PHYSICOCHEMICAL BIOLOGY

Original article

DOI: https://doi.org/10.21285/2227-2925-2023-13-1-99-106



Possibility the use biopreparations from coniferous raw materials in artificial reforestation of Scots pine

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Abstract. Ecologically safe biopreparations for agriculture are obtained from the waste of natural raw materials – greenery (needles), which remains after large-scale logging operations. These products (fir extract and spruce extract) contain natural triterpene acids, plant phenolic compounds and flavonoids. Although the growth regulators with similar characteristics are used in forest nurseries, there is still no data about the seedlings growth that were obtained under the influence of these preparations and then planted in the forest. The goal was to study the effect of biological preparations on the Scotch pine growth when it grown in a forest nursery, and then, in the forest culture. The experiments with pre-sowing treatment of pine seeds and its planting were conducted in the forest nursery. Seeds were soaked in preparation solutions (doses of 0.1 and 0.25 ml/kg of seeds) for 6 h. After growing of pine trees in the nursery, plot with cultures from these seedlings was created in the forest. Before planting in the forest, the seedlings were measured (stem height, root collar diameter). Measurements of pine trees were also taken three years in a row in the forest plot (stem height, root collar diameter, increment). Shown that a once seeds treatment by biostimulants ensured high survival and adaptation of pine when it was transplanted into the forest. The biometric characteristics of young trees were higher in the third year than in the ones from the control variant. Concluded that biostimulants obtained from coniferous raw materials are recommended for Scots pine growing in nurseries and increasing the forest cultures sustainability.

Keywords: biopreparation, Pinus sylvestris, coniferous raw, forest cultures, plant biostimulants, pine seedlings

Funding. This work was performed within the State program of the Botanical garden of the Urals branch of RAS and the Institute of chemistry of FRC Komi science center of the Ural Branch of RAS. The study of a biological product from spruce extract was supported by Russian Science Foundation (grant no. 21-73-20091).

For citation: Andreeva E.M., Stetsenko S.K., Terekhov G.G., Khurshkainen T.V., Kutchin A.V. Possibility the use bi-opreparations from coniferous raw materials in artificial reforestation of Scots pine. *Izvestiya Vuzov. Prikladnaya Khimiya i Biotekhnologiya = Proceedings of Universities. Applied Chemistry and Biotechnology.* 2023;13(1):99-106. https://doi.org/10.21285/2227-2925-2023-13-1-99-106.

ФИЗИКО-ХИМИЧЕСКАЯ БИОЛОГИЯ

Научная статья УДК 630*173/174:631.811.98

Возможности использования биопрепаратов, полученных из древесной зелени хвойных пород, в искусственном лесовосстановлении сосны обыкновенной

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Аннотация. Экологически безопасные биопрепараты для сельского хозяйства получают из отходов природного сырья – древесной зелени хвойных растений, которая остается после масштабных лесозаготовительных работ. Эти биопрепараты (экстракт пихты и экстракт ели) содержат натуральные тритерпеновые кислоты, растительные фенольные соединения и флавоноиды. Хотя в лесных питомниках используются регуляторы роста с аналогичными характеристиками, до сих пор нет данных о росте сеянцев, полученных под влиянием этих препаратов и затем высаженных в лесу. Цель работы – изучить влияние биопрепаратов на рост сосны обыкновенной при выращивании ее в лесном питомнике, а затем, после пересадки, – в лесных культурах. Опыты с

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предпосевной обработкой семян сосны и их посевом проводились в лесном питомнике. Семена замачивали в растворах препаратов (дозы 0,1 и 0,25 мл/кг семян) в течение 6 ч. После выращивания сосны в питомнике в лесу был создан участок с культурами из этих сеянцев. Перед посадкой в лес у них измеряли следующие показатели: высоту стволика и диаметр корневой шейки. Промеры деревьев сосны также проводились 3 года подряд на лесном участке (высота ствола, диаметр корневой шейки, прирост). Показано, что однократная обработка семян биостимуляторами обеспечила высокую приживаемость и адаптацию сосны при пересадке в лес. Биометрические характеристики молодых деревьев на 3-й год были выше, чем у контрольного варианта. Сделан вывод о том, что биостимуляторы, полученные из хвойного сырья, могут быть рекомендованы для выращивания сосны обыкновенной в питомниках и повышения устойчивости лесных культур.

Ключевые слова: биопрепарат, биостимуляторы растений, сосна обыкновенная, хвойное сырье, лесные культуры, сеянцы сосны

Финансирование. Работа выполнена в рамках государственного задания Ботанического сада УрО РАН и Института химии ФИЦ Коми НЦ УрО РАН. Исследование биопрепарата из экстракта ели проведено при финансовой поддержке Российского научного фонда (проект № 21-73-20091).

Для цитирования: Андреева Е.М., Стеценко С.К., Терехов Г.Г., Хуршкайнен Т.В., Кучин А.В. Возможности использования биопрепаратов, полученных из древесной зелени хвойных пород, в искусственном лесовосстановлении сосны обыкновенной // Известия вузов. Прикладная химия и биотехнология. 2023. Т. 13. N 1. C. 99–106. (In English). https://doi.org/10.21285/2227-2925-2023-13-1-99-106.

INTRODUCTION

Large-tonnage logging waste – coniferous tree greens – is a raw material for obtaining biologically active extracts, which can be the basis for the development of plant growth regulators [1–3]. These preparations are environmentally friendly, have a multifunctional effect, have low toxicity to humans, and can be widely introduced to agricultural and forest farms [4]. Preparations with a growth-stimulating effect are currently actively used in the cultivation of coniferous seedlings, which makes it possible to increase seed germination, increase biometric characteristics and seedling resistance when growing planting material in nurseries [5–7]. We found only a few studies that examined the fate of seedlings grown with the use of such biostimulants and subsequently transferred to forest sites [8].

However, the success in survival (preservation) and the active growth of conifers trees at the first years after transplanting to the forest environment is a very important aspect in the formation of a sustainable and productive artificial plantation.

A new biological product requires approbation not only in the laboratory, but also in the field experiments. Two preparations obtained by the emulsion extraction method from fir (fir extract) and spruce (spruce extract) tree greens using an aqueous alkaline solution as an extractant. This method makes it possible to simultaneously extract a complex of lipophilic and hydrophilic compounds with different polarity without the use of organic solvents [9].

The preparations were successfully tested for agricultural plants and received state registration¹ [4, 10]. Fir extract contains the natural triterpene acids, spruce extract – plant phenolic compounds and flavonoids. Currently, studies of these preparations for coniferous plant species are being carried out [11].

The goal was to study the effect of biological preparations on the Scotch pine growth when it grown in a forest nursery, and then, in the forest culture.

MATERIALS AND METHODS

Growing of pine seedlings (*Pinus sylvestris* L.) was carried out in the Berezovsky forest nursery (Sverdlovsk region). Pine seeds of the first quality class, collected in the Sverdlovsk region, were given by the Yekaterinburg Forest Seed Station Department of the Forest Protection Center of the Chelyabinsk Region. Experience was performed out in 5 variants: 1 – control and 4 – experimental (with biological products of fir extract (FE) and spruce extract (SE). Before sowing, the seeds were soaked in solutions of the preparations with doses of 0.1 and 0.25 ml/kg for 6 h. The seeds of the control variant were treated only with distilled water.

The size of the experimental and control sites in the nursery is 1×1 m, the repetition of each variant is three times. The seeding corresponded of the requirements for forest nurseries in the Ural region and was 1.5 g per running meter².

Agrotechnical care for plants was made according to the recommendations for growing seedlings in the nursery². The pine seedlings have grown for two years. At the end of the second vegetation season, some part of seedlings was dug up for measuring biometric parameters: stem height, root collar diameter (at least 50 seedlings); the safety of seedlings was carried out (number per 1 running meter).

The remaining seedlings in the next year spring were planted to the forestry area in the vicinity of Berezovskii city. Seedlings were planted in the control – 177 copies, with FE 0.1 – 118 ones, with FE 0.25 – 129 ones, with SE 0.1 – 158 ones, SE 0.25 – 118 ones. Every year at the end of the growing season in each variant the safety

¹Gosudarstvennyy katalog pestitsidov i agrokhimikatov, razreshennykh k primeneniyu na territorii Rossiyskoy Federatsii = State catalogue of pesticides and agrochemicals permitted for use on the territory of the Russian Federation. 2022. (In Russian). URL: www.agroxxi.ru/goshandbook (11.12.2022).

²Guidelines for the cultivation of tree and shrub planting material in forest nurseries of the Ural region. Moscow, 1998. 172 p. (In Russian).

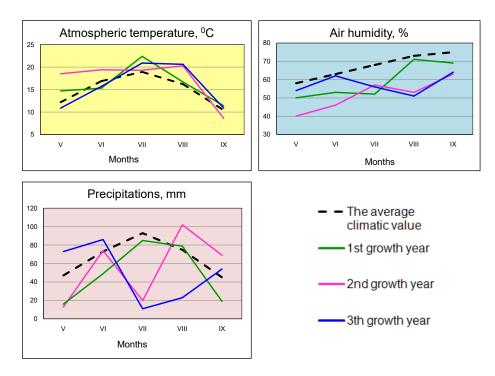


Fig. 1. The growing seasons weather characteristics on the researching plots in the experimental period^{3,4}

Рис. 1. Погодные характеристики вегетационного периода на исследуемых площадках в экспериментальный период^{3,4}

of the planted seedlings was fixed (number of survivors seedling from of the original ones, %), and also the diameter and height of the stems for all seedlings were measured.

The characteristics of the main weather factors affecting the growth of trees during the growing seasons at the study period are shown in Fig. 1.

As can be seen, the weather characteristics had deviations from the average climatic values – the some increasing of atmospheric temperature and a lack of humidity were noted.

Measurements of pine trees were also taken three years in a row in the forest plot (stem height, root collar diameter, increment). Also, there was counted the survival of cultures (the number of living trees remaining from planted ones, %).

Statistical data analysis was performed in the program Statistica 6.0. Arithmetic averages and standard errors are calculated for the obtained metric features. The reliability of the differences in the averages is established with the t-test.

RESULTS AND DISCUSSION

Pine seedlings in different variants at the end of the second year of growth in the nursery did not have significant differences in the root collar diameter (Table). Only variant with spruce extract, 0.1 ml/kg was thicker of control on 13.6%. The greatest height of the seedlings was noted in the variants using the studied biostimulants in small doses – fir extract, 0.1 and spruce extract, 0.1 (by 9.2 and 17.2% compared with the control, respectively).

Safety and averages (Mean + SE) of growth characteristics 2-year-old pine seedlings in nursery

Сохранность и показатели роста (среднее + SE) у 2-летних сеянцев сосны, выращенных в питомнике

Variant	Safety of seedlings, pcs/running m	Root collar diameter, mm	Stem height, sm
Control	120.6	2.2±0.08	8.7±0.30
Fir extract, 0,1	126.4	2.1±0.05	9.5±0.26
Fir extract, 0,25	134.3	2.1±0.07	8.0±0.29
Spruce extract, 0,1	121.3	2.5±0.07	10.2±0.36
Spruce extract, 0,25	137.7	2.2±0.07	8.3±0.30

³Archive weather in Yekaterinburg, URL: https://rp5.ru/Archive_weather_in_Yekaterinburg (11.12.2022).

⁴Klimaticheskie tablicy. Dannye dlja Ekaterinburga = Climate tables. Data for Yekaterinburg. (In Russian). URL: http://www.pogodaiklimat.ru/climate/28440.htm (11.12.2022).

The safety of seedlings in the experimental variants was at the control level or slightly higher, especially in the variants with the use of the spruce and fir extracts with a dose of 0.25.

The safety of pine seedlings a month after planting on the forest area was 91.1–100% in different variants. The survival of forest cultures was more in the first two years of growth, a slight decrease occurred in the third after planting (Fig. 2). Perhaps the influence of weather factors affected, because, all years of pine trees growth were accompanied by rather hot and dry weather with low precipitation in summer (Fig. 1). It should also be taken into account that the site on which the pine was planted is located in the outskirts of the city, near the private houses, so the influence of recreational load, including trampling, is not excluded. Generally, at the end of the third year of cultures growth, pine in the experimental variants had a 9-31.5% greater survival (with the exception of the FE, 0.25 ml/kg), compared to the control.

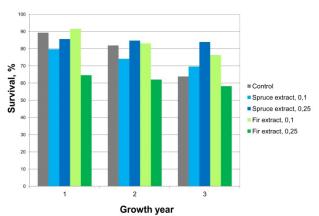


Fig. 2. The survival of pine trees in forest cultures at the first three growth year

Рис. 2. Приживаемость сосны в лесных культурах в первые 3 года роста

As well as when the growth of seedlings in the nursery, higher value of the pine stem height, compared with the control, was noted in the variants with the use of small doses of preparations (0.1 ml/kg) at the first two years of trees growth (Fig. 3). At the third year, the increase of height in all experimental variants was larger than the control: by using high doses of biostimulants by 24.3–26.1%, small doses – by 38.4–42.3%. Thus, the activity of biopreparations most likely helped to overcome the difficult conditions of drought for the pine in the experimental variants. At the end of the growing season, the largest value of the stem height significantly exceeded the control indicator in the variant with fir extract, 0.1 ml/kg by 35%, in the spruce extract variant, 0.1 ml/kg – by 25%.

Also, in experimental variants, the stem height exceeded the control values by 4.6% in the variant with fir extract (0.25 ml/kg) and by 11% with spruce extract (0.25 ml/kg), but the differences were not reliable.

The root collar diameter was significantly larger than the control values in the variants where small doses biopreperations were applied (Fig. 4); in the fir extract variant, 0.25 ml/kg was slightly more than the control (by 10.7%), there were no differences from the control in the spruce extract variant, 0.25 ml/kg.

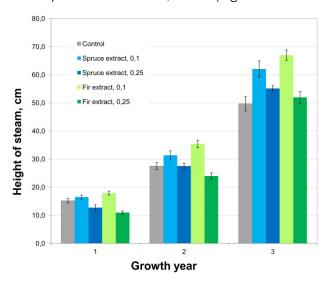
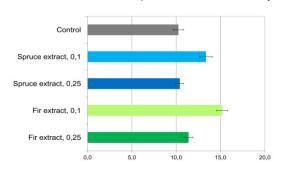


Fig. 3. The stem height of pine trees in forest cultures

Рис. 3. Высота стволика деревьев сосны в лесных культурах



Root collar diametr, mm

Fig. 4. The root collar diameter of pine trees in forest cultures at the third growth year

Рис. 4. Диаметр стволика сосны в лесных культурах на 3-й год роста

Since the biostimulants that we used in this experiment are based on extracts of plant materials, they are organic products consisting of a whole complex of various components [12, 13]. The conversion to a larger-scale use of natural compounds instead of artificially synthesized stimulants in the agro-industrial sector has begun relatively recently, so the composition and mechanism of action of these preparations is under study. Metabolic reactions of plants, leading to an increase in linear sizes, are also being studied - this is either stretching and an increase in the volume of plant cells, or an increase in their number due to more active synthesis. The latter case for long-growing conifers is more preferable, since a denser cellular structure ensures that the mechanical strength of growing trees is maintained. The pine response to the use of stimulants is supported by scientific data that the triterpene glycosides contained in the fir extract (which are one of the main active ingredients of this preparation) are able to

PROCEEDINGS OF UNIVERSITIES. APPLIED CHEMISTRY AND BIOTECHNOLOGY 2023 Vol. 13 No. 1 ИЗВЕСТИЯ ВУЗОВ. ПРИКЛАДНАЯ ХИМИЯ И БИОТЕХНОЛОГИЯ 2023 ТОМ 13 N 1

stimulate the most important biochemical processes in germinating wheat seeds (hydrolysis of storage starch, biosynthesis protein), which form the basis of active plant growth [14]. In addition, an increase in the dry weight of pine due to an increase in the content of macro elements under the influence of a coniferous preparation containing natural terpenoids was found [5]. The biological preparation from spruce extract is also a complex product introduced into agricultural practice later by fir extract, and its full composition and mechanism of action on plant metabolism is under study. However, it is already known that spruce phenolic compounds play an important role in growth and protection, including inhibition due to abiotic stresses [15]. In any case, the main factor that determines the study and practical use of such stimulants is their wide effectiveness, which is not inferior to the level of artificially synthesized products impact, their undoubted environmental safety, as well as their contribution to the development of resource-saving technologies in the timber industry.

The creation of forest cultures often occurs at the reason to the impossibility of restoring forest trees site in a natural way due to the lack of seed source trees or with difficult soil conditions. The experimental plot was located on the outskirts of the city, where there was no possibility of a quick forest restoration, although the place was characterized by optimal conditions for the growth of pine [16]. At the same, the faster growth of weedy herbaceous vegetation could cause the suppression of slower growing pine during its adaptation to the forest area. At the experiment, the planting of pine seedlings on the forest area was carried out in moist soil and in cool weather. This had a positive effect on the survival rate of seedlings after their transplantation into the forest. However, during the following years of trees growth, there were periods when the atmospheric temperature significantly exceeded the long-term average, and there was little precipitation. Observations at the first three years made it possible to establish that the biostimulants provided the more active young trees growth and helped to overcome negative abiotic factors affecting the survival of pine seedlings during their growth in the forest area. At the end of the third growing season, the pine parameters in the variants with fir extract and spruce extract exceeded the control ones, which may indicate a positive effect of the this biostimulants on pine growth.

Last time research on the use of biological preparations from the needles of fir, spruce, pine, larch for growing seedlings in nurseries has been actively conducted. These works are carried out in laboratories and field conditions in nurseries, where their influence on seed germination, growth and safety of seedlings, resistance to fungal diseases is studied [5, 6, 17–19].

According to the results of many years of research conducted in the nurseries of the Sverdlovsk region, the authors proposed a method "Method for stimulating the growth rate of Scotch pine seedlings" [20].

Obtaining of high quality planting material for the main forest-forming coniferous species is only the initial stage in the process of reforestation. The next important stage is the transplantation of seedlings into the forest environment, where the conditions are more stringent than in the nursery. Planting forest cultures from seedlings grown with the use of biostimulants is a pioneer work, especially in the Middle Urals conditions. Based on the results of further observations of the Scotch pine forest plantations, recommendations will be given for the use of these preparations for growing seedlings and forest plantations in other regions.

CONCLUSIONS

Ecologically safe preparations of fir extract and spruce extract, produced from fir and spruce tree greens, were once used for pre-sowing treatment of Scots pine seeds. Two-year pine seedlings grown in the nursery had higher stem heights in experimental variants with the applying of biostimulants in small doses.

Such growth trends persisted after the planting of seedlings to the silvicultural area for the first two years. At the end of third year, the highest values were noted in the experimental variants, where fir extract and spruce extract was used in the dose 0.1 ml/kg (+35 and +25% compared to the control, respectively).

The increased safety and biometric characteristics in the experimental variants, compared with the control ones, at the end of the third growing season, allow us to say that the survival rate and adaptation of the pine grown with the use of biostimulants in the forest area was successful. Concluded, there are the positive effect of fir extract and spruce extract plant growth regulators for obtaining Scots pine seedlings in nurseries and the subsequent applying of these plants for the forest cultures creation.

At further experiments, it is necessary to expand the range of applied doses of biostimulants in order to clarify their optimal doses for Scots pine. Also, it is necessary to study the effect of biopreparations not only on pine, but also on other widespread coniferous trees – spruce, larch, fir, etc.

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Andreeva E.M., Stetsenko S.K., Terekhov G.G., et al. Possibility the use biopreparations from coniferous raw ... Андреева Е.М., Стеценко С.К., Терехов Г.Г. и др. Возможности использования биопрепаратов, полученных ...

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The authors contributed equally to this article.

Conflict interests

The authors declare no conflict of interests regarding the publication of this article.

The final manuscript has been read and approved by all the co-authors.

Information about the article

The article was submitted 27.01.2023. Approved after reviewing 15.02.2023. Accepted for publication 28.02.2023.

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Вклад авторов

Все авторы сделали эквивалентный вклад в подготовку публикации.

Конфликт интересов

Авторы заявляют об отсутствии конфликта интересов.

Все авторы прочитали и одобрили окончательный вариант рукописи.

Информация о статье

Поступила в редакцию 27.01.2023. Одобрена после рецензирования 15.02.2023. Принята к публикации 28.02.2023.