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The use of biopreparation for artificial mycorhization seedlings of forest tree species in North – Eastern Kazakhstan

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Abstract. *The article presents the results of research carried out on the use of artificial mycorization of seedlings of forest tree species. The aim of the work is to study the mycotrophy of pinus sylvestris L. and betula pendula Roth. in forest ecosystems of North-Eastern Kazakhstan and mycorization of seedlings in open ground conditions. Experiments were made on 1160 seedlings of betula pendula Roth.and 1000 seedlings of pinus sylvestris L. in open ground conditions in the Shaldai branch of SFNRErtisormany " of Pavlodar region. In experimental seedlings, the survival rate in 2019 was 83.3% for betula pendula Roth., 55.2% for pinus sylvestris L., in 2020 it was 81.4% for betula pendula Roth., and 53.3% for pinus sylvestris L.*

The need to study mycorrhizal formation in coniferous seedlings in forest nurseries in Kazakhstan is caused as a way to improve the quality of planting material.

Keywords: *North-Eastern Kazakhstan, mycotrophy, ectomycorrhiza, seedlings, Pinus sylvestris L., Betula pendula Roth., mycorization.*

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Аннотация. *В статье представлены результаты проведенных исследований по применению искусственной микоризации сеянцев лесных древесных пород. Целью работы является изучение микотрофность pinus sylvestris L. и betula pendula Roth. in лесные экосистемы Северо-Восточного Казахстана и микоризация сеянцев в условиях открытого грунта. Опыты были проведены на 1160 сеянцах betula pendula Roth.и 1000 саженцев pinus sylvestris L. В условиях открытого грунта в Шалдайском филиале ГЛПР Ертіс орманы Павлодарской области. У опытных сеянцев приживаемость в 2019 году составила 83,3% для betula pendula Roth., 55,2% для pinus sylvestris L., в 2020 году она составила 81,4% для betula pendula Roth., и 53,3% для pinus sylvestris L.*

Необходимость изучения микоризообразования у сеянцев хвойных пород в лесных питомниках Казахстана обусловлена как способ повышения качества посадочного материала.

Ключевые слова: *Северо-Восточный Казахстан, микотрофность, эктомикориза, сеянцы, pinus sylvestris L., betula pendula Roth., микоризация.*

Introduction. The degree of michurinist of major forest forming species of the Earth is one of the most important indicators of prosperous as 8000 species of higher plants and 7000-10000 species of fungi on the planet form ectomycorrhiza (EcM) involved in the nutrient cycle, optimization of plant metabolism, enhancing mineral nutrition, induction of resistance to drought, salinity, heavy metals, pathogens [1].

There is no more interesting form of relationship between organisms than symbiotic relationships. The most important of the symbioses between plants and fungi is mycorrhiza. It is believed that about 80 % of higher plants have mycorrhizal formations on their roots.

The preservation of environmentally friendly human conditions in various regions of the world depends directly on the rational and careful use of forest resources. The forest ecosystems of Central and North-Eastern Kazakhstan are one of the most important components of the Earth's biosphere that support ecological balance on the entire planet. Ectomycorrhiza plays a leading role in forest biogeocenoses, since it is determined on the roots of dominant tree species [2,3].

Interest in the phenomenon of symbiosis of plant roots with fungi-macromycetes has not faded since Frank described almost all types of ectomycorrhizas in 1885 [4,5].

The term "mycorrhiza" refers to all forms of cohabitation of fungi with the root systems of higher plants. This term was introduced into the special literature by B. Frank [6].

There are more than 5,000 known species of fungi that form ectomycorrhizae, and several different types of ectomycorrhizae have been recorded. The hyphae of most mycorrhizal fungi are distributed in the litter horizon, but some prefer the upper mineral horizons of the soil.

A number of plant, fungi form above-ground fruiting bodies, which are suitable for human consumption. Most species of woody plants growing in the boreal zone form ectomycorrhiza [7].

The goal is to develop a technology for growing coniferous seedlings on substrates of mycorrhizal macromycetes during reforestation of technogenic disturbed territories of North-Eastern Kazakhstan in order to reproduce the natural symbiosis of coniferous roots with fungi to increase survival, stimulate growth, endurance, and improve the decorative qualities of coniferous and deciduous plants.

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Tasks: research activities included:

- planting seedlings and seedlings on prepared substrates. Conducting biological screening of coniferous plants.

- monitoring of seasonal dynamics of mycorrhizal-macromycetes development on the main forest-forming rocks, isolation of macromycete strains.

- conducting morphometric studies of collected samples.

- study of mycorrhizal activity of strains on seedlings of tree species.

Methodology: the material was collected in the Central and North-Eastern part of Kazakhstan. In the course of the work, we used route and stationary research methods *pinus sylvestris* L., *betula pendula* Roth.,

On a permanent trial area of 0.11 ha, 2-year-old seedlings of *pinus sylvestris* L.and 2-3-year-old seedlings of *betula pendula* Roth.,were planted in the pre-prepared soil in late April and early may 2019. The total amount of planting material is 2160: of these, ordinary pine – 1000 pieces, hanging birch-1160 pieces. *Suillus bovinus* culture was introduced under pine seedlings, and *Boletus edulis* culture was introduced under birch using the following technology:

1. For *pinus sylvestris* L ., mycorrhiza was introduced into the root system at the same time as planting. The sample of pine seedlings was 1000 pieces.Planting has 3 repetitions with mycorrhiza-750 PCs, and 1 control - 250 PCs. without mycorrhiza. The rate of application to the soil at the height of seedlings: up to 0.5 meters 50-100 ml. Planting scheme: 1x0. 75 m, with drip irrigation.

2. Mycorrhiza was introduced in the spring in the unfrozen warmed soil. Before planting, the damaged roots were cut off and the root system was renewed or shortened to 20-25 cm. After pruning, the roots were dipped in a chatterbox consisting of a liquid mixture of humus with earth and mycorrhizal mycelium. In order for the planted plants to have the earth firmly attached to their roots, they were trampled. This technique made it possible to avoid voids and bends of the roots. Seedlings were planted 1-2 cm deeper than the root neck .

In the future, the main agrotechnical care was carried out: loosening the soil, destroying weeds, fertilizing plants, watering, protection from diseases and pests.

The second object was a deciduous species, *betula pendula* Roth., hanging birch. In the warm days of may 9-11, 2019, 1160 seedlings of this species were planted: three repetitions with mycorrhiza - 880 pieces, and one control 280 pieces - without mycorrhiza. Landing scheme:1x0,75 m. Type of irrigation-drip irrigation. Ways to infect the root: "chatterbox containing live mycelium of the fungus, with 2-3 – fold immersion of the seedling root.

To maintain the identity of the calculations, groups of 250 plants were formed in each group: 1 group-control (without adding a substrate), 3 groups (3 repetitions) – experimental with the introduction of a mycorrhizal substrate.

The area of the experimental field is 0.11 ha.

Mycorrhiza was introduced in the spring in the unfrozen warmed soil. Before planting, the damaged roots were cut off and the root system was renewed or shortened to 20-25 cm. After pruning, the roots were dipped in a chatterbox consisting of a liquid mixture of humus with earth and mycorrhizal mycelium. In planted plants, the ground was trampled to fit snugly to the roots. At the same time, without allowing voids and bends of the roots. Seedlings were planted 1-2 cm deeper than the root neck.

In the future, the main agrotechnical care was carried out:loosening the soil, destroying weeds, fertilizing plants, watering, protection from diseases and pests.

Location of the object: State forest nature reserve "Ertis ormany" is located in the Eastern part of Pavlodar region on the right Bank of the Irtysh river and is located in the coordinates: North latitude 51^0 23′ - 52 ^{0}15′, East longitude 78 ^{0}01′ - 79 ^{0} 21. The area of the reserve is 277961 ha. It is located on the territory of Shcherbaktinsky district (117565 ha) and lebyazhinsky district (160396 ha) [8].

Discussion of the main results: Monitoring of seasonal dynamics of development of seedlings of *pinus sylvestris* L. and *betula pendula* Roth.

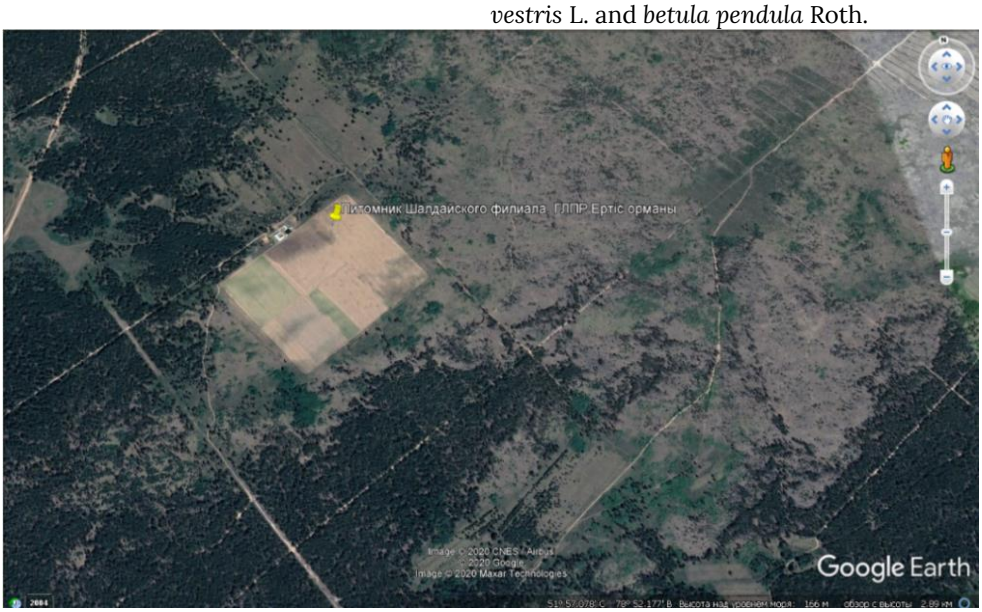

Figure 1. Location of the Shaldai nursery (Google Earth)

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Table 1-Biometric indicators of seedlings of *pinus sylvestris* L. and *betula pendula* Roth. by variants of the laid experiments as of 25.06. 2020

Name	Average height,cm	Crown		Average diameter,cm	increment
		lengthwise	crosswise		
Pinus sylvestris L. with mycorrhiza	26,3±1,0	19,2±0,6	17,4±0,5	1,2±0,5	15,0±1,2
Pinus sylvestris L. without mycorrhiza	16,0±1,0	13,0±0,5	12,1±1,0	0,8±0,2	11,0±0,5
The difference between the two samples is determined using a number of criteria: t ≥ 3	t = 6,4	7,9	4,7	0,8	3,1
Betula pendula Roth.with mycorrhiza	95,0±0,5	-	-	1,4±0,3	-
Betula pendula Roth.without mycorrhiza	71,5±1,0	-	-	1,2±0,6	-
The difference between the two samples is determined using a number of criteria: t ≥ 3	t = 20,9	-	-	-	-

The data shown in table 4 indicate that mycorrhiza had a positive effect on the growth of both coniferous and deciduous plants. In *pinus sylvestris* L., the differences in height, crown diameter along and across the row, and the current increase in comparison with the control are significant, more than three, and equal, respectively: 6,4; 7,9; 4,7; 3,1. The diameter of the root neck does not differ significantly - 0.8.

Significant differences in height were observed in the *betula pendula* Roth.: t = 20.9.

Figure 2,3,4. Measurement of biometric parameters of *pinus sylvestris* L. and *betula pendula* Roth.

Table 2-Biometric indicators of seedlings of *pinus sylvestris* L. and *betula pendula* Roth. by variants of the laid experiments as of 20.08. 2020

Name	Average height,cm	Crown		Average diameter,cm	Increment
		lengthwise	lengthwise		
Pinus sylvestris L. with mycorrhiza	30,4±18	20,2±0,6	18,4±0,2	1,4±0,1	16,7±1,2
Pinus sylvestris L. without mycorrhiza	18,0±1,0	15,0±0,5	14,1±1,0	0,9±0,2	14,0±0,5
Betula pendula Roth.with mycorrhiza	126±0,2	-	-	1,7±0,1	-
Betula pendula Roth. without mycorrhiza	83,1±1,0	-	-	1,4±0,4	-

Average height of pine with mycorrhiza 30.4 cm, diameter 1.4 cm, growth 16.7 cm, without mycorrhiza height 18.0 cm, diameter 0.9 cm, growth 14.0 cm. The data shown in table 4 indicate that with mycorrhiza, the biometric indicators of the birch were average height 126 cm, average diameter, cm 1.7, without mycorrhiza average height 83.1 cm, average diameter 1.4 cm.

Table 3. Survival ability of seedlings of common pine and hanging birch by variants of laid experiments as of spring 2020

	Planting spring 2019			2020	
	Planted seedlings, pieces	Healthy, PCs.	Survival, %	Healthy, PCs.	Survival, %
Pinus sylvestris L.	750	414	55,2	400	53,3
Betula pendula Roth.	780	650	83,3	635	81,4

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Studies on the effect of artificial mycorrhiza on the growth of seedlings of common pine and hanging birch in the first year of growth in the conditions of the nursery SFNR "Ertis ormany" showed a positive effect on survival, growth in height and diameter.

In the first year of growth, mycorrhiza had a more effective influence on survival on deciduous trees than on coniferous trees. Survival rate in 2019 was 83.3% for *betula pendula* Roth., 55.2% for *pinus sylvestris* L., and 81.4% for *betula pendula* Roth.and 53.3% for *pinus sylvestris* L., in 2020.

We have studied changes in the physical and chemical properties of chestnut soils of forest nurseries of the Karaganda and Pavlodar regions under seedlings of tree crops when applying mycorrhizal biologics. Studies have shown that when applying this preparation to the soil under seedlings, there were small changes in the amount of absorbed bases and the reaction of the soil solution (table 4).

Table 4-Changes in the physical and chemical properties of chestnut soils under tree seedlings after the introduction of mycorrhizal biopreparation.

The depth of sampling, cm	pH	Absorbed bases, mg-EQ per 100 g of soil			Absorbed bases, in % of the amount or capacity	
		Ca^{2+}	Mg^{2+}	the amount	Ca^{2+}	Mg^{2+}
Option *Pinus sylvestris* L. without making a biological product, Pavlodar region						
0-20	7,1	5,60	1,60	7,20	77,78	22,22
Option *Pinus sylvestris* L. with making a biological product, Pavlodar region						
0-20	7,0	5,7	1,55	7,25	78,62	21,38
A variant of the *Betula pendula* Roth.without any biological product, Pavlodar region						
0-20	7,15	5,50	1,50	7,00	78,57	21,43
A variant of the *Betula pendula* Roth. introduction a biological product into the soil, Pavlodar region						
0-20	7,1	5,60	1,50	7,10	78,87	21,13

The studied chestnut soils of forest nurseries have a neutral, weakly alkaline reaction of the upper horizons. As a result of the research, it was found that the pH of the soil varies slightly in all the studied variants. The soils where mycorrhizal biologics were introduced under the seedlings of tree crops led to a very weak decrease in the reaction of the soil solution in the studied depths. It is shown that mycorrhiza reduces the pH in the rhizosphere due to the selective absorption of ammonium NH+ ions and the release of h+ ions [9.10].

It is also observed in the indicator of the amount of absorbed bases of chestnut soil of the forest nursery of the Pavlodar region, where the amount of exchange cations increased by 0.25 mg-EQ per 100 g of soil under the seedlings of ordinary pine and slightly lower by 0.10 mg-EQ per 100 g of soil under the seedlings of hanging birch compared to the control variants. The share of calcium cation in the soil-absorbing complex when using the biopreparation is 78.62% under pine seedlings, 78.87% under birch seedlings, these indicators are higher by 0.84% and 0.30%, respectively, compared to the controls.

The free mycelium of mycorrhizal fungi contributes to the aggregation of soil particles and modifies the soil structure, which affects the overall physical properties of the soil. Hyphae of mycobionts are involved in the stabilization of soil micro aggregates by binding soil particles and accumulating organic compounds. Aggregation allows you to maintain a porous but stable soil structure and prevent erosion. One of the most important compounds secreted by fungal hyphae is glomalin glycoprotein [11].

Conclusion: Specific characteristics of sapling development parameters depend on the type of plant and age: the average current growth of the the *betula pendula* Roth.was higher than of the *pinus sylvestris* L.

Studies on the effect of artificial mycorrhiza on the growth of seedlings of *pinus sylvestris* L.and *betula pendula* Roth.in the first year of growth in the conditions of the nursery SFNR "Ertis ormany" showed a positive effect on survival, growth in height and diameter.

In the first year and second year of growth in 2019-2020, mycorrhiza had a more effective influence on survival on deciduous trees than on coniferous trees. The survival rate of *betula pendula* Roth.was 83.3%, and *pinus sylvestris* L. 55.2%.

When conducting research on mycorization of seedlings during planting, a positive effect of mycorrhiza on the growth of both coniferous and deciduous plants was noted. In common pine, the differences in height, crown diameter along and across the row, and the current increase in comparison with the control are significant, more than three, and equal, respectively: 6,4; 7,9; 4,7; 3,1. The diameter of the root neck does not differ significantly - 0.8.

Significant differences in height were observed in the hanging birch: t = 20.9.

The need to study mycorrhizal formation in coniferous seedlings in forest nurseries in Kazakhstan is caused as a way to improve the quality of planting material.

Further research should be directed to expanding the range of nurseries studied to make a reasonable judgment about the relationship between soil characteristics and the technologies used for growing planting material with the success of mycorization; to study possible correlative (or functional) relationships between the development of ectomycorrhizas and the success of seedling development at the nursery level as a whole and at the level of individual correlations.

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