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THE INFLUENCE OF *AZOTOBACTER VINELANDII* IMV B-7076 ON THE BUCKWHEAT DEVELOPMENT AND EXOMETABOLITE COMPOSITION IN THE ROOT ZONE

During the growth and development of plants, a large quantity of root exudates is released into the rhizosphere, forming a microbiome capable of stimulating plant growth and productivity. The application of microbial preparations will be the cornerstone of soil improvement and obtaining high-quality plant products. The ***aim*** *of this study was to determine the influence of Azotobacter vinelandii IMV B-7076, a component of the highly effective complex bacterial preparation Azogran, on the buckwheat development and exometabolite composition in the root zone.* ***Methods.*** *The effect of azotobacter on the growth of buckwheat plants and their exometabolite synthesis was investigated during a 14-day cultivation period in a Farreus medium under phytotron conditions. The content of photosynthetic pigments in plant leaves was determined using spectrophotometric methods. The protein content in the medium was determined by the Bradford method, carbohydrates by their interaction with phenol and sulfuric acid, and phenols by the Folin-Chokalteu reagent.* ***Results.*** *It was found that the cultivation of buckwheat in a medium with azotobacter significantly stimulated the growth activity of plants and the content of chlorophyll a in their leaves. Under these conditions, the content of chlorophyll b and carotenoids in buckwheat leaves increased less noticeably. During the 14-day cultivation of plants in a medium containing 10^7 CFU/mL of these bacteria, the protein content increased by 110.4% compared to the control, phenolic compounds by 48.8%, and carbohydrates by 266.4%.* ***Conclusions.*** *Azotobacter vinelandii IMV B-7076 noticeably improves the growth of buckwheat, increases the concentration of exometabolites in the medium during hydroponic cultivation.*

Keywords: *Azotobacter vinelandii IMV B-7076, buckwheat plants, morphometric indicators, content of photosynthetic pigments, protein, carbohydrates, phenolic compounds.*

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The widespread use of chemical fertilizers, pesticides, and other chemical agents in agriculture leads to environmental pollution, decreased food quality, and negative impacts on human health. Considering this, organic farming emerges as a promising direction for obtaining high-quality agricultural products. The idea behind organic farming involves refraining from the use of GMOs, chemical fertilizers, pesticides, and other harmful substances (Francis, 2005).

An important aspect of organic farming is the application of microbial preparations in crop cultivation. These preparations stimulate the growth, development, and productivity of plants by improving their nutrition and protecting them from phytopathogens, pests, and other negative factors, thus enhancing their productivity (Kurdish, 2010; Innerebner et al., 2011; Roy et al., 2012; Kurdish, 2019). The complex bacterial product Azogran, developed by us, which contains nitrogen-fixing bacteria *Azotobacter vinelandii* IMV B-7076 and phosphate-mobilizing *Bacillus subtilis* IMV B-7023 is one of such preparations. It has been shown to significantly increase the productivity of various plant species (Kurdish, 2010). For example, when buckwheat seeds were inoculated with the nanocomposite preparation Azogran, the yield of these plants in organic farming conditions increased by 30.6% (Hryshchenko et al., 2020).

The functioning of microbiota in the rhizosphere is made possible by the secretion of root exudates by plants, the composition of which depends on the species-specific characteristics of the plants and the environmental conditions (Gray & Smith, 2005; Badri & Vivanco, 2009; Sasse et al., 2018). These exudates can constitute up to 40% of the products of plant photosynthesis (Badri & Vivanco, 2009; Kuijken et al., 2015). The release of root exudates forms the basis for the establishment of the microbial community in the rhizosphere (The Rhizosphere, 2007; Sasse et al., 2018). The content of microorganisms in the rhizosphere of plants is significantly higher than in the bulk soil (Kuske et al., 1997).

It has been demonstrated that microorganisms in the rhizosphere of plants act as potent absorbers of carbon compounds from root exudates, leading to an increase in the concentration gradient of metabolites and thus influencing root exudation (Canarini et al., 2019). It has also been found that *Bacillus subtilis* bacteria are capable of influencing the exudation of acetylated carbohydrates by plants, which are characterized by insecticidal activity (Korenblum et al., 2020). However, currently, the impact of microbial preparations on the synthesis of root exudates has not been sufficiently investigated. The **aim** of this study was to determine the influence of *Azotobacter vinelandii* IMV B-7076 on the synthesis of exometabolites by buckwheat plants.

Materials and Methods. The strain *Azotobacter vinelandii* IMV B-7076 was isolated at the Zabolotny Institute of Microbiology and Virology, NAS of Ukraine (Kurdish & Bega, 2006). The bacteria were cultured in 700 mL Erlenmeyer flasks containing 100 mL of Ashby medium (Rubenchik, 1960) and incubated on orbital shakers at 220 rpm at 28 °C for two days. The population of azotobacter in the suspension was determined by serial dilution and plating onto agarized Ashby medium, followed by cultivation and colony counting.

In the experiments, seeds of buckwheat variety Syn 2/3 (*Fagopyrum esculentum*) provided by the National Scientific Center «Institute of Agriculture» of the National Academy of Agrarian Sciences of Ukraine were used. Before application in the experiments, they were soaked for 1 minute in 70% ethanol (Avksentyeva & Petrenko, 2011), washed three times in sterile water, and then sterilized for 40 minutes in 25% hydrogen peroxide (Pogorelova et al., 2012). The seeds treated in this manner were rinsed five times with sterile water, after which they were placed on the surface of meat-peptone agar in Petri dishes to determine their germination and identify contaminated seeds.

The buckwheat plants were grown in cylindrical glass vessels with a volume of 1.5 liters, a diameter of 115 mm, and a height of 160 mm.

Stainless steel meshes were placed in these vessels at a distance of 5 mm from the bottom. The vessels were sterilized at 160 °C, after which 75 mL of sterile Farreus medium of the following composition per liter was added: $CaCl_2$ — 0.1g, $MgSO_4 \times 7\ H_2O$ — 0.12 g, KH_2PO_4 — 0.1g, $Na_2HPO_4 \times 12H_2O$ — 0.15 g, Fe citrate — 0.005g, and trace amounts of Cu, Zn, B, Mn, Mo, pH6.5 (Wilson, 1995). This medium was distributed in four variants. In three variants, a suspension of azotobacter was added to achieve concentrations of 10^5, 10^6, and 10^7 CFU/mL**.** No bacteria were added to the control Farreus medium. Subsequently, 5 germinated uncontaminated seeds were placed on the surface of the stainless-steel meshes in each vessel. Each variant included four replicates. The plants were grown in a phytotron for 14 days with a light phase of 16 hours at 20 °C.

Upon completion of the plant growth process, the medium containing root exudates was sterilely collected and centrifuged at 6600 g for 15 minutes. The resulting supernatant was lyophilized (Lyoquest-55 Plus, Spain) and stored at 20 °C for further biochemical analysis. The mass, morphometric parameters, and content of photosynthetic pigments were determined in the grown plants, while the content of protein, carbohydrates, and phenolic compounds was analyzed in the root exudates.

The determination of the chlorophyll and carotenoid content in buckwheat leaves was carried out using the spectrophotometric method (Lichtenthaler & Buschmann, 2001; Korus, 2012). For pigment extraction, 0.1 g of plant leaves was ground in a porcelain mortar containing 25 mL of 96% ethanol. The extraction of pigments was repeated, bringing the volume of the extract to 50 mL.

The optical density was measured using an SF—46 spectrophotometer manufactured by LOMO. The maximum absorption of chlorophyll a was determined at a wavelength of 665 nm, and chlorophyll b at 649 nm. Carotenoids were determined at a wavelength of 441 nm.

The concentrations of chlorophyll a (C_a, mg/L) and b (C_b, mg/L) were calculated using the following formulas:

$$C_a = 13.70 \times A_{665} — 5.76 \times A_{649},$$
$$C_b = 25.80 \times A_{649} — 7.60 \times A_{665},$$

where A_{665} is the optical density of the solution at a wavelength of 665 nm, and A_{649} is the optical density of the solution at a wavelength of 649 nm.

The concentration of carotenoids (C_c, mg/L) was calculated using the formula:

$$C_c = 4.695 \cdot A_{441} — 0.268\ (C_a + C_b),$$

where A_{441} is the optical density of the solution at the wavelength of 441 nm, and $(C_a + C_b)$ is the total content of chlorophyll a and b in the solution, mg/L.

After determining the pigment concentrations in the extract, the quantitative content of the pigments (X, mg/mL) in the raw material was calculated using the formula:

$$X = V \cdot C/m \cdot 1000,$$

where V is the volume of the alcoholic extract, mL; C is the concentration of chlorophyll in the alcoholic solution, mg/L; m is the mass of the raw material, g.

The determination of protein content in cell-free supernatants was carried out according to the Bradford method (Bradford, 1976). This method is based on the reaction of Coomassie with arginine and hydrophobic amino acid residues.

Quantitative determination of carbohydrates was carried out using the colorimetric method based on the ability of both free sugars and those composed of monosaccharide residues of homo- and heteropolymers to create a yellow-brown color during interaction with phenol and sulfuric acid (Varbanets et al., 2006).

The total content of phenols was determined using the Folin-Ciocalteu reagent (Maurya & Singh, 2010). The reaction mixture contained the following components: the test sample — 1 mL and Folin-Ciocalteu reagent: H_2O = 1: 9 —

2.5 mL. After mixing, the samples were placed in a dark place for three minutes, then 2 mL of 3.5% sodium carbonate was added to each sample and placed in a dark place again for two hours. The total content of phenols was determined at a wavelength of 765 nm. The calibration curve was constructed using gallic acid.

Statistical analysis of experimental data was performed using Microsoft Excel 2010.

Results. It has been established that the cultivation of buckwheat of the Syn 2/3 variety in the Farreus medium with the addition of *A. vinelandii* bacteria at a concentration of 10^5 cells/mL significantly stimulates the growth activity of the plants (Table 1).

After 14 days of cultivation, the root length increased by 30.5% compared to the control, while the stem length increased by 67.4%. The less pronounced stimulatory effect of bacteria on plant growth was observed when introduced into the environment at a concentration of 10^6 cells/mL, whereas at a higher concentration of this strain in the environment (10^7 cells/mL), the investigated parameters were lower than those in the control variant where azotobacter was not introduced.

The cultivation of buckwheat in the presence of *A. vinelandii* in the environment significantly influenced the chlorophyll content in the plants (Table 2). In the control variant, its quantity was 0.72 mg/g of plant fresh mass, whereas at a concentration of 10^5 cells/mL of azotobacter, this parameter increased by 22.2%, and at a concentration of 10^7 cells/mL, it increased by 34.7%. The influence of this strain on the content of chlorophyll b and carotenoids in the plant leaves under these conditions was insignificant.

The addition of azotobacter cells significantly influenced the accumulation of protein in the Farreus medium during the cultivation of buckwheat (Fig. 1). The highest amount of protein was observed during the cultivation of these plants for 14 days with a concentration of *A. vinelandii* in the medium of 10^7 cells/mL (Fig. 1).

Under these conditions, the protein content in the environment increased by 110.42% compared to the control. At the same time, upon introducing

Table 1. **Influence of *A. vinelandii* IMV B-7076 on the morphometric parameters of buckwheat plants**

Research options	Average root length (cm)	% to control	Average stem length (cm)	% to control
Control — medium without *A. vinelandii*	12.47 ± 0.27	100.0	13.5 ± 1.61	100.0
A. vinelandii (3.00±0.06) ·10^5 cells/mL	16.27 ±4.26	130.5	22.6 ± 6.81	167.4
A. vinelandii (2.32±0.02) ·10^6 cells/mL	12.83 ± 1.44	102.9	18.72± 8.43	138.7
A. vinelandii (2.23±0.03) ·10^7 cells/mL	10.09 ± 1.42	80.9	12.98± 4.36	96.1

Table 2. **Determination of contents of chlorophyll a and b and carotenoids in leaves during plant cultivation in the presence of varying amounts of azotobacter**

Research options	Content of photosynthetic pigments in plant leaves, mg/g		
	chlorophyll a	chlorophyll b	carotenoids
Control — medium without *A. vinelandii*	0.72 ±0.26	0.93 ±0.28	0.17 ± 0.01
A. vinelandii (3.00 ± 0.06) · 10^5 cells/mL	0.88 ± 0.06	0.94 ± 0.38	0.21 ± 0.01
A. vinelandii (2.32±0.02) · 10^6 cells/mL	0.97 ± 0.25	0.89 ± 0.49	1.19 ± 0.09
A. vinelandii (2.23±0.03) · 10^7 cells/mL	0.92 ± 0.16	0.95 ± 0.05	0.21± 0.05

azotobacter into the buckwheat growth medium in quantities of 10^5 — 10^6 cells/mL, the protein content was slightly higher than in the control.

The influence of azotobacter on the accumulation of phenols in the plant growth medium has been investigated. The highest amount of these compounds in the Farreus medium was found after 14 days of co-cultivation of buckwheat and *A. vinelandii* at a quantity of $1 \cdot 10^7$ cells/mL (Fig. 2). Under these conditions of plant growth in the medium, 48.78% more phenolic compounds were accumulated compared to the control.

A. vinelandii IMV B-7076 exerted a noticeable influence on carbohydrate accumulation in the buckwheat growth medium (Table 3).

If during the 14-day cultivation of these plants in the control variant (without azotobacter), carbohydrate accumulation amounted to 4.59 μg/mL, then upon introducing 10^5 cells/mL of *A. vinelandii* into the medium, its content increased to 6.51 μg/mL (by 41.8%). With the introduction of 10^7 cells/mL of azotobacter into the medium, the carbohydrate content increased to 16.8 μg/mL (by 266% compared to the control).

Discussion. Root exudates of plants have a noticeable impact on the formation of the microbial community in the rhizosphere and its functioning in this zone (The Rhizosphere, 2007; Canarini et al., 2019; Shaposhnikov et al., 2020; Wiesenbauer et al., 2024). Profiles of root exudates significantly differ among different plant species (Seitz et al., 2022), leading to distinct differences in the rhizosphere microbiome.

Functioning within the rhizosphere, microorganisms are capable of influencing plant growth to a certain extent by improving their nitrogen and phosphorus nutrition, stimulating their growth through the secretion of various biologically active substances, protecting plants from phytopathogens and herbivores, and increasing the availability of mineral compounds (Bulavenko et al., 2000; Kravchenko et al., 2003; Volkogon et al., 2006; Krewulak & Vogel, 2008; Kurdish et al., 2021). However, the potential impact of rhizosphere mi-

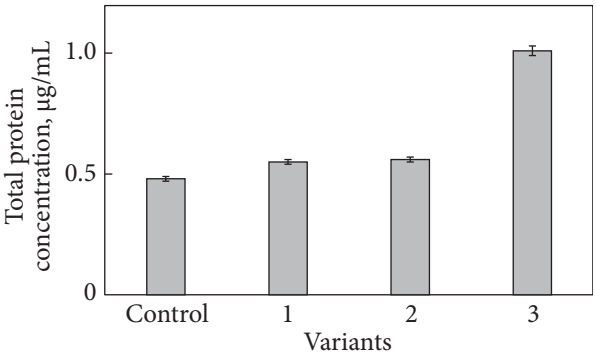

Fig. 1. Total protein content in the Farreus medium during buckwheat cultivation over 14 days with the addition of varying amounts of *A. vinelandii*: *1* — 10^5 cells/mL; *2* — 10^6 cells/mL; *3* — 10^7 cells/mL

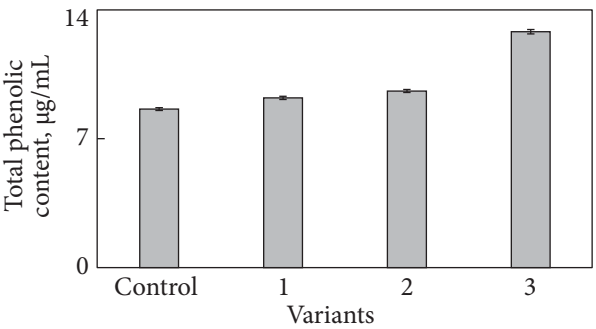

Fig. 2. Total phenol content in the Farreus medium during buckwheat cultivation over 14 days with the addition of varying amounts of *A. vinelandii*: *1* — 10^5 cells/mL; *2* — 10^6 cells/mL; *3* — 10^7 cells/mL

Table 3. **Influence of different doses of *A. vinelandii* IMV B-7076 on the accumulation of carbohydrates by buckwheat plants in the Farreus medium**

Research options	Carbohydrate content	
	μg/mL	%
Control — medium without *A. vinelandii*	4.59 ± 0.80	100.0
A. vinelandii (3.00±0.06) · 10^5 cells/mL	6.51 ± 0.70	141.8
A. vinelandii (2.32±0.02) · 10^6 cells/mL	4.57 ± 0.10	104.8
A. vinelandii (2.23±0.03) · 10^7 cells/mL	16.82 ± 0.90	366.4

crobiota on plant root exudation remains inadequately researched (Korenblum et al., 2020).

We have demonstrated that the introduction of azotobacter into the buckwheat growth medium in hydroponic culture significantly influences the growth activity of these plants, the content of photosynthetic pigments in their leaves, as well as the accumulation of protein, carbohydrates, and phenolic compounds in the medium of cultivation. For instance, the maximum indicators of root and stem length were observed when buckwheat was grown in a medium where the content of azotobacter during the inoculation was at a level of 10^5 cells/mL. With a higher content of these bacteria in the medium, the morphometric indicators of plants decreased.

The investigated quantities of azotobacter increased the chlorophyll content in the leaves of plants when grown in the Farreus medium. The highest chlorophyll content was determined when azotobacter was introduced at 10^6 cells/mL (34% higher than the control). Application of *A. vinelandii* under these conditions slightly increased the content of chlorophyll b and carotenoids.

All investigated doses of azotobacter increased the protein, phenolic, and carbohydrate content in the buckwheat growth medium. The highest levels of these compounds were determined when 1 mL of medium contained 10^7 cells of *A. vinelandii* during inoculation. Thus, the results obtained indicate a significant influence of *A. vinelandii* IMV B-7076 bacteria on the concentration of exometabolites in the medium when buckwheat plants grow under hydroponic conditions.

Conclusions. Introduction of *A. vinelandii* IMV B-7076 into the buckwheat growth medium significantly affects the growth of plants and the content of photosynthetic pigments and exometabolites in the root zone. The most noticeable stimulation of plant growth by azotobacter was observed when bacteria were introduced into the medium at a concentration of 10^5 cells/mL. In the medium where 10^6 cells/mL of azotobacter was introduced, its stimulating effect on buckwheat growth decreased, and at higher concentrations of these bacteria (10^7 cells/mL), a certain suppression of plant growth activity occurred. However, in plants, the chlorophyll a content increased by 22 — 40%, and chlorophyll b and carotenoids slightly increased as well. Additionally, the highest levels of protein, phenolics, and carbohydrates were detected. The results obtained indicate a significant influence of *A. vinelandii* IMV B-7076 on the root exudation of buckwheat plants under conditions of hydroponic cultivation.

Conflicts of interest. The authors declare that there are no conflicts of interest.

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ВПЛИВ *AZOTOBACTER VINELANDII* IMB B-7076 НА РОЗВИТОК ГРЕЧКИ ТА СКЛАД ЕКЗОМЕТАБОЛІТІВ У КОРЕНЕВІЙ ЗОНІ

У процесі росту і розвитку рослини виділяють в кореневу зону велику кількість кореневих ексудатів, за рахунок чого формується мікробіом ризосфери, який здатний стимулювати ріст і продуктивність рослин. Застосування мікробних препаратів стає основою оздоровлення ґрунтів, отримання високоякісної рослинної продукції. **Метою** даної роботи було визначити вплив *Azotobacter vinelandii* IMB B-7076, компонента високоефективного комплексного бактеріального препарату Азогран, на розвиток рослин гречки та склад екзометаболітів у кореневій зоні. **Методи.** Вплив азотобактера на ріст рослин гречки та синтез нею екзометаболітів досліджували при її вирощуванні впродовж 14 діб в середовищі Фарреуса в умовах фітотрону. Вміст у листі рослин фотосинтетичних пігментів визначали спектрофотометричним методом. Вміст білку в середовищі визначали методом Bredford, вуглеводів — за взаємодією їх з фенолом і сірчаною кислотою, а фенолів — з допомогою реактиву Фоліна-Чокальтеу. **Результати.** Встановлено, що вирощування гречки в середовищі з азотобактером значно стимулювало ростову активність рослин, вміст в їх листі хлорофілу a. За цих умов вміст у листі гречки хлорофілу b і каротиноїдів зростав менш помітно. При вирощуванні рослин протягом 14 діб у середовищі, що містило 10^7 кл/мл цих бактерій, вміст білку в ньому зростав, у порівнянні з контролем, на 110,4%, фенольних сполук — на 48,8%, а вуглеводів — на 266,4%. **Висновки.** *A. vinelandii* IMB B-7076 помітно покращує ріст гречки, підвищує концентрацію екзометаболітів у середовищі при її гідропонному вирощуванні.

***Ключові слова**: Azotobacter vinelandii IMB B-7076, рослини гречки, морфометричні показники, вміст фотосинтетичних пігментів, білок, вуглеводи, фенольні сполуки.*