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**COMPREHENSIVE TREATMENT OF SPRUCE (PICEA PUNGENS)  
SUBSTRATE AND SEEDS AS A FACTOR TO INCREASE GERMINATION  
КОМПЛЕКСНАЯ ОБРАБОТКА СУБСТРАТА И СЕМЯН ЕЛИ КОЛЮЧЕЙ  
(PICEA PUNGENS) КАК ФАКТОР ПОВЫШЕНИЯ ВСХОЖЕСТИ**



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**Abstract.** High cost of cell-space using modern cassettes for growing conifers and low germination of spruce seeds makes it relevant to discover methods to increase the germination of the crop. The purpose of the experiment was to find the possibility to increase the germination of picea pungens seeds by pre-sowing treatment of seeds and substrate with biological products based on humates, boron and microelements, as well as with bacteria *Bacillus subtilis* and mycorrhiza. Seed treatment with Borogum-M and

Phytosporin-M statistically significantly increased the germination of picea pungens seeds by 6.4%. The treatment and supplemental application of 33 Bogatyrs and Kormilitsa Mycorrhiza products to the turfy substrate increased the germination to 68.1 %, which exceeded the control by 29.8 %.

**Аннотация.** Высокая стоимость ячейка-места при использовании современных кассет для выращивания хвойных и низкая всхожесть семян ели колючей делает актуальным является выявление способов повышения всхожести этой культуры. Целью опыта было выявление возможности повышения всхожести семян ели колючей путем предпосевной обработки семян и субстрата биопрепаратами на основе гуматов, бора и микроэлементов, а также бактериями *Bacillus subtilis* и микоризой. Обработка семян препаратами Борогум-М и Фитоспорин-М статистически значимо повысила всхожесть семян ели колючей на 6,4 %. При данной обработке и дополнительном внесении в торфяной субстрат препаратов 33 богатыря и Кормилица Микориза всхожесть увеличилась до 68,1 %, что превысило контроль на 29,8 %.

**Keywords:** Picea pungens, seeds, germination, pre-sowing treatment, substrate, biological products, mycorrhiza.

**Ключевые слова:** ель колючая, семена, всхожесть, предпосевная обработка, субстрат, биопрепараты, микориза.

## INTRODUCTION

Recently, growing of conifer seedlings in nurseries has been significantly changing. The problem of low survival rate of conifer seedlings after planting in reforestation was expected to be solved by growing with root-balled tree system in cassettes. However, the use of conventional seedling cassettes (by analogy with seedling cultivation of vegetables and flowers) did not bring the expected result, since long roots of seedlings were twisted in the cells with negative consequences for further plant growth (Gough A.A. et al., 2019). Therefore, more expensive recessed cassettes with slots for air root cutting are used for this purpose now. However, significant increase of cassettes cost led to the following

problem - cost price of cell-space increased, which caused the need for high seed germination.

Meanwhile, in nurseries when propagating *picea pungens* (its popular form with blue needles) low seed germination is often observed. The reasons may be hidden in self-pollination of mother plants and lack of seed stratification (Cram, W.H., 1984). However, even if the technology of seeding with stratification is followed, the germination of spruce seeds is far from 100%, since the germination is affected by other factors - possible infestation of seeds by pathogenic microbial flora (Boiko T.A. et al., 2012), absence of symbiont fungi in sterile turfy substrate, etc.

Spruce seed germination can be increased by modern products (Muraya L.S. et al., 2017), which makes further optimization of production processes in nurseries possible. Therefore, it is relevant to find possible ways to increase the germination of spruce seeds through a comprehensive treatment of seeds and soil by the products that increase seed germination in other plants and restrain the development of pathogenic microbial flora and ensure the development of symbiotic mycorrhiza.

The purpose of the experiment was to find the possibility to increase the germination of *picea pungens* seeds by pre-sowing treatment of seeds and substrate with biological products based on humates, boron and microelements, as well as with bacteria *Bacillus subtilis* and mycorrhiza.

## **LITERATURE REVIEW ON PRE-SOWING TREATMENT OF PICEA PUNGENS SEEDS AND SUBSTRATE**

One of the described promising ways to increase the germination of spruce seeds is to treat them with humic products. P.V. Tupik (2010) notes increase in spruce germination by 9 % and germination energy by 20 % when treated with humates. Ustinova T.S. and Zurov R.N. (2010) obtained the similar data for treatment of spruce and pine seeds with humic products.

Interesting results can be obtained by combining the effect of humic acids with the trace element boron, particularly in Borogum-M, which increased the germination energy and germination even of such a cereal as barley, which initially has good characteristics

of germination (Panfilov A.L., Abdrashitov R.R., 2022). Here it should be noted that the quantitative increase of field germination when treating seeds with boron depends on the initial germination capacity of seeds. In case with high germination seeds, boron increases this indicator insignificantly: in case with wheat seeds increase of germination was noted by 2,5 % (Bazarbaeva S.M. et al., 2006; Myazin N.G. et al., 2019). In case of seeds with lower initial germination, boron treatment increased germination significantly higher: by 5% - vetch (Zolotarev V.N., 2015), 8% - shamrock (Palchikova M.M., 2003), 10-12% - pumpkin (Bezruchenok N.N. et al., 2017), 13% - pea (Gromov A.A. et al., 2009), 18% - oil radish (Novak S.O. et al., 2019).

As reflected by literature sources, studies of the combined effect of humic acids and boron in Borogum-M on the germination of spruce seeds have not been conducted before us yet.

One of the ways to increase the germination of seeds of some plant species is to treat them with mycorrhiza of symbiotic fungi. For instance, symbiotic mycorrhiza has a stable positive effect on germination of orchid seeds (Masuhara G.A.K.U., Katsuya K., 1989; Porras-Alfaro A., Bayman P., 2007; Herrera, H. et al., 2017). In millet (*Panicum virgatum* L.) of c.v. Kanlow, symbiotic mycorrhiza increased seed germination by 52% (Ghimire S.R. et al., 2009), in radish (*Brassica rapa*) it reduced seed germination period (Gutowski, V., 2015). Pepper (*Capsicum annum* L. var. *aviculare*) has a positive effect of mycorrhiza on seed germination (Rueda-Puente E.O. et al., 2010). However, the effect of mycorrhiza on seed germination of conifers has not been sufficiently studied yet.

Fungi get carbohydrates from the roots and, in turn, supply plants with phosphorus, nitrogen and other elements of mineral nutrition, as well as perform the function of protecting the root systems from phytogenic organisms. Therefore, mycorrhizal plants are characterized by higher viability and biomass than non-mycorrhizal plants. In addition, the ability of mycobionts to accumulate mineral and organic substances in their cells ensures the stability of woody plants when growth conditions deteriorate (Zagirova S., Tworozhnikova T., 2008).

According to Boyko T.A. (2007), when growing spruce seedlings in nurseries on natural soils, abundance of mycorrhizae can serve as a reliable criterion for assessment of seedlings condition. However, when growing conifers according to modern technology with a closed root system (seeding in cassettes) in substrates such as peat, vermiculite or perlite, plants are deprived of their usual mycorrhizal symbiosis. The problem can be solved by supplemental application of mycorrhizal products to the substrate. It is relevant to find the practical effect of such products in order to improve the technologies of coniferous trees propagation in nurseries.

One of the new microproducts to use in agriculture and forestry is Kormilitsa Mycorrhiza biological product, consisting of mycelium and spores of the fungus genus *Glomus*, colonized by it fragments of roots and turf. The usage of the products showed a positive effect on the biometric indicators of wheat, pea, garden onion and increased the indicators of plant root mycorrhization (K.V. Sviridova, 2021).

It should be noted that in addition to mycorrhizal products, spores of natural forest strains can be transmitted to the substrate used for sowing together with the seeds, but provided the seeds are not dressed with chemical products. However, it is a negative factor not to treat seeds before sowing, since they might have spores of harmful phytopathogens on them. Protection from phytopathogenic fungi is especially important for the seeds of coniferous plants. For instance, according to Boyko T.A. et al. (2012), severe contamination of the seed surface of common spruce with pathogenic fungi spores was observed in 80% of seed batches in Perm Krai. In this regard, it is important to find the possibilities of seed pre-sowing treatment with biological products that have a repulsive effect on pathogens of seedlings.

One of the most common microorganisms used in agriculture for these purposes is the bacterium *Bacillus subtilis* (hay bacillus). Several biological products are based on it, including Fitosporin-M. The protective effect of this bacterium is based on the repulsive effect of its products on pathogenic microflora, which might positively affect all stages of plant development, including seed germination. Turner J.T. and Backman P.A. (1991) noted increase in germination energy and germination of peanut seeds when treated with *Bacillus subtilis*.

In laboratory conditions, pre-sowing treatment of pine seeds with a suspension of *Bacillus subtilis* increased the germination of pine seeds by 4% (Grodnitskaya I.D., Kondakova O.E., 2014). The authors noted high antagonistic activity of *Bacillus subtilis* to phytopotogenic fungi of *Fusarium* genus.

The effect of *Bacillus subtilis* on useful mycorrhizal fungi is unclear, but under certain conditions, for instance, under a lack of moisture, treatment of seeds with strains of these phyto-bacteria led to an increase of mycorrhization of wheat and awnless brome roots (Kuramshina Z.M. et al., 2015).

From new complex microbiological products we can mention 33 Bogatyrs, containing 40 strains of soil microorganisms that provide biological protection against pathogenic infections and involve in the transfer of nutrient elements of the soil complex into easily digestible by plants.

The effectiveness of 33 Bogatyrs product was noted in carrot crops: when the drug was applied to the soil at the rate of 20 kg/ha, the plant infestation with powdery mildew and *Alternaria* was reduced by 2.5 times, the yield of root crops increased by 7 t/ha (Abdulvaleeva G.R. et al., 2022). The application of 33 Bogatyrs product at a rate of 2 g per plant in a hole when planting garden strawberry seedlings increased berry yield for more than 2 times, increased vitamin C content in fruits by 43 %, the sugar content by 27 % compared to control (Davletov A.M. et al., 2022).

## **STUDY METHODOLOGY**

Study subjects: spruce seeds (*Picea pungens*, blue form), turfy substrate, Borogum-M, Fitosporin-M, 33 Bogatyrs, Kormilitsa Mycorrhiza products.

The turfy substrate consisted of highmoor peat deoxidized to pH = 5.5-6.0, 0-20 mm fraction.

The products for seed and substrate treatment have not been chosen randomly, they are the part of Anti-stress High Yield Farming technology, developed by BashIncom company to increase the germination of crop seeds (Kuznetsov V.I. et al., 2011; Sergeev, V.S., Dmitriev A.M., 2014) and its further development - AC-35 technology of the same company.

Borogum-M is a product containing boron in organic-humic form (4%) and a set of other trace elements at the following concentrations: S - 0,17 %, Fe - 0,05 %, Cu - 0,2 %, Zn - 0,01 %, Mn - 0,02 %, Mo - 0,05 %, Co - 0,005 %, Ni - 0,001 %, Li - 0,0002 %, Se - 0,0001 %, Cr - 0,0002 %. Fe, Cu, Zn, Mn, Co, Ni, Li, Cr are contained in chelate form. Borogum-M contains potassium salts of BMV-humic acids - 1 % and Phytosporin-M (titer no less than  $5 \times 10^8$  CFU/ml).

Phytosporin-M is a live symbiotic bacterial culture *Bacillus subtilis* 26D strain (titer of live spores and cells no less than 1 billion/ml).

33 Bogatyrs is a complex of 40 strains of soil microorganisms in spore form, which improve the availability of nutrients to plants and perform protective functions against pathogenic microflora.

Kormilitsa Mycorrhiza is a bio product that consists of peat colonized by mycelia and spores of the fungus of the genus *Glomus*.

The scheme of the experiment included the following variants:

Variant 1 - seeds and peat without treatment (control);

Variant 2 - seeds treated with Borogum-M and Fitosporin-M, without adding products to the peat.

Variant 3 - seeds treated with Borogum-M and Fitosporin-M, adding 33 Bogatyr and Kormilitsa Mycorrhiza products to the peat.

Microdividing experiments (1 plot - 1 cassette with 81 cells) were laid in 7-fold repetition, the arrangement of the cassettes was randomized.

The experiment was conducted in Tsvetnik Urala nursery located in Blagoveshchensky district of the Republic of Bashkortostan, vil. Ukman, in the greenhouse complex and container sites of the nursery.

Early winter sowing was performed in greenhouses in November 2021, with further placement of cassettes in the open area under the snow for natural stratification of seeds. Variants 1 and 2 were used for pre-sowing soaking of seeds for 12 hours (with periodic airing) in Borogum-M (5 ml per 1 liter of water) and Fitosporin-M (5 ml per 1 liter of water) products. In addition, in variant 3 we introduced 33 Bogatyrs (15 ml per 1 L of



peat) and Kormilitsa Mycorrhiza (10 ml per 1 L of peat) cassettes products into the peat. Evaluation of full sprouts was performed on May 20, 2022.

## RESULTS AND DISCUSSION

In spring evaluation of germination showed that spruce seed germination (blue form) was significantly affected by both seed treatment and application of bio products to the peat substrate (table).

**Table. Spruce (*Picea pungens*) seed germination depending on seed and peat treatment with bio products (Blagoveshchensky district of the Republic of Bashkortostan, Nursery "Tsvetnik Urala", 2022)**

Variants of the experience	Seed germination, %
Control (seeds and peat without treatment)	38,3
Seeds treated with Borogum-M and Fitosporin-M	44,7
Seeds treated with Borogum-M and Fitosporin-M, adding 33 Bogatyrs and Kormilitsa Mycorrhiza products to the peat	68,1
LSD <sub>05</sub>	3,1

Without the treatment, the seeds germinated only by 38.3 % - apparently affected by the factors described in the literature review. It should be noted that this low germination rate is a rather common phenomenon, which causes the need to sow 3 seeds in the cell cassette at once (nested seeding) with subsequent breakthrough.

Treatment of seeds with Borogum-M and Fitosporin-M statistically significantly increased the germination of spruce seeds, but only by 6.4 %. However, this treatment and supplemental application of microorganisms (33 Bogatyrs product) and mycelium (Mycorrhiza kormilitsa) to the peat allowed germination to increase more significantly, amounting to 68.1 %, which was higher than the control by 29.8 %.



The production experience has shown that germination of spruce seeds (*Picea pungens*) can be improved by treating seeds and substrates with micronutrients and bio products. For production cultivation of *Picea pungens* seedlings we can recommend pre-sowing treatment of seeds with Borogum-M, Fitosporin-M and introduction of 33 Bogatyrs and Kormilitsa Mycorrhiza products into peat substrate.

## CONCLUSION

Treatment of spruce seeds (blue form) with Borogum-M and Fitosporin-M, with the addition of 33 Bogatyrs and Mycorrhiza Kormilitsa products into the peat substrate increases the germination of seeds by 29.8 %.

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