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BIOLOGICAL ACTIVITY OF AQVEOUS SOLUTION OF AMBER

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The aim of the research was to detect the biological activity of various samples of amber from the Trancedniestria province, and to characterize their effect on the physiological processes of higher plants. The allelopathic and cytostatic effects of water solutions of amber were studied using biotesting methods in open soil conditions and in culture *in vitro*. The test-object was the seedlings of cucumber *Cucumis sativus* L. It was shown that the activity and nature of the action of the samples depend on the region of origin of the amber, the degree of shredding and concentration of the solution. According to the results, fine fractions of amber show biostimulating effects on plants. Thus, amber can be effectively used for cultivating agricultural plants *in vivo* and *in vitro* conditions. For stimulation of the increasing of the plant stem mass it is recommended to use amber B1, to increase the mass of roots — amber B4, and amber B2 — to stimulate stem growth in length.

Key words: amber, succinic acids, allelopathic and cytostatic action.

A common challenge of our time is achieving a healthy living style. A prominent feature of the approach is the consumption of wholesome foods, grown without fertilizers or pesticides.

One of such natural organic compounds might be amber, natural organic yellow, brown and red substance containing succinic acid. It is fossilized coniferous resin of Upper Cretaceous and Paleogenic periods, 15–40 MY in age. It occurs as stalactites, drops, lens conglomerates of "resin pockets" and their chips, ranging in size from 0.02 to 50 cm (usually 2–30 cm). In Europe, amber is found in the range from Southern Sweden to the shores of the Black and Azov Seas, including the part of Ukraine to the west of the Dnieper river. The region known as "the Baltic-Dnieper amber-bearing province" [1] is the main source and provider of the stone to the world markets [2]. In Ukraine, amber occurs in Zhytomyr, Lviv, and Kharkiv regions [3]. The identification of those amber samples according to the characteristic patterns of infra-red light absorption spectra did not show substantial differences between them and the samples from the Baltic states and Belarus, which supports their placing in the Baltic-Dnieper amber-bearing province [1, 4, 5].

The hypothesized possibility to use amber to rehabilitate and protect plants from various kinds of damage is based on the very nature of resin flow. Amber contains a stimulator of plant growth, succinic acid; terpenes with bactericide properties; organo-mineral complexes and microelements, various resinous acids with fungicide properties; and phytosteroids which are structurally similar to steroids (hormones) of the human body and have powerful anti-inflammatory and antibacterial effect.

The biological activity of succinic acid was revealed a long time ago. Nowadays, spurred by the search for human- and environment-friendly agents, it became increasing important as a natural growth and productivity enhancer and stress protectant [6–13].

Besides amber, succinic acid ($HOOCCH_2CHCOOH$) is found free and bound in the tissues of many plants and animals, especially in unripe fruit, and in brown coal. It is a colorless crystalline powder tasting much like citric acid.

The research of Zaimenko [14] showed the possibility of managing the growth processes, physiological properties of plants of different ecological morphotypes and of different carbon metabolism by adding succinic acid. Also, the acid positively influences the biosynthesis of aminoacids in plants and can be a substrate for this biosynthesis. It was found that succinic acid content in the leaves of barley seedlings, cultured on 0.005 M solution of succinic acid, increased by 51%, while the water control increased by 39% [15].

Amber which contains succinic acid according to Elroy Rice's classification of the chemical nature of allelopathic agents is a compound of secondary posthumous origin in dead plant tissue [16]. Biogenic excretions are extraordinarily significant for the chemical interaction between organisms at different levels of their existence. They can inhibit or facilitate various vitally important functions (germination of spores and pollen, seed vitality, seedling growth etc.), affecting the life of whole biogeocoenoses and the function of balanced processes in the biosphere [17].

The aim of our work was to study the biological activity of aqueous solutions of various samples of amber from the Baltic-Dnieper province using classic techniques, and to characterize their effect on the physiological processes of higher plants.

Bearing in mind that more than 70% of amber are of value for jewelry, and there is a lot of waste when it's developed, the amber's biological properties and the possibilities of using it as an efficient and safe bioregulatory agent for cultured plants is a matter of practical importance. Research in the field of rational use of minerals obsoletes the practice of selling the raw materials and expedites the introduction of the end products into environmentally safe horticulture.

Materials and Methods

For this study, the samples of amber from Ukrainian deposits were differentiated by color into four groups: B1 darker, opaque dark-amber (or transparent, brown) color; B2 lighter, yellowish, lemon, light-amber color, translucent and transparent; B3 dark red or cherry-red, translucent and transparent; B4 mechanical mixture of all of the above. Most of the samples came from the deposits of Zhytomyr region (Olevskiy district) and Rivne regions (Klyosove and Volodymyrets) and belonged to the B1, B2 and B3 groups. Most of the B4 amber came from Volhyn region.

Fragments of B1 amber (dark-brown to reddish) were milled to 300–500 μm, lighter B2 (amber, lemon-yellow) to 100–200 μm. According to [3], dark ambers have higher ratios of resin acids (abietic acid and its derivatives) to succinic acid, and more different microelements.

The samples were analyzed using infra-red spectroscope Vertex-70, Bruker. Fragments of Ukrainian amber were milled en masse using laboratory mill Kinematica AG, model Polymix® PX-MFC 90 D to obtain particles of 50–500 μm.

Studies of the allelopathic and cytostatic activities of aqueous solutions of various amber samples were conducted after the widely used techniques of Grodzinsky and Ivanov [17, 18]. We studied the effect of the various samples on the rates of biomass growth of test plants, both above and below ground.

As the test culture, we used seedlings of cucumber (*Cucumis sativus* L.), sort Konkurent. Biotests to reveal allelopathic activity were replicated on 1– and 3-4-days-old seedlings, using 0.1, 0.5, 1.0 g/l concentrations of aqueous solution of amber of the B1-B4 groups. Cytostatic activity of amber was evaluated by counts of lateral roots.

To conduct *in vitro* tests, cucumber seeds were sterilized for 1 min in 70% ethanol, then for 2 min by 0.05% sodium merthiolate, and then planted in MS cultural medium with the addition of amber powder at the concentrations of 0.15 and 1.5 g/l. The experiment was carried out in two replicas. The results were visually evaluated by morphometry. All laboratory research was carried out at +26 °C.

The data were statistically treated using EXCEL 7.0.

Results and Discussion

Allelopathic and cytostatic activity of amber. Studying allelopathic and cytostatic activities of the four amber sample groups revealed that the aqueous solution of B2 amber, regardless of the concentration, also had significant the most effective allelopathic activity, it stimulated the growth of one-day-old cucumber seedlings in the range of 126.0–133.8%. Meanwhile, on three-days-old seedlings we saw significant depression of root growth by the solution at 0.1 g/l and notable stimulation at 0.5 g/l and 1.0 g/l. The most efficient was aqueous solution of B2 amber at 0.5 and 1.0 g/l (Fig. 1).

The solution also was high effective (131.48%) for B1 amber at 0.5 g/l. The effect was recorded during all three days of the experiment (Fig. 1).

Aqueous solution of B3 amber, regardless of the concentration, exhibited an inhibitory effect on one-day-old cucumber seedlings, and at the concentration of 1.0 g/l, it inhibited also the growth of four-days-old ones. At 0.1 g/l and 0.5 g/l, we saw slight stimulation of the growth of four-days-old seedlings (Fig. 1).

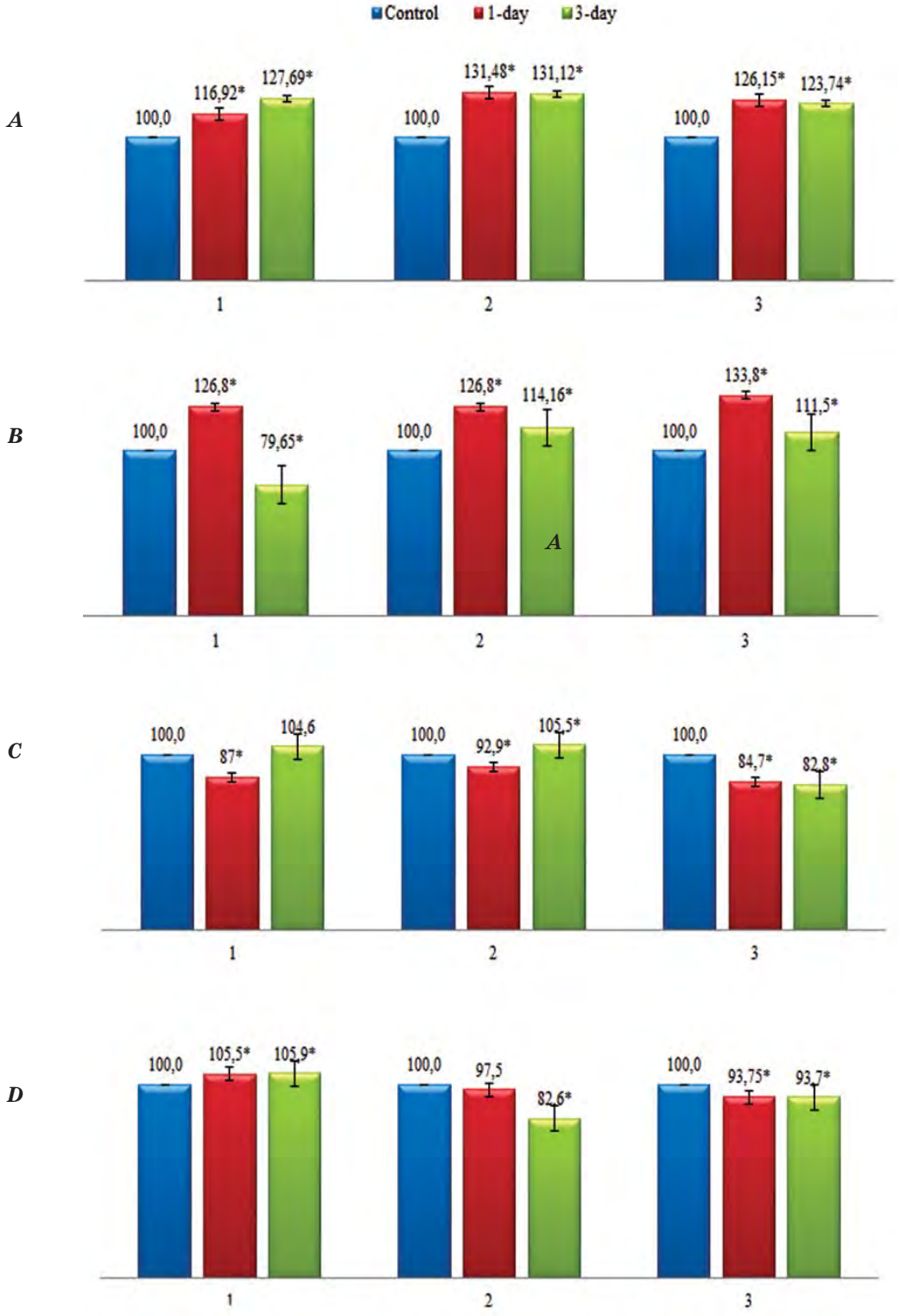

Fig. 1. **Allelopathic activity of the aqueous amber solutions (% compared to control), depending on the concentration depending on the concentration**

Hereafter: (% to control — 100%);1 — 0.1 g/l, 2 — 0.5 g/l, 3 — 1.0 g/l:
A — sample B1; *B* — sample B2; *C* — sample B3; *D* — sample B4; * $P < 0.05$

The solution of B4 amber revealed slight inhibitory effect on the cucumber seedlings at 0.5 g/l and 1.0 g/l, while at 0.1 g/l it had a slight stimulating effect.

Functional activity of amber solutions can occur not only as the ability to have an allelopathic effect on the plant as a whole and its organs, but on the cells, too, through its ability to impact their proliferation, that is, in its cytostatic activity [19]. The cytostatic activity lies in the selective inhibition of mitosis in the main roots of the seedlings, and in the strongly inhibited growth of lateral roots.

Studying cytostatic activity of the aqueous solution of B1 amber at different concentrations, we saw significant stimulatory effect on the cellular proliferation during the first day of the experiment, in particular, the initiation of lateral roots. Immediately after germination, the lateral roots in the experimental plants were initiated at 474.4–566.7%, compared to control (Fig. 2). However, on the third day the level of activity decreased to the level of 121.0–142.9%.

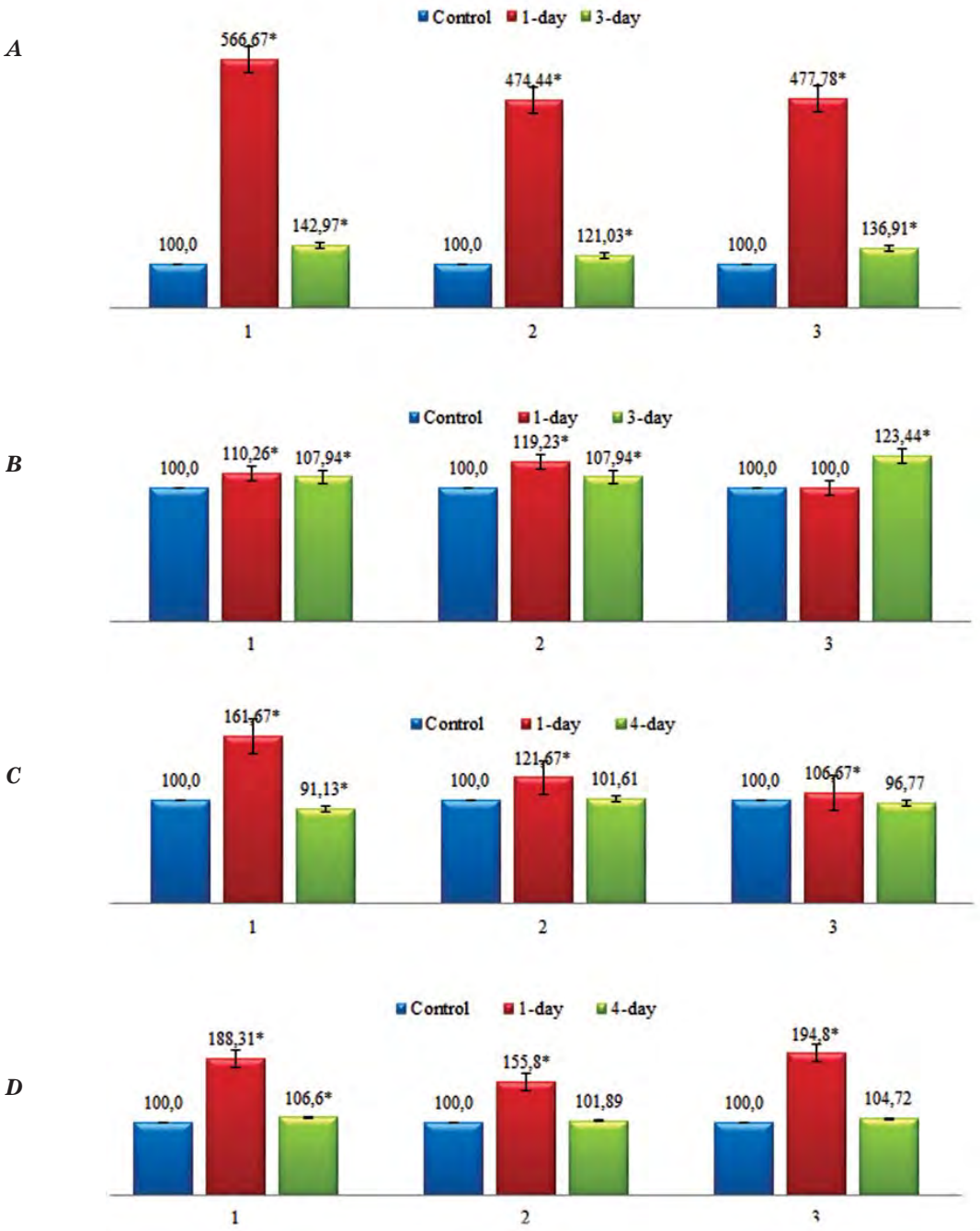

Fig. 2. **Cytostatic activity of amber solutions depending on the concentration**

B2 amber exhibited a relatively small stimulating action, 119.2% on the first day at 0.5% amber solution and 123.4% at 0.1% (Fig. 2). Significant stimulating action of the B3 amber solution in the range of 106.7–161.7% was seen on the first day of seed germination. However, on the fourth day, at 0.1 g/l and 1.0 g/l, there was a slight cytostatic action in the range of 91.1–96.8%. A significant activation of proliferation by the B4 solution in the range of 155.8–194.8%, independently of concentration was seen on the first day of seed germination, but leveled out on the fourth day.

Thus, amber and its water solutions are active stimulating substances towards plants during germination. However, only slight cytostatic activity was revealed under the influence of B3 amber. The effect's size and character depend on the amber's origin and the solution's concentration.

Testing the solution's effect on the growth and development of cucumber seedlings in vitro. Currently, urgent biotechnological research includes studying genetically transformed medicinal plants which produce their own biologically active compounds (BAC), including fructans, artemisinin and recombinant substances (interferon, micobacterial antigens) with prebiotic, hepatoprotectant, antidiabetic, antitumour, antimalarial, antioxidant, and UV-protectant properties, useful in the treatment of viral, bacterial and other diseases [20]. There are collections of such plants which produce a whole range of natural and recombinant BAC for cosmetology, medicine and veterinary. For successful culture of such plants in order to obtain industrial quantities of BAC, it's important to search for new natural growth factors to compose cultural media.

The experiments showed that B2 amber, regardless of concentration, is the most fficient factor promoting plant growth and development *in vitro*, compared to B1, B3 and B4. This advantage is seen in greater size, number of leaves and leaf size in experimental plants. The average values were obtained for B3 samples, and the lowest for B1 and B4.

Test plants on MS medium with B2 amber topped control plants in height by 28–38% and by 10–48% on MS medium with B1, B3 and B4 ambers (Fig. 3, 4).

Thus, our research showed that amber solutions did strongly stimulate the growth and development of test plants during early ontogenesis. The level of such stimulation depends not only on the concentration of amber in the cultural medium, but also on the region of its origin. Probably, the difference in activity is tied to the difference in composition (% of succinic acid and other components of amber), as well as to the degree of milling.

It was found that *in vivo* the solutions of studied samples of amber also differ in influence on the growth of above- and belowground parts of test plants, stimulating or inhibiting it (Fig. 5).

To establish the action of amber solutions we chose the parameters of growth of above- and belowground parts of test plants: mass and the increase of the relevant parts compared to dry substance, %.

Maximal effect on growth activation of the aboveground parts of the seedlings was seen in the B1 amber solutions, somewhat lower in B4. Clearly, these amber samples at a certain dilution have the optimal concentration and ratio of resin acids (abietic acid and its

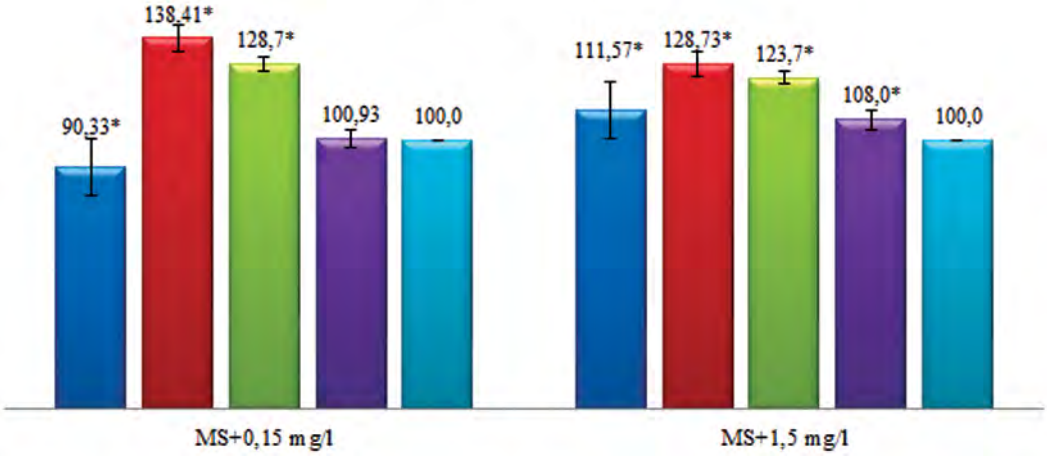

Fig. 3. **Morphometric characteristics of test plants *in vitro* depending on the concentration of ambers B1 – B4 (% to control)**

derivatives) to the succinic acid and the microelements suitable to stimulate the growth of the aboveground part of the plants.

We observed a tendency, for B1 and B2 samples, independently of the amber concentration, to activate the aboveground growth of the test plants and also to significantly inhibit their root systems. It should be noted that 0.1 g/l and 0.5 g/l solutions of B3 amber strongly inhibit both aboveground and root mass compared to control. Meanwhile, at 1.0 g/l its action is practically invisible.

All in all, the highest aboveground growth of the test plants in the range of 18–21% was observed in B1 solutions, regardless of concentration. However, the highest growth of root system of about 8% is expected in a B4 solution at 0.5 g/l.

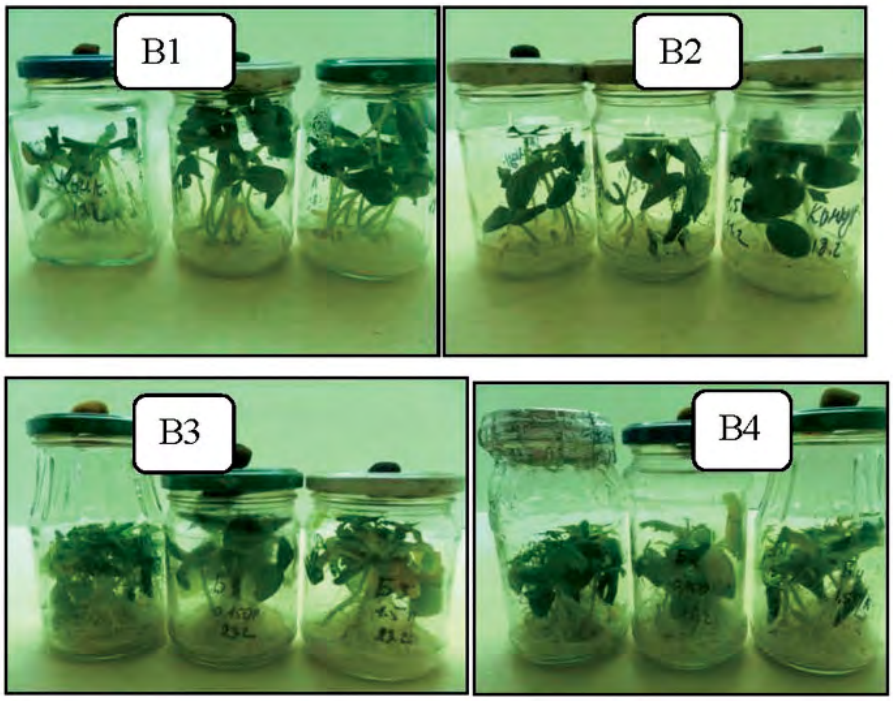

Fig. 4. **Eight-days-old cucumber seedlings on MS+amber medium (from left to right):** control; 0.15 g/l; 1.50 g/l

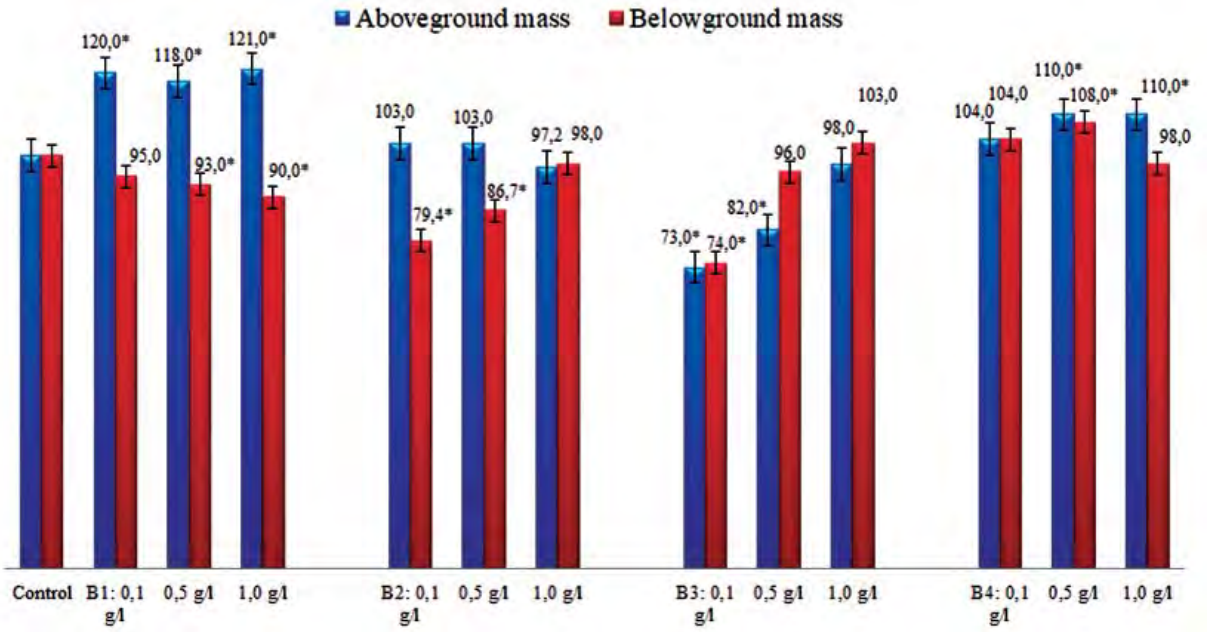

Fig. 5. **The growth of above- and belowground mass of test plants depending on concentrations of the B1–B4 amber solutions**

Studying the effect of water solutions of varying concentrations of different ambers on allelopathic and cytostatic activity, and also on cultural plants in vitro showed the following:

– the highest allelopathic activity was seen on a water solution of B2 amber, while its 0.1 g/l solution had an inhibitory effect;

– during the first day, the highest stimulatory activity of was seen in the B1 amber sample, regardless of the concentration;

– *in vitro* the highest stimulating effects were seen when we added to the MS medium a sample of B2 amber at 0.15 g/l and 1.5 g/l; if we added B1 amber at 0.15 g/l we saw the maximal inhibition of the plant life processes;

– amber solutions *in vitro* act differentially on the aboveground parts of the test plants and on their root systems, influencing growth parameters and the intensity of growth of the green mass of the plants.

To stimulate the growth of stem mass of the plants B1 amber should be taken, to increase the mass of the root — amber sample B4 and B2 — to stimulate the stem growth in length.

Therefore, the research demonstrated that the wastes and fine fractions of amber are a valuable source of natural complex chemical compound with growth stimulation for plants in *vivo* and *in vitro*. Amber in general, and its water solutions in particular could be very efficiently applied to plant cultivation.

REFERENCES

1. *Trofimov V. S.* The amber. *Moskva: Nedra*. 1974, 184 p. (In Russian).
2. *Matsuy V. M.* Marine stage of fossilization of resinous coniferous isolates on the path of transformation into amber-succinite. *Geologiya i poleznyie iskopaemyie Mirovogo okeana*. 2013, V. 2, P. 101–108. (In Russian).
3. *Srebrodolskiy B. I.* The amber of Ukraine. *Kyiv: Naukova dumka*. 1980, 124 p. (In Russian).
4. *Konovalenko S. I., Bogdasarov M. A.* IR-spectroscopy of fossil resins of the Baltic-Dnieper and Chulym-Yenisei sub-provinces of northern Eurasia. *Vestnik Tomskogo gosudarstvennogo universiteta*. 2008, V. 314, P. 201–203. (In Russian).
5. *Mironov O. L., Kachalova N. M., Dzyuba O. I., Bogza S. L.* Complex of biologically active compounds of amber: method of obtaining, properties and applications. *International Theoretical and Practical «The modern aspects of human's health maintenance»*. 2017. (In Ukrainian).
6. *Gizbullin N. G.* Amber acid is an effective plant growth regulator. *Tsukrovi buriaky*. 2009, V. 2, P. 4–5. (In Ukrainian).
7. *Golovatskaya I. F.* Morphogenesis of plants and its regulation. Part 1. *Tomsk: ID TGU*. 2016, 172 p. (In Russian).
8. *Klochkova N. M.* The iInfluence of succinic acid, epine and their combined effect on gas exchange of various morphotypes of peas (*Pisum sativum* L.) in optimal conditions and conditions of water deficiency (Doctoral dissertation). *Moskva*. 2004, 202 p. (РГБ ОД, 61:04-3/1129). (In Russian).
9. *Margitaj M.* Influence of growth regulators on rooting of *Buddleja davidii Franch*. cuttings. *Introduktsiia ta zberezhennia riznomanittya*. 2010, V. 28, P. 57–59. (In Ukrainian).
10. *Shakirova F. M.* Nonspecific resistance of plants to stress factors and its regulation. *Ufa: Gilem*. 2001, 160 p. (In Russian).
11. *Kropotkina V. V., Vereshchagin A. L.* Stimulating effect of the superslow doses of the mixture of organic acids to acclimatization cuttings of grapes. *Book of Abstracts Posters Session 7. Hormones and synthetic plant growth regulators in agriculture*. P. 136.
12. *Sydorovych M. M., Kuldelchuk O. P., Kot S. Yu.* Phytotesting of biological properties of a new synthetic plant growth stimulator — a spirocarbon complex with amber acid. *Pryrodnychij almanakh. Ser. Biologichni nauky*. 2016, V. 23, P. 108–116. (In Ukrainian).
13. *Tumiłowicz P., Synoradzki L., Sobiecka A.* Bioactivity of Baltic amber – fossil resin. *Polimery*. 2016, 61 (5), 347–356. doi: dx.doi.org/10.14314/polimery. 2016.347.
14. *Zaimenko N. V., Ivanyczka B. O.* Influence of organic acids on growth processes of plants of various ecomorphotypes. *Introduktsiia roslyn*. 2013, V. 3, P. 108–114. (In Ukrainian).
15. *Chupahina G. N.* The system of ascorbic acid of plants. *Kaliningrad: izd-vo Kaliningradskogo un-ta*. 1997, 120 p. (In Russian).
16. *Rays Elroy L.* Allelopathy. *Moskva: Mir*. 1978, 392 p. (In Russian).
17. *Grodzinskiy A. M.* The experimental allelopathy. *Kyiv: Naukova dumka*. 1987, 236 p. (In Russian).
18. *Ivanov V. B., Byistrova E. N., Dubrovskiy I. G.* Cucumber seedlings as a test object for the detection of effective cytotoxic agents. *Fiziologiya rasteniy*. 1986, 33 (1), 195–199. (In Russian).
19. *Mamaeva A. S.* The proliferation of plant cells and its regulators. *Fiziologiya rasteniy*. 2013, 60 (4), 529–536. (In Russian).
20. *Matvyeyeva N. A.* Creation of plant-producing biologically active compounds via Agrobakterium-mediated transformation (Doctoral dissertation). *Kyiv*. 2015, 361 p. (In Ukrainian).

БІОЛОГІЧНА АКТИВНІСТЬ ВОДНИХ РОЗЧИНІВ БУРШТИНУ

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Метою дослідження було виявити біологічну активність різних зразків бурштину з Придністровської провінції й охарактеризувати їх вплив на фізіологічні процеси вищих рослин. Вивчали алелопатичну і цитостатичну дію бурштину методами біотестування за умов відкритого ґрунту та в культурі *in vitro*. Тест-об'єктом слугували проростки огірка *Cucumis sativus* L. Показано, що активність і характер дії зразків залежать від області походження бурштину, ступеня подрібнення й концентрації розчину. Згідно з результатами дрібні фракції бурштину виявляють біостимулювальну дію на рослини. Таким чином, бурштин може ефективно застосовуватися для культивування сільськогосподарських рослин за умов *in vivo* та *in vitro*. З метою стимулювання збільшення маси стебла рослин доцільно використовувати бурштин В1, для збільшення маси коренів — бурштин В4, а бурштин В2 — для стимулювання росту стебла у довжину.

Ключові слова: бурштин, бурштинова кислота, алелопатична та цитостатична дія.

БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ ВОДНЫХ РАСТВОРОВ ЯНТАРЯ

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Целью исследования было выявить биологическую активность различных образцов янтаря из Приднестровской провинции и охарактеризовать их влияние на физиологические процессы высших растений. Изучали аллелопатическое и цитостатическое действие янтаря методами биотестирования в условиях открытого грунта и в культуре *in vitro*. Тест-объектом служили проростки огурца *Cucumis sativus* L. Показано, что активность и характер действия образцов зависят от области происхождения янтаря, степени измельчения и концентрации раствора. Согласно результатам мелкие фракции янтаря проявляют биостимулирующее действие на растения. Таким образом, янтарь может эффективно применяться для культивирования сельскохозяйственных растений в условиях *in vivo* и *in vitro*. С целью стимулирования увеличения массы стебля растений рекомендовано использовать янтарь В1, для увеличения массы корней — янтарь В4, а янтарь В2 — для стимулирования роста стебля в длину.

Ключевые слова: янтарь, янтарная кислота, аллелопатическое и цитостатическое действие.