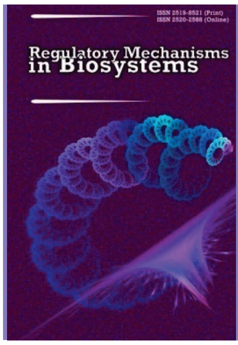


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# Prospects for the use of growth regulators in vegetative propagation technology of *Lavandula angustifolia*

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***Kobets, O., Derevianko, N., Zavhorodnii, M., & Berezovska, M. (2024). Prospects for the use of growth regulators in vegetative propagation technology of Lavandula angustifolia. Regulatory Mechanisms in Biosystems, 15(2), 198–206. doi:10.15421/022429***

Lavenders include some 28 species of evergreen aromatic shrubs and subshrubs belonging to the mint family, the Lamiaceae. They occur mainly around the Mediterranean and their flowers range from pink to purple and white. It is known that *Lavandula angustifolia* Mill. is an unpretentious decorative and essential oil crop that is widely used in the pharmaceutical, cosmetic, and food industries. Therefore, the planting material of this crop is in great demand in Ukraine. Cultivated varieties of lavender are very different for vegetative reproduction. A promising path is the testing for new effective and environmentally friendly rooting stimulants. The experiments were conducted in the Horticulture Laboratory of the Khortytsia National Academy. The study tested the influence of aqueous solutions of a complex of amino acids, polypeptides, amino sugars, hexuronic acids, and microelements called “Bioglobin”, a complex of inorganic compounds containing (nitrogen – 4.7%, phosphorus – 3.4%, potassium – 4.6%, iron – 1.6%, zinc – 1.6%) and organic compounds (alginate acid, betaine, polysaccharides, vitamin complex of groups B, C, D) called “Ferti Root”, a complex of saturated and unsaturated fatty acids ($C_{14}$–$C_{28}$), polysaccharides, 15 amino acids, analogs of cytokinin and auxin nature phytohormones called “Emistim S”, a complex of 2.6-dimethylpyridine-1-oxide with α-phenylacetic acid called “Charkor”, disodium salt of 2-(quinolin-4-ylthio) succinic acid on the rooting and development of varieties "Stepova", "Vdala", "Sineva" one-year woodcuttings. The indicators were evaluated in comparison with the control without processing the cuttings before planting, and with the use of (4-(indol-3-yl) butyric acid) called “Kornevin”. Mother plants are 2–3 years old. The experiment was repeated three times, 30 cuttings each. Woodcuttings were planted in open-door ground at early October. In May, the number of rooted cuttings, as well as the number and length of roots was counted. It was found that two preparations had the maximum positive effect on the cuttings rooting: disodium salt of 2-(quinolin-4-ylthio) succinic acid at a concentration of 5 mg/L and “Ferti Root”. Consequently, the rooting percentage increased by 18–20% in all researched varieties compared to the control. The length and number of new formed roots in these experimental versions exceeded the control indicators by 1.5–2.0 times. Other researched preparations did not show a positive effect on the rooting cuttings indicators.

*Keywords*: propagation of lavender; derivatives of quinoline; succinic acid; rooting; lipophilicity; grafting; survivability; biological activity.

## Introduction

The beneficial qualities of *Lavandula angustifolia* Mill., commonly known as narrow-leaved lavender, have been known to humanity for a long time. This plant is widely used in the perfume, cosmetics, pharmaceutical, food industry, and other sectors (Tsygankova et al., 2016; Aruna et al., 2018; Lieshchova & Brygadyrenko, 2021; Bilan et al., 2023). One significant advantage of this crop is its ability to be grown on land unsuitable for other agricultural crops (Mohilnikova et al., 2021). This plant is also of great interest as a decorative, long-blooming border plant. Many decorative varieties have been developed with various flower colors, including "Dwarf Blue," "Hidcote Blue," "Munstead" with dark purple, "Hidcote Giant" with bright purple, "Atropurpurea", "Twickel Purple" with dark purple, "Dutch," "Stepova" with light lilac, "Sineva" with blue, "Vdala" with lilac, "Nana Alba" with white, "Loddon Pink," "Rosea" with lavender-pink flower colors (Mykhalska et al., 2020). Therefore, this crop with its diverse uses attracts the attention of landscape designers, estate owners, photographers, as well as farmers who wish to obtain high and consistent profits from lavender perennial plantings over many years. However, the main limiting factor for the development of this direction in agriculture is the shortage of planting material. Plants of seed origin do not always inherit the qualities of the mother plants, and there is significant variability in morphological, biochemical, and ecological characteristics in the offspring (Tsygankova et al., 2022). Therefore, the only way to obtain uniform planting material with specific qualities is vegetative propagation. Lavender can be propagated by division, cuttings, and tissue culture. Despite its effectiveness, tissue culture requires significant financial investments and the availability of specialists and equipment, making it inaccessible to most domestic planting material producers. The division method has a very low multiplication coefficient, so it is not suitable for mass propagation. Therefore, cuttings are the most suitable method for obtaining lavender planting material.

The traditional method of propagation involves the use of root-inducing stimulants, typically auxins (β-IBA, heteroauxin, etc.). However, quality preparations have moderate toxicity and high costs. Therefore, a promising direction is to test substances of natural origin (derivatives of humic acids, carbonic acids, retardants, biologically active substances of plant and animal origin, etc.) as root-inducing stimulants that would be environmentally safe, cost-effective, and not inferior in efficacy to synthetic auxins.

In recent years, climate change has been observed worldwide, including in Ukraine, with increasing average temperatures and drought, which opens up opportunities for successful cultivation of crops that previously faced difficulties due to sensitivity to winter temperatures. Narrow-leaved lavender, an essential oil and decorative plant that was previously cultivated only in Crimea and southern Ukraine, now has the potential to be grown throughout Ukraine, leading to a growing demand for its planting material. Propagation of lavender by woody one-year-old cuttings is considered the most acceptable method, as it allows for the use of cuttings left after the autumn shaping pruning of mother plants (Fig. 1). Recent research has shown that the survival rate of cuttings from decorative and essential oil lavender varieties varies widely, making the use of root-inducing stimulants an essential element of this crop's propagation technology. Scientists have been actively searching for the most effective growth regulators for vegetative propagation of narrow-leaved lavender, including (Tsvilyiuk, 2018; Kremenchuk, 2020; Lozinska, 2022; Bahan et al., 2022).

One of the promising areas in the creation of new compounds for plant reproduction is the modeling of compounds that combine in their composition known pharmacophores of known natural and synthetic growth stimulants. Pyridine, quinoline, pyrimidine and anthracenedione occupy an important place (Brazhko et al., 2013; Tsygankova et al., 2018; Tsygankova et al., 2021). Various derivatives of these heterocycles are used both as synthons in organic synthesis and molecular design, and as known effective biologically active compounds.

One of the modern approaches in creating bioregulators for plant propagation involves the molecular design of natural and synthetic compounds that combine derivatives of nitrogen-containing heterocycles and residues of carboxylic acids. The heterocyclic systems of nitrogen-containing heterocycles have highly reactive positions, allowing for molecular design and the generation of libraries of new promising biologically active compounds (Brazhko et al., 2013, 2018; Kobets, 2022). The study of characteristic pharmacophores and molecular descriptors of the structure of derivatives 2-((6-R-quinolin-4-yl)thio)carboxylic acids suggests the possibility of a fairly wide range of biological activity of these compounds. It was found that the studied derivatives of (quinolin-4-ylthio)acetic and propionic acids are promising as potential growth regulators. Through molecular design, potentially bioactive molecules based on succinic acid were created for experimental research (Kornet et al., 2021; Yakovleva-Nosar et al., 2022; Zavhorodnii et al., 2022).

Furthermore, experiments are currently underway in Ukraine to synthesize new substances that could potentially have plant growth regulator properties. Some success in this direction has been achieved by scientists from Zaporizhzhia National University. According to recent research, one such substance could be dinatrium salt of 2-(quinolin-4-ylthio) succinic acid (Brazhko et al., 2013; Metelytsia et al., 2020).

The aim of this study is to determine the effectiveness of using a new growth stimulant, as root-inducing stimulants in the technology of vegetative propagation by woody one-year-old cuttings of narrow-leaved lavender varieties of Ukrainian selection, in comparison with traditional technology using synthetic auxins.

## Materials and methods

A new artificially synthesized substance, a derivative of quinolin-succinic acid with potential growth regulator properties – dinatrium salt of 2-(quinolin-4-ylthio) succinic acid (DQS) (Brazhko et al., 2013; Yakovleva-Nosar et al., 2022; Zavhorodnii et al., 2022).

Table 1 describes the composition and biological properties of the growth regulators that were used to perform the work.

*Lavandula angustifolia* varieties of Ukrainian selection on which the above-mentioned substances were tested:

*Narrow-leaved lavender "Stepova"*. Obtained at the Institute of Essential Oil and Medicinal Plants of the Ukrainian Academy of Agrarian Sciences, Simferopol, in 1957. The variety was registered in the State Register of Varieties in 1962. The bush is semi-spreading, up to 60 cm tall, with silvery elongated lanceolate leaves and flower spikes up to 35 cm long. The flowers are light lilac. The variety has a medium flowering period (mid-June to early July) and blooms for 5–6 weeks. It has a high essential oil content (2.0–2.2%).

*Narrow-leaved lavender "Vdala"*. Obtained at the Institute of Essential Oil and Medicinal Plants of the Ukrainian Academy of Agrarian Sciences, Simferopol. The variety was registered in the State Register of Varieties in 2008. The bush is compact, 55–60 cm tall, with silvery narrow-lanceolate leaves and flower spikes up to 30 cm long. The flowers are lilac. The variety has an early flowering period (early June to July) and blooms for 6 weeks. It has an essential oil content of 2.35%.

*Narrow-leaved lavender "Sineva"*. Obtained at the Institute of Essential Oil and Medicinal Plants of the Ukrainian Academy of Agrarian Sciences, Simferopol. The variety was registered in the State Register of Varieties in 1997. The bush is spreading, 65–70 cm tall, well-branched. The leaves are narrow, 2–6 cm long, and the flowers are blue. The variety has a medium flowering period (mid-June to July) and blooms for 6–8 weeks. It has an essential oil content of 2.1–2.3% (Kremenchuk, 2017).

**Table 1**
Description of the growth regulators used in the study

| No. | Commercial name | Solubility | Composition | Biological activity |
|---|---|---|---|---|
| 1 | "Bioglobin" | a water-soluble | a complex of amino acids, polypeptides, amino sugars, hexuronic acids, and microelements | the preparation stimulates root system growth in plants and reduces stress during transplantation |
| 2 | "Ferti Root" | a water-soluble | a complex of inorganic compounds containing (nitrogen – 4.7%, phosphorus – 3.4%, potassium – 4.6%, iron – 1.6%, zinc – 1.6%) and organic compounds (alginate acid, betaine, polysaccharides, vitamin complex of groups B, C, D) | the product is used to improve the survival of plants during transplantation, enhance nutrient absorption, and nutrition |
| 3 | "Emistim C" | a water-alcohol, transparent solution | a complex of saturated and unsaturated fatty acids ($C_{14}$–$C_{28}$), polysaccharides, 15 amino acids, analogs of cytokinin and auxin nature phytohormones | it increases seed germination energy and field similarity, plant resistance to diseases (brown rust, root rot, etc.), and stress factors (high and low temperatures, drought, phytotoxic effects of pesticides), increases yield, and improves the quality of plant products |
| 4 | "Charkor" | a water-soluble | a complex of 2.6-dimethylpyridine-1-oxide with α-phenyl-acetic acid | it promotes the growth of the root system in cuttings, improves their survival and rooting, stimulates plant immunity against pathogenic microflora, and reduces stress during transplantation |
| 5 | "Kornevin" | a water-soluble | 4-(indol-3-yl) butyric acid | it is used as a root-inducing stimulant in the traditional technology of plant propagation by cuttings |

Testing the above-mentioned substances as activators of rhizogenesis in the technology of cutting propagation of narrow-leaved lavender varieties of Ukrainian selection.

The plant growth regulators used in the study were as follows: dinatrium salt of 2-(quinolin-4-ylthio) succinic acid in the form of aqueous solutions with concentrations of 1, 5, and 10 mg/L (referred to as DQS). "Bioglobin" – in the form of an aqueous solution with concentrations of 1 and 2 mg/L. "Ferti Root" – in the form of aqueous solutions – 2.5, 2, 2 mg/L; 5, 3, 3 mg/L. "Emistim C" – in the form of an aqueous solution – 0.1 and 0.2 mg/L. "Charkor" – in the form of an aqueous solution with a concentration of 1 mg/L. "Kornevin" in the form of a powder. The content of the active substance was 5 g/kg.

Cuttings were taken from mother plants at the beginning of October during the fall pruning of the lavender plantation (Fig. 1). The age of the mother plants was 2–3 years. The cuttings were one-year-old apical shoots with a woody base and green tip, measuring 10–15 cm in length. In the control group, cuttings for rooting were planted without any prior treatment.

The methodology for preparing cuttings for planting in the experimental variants depended on the plant growth regulator used: DNHBK (2-(quinolin-4-ylthio) succinic acid dinatrium salt): cuttings were immersed with their basal parts in a water solution of DNHBK with the following concentrations for 12 hours before planting: 1st variant – 1 mg/L, 2nd variant – 5 mg/L, 3rd variant – 10 mg/L. "Bioglobin": cuttings were soaked for 24 hours with their basal parts immersed in solutions of the preparation with concentrations: 1st variant – 1 mg/L, 2nd variant – 2 mg/L. "Ferti Root": cuttings were planted for rooting without prior treatment. After planting, the cuttings were watered three times with a water solution of the preparation at one-week intervals. The working solution consumption was 20 mL per cutting. The first watering was done immediately after planting, with a concentration of 2.5 mg/L for the 1st variant and 5 mg/L for the 2nd variant. The second and third waterings had concentrations of 2 mg/L for the 1st variant and 3 mg/L for the 2nd variant. "Emistim C": cuttings were soaked with their basal parts for 12 hours in a water solution of the preparation with the following concentrations: 1st variant – 0.1 mg/L, 2nd variant – 0.2 mg/L. "Charkor": cuttings were soaked with their basal parts for 20 hours in a water solution of the preparation with a concentration of 1 mg/L. "Kornevin": just before planting, cuttings were first immersed with their basal ends in pure water and then in the powder preparation. This variant was used as an additional control since the use of auxins is part of the traditional cutting technology.

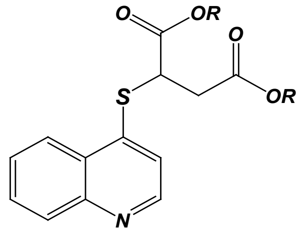
*a* *b* *c*

**Fig. 1.** Mother plants after the fall pruning: *a* –"Stepova", *b* – "Vdala", *c* – "Sineva"

The experiment was conducted with three repetitions, and each repetition included 30 cuttings. The cutting technique used was commonly accepted. The cuttings were planted for rooting in open ground in prepared beds. A substrate mixture of lowland peat with sand and garden soil (1:2:4) was used for rooting. The thickness of the substrate layer was 30 cm. The planting scheme for cuttings was 2.5 x 10 cm. The beds were covered with white agricultural fabric with a density of 60 g/m². In spring, after the establishment of an average daily temperature above +5 °C, the covering was removed. In May, assessments were made for cutting survival rates, the number, and length of roots.

The derivative of quinolinic-amber acid with potential growth regulator properties, dinatrium salt of 2-(quinolin-4-ylthio) succinic acid, was synthesized by the Department of Chemistry at Zaporizhia National University in collaboration with the Department of Horticulture at Khortytsk National Academy (Brazhko et al., 2013; Metelytsia et al., 2020; Zavhorodnii et al., 2022) (Fig. 2).

The 4-chloroquinolines ("IBS") were used as starting materials, as well as reagents and solvents ("UkrOrgSynthesis", Ukraine) for the synthesis of derivatives 2-(quinolin-4-ylthio) succinic acid. The general reaction scheme followed for the synthesis of selected 4-thioquinolines is presented in Figure 2. The reactions and the purity of the synthesized compounds were controlled by the TLC on Sorbton-2 plates. As an eluent, mixtures of chloroform-methanol (1:1) and acetate-water (1:1) were used. Manifestations of chromatograms were performed using UV rays. The 1H NMR spectra were recorded on the "Bruker AC-300", manufacturer Bruker, 2010, (300 MHz) device in DMSO-d6 and D2O. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane (TMS). The coupling constants (J) are reported in Hertz (Hz).




*R = H, Na, K*

**Fig. 2.** Structure of derivatives 2-(quinolin-4-ylthio) succinic acid

LC-MS spectra were recorded on a high-performance liquid chromatography module of the HPLC system for Agilent 1260 Infinity and a proton-ionization diode-matrix probe. Disodium salt of 2-(quinolin-4-ylthio) succinic acid was synthesized according to the well-known method (Brazhko et al., 2013; Metelytsia et al., 2020) with the corresponding physico-chemical and spectral data, which correspond to the literature. It is presented in Figure 3.

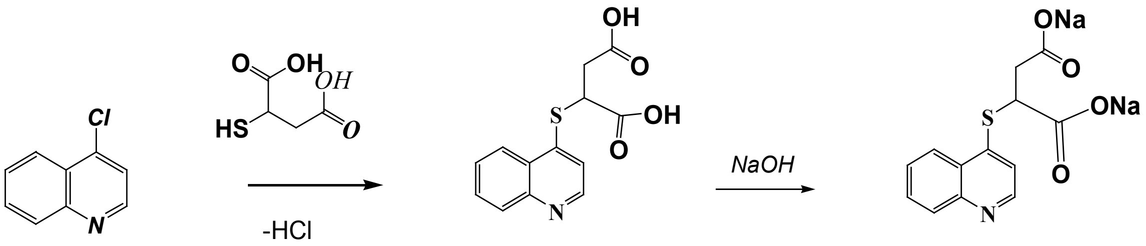



**Fig. 3.** Synthesis of  of the dinatrium salt of 2-(quinolin-4-ylthio) succinic

The researched compound is a hybrid molecule of the heterocycle quinoline and the residue of thiocarboxylic acid (mercap-topropanoic acid). It consists of a quinoline ring fused with the thioacid residue, forming a unique molecular structure. Molecular descriptors of structure: gross formula, elemental composition, molecular weight, molecular refractive index, Log P, Log D, investigated compounds were determined using the computer software package ACD-I-Labs. LogP is the partition coefficient of the compound between n-octanol and water, and Log D is the lipophilicity of the compound depending on the pH of the medium (Brazhko et al., 2018).

A key parameter in the study of the relationship between the structure and biological activity of organic compounds is the partition coefficient in the system of n-octyl alcohol – water. Correlations between the value of Pow and toxicity, penetration of artificial and natural membranes, biological activity of non-specific drugs, bioaccumulation, soil adsorption, etc. were found. However, the experimental determination of Pow is very time consuming. Therefore, it is generally accepted to use calculation methods for their evaluation. We confirmed the adequacy of additive methods of calculation of distribution coefficients, and completeness of a set of experimental values of Pow on which this model is built.

Toxicity studies of researched growth stimulants were carried out virtually and experimentally. To evaluate the toxic effect of in silico compounds, software solutions were used to build structure-toxicity models and predict $LD_{50}$ using models Gusar (Germany) (Martin, 2016; Brazhko et al., 2018b). To perform individual stages of calculation of molecular descriptors of the structure we used a number of software tools, such as: framework JSDraw, OpenBabel, PaDEL-Descriptor, McQSAR, Pandoc, ACD-I-Labs. The following molecular descriptors of the structure were calculated: gross formula, elemental composition, molecular weight, molecular refractive index, Log P, Log D (Table 1).

A technique was employed to calibrate the poorly water-soluble substance quickly and improve the accuracy of the result. This involved adding a co-solvent (hydrochloric acid or ethanol) to a specific volume of the aqueous phase, which increased the compound's solubility limit and eliminated calibration errors. Importantly, the co-solvent was not introduced into the extraction system but added to the sample of the equilibrium aqueous phase (Petrusha, 2010; Brazhko, 2020). The determination of the distribution coefficient was conducted by the "shaking" method, which involved extracting the substance with n-octanol (concentration range: $10^{-2}$ to $10^{-4}$ mol/L) from water (with a volume ratio of 5:1 water to octanol) (Petrusha, 2010; Brazhko, 2020). The system was shaken for one hour and left to stand for 24 hours at 25 ± 1 °C. After phase separation, a sample of the aqueous phase was collected and centrifuged at 3000 rpm to separate tiny droplets of n-octanol. Then, 10 mL of the aqueous phase was taken, avoiding any n-octanol contamination. The aqueous phase sample was mixed with the co-solvent (ethanol or hydrochloric acid) and analyzed in one- or five-centimeter cuvettes using a spectrophotometer SF-46 in the ultraviolet range.

Calibration was performed for 8–10 concentrations covering the studied range for the extraction system ($10^{-7}$ to $10^{-4}$ mol/L). The distribution coefficient was calculated as the tangent of the slope of the isotherm Sost = f(Cw) based on 7–12 data points (Fig. 4). This procedure allowed the determination of distribution coefficients for the substance of interest under the specified conditions.

Usually, 3–4 measurements at similar concentrations are conducted. In our experiments, the relatively large number of points on the extraction isotherm was motivated by the need to confirm the absence of possible compound dissociation, as well as to enhance the precision of the results (Table 2).

The partial coefficient P often refers to the partition coefficient, which is a measure of the solubility of a substance between two solvents, usually between octanol and water. It is expressed as the logarithm of the ratio of the substance's concentration in octanol to its concentration in water at equilibrium. Log P, known as the octanol-water partition coefficient, is calculated as the logarithm of the ratio of the substance's concentration in octanol to its concentration in water at equilibrium. Initially, it is necessary to bring the system to phase equilibrium, where the substance is dissolved in both water and octanol. It is important that both phases are saturated with the substance and do not chemically react with each other. The experimental calculation of Log P typically includes direct measurements of the substance's concentrations in octanol and water after reaching equilibrium. This indicator is important for assessing the hydrophobicity of chemical compounds, which affects their ability to penetrate through biological membranes and their bioavailability.

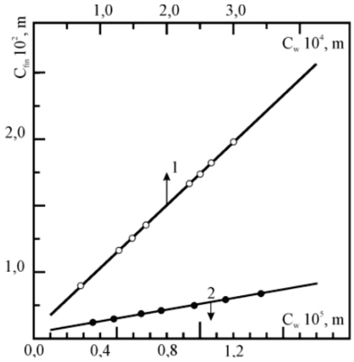



**Fig. 4.** Calibration for determining the pKa of growth stimulants was performed for 8–10 concentrations covering the studied range for the extraction system (from $10^{-7}$ to $10^{-4}$ mol/L)

It is known that organic compounds are generally weak acids and bases. Structural changes in their molecules lead to an increase or decrease in acidity and basicity. Therefore, by varying substituents in acids and bases, it is possible to control the degree of ionization, i.e., the number of ionized forms in solutions, which is important for the manifestation of biological activity. This control can affect substance permeability through membranes and interactions with membranes of neutral and ionized molecules.

It is also known that the activity of many antibacterial agents of the acridine group depends on the ionization constant of these compounds (Orlov, 2005). The most ionized compounds exhibit the highest biological activity. It is the concentration of the cation (rather than the total amount of the substance) that determines the antibacterial action of quinoline derivatives. We experimentally determined the pKa of the investigated growth stimulants using the potentiometric titration method (Fig. 5, Table 2). The value of pKa1 corresponds to the heterocyclic nitrogen, while the values of pKa2 and pKa3 correspond to the carboxyl group residue of the acid in the respective compounds.

For both compounds, there is a decrease in the basic properties of the heterocyclic nitrogen due to the influence of the electron-withdrawing substituent – the carboxyl group (negative inductive effect).

A particularly important characteristic of any biologically active substance is lipophilicity (hydrophobicity) – a model of the distribution of the substance studied between two phases that do not mix (most often used octanol:water). This characteristic is easily modulated by the use of an appropriate descriptor and is most often used to assess the ability of a substance to overcome the biological membranes of cells.

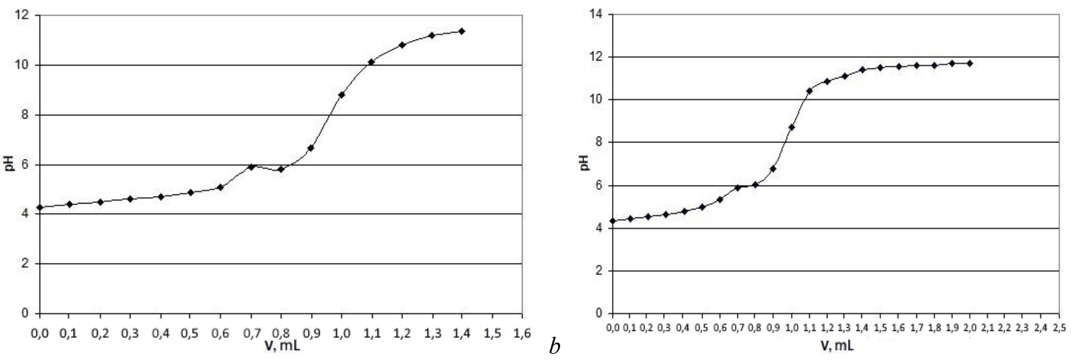



**Fig. 5.** Titration curves 2-(quinolin-4-ylthio) succinic acid (*a*) and 1H-indole-3-acetic acid (*b*)

**Table 2**
Molecular descriptors and toxicity tests of researched growth stimulants for treating winter lavender cuttings of the *L. angustifolia* variety "Stepova"

| Compounds | $M_r$, g/mol | C log P (neutral form) | log D (pH = 7) | Experimental Log P | pKa | $LD_{50}$ mg/L (calculations computer program GUSAR) | $LD_{50}$,DI*, mg/L (n = 3, experimental studies) |
|---|---|---|---|---|---|---|---|
| 2-(quinolin-4-ylthio) succinic acid | 321.26 | 1.08 | – 2.90 | 1.84 ± 0.48 | pKa1 = 5.04 ± 0.16<br>pKa2 = 4.49 ± 0.19<br>pKa3 = 2.84 ± 0.13 | 1217.3 | > 1200 (95%: 1100–1300) |
| 4-(indol-3-yl) butyric acid | 203.24 | 2.03 | – 0.80 | 2.34 ± 0.22 | pKa1 = 4.83 ± 0.10 | 2519.1 | > 1200 (95%: 1100–1300) |
| 1H-indole-3-acetic acid | 175.18 | 1.40 | – 0.81 | 1.43 ± 0.23 | pKa1 = 4.54 ± 0.10 | 2724.1 | > 1200 (95%: 1100–1300) |

*Note*: LogP (is a theoretically calculated partition coefficient in the ChemSpider software); ClogP (is a theoretically calculated partition coefficient in the ACD/Labs software); $LD_{50}$ (lethal dose 50%) – calculated in the program from databases and models based on studies on standard laboratory animals, such as rats and mice; $LD_{50}$, DI*(lethal dose 50%) – refers to experimental studies on the concentration of a substance at which 50% of zebrafish (*Danio rerio*) have died; LogD (the logarithm of the distribution coefficient) determines the solubility of a substance between two solvents at a given pH.

When the test substance is in the aqueous phase in the form of molecules (uncharged particles) to characterize lipophilicity we use the indicator log P (P – partition coefficient at the boundary of octane:water). If the test substance in the aqueous solution is partially dissociated in the form of charged particles (ions), there will be a certain dynamic equilibrium between the different forms of the compound, which will vary depending on the pH of the medium. The lipophilicity of such a system will be determined by the partition coefficient log D – the ratio of the sums of activities of all components of the organic and aqueous phases.

This characteristic is most often used to assess the ability of the substance to overcome biological membranes of cells of the root system depending on the pH of the medium. The pH of most plant cloning media is maintained in the range of 6.5 to 7.5.

It was found that the values of log D for the tested compounds are much smaller than the values of log P, this is due to the consideration in the second case of acid-base equilibrium, which is in solution of the test substances. The change in lipophilicity of the substance from the ability to dissociate into ions in aqueous solution is explained as follows. Since water is a polar solvent (μ = 1.86 D) and the dipole moment of octanol is much smaller (it can be taken as a non-polar solvent), the ions that will be formed in the aqueous medium will hardly diffuse into the organic layer and the concentration of ions in it will be caused mainly by the transition of uncharged molecules of matter, resulting in a significant reduction in the concentration of matter in the organic phase.

Dinatrium salt of 2-(quinolin-4-ylthio) succinic acid according to Lipinski's "rule five" can show high biological activity.

The acute toxicity ($LD_{50}$) of the investigated plant growth regulators was determined in vivo using the zebrafish (*Danio rerio*) hydrobiont model, following the OECD instructions for chemical testing.

In the experiment, 2-month-old fish with a length of 11.8 ± 0.1 mm and a weight of 2.6 ± 0.2 g were used. The concentrations of the tested compounds ranged from 0.1 to 100 mg/L. Adult *D. rerio* were kept in aerated aquariums with carbon-filtered tap water at a temperature of 26.5 ºC. This oxygen-enriched water was also used for the experiments. Before conducting the experiments, the fish were acclimatized, with a mortality rate of no more than 1 in 500 individuals. Water-soluble IP compounds were dissolved in DMSO, while water-soluble compounds were dissolved in distilled water. Each mini-aquarium with a specific compound dose contained at least 7 *D. rerio* individuals. During the experiments, the fish were kept on a diet for 96 hours, and their mortality was checked every 24, 48, 72, and 96 hours. The degree of toxicity of the tested compounds was determined using the classification by Passino et al. (1987). Data processsing was performed using SPSS (IBM SPSS Statistics 19) statistical software. The tables and figures show the arithmetic mean values and their standard error (x ± SE). Dunnett's Test was utilized for comparison between several experimental groups and one control group. In the SPSS program, the "comparing means" module was used. Differences were considered certain at P < 0.05.

**Results**

In the control variant of lavender cuttings of the "Stepova" variety (Table 3), rooting was observed at a level of 68.8%. The quality of the newly formed root system was good, with an average number of roots of 8.3 and a length of 7.2 cm. The use of traditional auxins ("Kornevin") improved both the survival rate (by 20.6%) and the quality of the root system. The average number of roots increased to 11 (an increase of 32.4%), and the root length increased to 10.4 cm (an increase of 59.6%).

**Table 3**
Effect of treatment of winter lavender cuttings of *L. angustifolia* variety "Stepova" with growth regulators on their survival and development (x ± SE, n = 7)

| Preparation | Solution concentration, mg/L | Processing times, hours | Rooting, % | Number of roots, piece | Length of roots, cm |
|---|---|---|---|---|---|
| Control | 0 | 24 | 68.8 | 8.34 ± 0.70 | 7.16 ± 0.66 |
| "Rootone" – indole-3-butyric acid (IBA): 0.1–1.0% naphthaleneacetic acid (NAA): 0.1–1.0% | 1 | 24 | 88.2 | 11.04 ± 0.54*** | 10.43 ± 0.34*** |
| "Bioglobin" – 1 mL of solution contains Bioglobin-U 5000 IU; in the list for dry residue – 20 mg: polypeptides 3.5-7%, amino acids 50–60%, aminosugars 4–5%, hexuronic acids 8–9% | 1 | 24 | 34.6 | 7.98 ± 0.34 | 8.88 ± 0.80 |
| | 2 | 24 | 53.4 | 8.65 ± 0.59 | 8.12 ± 0.45 |
| Ferti Root – alginic acid; betaine; polysaccharides; phytohormones; vitamin complex of groups B, C, D; N, $P_2O_5$, $K_2O$, Fe, Zn | 2.5+2+2 | 168 | 88.6 | 12.12 ± 0.17*** | 10.54 ± 0.60*** |
| | 5+3+3 | 168 | 90.0 | 12.67 ± 0.24*** | 10.98 ± 0.65*** |
| "Emistim C" – 1 L contains 1 g of saturated and unsaturated fatty acids ($C_{14}$–$C_{28}$), polysaccharides, 15 amino acids, analogues of cytokinin and auxin phytohormones | 0.1 | 24 | 44.8 | 11.40 ± 0.71*** | 8.67 ± 0.40 |
| | 0.2 | 24 | 65.4 | 12.42 ± 0.62*** | 9.33 ± 0.20** |
| "Charcor" – a complex of 2.6-dimethylpyridine-1-oxide with α-phenylacetic acid at a concentration of 8.3 g/L. | 1 | 24 | 65.5 | 10.05 ± 0.44*** | 8.44 ± 0.70* |
| "DNHBK" – dinatrium salt of 2-(quinolin-4-ylthio) succinic acid | 1 | 24 | 55.2 | 8.56 ± 0.70 | 8.84 ± 0.45* |
| | 5 | 24 | 81.8 | 12.44 ± 0.50*** | 12.80 ± 0.51*** |
| | 10 | 24 | 42.7 | 9.60 ± 0.81** | 8.04 ± 0.55* |

*Note*: this Table takes into account statistically significant differences compared to controls: * – P < 0.05; ** – P < 0.01; *** – P < 0.001.

The use of "Bioglobin" did not yield the expected results. The percentage of cuttings that successfully rooted was observed to be lower than the control, at 34.1% and 53.4%, respectively, for the two tested concentrations. The number of formed roots and their length remained at the same level as the control group. The "Ferti Root" treatment was effective in both variants of the experiment. The survival rate of the lavender cuttings reached 90.0%, which was 21.2% better than the control group. The number of roots formed increased to 12.1–12.7, which was 50% more than the control, and the length of the newly formed roots also increased by 50%. Lavender cuttings treated with "Emistim C" rooted less successfully than the control, with a rooting rate not exceeding 65.4% in both concentration variants. However, the quality of the newly formed root system was better than in the control group, with a 37% increase in the number of roots in the 0.1 mg/L concentration variant and a 49% increase in the 0.2 mg/L concentration variant. The length of the roots also increased by 21% and 30%, respectively, compared to the control. After treatment with "Charkor," the rooting parameters of the cuttings remained at the same level as the control. Among the three tested concentrations of the dinatrium salt of

2-(quinolin-4-ylthio) succinic acid preparation, the maximum positive effect was observed in the 5 mg/L variant. The rooting rate reached 81.8%, and the quality of the roots significantly improved, with a 49% increase in length and a 79% increase in the number of roots. Other concentration variants of this preparation provided only minor improvements in the root system's quality.

For the variety "Vdala," in the control group, 55% of the cuttings successfully rooted. The newly formed root system was weak, with an average of 6.4 roots and a length of 5.8 cm. Predictable results were achieved with the treatment of cuttings with "Kornevin," which increased survival (up to 78%) and significantly improved the length and number of roots (by 45.5% and 77.5%, respectively). Similar to the "Stepova" variety, "Bioglobin" did not produce the expected effect, with rooting percentages being 1.5–2.5 times lower than the control. The quality of the roots remained at the control level. The results of cutting rooting using "Ferti Root" significantly exceeded the control. In both concentration variants, the survival rate increased (up to 68.3–70.0%), and the number of roots increased 1.8–1.9 times, while the length of the formed roots increased by one and a half times.

The use of "Emistim C" and "Charkor" did not have a significant positive impact on the rooting of cuttings of this variety. The best result was achieved with "Emistim C" at a concentration of 0.2 mg/L and "Charkor," both of which provided rooting and root quality similar to the control group.

"DNHBC" was effective at a concentration of 5 mg/L: both the rooting percentage and the quality of the newly formed roots improved. Rooting was observed at a level of 72.2%, which was 18.2% better than the control group. The length of the roots increased to 10.9 cm, which was 1.7 times better than the control. The number of roots increased by 1.5 times. Smaller and larger concentrations provided rooting similar to the control group.

On the "Sineva" variety (Table 5), the same trend was observed. The percentage of rooting in the control group was 48.6%, and the root system was weak, with fewer than 7 roots and a length of less than 5 cm. Significant improvement in rooting (up to 72.6%) was achieved with "Kornevin." In this treatment, the quality of roots also improved: the number of roots increased by 36.5%, and their length by 73.1%. "Bioglobine," "Emistim C," and "Charcor" did not provide any improvement in rooting parameters.

**Table 4**
Effect of treatment of winter lavender cuttings of *L. angustifolia* variety "Vdala" with growth regulators on their survival and development (x ± SE, n = 7)

| Preparation | Solution concentration, mg/L | Processing times, hours | Rooting, % | Number of roots, piece | Length of roots, cm |
|---|---|---|---|---|---|
| Control | 0 | 24 | 55.0 | 6.40 ± 0.33 | 5.80 ± 0.23 |
| "Rootone" – indole-3-butyric acid (IBA): 0.1–1.0% naphthaleneacetic acid (NAA): 0.1–1.0% | 1 | 24 | 78.3 | 11.36 ± 0.51*** | 8.44 ± 0.34*** |
| "Bioglobin" – 1 mL of solution contains Bioglobin-U 5000 IU; in the list for dry residue – 20 mg: polypeptides 3.5–7.0%, amino acids 50–60%, aminosugars 4–5%, hexuronic acids 8–9% | 1 | 24 | 19.8 | 6.32 ± 0.54 | 5.12 ± 0.27* |
| | 2 | 24 | 43.3 | 6.36 ± 0.84 | 6.28 ± 0.74 |
| Ferti Root – alginic acid; betaine; polysaccharides; phytohormones; vitamin complex of groups B, C, D; N, $P_2O_5$, $K_2O$, Fe, Zn | 2.5+2+2 in a week | 168 | 68.3 | 12.34 ± 0.44*** | 8.34 ± 0.32*** |
| | 5+3+3 in a week | 168 | 70.0 | 11.65 ± 0.63*** | 8.75 ± 0.61*** |
| "Emistim C" – 1 L contains 1 g of saturated and unsaturated fatty acids ($C_{14}$–$C_{28}$), polysaccharides, 15 amino acids, analogues of cytokinin and auxin phytohormones | 0.1 | 24 | 30.8 | 5.58 ± 0.71** | 7.12 ± 0.34*** |
| | 0.2 | 24 | 61.0 | 6.74 ± 0.27 | 7.86 ± 0.71*** |
| "Charcor" – a complex of 2.6-dimethylpyridine-1-oxide with α-phenylacetic acid at a concentration of 8.3 g/L | 1 | 24 | 49.8 | 8.32 ± 0.33*** | 8.17 ± 0.39*** |
| "DNHBK" – dinatrium salt of 2-(quinolin-4-ylthio) succinic acid | 1 | 24 | 45.6 | 7.44 ± 0.54*** | 6.34 ± 0.37*** |
| | 5 | 24 | 72.2 | 10.85 ± 0.39*** | 8.76 ± 0.42*** |
| | 10 | 24 | 40.0 | 8.54 ± 0.59** | 5.44 ± 0.61 |

*Note*: see Table 3.

**Table 5**
Effect of treatment of winter lavender cuttings of *L. angustifolia* variety "Sineva" with growth regulators on their survival and development (x ± SE, n = 7)

| Preparation | Solution concentration, mg/L | Processing times, hours | Rooting, % | Number of roots, piece | Length of roots, cm |
|---|---|---|---|---|---|
| Control | 0 | 24 | 48.6 | 6.43 ± 0.36 | 4.76 ± 0.27 |
| "Rootone" – indole-3-butyric acid (IBA): 0.1–1.0% naphthaleneacetic acid (NAA): 0.1–1.0% | 1 | 24 | 72.5 | 8.78 ± 0.49*** | 8.24 ± 0.36*** |
| "Bioglobin" – 1 mL of solution contains Bioglobin-U 5000 IU; in the list for dry residue – 20 mg: polypeptides 3.5–7.0%, amino acids 50–60%, aminosugars 4–5%, hexuronic acids 8–9% | 1 | 24 | 34.8 | 6.90 ± 0.39 | 3.98 ± 0.56* |
| | 2 | 24 | 33.3 | 7.04 ± 0.51*** | 4.12 ± 0.42* |
| Ferti Root – alginic acid; betaine; polysaccharides; phytohormones; vitamin complex of groups B, C, D; N, $P_2O_5$, $K_2O$, Fe, Zn | 2.5+2+2 in a week | 168 | 58.8 | 9.34 ± 0.71*** | 9.56 ± 0.66*** |
| | 5+3+3 in a week | 168 | 62.4 | 10.04 ± 0.47*** | 9.14 ± 0.79*** |
| "Emistim C" – 1 L contains 1 g of saturated and unsaturated fatty acids ($C_{14}$–$C_{28}$), polysaccharides, 15 amino acids, analogues of cytokinin and auxin phytohormones | 0.1 | 24 | 28.9 | 8.65 ± 0.64*** | 7.67 ± 0.67*** |
| | 0.2 | 24 | 48.2 | 6.88 ± 0.75* | 8.12 ± 0.72*** |
| "Charcor" – a complex of 2.6-dimethylpyridine-1-oxide with α-phenylacetic acid at a concentration of 8.3 g/L | 1 | 24 | 50.6 | 6.04 ± 0.70* | 8.45 ± 0.68*** |
| "DNHBK" – dinatrium salt of 2-(quinolin-4-ylthio) succinic acid | 1 | 24 | 49.6 | 6.65 ± 0.39 | 9.88 ± 0.79*** |
| | 5 | 24 | 68.1 | 12.40 ± 0.69*** | 10.54 ± 0.58*** |
| | 10 | 24 | 20.9 | 4.90 ± 0.55** | 5.76 ± 0.56** |

*Note*: see Table 3.

As with the previous varieties, "Ferti Root" in both concentrations and dinatrium salt of 2-(quinolin-4-ylthio) succinic acid in a concentration of 5 mg/L had a positive impact on the rooting process of lavender cuttings. "Ferti Root" increased the percentage of rooted cuttings to 58.8–62.4%, which is almost 25% better than the control. The number of roots increased to 9.3–10.0. The length of the roots increased 1.9–2.0 times compared to the control. The use of dinatrium salt of 2-(quinolin-4-ylthio) succinic acid (5 mg/L) improved the quality of the roots by two times, and the percentage of rooted cuttings increased to 68.1%, which is 19.5% more than the control.

Thus, on all examined varieties of *L. angustifolia*, there is a positive effect of using "Ferti Root" in both concentrations and dinatrium salt of 2-(quinolin-4-ylthio) succinic acid at a concentration of 5 mg/L on both the rooting percentage and the quality of the root system. The rooting percentages after using these preparations approach the level of effectiveness of traditional auxin-based preparations like "Kornevin" (Rootone).

## Discussion

Recently, new classes of synthetic compounds based on low-molecular-weight heterocyclic compounds, derivatives of pyridine, pyrimidine, pyrazole, triazine, oxazole, oxazolopyrimidine, and isoflavonoids have also been tested for growth regulatory effects similar to auxins and cytokinins. The most promising among these classes of synthetic compounds for practical use are pyridine and pyrimidine derivatives, which are used in agriculture as herbicides, fungicides, and plant growth regulators. The main advantage of using pyridine and pyrimidine derivatives is the broad specificity of their regulatory effects on the growth and development of different plant species and varieties during ontogenesis and on the organogenesis of plant shoots and roots *in vitro* at low concentrations from $10^{-9}$ to $10^{-5}$ M, which do not have toxic effects on humans, animals, and the environment (Tsygankova et al., 2016; Tien, 2020).

The results of several studies have confirmed the possibility of practical application of new environmentally safe plant growth regulators based on sodium and potassium salts of 6-methyl-2-mercapto-4-hydroxypyrimidine (Methiur and Kametur) and N-oxide-2,6-dimethylpyridine (Ivin) to improve growth and increase the productivity of sorghum. Thanks to the use of Methiur, Kametur, and Ivin plant growth regulators, it is possible to increase the productivity of agricultural crops and their adaptive properties to stress factors of abiotic nature (Tsygankova et al., 2023).

In a number of studies, it is noted that commercial producers of ornamental plants use plant growth regulators as a necessary component of the technological process. Plant growth regulators have a rapid effect on both vegetative growth and the yield of flowers in flowering crops. One of the most important characteristics of a modern plant growth regulator is that it requires less time to treat the plant and is harmless to the environment. The effectiveness of various classes of plant growth regulators depends on different factors, among which one of the most important is the method of application, which plays a significant role in the effectiveness of plant growth regulators (Janick, 2007; Bharathi, 2009; Kumar, 2015; Kumar, 2018; Tsygankova et al., 2018).

The use of plant growth hormones in flowering crops must be specific, and the plant growth regulator must be safe for health, non-toxic, and harmless to the environment. The physiological activity and decorative qualities of flowering crops depend on the applied plant growth regulators. Therefore, the growth and profitability of flower production in flowering crops depend on the use of non-toxic plant growth regulators at low concentrations from $10^{-9}$ to $10^{-5}$ M (Lawson, 1996; Davies, 2010; Tsygankova et al., 2023). To increase production and income from the flower production sector, a scientific approach and proper management methods are applied, including improving the quality of flower germplasm, proper nutrient management, highly effective modern growth stimulators, high-tech production technologies, and quality seeds and planting material (Wani, 2017; Tsygankova et al., 2018).

Virtual compound databases for the molecular design of new plant growth regulators include both natural compounds and synthetic growth regulators. The natural compounds considered are IAA (indole-3-acetic acid), natural cytokinin – zeatin, 4-Cl-IAA (4-chloro-IAA), PAA (phenylacetic acid), IPA (indole-3-pyruvic acid), IBA (indole-3-butyric acid). The synthetic growth regulators include NAA (1-naphthylacetic acid), 2,4-D (2,4-dichlorophenoxyacetic acid), 3,4-D (3,4-dichlorophenoxyacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), 4-CPA (4-chlorophenoxyacetic acid), dicamba (3,6-dichloro-2-methoxybenzoic acid), picloram (4-amino-3,5,6-trichloropyridine-2-carboxylic acid), BSAA (3-(benzo[b]selenienyl)acetic acid), 5,6-Cl2-IAA-Me (5,6-dichloroindole-3-acetic acid methyl ester), TA-12 (1-[2-chloroethoxycarbonylmethyl]-4-naphthalenesulfonic acid calcium salt), TA-14 (1-[2-dimethylaminoethoxycarbonylmethyl] naphthalene chloromethylate), and synthetic cytokinins such as kinetin (6-furfurylaminopurine), 2iP (N6-(2-isopentenyl)adenine (Voytsehovska, 2010; Tsygankova et al., 2022).

Recent studies have provided the opportunity to evaluate new classes of synthetic compounds based on low molecular weight heterocyclic compounds, derivatives of pyridine, pyrimidine, pyrazole, triazine, oxazole, oxazolopyrimidine, and isoflavonoids for regulatory effects similar to auxins and cytokinins. (Ross, 2014; Mohilnikova, 2021). The most promising among these classes of synthetic compounds for practical use are derivatives of pyridine and pyrimidine, which are used in agriculture as herbicides, fungicides, and plant growth regulators. They are non-toxic and used at low concentrations (Saito, 2006; Namitha, 2021; Salaün, 2021; Tsygankova et al., 2022). New derivatives of pyridine and pyrimidine offer a range of advantages over known compounds. These include a broad specificity of their regulatory effects on the growth and development of various species and varieties of plants during ontogenesis and on the organogenesis of plant shoots and roots in vitro at low concentrations from $10^{-9}$ to $10^{-5}$ M, which do not have toxic effects on humans, animals, and the environment (Tsygankova et al., 2023). Screening synthetic compounds, derivatives of pyridine and pyrimidine, as new effective substitutes for the phytohormones auxins has yielded new compounds for improving the vegetative propagation of plants (Boussemghoune, 2012; Minn, 2013; Tsygankova, 2022).

Previous studies by various authors have demonstrated the possibility and effectiveness of using the investigated biologically active substances as root formation stimulants for *Lavandula angustifolia* cuttings instead of traditional auxins. These substances include "Foliar Concentrate," "1R Seed treatment," succinic acid, "Charcor," "Emistim S," "Epin-extra," *Aloe vera* juice, gibberellic acid, among others. The dinatrium salt of 2-(quinolin-4-ylthio) succinic acid has also been considered a biologically active substance with previous research supporting its root-forming activity on different plant species, such as *Thuja occidentalis* and *Platycladus orientalis*.

The current research provides evidence for the potential use of "Ferti Root" and the dinatrium salt of 2-(quinolin-4-ylthio) succinic acid (at a concentration of 5 mg/L) as effective root formation stimulants for *L. angustifolia* cuttings. Importantly, their effectiveness is comparable to traditionally used auxin-based preparations like "Rootone".

Unlike previous studies that showed positive effects of "Bioglobin", "Charcor", and "Emistim S" when used on green lavender cuttings under mist propagation conditions, our research did not observe such benefits when using these substances with woody one-year-old cuttings for open-field rooting.

In conclusion, the results of this study highlight both the scientific novelty and practical significance of using alternative substances, such as "Ferti Root" and the dinatrium salt of 2-(quinolin-4-ylthio) succinic acid, as root formation stimulants for lavender cuttings, which can have a positive impact on lavender propagation and cultivation on a commercial scale.

Scientific novelty of the obtained research results – for the first time, a comprehensive comparison of the effectiveness of using a variety of preparations of different origins (biologically active substances of plant, animal, and other origins, derivatives of quinoline-carboxylic acid, etc.) as root formation stimulators in the propagation technology of *L. angustifolia* using one-year-old woody cuttings has been conducted.

Practical significance of the research results – it has been demonstrated that dinatrium salt of 2-(quinolin-4-ylthio) succinic acid and the "Ferti Root" preparation can be used as root formation stimulators in the propagation technology of *L. angustifolia* varieties using one-year-old woody cuttings instead of traditional auxins.

## Conclusion

The positive effect of preparing *L. angustifolia* varieties "Stepova," "Vdala," and "Sineva" cuttings for rooting using the biologically active preparation "Ferti Root" and the novel substance DNHBK was observed on all tested varieties.

For the "Stepova" variety, the use of "Ferti Root" resulted in a rooting percentage of 90.0%, which was 20.0% better than the control. The number of roots increased by 50.0%, and the length of newly formed roots almost doubled compared to the control. Dinatrium salt of 2-(quinolin-4-ylthio) succinic acid at a concentration of 5 mg/L provided the maximum positive effect: rooting reached 80.0%, and both the length and the number of roots increased by 49.0% and 79.0%, respectively.

For the "Vdala" variety, "Ferti Root" significantly outperformed the control: both concentrations increased rooting (up to 68.3–70.0%), the number of roots almost doubled, and the length of the roots increased by one and a half times. Dinatrium salt of 2-(quinolin-4-ylthio) succinic acid at a concentration of 5 mg/L also positively affected rooting, with a 72.2%

survival rate, 18.2% better than the control. The length and number of roots increased by more than 1.5 times.

Similar trends were observed for the "Sineva" variety. "Ferti Root" in both concentrations and dinatrium salt of 2-(quinolin-4-ylthio) succinic acid at a concentration of 5 mg/L had a positive impact on rooting, resulting in a 25% increase in survival and more than a 50% increase in the number and length of roots compared to the control.

Other tested preparations, such as "Bioglobin," "Emistim C," and "Charkor," did not provide the expected improvement in rooting on all lavender varieties. In the best-case scenarios, the survival and root quality remained at the control levels, possibly due to lower autumn-winter temperatures that negated the effects of these biologically active preparations. In conclusion, "Ferti Root" and dinatrium salt of 2-(quinolin-4-ylthio) succinic acid can be successfully used as alternatives to traditional auxins in the vegetative propagation process of Ukrainian *L. angustifolia* varieties "Stepova," "Vdala," and "Sineva" using one-year-old woody cuttings in open soil.

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