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Growth stimulant influence on biometric indicators of oak seedlings in the Bukovyna Sub-Carpathian region

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Abstract. The use of growth stimulants for the cultivation of oak seedlings in the Bukovyna Sub-Carpathian region allows to accelerate their development and increase their sustainability in an environment where there is no natural forest regeneration. The aim of the study was to evaluate the effect of growth stimulants at different multiplicity of treatments during the growing season

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on the biometric parameters of *Quercus robur* L. Seedlings were grown in Ecotherm containers in the glasshouse of the base nursery. The composition of the substrate for filling the containers is a mix of peat, sand and black soil in equal proportions with mycorrhiza from under the forest canopy of oak stands. The results of the effect of growth stimulants on the biometric parameters of one-year-old seedlings of common oak with a closed root system are presented. Plant growth stimulants were used for spraying and irrigation seedlings during their cultivation. The results of the research indicate a positive effect of the use of growth stimulants on the seedlings of common oak. All experimental variants showed a positive effect on the biometric parameters of one-year-old oak seedlings at three, six and nine times of feeding with growth stimulants during the growing season. The difference in the variants with the use of plant growth stimulants compared to the control in height is up to 27%, in the length of the root system – up to 43%, and in the total length – up to 29%, accordingly, depending on the dose of the stimulants and the frequency of treatment during the growing season. Refined data on the biometric parameters of one-year-old seedlings of common oak with a closed root system grown in closed ground conditions were obtained. The data obtained are useful for forest restoration in regions with damaged or degraded ecosystems where natural regeneration is limited

Keywords: planting material; seedling height; root system length; seedling feeding; cultivation

Introduction

Improving the efficiency of forest plantations and preserving the forest fund are key tasks for ensuring environmental sustainability and biodiversity. Optimal methods of growing and caring for forest crops contribute to the rapid growth and health of trees, which ensures their resistance to diseases, pests and climate change. Biometric parameters of tree seedlings include growth parameters such as height, trunk diameter, number of leaves and root mass, which are important for assessing their development and adaptation to local conditions.

The reproduction methods of plantations involving common oak (artificial, natural or combined) are among the methods of plantation reproduction. According to M. Rumiantsev *et al.* (2022), the most common is artificial, i.e. the creation of forest crops. The production of *Quercus robur* L., which accounts for most hardwood plantations in the Bukovyna Sub-Carpathian region, continues to increase, which will continue to grow in the future (Tkach *et al.*, 2019), so the success of reforestation largely depends on the type and quality of planting material, which can be improved by certain growth stimulants. In Ukraine and abroad, various biologically active substances are actively used to improve the efficiency of growing planting material, in particular plant growth and development regulators (Tkaczyk *et al.*, 2022). They can significantly improve the resistance of plants to biotic and abiotic stress factors, which contributes to yield increase along with quality improvement (Raspopina *et al.*, 2022). The use of growth stimulants for growing oak seedlings is not widespread, which is confirmed by only a few scientific studies on this issue. Therefore, the studies on the influence of growth stimulants on the biometric parameters of one-year-old containerized seedlings of common oak with a closed root system in the conditions of Bukovyna Sub-Carpathian region are relevant.

The use of growth stimulants is a means of reducing the negative impact of the environment on plants to accelerate the formation of generative organs and roots, which improves important physiological processes, intensifies the hydrolysis of sugars and proteins, and activates photosynthesis. Modern plant growth regulators and other biological products consist of a complex of biologically active substances that activate metabolic processes in soil and plants, increase plant resistance to adverse conditions and contribute to the most efficient use of their productive potential. Following N. Paradikovic *et al.* (2019), growth stimulants include a variety of substances applied to the seed surface, root system or leaf surface. They can improve plant nutrition, and increase their resistance to various stresses, regardless of the supply of nutrients to plants. Modern, highly effective growth stimulants include herbal preparations, as well as humic and fulvic acids. Preparations based on amino acids, chitosan, seaweed extract, and humic substances also have high effectiveness on the plant (Lymar & Kholodnyak, 2021).

Preparations containing protein hydrolysates have an anti-stress effect on plants. In addition, they stimulate growth processes, improve the absorption and assimilation of nutrients, increase yields, and improve the development of the root system and leaf mass. Following S. Corsi *et al.* (2020), growth regulators increase plant resistance to adverse environmental conditions, such as low air and soil temperatures, significant daily temperature fluctuations, lack of moisture, negative effects of pesticides, etc.

N. Boiko *et al.* (2021) stated that all stimulants, due to the complex of biologically active substances, have high physiological activity and are capable of regulating plant growth and development. Modern growth regulators on a natural basis are safe for the environment, humans and insects, increase metabolic processes in the soil and improve its physical, chemical and biological properties.

The main components of plant growth promoters are auxin, cytokinin, gibberellin, abscisic acid and ethylene, as well as non-traditional phytohormones such as brassinosteroids, salicylic acid and jasmonic acid. Auxins are one of the most extensively studied phytohormones and are known to be involved in the regulation of growth and shape formation stimulating cell elongation and activating enzymes responsible for cell wall strength (Bilous *et al.*, 2023). According to H. Boiko *et al.* (2021), microorganisms can be potential producers of auxins, gibberellins and vitamins through the release of biologically active substances. The production of growth-stimulating substances by microbial strains can have a positive effect on the quality and quantity of seeds.

G. Benitez *et al.* (2020) determined that the complex effect of plant growth regulators on physiological, biochemical and metabolic processes in plant organisms has an anti-stress effect and unlocks the productivity potential inherent in plants. The use of growth stimulants in silviculture was studied by M. Savushchyk *et al.* (2020). They concluded that the need to use these products is determined by a decrease in the yield of high-quality planting material in nurseries as a result of prolonged pressure on the soil, especially with unjustifiably high doses of various herbicides, which reduces soil fertility.

Growth simulators are widely used in agriculture to increase the yield and quality of grain, vegetable, melon, and berry crops (Palamarchuk, 2023). However, there is little experimental data in the literature on the effect of such agents on the development of seedlings, cuttings and seedlings of woody plants. Therefore, research on the impact of promising growth stimulants and complex mineral fertilisers for growing seedlings of common oak can be used as a scientific basis for the creation of environmentally friendly and intensive technologies for growing high-quality planting material capable of withstanding plant diseases and pests in a short time.

The study aims to determine the effect of the multiplicity of treatments with growth stimulants of common oak seedlings on the biometric parameters of one-year-old containerized seedlings with a closed root system in the conditions of the Bukovyna Sub-Carpathian region.

Materials and Methods

To assess the impact of plant growth stimulants on the quality of common oak planting material, a series of experimental plots were laid in 2023 in the greenhouse of the basic forest nursery of the State Enterprise "Hertsaiv State Specialised Forestry of the Agro-Industrial Complex" (Chernivtsi region). The influence of different concentrations of growth stimulants at different multiplicity of treatments on the seedlings of common oak was addressed.

The following growth stimulants were selected for the study: Vermibiomag NPK (Herbicom LLC, Ukraine) and Ecostim-1 (Agrosvit LLC, Ukraine). These are products are characterised by a low consumption rate, which has an optimal impact on the environment. Vermibiomag NPK is a soluble preparation with organic and mineral biogenic microelements, humic and fulvic acids, as well as biostimulants. It reduces the negative impact of stress factors, such as drought, extreme temperatures, pesticides and soil salinity and the contents of nitrites, radionuclides and heavy metals in the soil, and has the properties of a natural immunostimulant with rapid inhibition of the development of pathogens and pathogens.

Ecostim-1 with auxin complex promotes intensification and stimulation of plant growth due to the rational correlation of growth and development of plant cells and organs. The product is suitable for the treatment of plants in any conditions – from open and closed ground to hydroponic systems and can also be used for the treatment of seedlings.

The treatment of common oak seedlings was carried out for one, two and three months with the studied preparations in different doses with the addition of a growth stimulator. A total of 45 containers were selected (Fig. 1): 5 per 1 experiment variant. Vermibiomag NPK was applied in doses of 1000 ml, 2000 ml and 3000 ml per 1 ha, and Ecostim-1 – 100 ml, 200 ml and 300 ml per 1 ha with the addition of microelements at the rate of 200, 400 and 600 ml per 1 ha, respectively. One variant was used as a control (without treatment).

Figure 1. Containers with common oak seedlings

Source: authors' photo

The study used cassette trays Ecotherm (Energy Saving Technologies, Kyiv, Ukraine) for sowing seeds and obtaining planting material. The overall dimensions of the tray are 650×312×180 mm. The trays are intended for use in forest nurseries for planting seedlings of deciduous and coniferous tree species, shrubs and other planting materials. These trays are made of a special material with low moisture absorption properties. Substrate composition for filling containers: a mixture of peat in the proportions of 33.3% peat, 33.3% sand, and 33.3% black soil with mycorrhiza from under the canopy of oak trees. To obtain the desired fraction, the mixture was sieved before mixing.

For all research variants, the treatment began on June 23 with Vermibiomag NPK, 24 hours later – with Ecostim-1 solution with the addition of trace elements and was carried out every 15 days. Thus, the seedlings in trays 1, 2, and 3 were treated three times within a month, 4, 5, 6 – six times within two months, and 7, 8, 9 – nine times within three months.

After the end of the growing season, a part of the middle seedlings in each variant (control batch) was selected from the grown oak seedlings, laboratory measurements of biometric parameters (height of the aerial part, length of the root system and total length of seedlings) and the yield of standard seedlings were carried out. For an objective assessment of the effectiveness of growth stimulants during the cultivation of common oak seedlings, the average values of the control (without treatments) were addressed. They were used to compare all the indicators of the experimental seedlings. Experimental plant research complied with national and international guidelines. The standards of the Convention on Biological Diversity (1992) were used in the study. The data obtained were processed using the methods of variation statistics using the MS Excel software package.

Results and Discussion

The results of studies on the effect of plant growth regulators on the growth characteristics of common oak seedlings are presented in Table 1.

Table 1. Average biometric parameters of *Quercus robur* seedlings

Experiment variants	Height of the aerial part of the seedlings, cm	Deviations from control, %	Length of the root system of seedlings, cm	Deviations from control, %	Overall length of seedlings, cm	Deviations from control, %
Vermibiomag NPK 0.1 ml/m², Ecostim-1 0.01 ml/m² + micronutrients 0.02 ml/m²						
Container No. 1 (triple processing)	15.4	+20	27.2	+14	42.6	+16
Container No. 4 (six times processing)	14.2	+11	23.1	-3	37.3	+2
Container No. 7 (nine times processing)	14.2	+11	23.9	+0.1	38.1	+4
Vermibiomag NPK 0.2 ml/m², Ecostim-1 0.02 ml/m² + micronutrients 0.04 ml/m²						
Container No. 2 (triple processing)	13.0	+2	34.1	+43	47.2	+29
Container No. 5 (six times processing)	13.3	+4	21.9	-8	35.2	-4

Table 1, Continued

Experiment variants	Height of the aerial part of the seedlings, cm	Deviations from control, %	Length of the root system of seedlings, cm	Deviations from control, %	Overall length of seedlings, cm	Deviations from control, %
Container No. 8 (nine times processing)	14.0	+9	24.0	+1	38.0	+4
Vermibiomag NPK 0.3 ml/m², Ecostim-1 0.03 ml/m² + micronutrients 0.06 ml/m²						
Container No. 3 (triple processing)	12.5	-2	26.1	+10	38.6	+5
Container No. 6 (six times processing)	14.1	+10	20.4	-14	34.5	-6
Container No. 9 (nine times processing)	16.2	+27	20.5	-14	36.7	+0.1
Control	12.8	–	23.8	–	36.6	–

Source: compiled by the authors

The average height of the seedlings ranged from 14.2 cm to 15.4 cm under the treatment with Vermibiomag 0.1 ml/m², Ecostim-1 0.01 ml/m² + microelements 0.02 ml/m², the length of the root system – from 23.1 cm to 27.2 cm, the total length – from 37.3 cm to 42.6 cm (Fig. 2). The best result of the application of the preparations was recorded at three treatments within 1 month (container 1). Notably, the seedlings from container 1 outperformed the control for all biometric parameters, while the six-fold (container 4) and nine-fold treatments (container 7. The growth of only the aerial part of the seedlings was stimulated, and the length of the root system was the same as in the control.

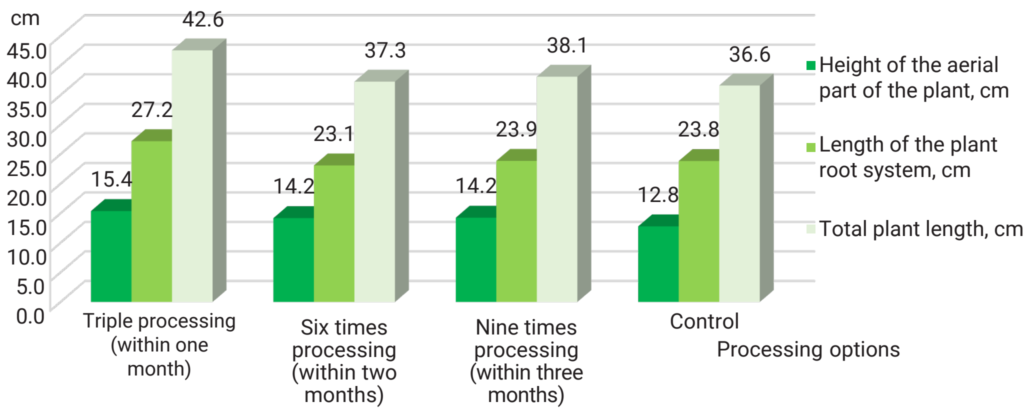

Figure 2. Average biometric parameters of seedlings when treated with Vermibiomag NPK 0.1 ml/m², Ecostim-1 0.01 ml/m² + microelements 0.02 ml/m²
Source: compiled by the authors

In general, the seedlings grown under such conditions exceeded the control by 20% in height, by 14% in root length, and by 16% in total length, respectively. All experimental variants significantly exceed the control both in height and length of the root system. A significant improvement in the overall condition of the plants, including an increase in leaf number and viability, was noted. The use of growth stimulants also contributed to a more uniform development of seedlings and increased their resistance to stressful conditions.

The average height of seedlings treated with Vermibiomag NPK 0.2 ml/m², Ecostim-1 0.02 ml/m² + microelements 0.04 ml/m² ranged from 13.0 cm to 14.0 cm, the length of the root system from 21.9 cm to 34.1 cm, and the total plant length from 35.2 cm to 47.2 cm (Fig. 3). At this concentration, three treatments within 1 month (container 2) are optimal, and at six treatments within 2 months, the total length of the seedling was less than the control (container 5). The study also noted that more frequent cultivation led to inhibition of root growth and a decrease in the overall viability of seedlings. This indicates the need for careful selection of processing modes to achieve optimal results.

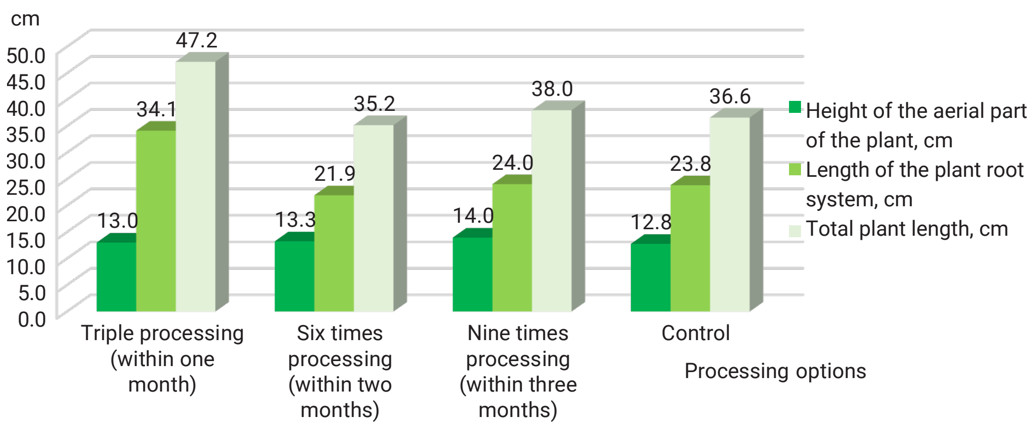

Figure 3. Average biometric parameters of seedlings when treated with Vermibiomag NPK 0.2 ml/m², Ecostim-1 0.02 ml/m² + microelements 0.04 ml/m²
Source: compiled by the authors

The tested preparations had a positive effect on the growth performance of common oak seedlings. In general, the seedlings grown under the three-treatment method outperformed the control by 9% in height, 43% in root length, and 29% in total length, respectively. The biometric parameters of seedlings do not fully characterise their quality, since the viability of plants depends on the size of the assimilation apparatus. In the experimental variants using concentrations of Vermibiomag NPK 0.2 ml/m², Ecostim-1 0.02 ml/m² + microelements 0.04 ml/m², a significantly better condition of plants was observed during the cultivation of oak seedlings with the use of three times fertilisation (Fig. 4).

The average height of the seedlings under the treatment with Vermibiomag NPK 0.3 ml/m², Ecostim-1 0.03 ml/m² + microelements 0.06 ml/m² ranged from 12.5 cm to 16.2 cm, the length of the root system from 20.4 cm to 26.1 cm, the total length of the plant from 34.5 cm to 38.6 cm (Fig. 5).

Figure 4. The general appearance of seedlings in variants treated with Vermibiomag NPK 0.2 ml/m^2, Ecostim-1 0.02 ml/m^2 + microelements 0.04 ml/m^2

Source: authors' photo

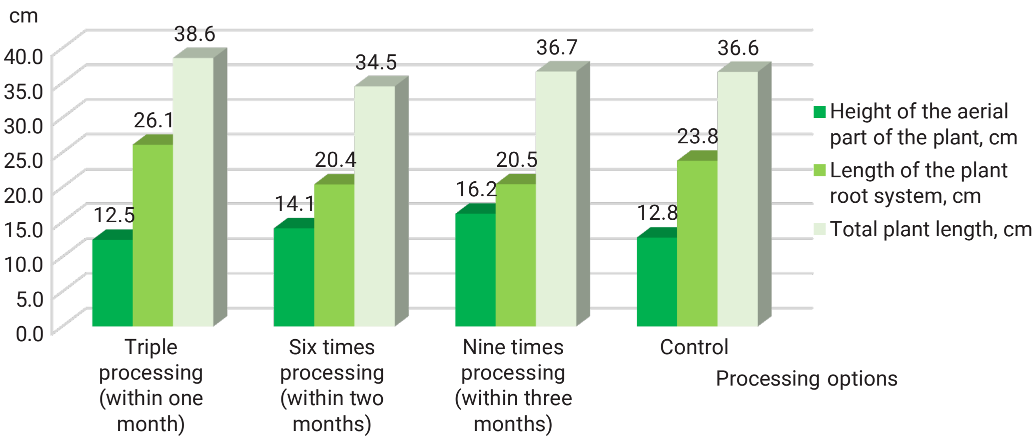

Figure 5. Average biometric parameters of seedlings when treated with Vermibiomag NPK 0.3 ml/m^2, Ecostim-1 0.03 ml/m^2 + microelements 0.06 ml/m^2

Source: authors' photo

In general, at this concentration, with nine treatments over 3 months, the height of the aboveground part exceeded the control by 27%, but the length of the root system was 14% shorter than the control (container 9).

The average total length of common oak seedlings is close to the control at different concentrations at six- and nine-times treatment, the best results were observed in all variants at three times treatment with Vermibiomag NPK, Ecostim-1 with the addition of microelements. Seedlings from container 2 (43%) significantly outperformed the control in terms of root length, and seedlings from containers 6 and 1 in terms of height (27% and 20%, respectively), but were significantly inferior to the control in terms of root length were seedlings from containers 6 and 9 (14%).

At the chosen concentrations, plant growth stimulants activated the growth of roots to a greater extent than the aerial part of seedlings, and according to the studies of O. Danylenko *et al.* (2021), M. Rumiantsev *et al.* (2022), plant growth regulators contribute to a more intensive increase in the mass of the aerial part and roots than in the height and diameter of the root collar of oak seedlings with a closed root system. S. Bhatla (2018) noted that plants respond to external stimuli, and feel changes in the environment, in particular temperature (Neill *et al.*, 2019). S. Fahad *et al.* (2016) noted the effectiveness of the use of growth stimulants at high temperatures, which affect plant morphology by reducing leaf area, aboveground and underground biomass, photosynthesis, and water use efficiency.

L.K. Abbott *et al.* (2018) believe that stimulants regulate many plant development processes, such as accelerating or delaying seed germination, stimulating or inhibiting shoot growth, inducing flowering and fruiting, etc. W. Rademacher (2015) studied plant growth regulators, which are important tools in modern agriculture that allow for controlling and optimising plant growth and development. They are used to improve product quality, increase yields and plant resistance to stressful conditions. Growth regulators can achieve sustainable results in the production of plant products. According to the research of several authors (Colla *et al.*, 2017; Paradikovic *et al.*, 2019), biostimulants increase the energy of seed germination, stimulate vegetation, improve the absorption and distribution of nutrients in the plant, increase the antioxidant capacity of plant tissues, increase resistance to stress factors and plant yields, and promote the rigosis of rooted cuttings during vegetative propagation. A. Pinchuk & Yu. Kosenko (2015) proved that the use of growth stimulants promotes the rooting of lignified cuttings of ornamental deciduous species, which significantly increases their survival rate and development speed. These products stimulate the formation of root hairs, improving the absorption of nutrients and water from the soil. The use of growth stimulants can help to achieve faster rooting and improve the overall quality of ornamental plants.

Thus, feeding seedlings with plant growth regulators increases their resistance and contributes to an increase in their biometric parameters compared to the control. However, it should be noted that the stimulation of seedling growth due to the influence of growth regulators sometimes occurs only after a certain period of general inhibition, and the most noticeable effect of the stimulator is observed 2-3 years after its application.

The use of growth regulators in forestry practice is driven by the decline in the production of high-quality nursery stock. This decline is attributed to signs of soil depletion and a progressive decline in fertility caused by long-term chemical exposure to the soil. This is especially true in cases of using increased doses of herbicides, which have proven to be harmful to soil biocenosis. This conclusion was reached by Yu. Taranenko (2012) and M. Savushchyk *et al.* (2020).

The use of plant growth stimulants has become a fairly common practice in the forestry sector of Ukraine when growing coniferous seedlings. This practice has been going on for about 20 years and covers different regions of the country, which is confirmed by numerous publications, such as V.A. Veshchytsky (2006) originating from Ukraine. Studies by V. Hudyma *et al.* (2014) determined a significant effect of growth stimulants on germination energy, seed germination, survival rate and biometric parameters of coniferous tree seedlings up to three years of age, compared to the control without growth stimulant treatment. V.V. Siryk *et al.* (2006) found that after treatment of Scots pine seeds with Emistim C, germination energy increased by 30-50%, and after treatment with Triman-1, germination increased by 5-37%.

O. Danylenko *et al.* (2021) proved that the most effective variants for the pre-sowing treatment of oak acorns were those in which Agrostimulin, Charcor, and Triman-1 were used. The greatest positive effect on biometric parameters and weight of one-year-old containerized seedlings during cultivation was noted in the variants where Megafol and Radifarm were used. The conducted studies evaluated the results of the use of plant growth stimulants in the cultivation of seedlings of common oak with a closed root system in open ground conditions, as well as for reforestation and afforestation. According to M. Rumiantsev *et al.* (2022), three times feeding of annual oak seedlings with a closed root system using plant growth stimulants Aminostim, Stovit TURBO, Radifarm plus, Megafol had a positive effect on their biometric parameters and general condition compared to the control (seedlings grown without the use of stimulants), which corresponds to the results of the current experiment using Vermibiomag NPK and Ecostim-1.

Therefore, to evaluate the effectiveness of growth stimulants in the cultivation of common oak seedlings, a series of experiments with different doses and frequency of treatments was conducted. The results showed that the most effective treatments were three times within a month, which contributed to a significant increase in the biometric parameters of seedlings compared to the control. The use of growth stimulants proved to be a promising tool for improving the quality of planting material in the conditions of the Bukovyna Sub-Carpathian region.

Conclusions

The study was conducted to improve the methods of growing oak seedlings with a closed root system using growth stimulants of different concentrations at different cultivation rates and to study their effectiveness. A positive effect on the height of annual oak seedlings during cultivation with a closed root system (six and nine times feeding of seedlings with plant growth stimulants during the growing season) was noted in all experimental variants. The results of the research show that almost all experimental variants prevailed over the control of the length of the aerial part of seedlings, but the effect of stimulants on the length of the root system was less effective in the variants of Vermibiomag NPK 0.1 ml/m^2, Ecostim-1 0.01 ml/m^2 + microelements 0.02 ml/m^2 and Vermibiomag NPK 0.2 ml/m^2, Ecostim-1 0.02 ml/m^2 + microelements 0.04 ml/m^2 at six times of treatment, and concentrations of Vermibiomag NPK 0.3 ml/m^2, Ecostim-1 0.03 ml/m^2 + microelements 0.06 ml/m^2 – at six and nine times of treatment. According to the results obtained, the use of Vermibiomag NPK and Ecostim-1 with treatment doses of Vermibiomag NPK 0.2 ml/m^2, Ecostim-1 0.02 ml/m^2 + microelements 0.04 ml/m^2, respectively, and three times treatment within a month has a positive effect on growth processes in the cultivation of oak seedlings. Thus, the use of physiologically active substances allows for the regulation of vital processes in the plant organism, activates its genetic potential and increases resistance. The results obtained during the research confirm the feasibility of using the studied plant growth stimulants for growing seedlings of common oak with a closed root system in closed-ground conditions and for reforestation and forestry in the conditions of the Bukovyna Sub-Carpathian region region.

It is worth noting that the most noticeable effect of stimulants is observed several years after their application, the study of biological bases, development of new and improvement of existing technological parameters of growing common oak saplings with a closed root system using growth stimulants, which allows to obtain high-quality and sustainable planting material, is a relevant task that requires in-depth scientific research.

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None.

Conflict of Interest

None.

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Вплив стимуляторів росту на біометричні показники сіянців дуба в умовах Буковинського Прикарпаття

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Анотація. Використання стимуляторів росту для вирощування сіянців дуба в умовах Буковинського Передкарпаття дозволяє прискорити їх розвиток та підвищити стійкість у середовищі, де природне поновлення лісу відсутнє. Мета дослідження полягала в оцінці впливу стимуляторів росту за різної кратності обробок протягом вегетаційного періоду на біометричні показники сіянців *Quercus robur* L. Сіянці вирощено в контейнерах Ecotherm в теплиці базисного розсадника. Склад субстрату для заповнення контейнерів – суміш в рівних пропорціях торфу, піску та чорнозему з мікоризою з-під намету дубових деревостанів. Наведено результати впливу стимуляторів росту на біометричні показники однорічних сіянців дуба звичайного з закритою кореневою системою. Стимулятори росту рослин використовували для обприскування і поливу сходів під час вирощування сіянців. Результати дослідження вказують на позитивний вплив застосування стимуляторів росту на сіянці дуба звичайного. Усі дослідні варіанти показали позитивний вплив на біометричні показники однорічних сіянців дуба при триразовому, шестиразовому та дев'ятиразовому підживленні стимуляторами росту протягом вегетаційного періоду. Різниця у варіантах із застосуванням стимуляторів росту рослин порівняно з контролем за висотою становить до 27 %, за довжиною кореневої системи – до 43 %, та за загальною довжиною – до 29 %, відповідно, залежно від дози препаратів та кратності обробки протягом вегетаційного сезону. Отримано уточнені дані щодо біометричних показників однорічних сіянців дуба звичайного з закритою кореневою системою, вирощених в умовах закритого ґрунту. Отримані дані корисні для відновлення лісів у регіонах з пошкодженими або деградованими екосистемами, де природне поновлення є обмеженим

Ключові слова: садивний матеріал; висота сіянців; довжина кореневої системи; підживлення сіянців; обробіток