvement strategy. Such practical projects should be implemented as soon as possible.

In this research, the granulometric composition of the chestnut soils gradually decreased the content of physical clay, i.e., from the horizon $A_1$ of the soil to the parent rock. A similar change in the content of physical clay along the profile has occurred in the soil by applying the mycorrhizal product. In spite of the same humus content, it was found that there are minor changes in the amount of absorbed bases and pH by applying the mycorrhizal activator. The exchange ability of the soils was low due to the light particle size distribution. A slight change in the increase in the amount of exchange bases occurs either 0-20 cm or 20-40 cm by applying mycorrhizal activator. The $Ca^{2+}$ cation has been increased very slightly by using the mycorrhizal product. However, the value of P in the A horizon has been found to increase twice due to the mycorrhizal activator. This result is very important. Cosgrove (1967) stated that in the boreal forest ecosystem, P is second only to N as a major limiting nutrient for plant growth and a major part of soil P, sometimes as much as 90%, is sequestered in organic compounds. The majority of P in boreal forest soils is present as organic P, and phosphatase activity is therefore significant for the ability of ectomycorrhizal fungi to obtain P in these systems (Haussling and Marschner, 1989). In addition, the soils of the nursery have a neutral, slightly alkaline reaction of the lower horizons. As a result of this study, it was found that the soil pH varies slightly in all studied options. The relationship between mycorrhizal fungi and soil acidity has long been discussed in the literature (Kalliokoski *et al.*, 2010; Pena *et al.*, 2017). Ectomycorrhizal fungi are important source of organic acids in soil (Griffiths *et al.*, 2009) and affect the pH of the rhizosphere (Smith and Read, 2008). Eremin and Popova (2016) emphasized that soils, where mycorrhizal biological products are applied under the seedlings, have led to a very slight decrease in the reaction of the soil solution in the studied depths. Mycorrhiza has been noted by Eremin and Popova (2016) to decrease the pH in the rhizosphere, because of the selective absorption of ammonium NH+ ions and the release of H+ ions. Smith *et al.* (2003) stated that the low effectiveness of root and mycorrhizal functions in the cold soils is due to insufficient mineral elements supply. The results obtained in this study are consistent with the general results of previous studies.

A negligible amount of soil density has been decreased by using the biological product on the spruce seedlings and the soil beneath. Against it, a slight increase has been determined in the aggregates. According to Rillig and Steinberg (2002), the free mycelium of mycorrhizal fungi promotes the aggregation of soil particles and modifies the soil structure, which affects the general physical properties of the soil. Mycobiont hyphae are involved in the stabilization of soil micro aggregates by binding soil particles and the accumulation of organic compounds. Aggregation helps maintain a porous but stable soil structure and prevent erosion. However, these data are for a one-year investigation. Mycorrhization of seedlings did not lead to significant changes in soil properties within one growing season. Therefore, the expected positive results of this research will be more clearly observed in further stages.

The presence of mycorrhiza on the roots of seedlings, trees and shrubs and the degree of its development is an essential indicator of their quality. In fact, plants with developed mycorrhiza form better root system and growth, especially on poor soils. It was experimentally found that seedlings without mycorrhiza die or have low survival rate (9–12%), while plants with well-developed mycorrhiza are characterized by high survival rate (70–100%) (Meshkov *et al.*, 2009a). In this research, the survival rate of the mycorrhizal seedlings is found higher than in the control seedling. Research supporting this result was conducted by Gryndler *et al.* (2004), Ortega *et al.* (2004), Pešková and Tuma (2010). Gryndler *et al.* (2004), Pešková and Tuma (2020) highlighted that inoculation with mycorrhizal fungi has a positive effect on plants' survival rate and growth, and it increases resistance to various abiotic factors and biotic harmful agents.

In this research, significant differences were determined between control and mycorrhizal seedlings for the lignified stem of the seedlings in the periods of mid of July and late August. Particularly, in late August observation, mycorrhizal seedlings showed 14.2% more lignification than the control treatment. These results reflect that the survival percentage and growth of mycorrhizal seedlings would be better in the next years. Tvorozhnikova *et al.* (2009) emphasized that the formation of symbiotic complexes of Siberian spruce with mycorrhizal fungi favors this species tolerance to the conditions of cold climate. The studies by Qiang-Sheng and Ren-Xue (2006) showed that mycorrhized plants are most resistant to drought, they have higher evaporation and photosynthesis rates, lower leaf temperatures, and higher levels of dissolved sugars and starch (Selivanov, 1975).

## CONCLUSIONS

It can be concluded that the most important value of mycorrhiza is that it protects the plant from the effects of adverse environmental factors and induces the plant's immunity to numerous stress factors. Particularly, mycorrhizal application as a tool would play an even more important role for Kazakhstan, which has severe climatic and unfavorable ecological conditions, especially under the influence of climate change. Moreover, results of this research give us valuable information about adaptation of ecosystems to the extreme environment. According to the initial results of this research, it was found that artificial mycorrhization had positive effects on the survival percentage of seedlings and the lignification of the seedlings. However, in the long-term, the research will be able to give more reliable results for the practices of afforestation and forest regeneration in marginal areas.

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