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EFFECT OF *BACILLUS MEGATERIUM* ONU 500 ON THE GERMINATION AND GROWTH OF SUNFLOWER SEEDLINGS

The aim *of the study was to evaluate the effect of Bacillus megaterium ONU 500 on the germination and growth of sunflower seedlings.* ***Materials and methods.*** *Sunflower seeds were sown in containers with soil inoculated with a suspension of bacteria B. megaterium ONU 500 in concentration of 10^6 CFU/ml. After 21 days growth characteristics of seedlings were compared: germination, average height, root length, leaf area, dry weight. Effect of these microorganisms on biofilm formation on roots was investigated with light microscopy.* ***Results.*** *Inoculation of soil with B. megaterium ONU 500 resulted in the positive effect on roots length (increase by 21%), leaf area (increase by 22%) and dry weight of seedlings (increase by 17.8%). No significant effect on germination and plant height was observed. On the roots of plants grown in soil treated with B. megaterium ONU 500 more developed biofilms with well formed matrix were observed, which indicates the stimulation of ability of plant and soil microbiota to form biofilms in presence of bacilli from the studied strain.* ***Conclusion.*** *The investigations demonstrated that bacteria of B. megaterium ONU 500 strain caused a positive effect on development of sunflower seedlings and formation of biofilms on their roots.*

Key words: B. megaterium, sunflower, growth characteristics of seedlings, biofilm.

Bacillus megaterium de Bary – representatives of plant and soil microbiota. Antagonistic and phytostimulative properties of these microorganisms make them useful for application in agriculture [11; 12]. Use of *B. megaterium* as a biofertilizer helps to stimulate plant growth, promotes better decomposition of organic phosphorus and increases resistance to various stresses and a wide range of pathogens [10; 13].

Nowadays, protection of the environment against contamination with chemical fertilizers and pesticides becomes increasingly important. Use of different chemicals leads to depletion of soil fertility and reduced quality of agricultural products. Biological products based on beneficial soil- and plant-associated bacteria can be used as alternative methods to stimulate plant growth, protect them from pathogens and increase chances of survival under stressful conditions [14; 17].

Microorganisms *B. megaterium* are able to synthesize phytohormones that promote plant growth and increase yield of horticultural crops. They also cause

visible improvement of morphological characteristics of plants and reduce seed germination time. *B. megaterium* produce cytokinins, indole-3-acetic and abscisic acids, which play an important role in plant growth regulation, cytokinesis and in the regulation of environmental stress [16; 19].

Modern crop production is based on the ideas of using biological preparations containing microbial phytohormones due to the simplicity and cheapness of their production [14]. In addition, an important factor in yielding a high-quality crop is the control of diseases. Some strains of *B. megaterium* are described as agents of biological control – they can colonize the rhizosphere of plants and provide protection against pathogenic microorganisms [7; 10; 13; 16].

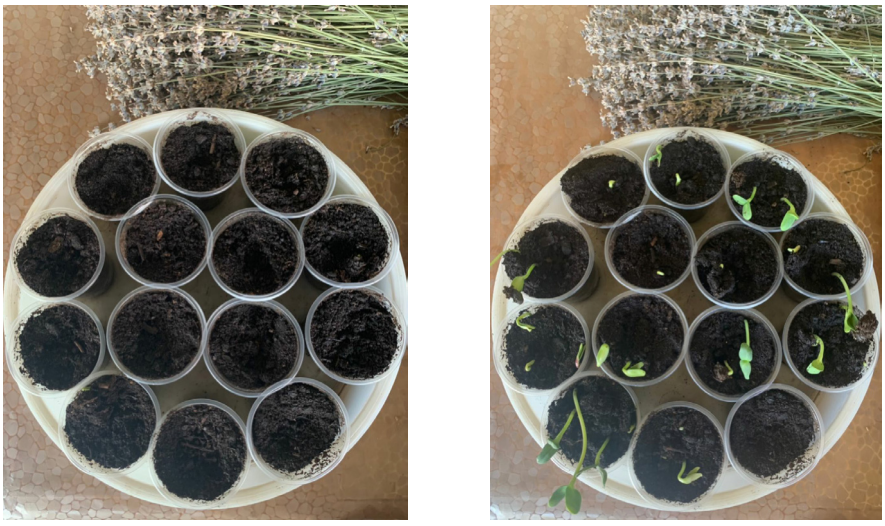
B. megaterium are phosphate-mobilizing bacteria that are able to absorb phosphorus from compounds inaccessible to plants and transport them to roots. They also regulate water and minerals metabolism of plants. As a result, increased resistance of plants to drought, heat and salt stress is observed, which makes *B. megaterium* extremely attractive for use in arid regions [19].

Due to phytostimulating properties useful for agriculture, the aim of the work was to evaluate the effect of *Bacillus megaterium* ONU 500 on the germination and growth of sunflower seedlings.

Materials and methods

The culture of *B. megaterium* ONU 500 was cultivated overnight at 28 °C in liquid LB medium (trypton – 10 g/l, yeast extract – 5 g/l, NaCl –10 g/l) [9]. The concentration of overnight culture was measured using a SmartSpec spectrophotometer (BioRad). The culture was diluted with tap water to 10^6 CFU/ml according to Tverdokhlib et al. [18].

Sunflower seeds [4] were sown in plastic containers with peat soil (4 seeds in each container) to a depth of 0.5–1.0 cm (fig. 1).



A B

Fig. 1. Sunflower seedlings on the first (A) and third (B) day after sowing

The soil was inoculated with a suspension (50 ml) of *B. megaterium* ONU 500 in concentration of 10^6 CFU/ml on the first day of the experiment. The soil in a control variant was poured with the equivalent volume of water. Three independent experiments of 200 seeds in each variant were conducted.

Sunflower plants growth was observed for three weeks under the next conditions: open air, partial shade, 12-hour daylight, temperature 28 ± 2 °C, moderate humidification. Biometric indexes were measured on 22nd day of the experiment – on the phase of 5–6 leaves. Seed germination, average seedling height, seedling root length and seedling leaf area were evaluated [8]. Average dry weight of seedlings was measured. Average values and confidence intervals were calculated using the Excel application package.

To study the effect of *B. megaterium* ONU 500 on biofilm formation, microscopy of biofilms was performed. Roots of sunflower seedlings were dyed with 1% solution of acridine orange for 10 minutes. The stained roots were dried on a glass slide and examined under a light microscope with a total magnification of 100x. The level of biofilm formation was assessed on a scale according to Galkin et al. [1]:

Table

The evaluation criteria of the formation of biofilms

Criteria	Description
–	Biofilms are not formed
+	Individual attached cells without formation of biofilms
+ +	Individual developed microcolonies
+ + +	Developed biofilms with gaps in the structure
+ + + +	Developed biofilms with matrix and without gaps

Biofilm studies were performed in each independent experiment.

Results and discussion

Germination of plants grown in the soil inoculated with *B. megaterium* ONU 500 (87.3 ± 2.9%) did not significantly differ from the control (85.0 ± 2.5%).

Similarly, inoculation of soil with bacilli of this strain did not significantly affect the height of sunflower seedlings (fig. 2, a).

At the same time, a significant positive effect of inoculation with *B. megaterium* ONU 500 on the root length was observed. The average root length increased by 21.08% (fig. 2, b).

The results of Dahmani et al. confirmed that strains of *B. megaterium* had positive effect on the growth and development of plant roots [10]. These micro-

organisms also contribute to the formation of lateral roots and increase the length of root hair, which is necessary for collection and transfer of water and minerals present in soil to roots [10; 13; 15].

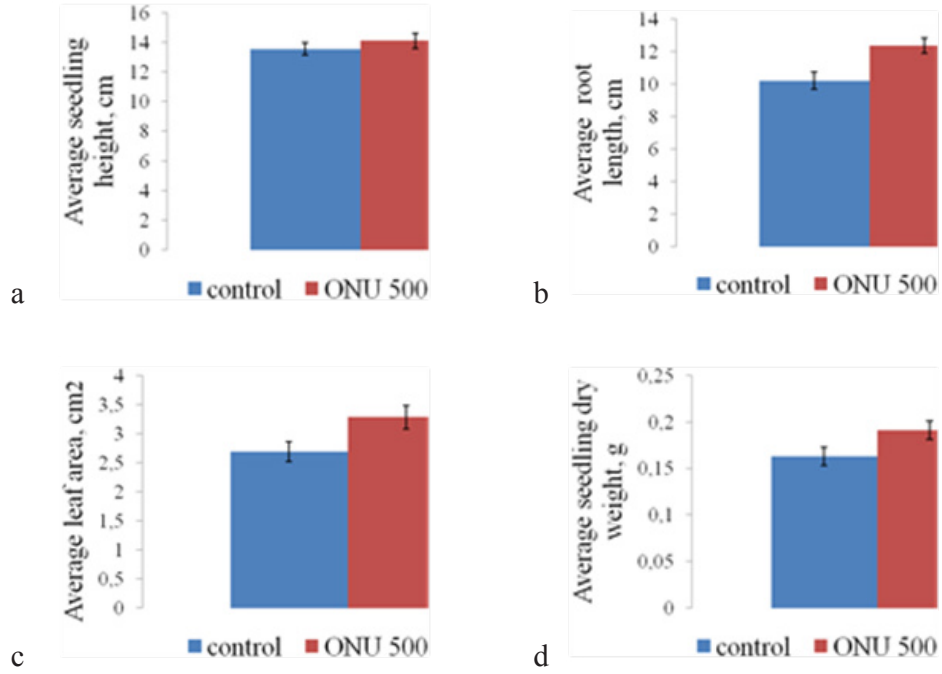


Fig. 2. Biometric indexes of the sunflower seedlings:
a – average height (cm); b – average length of roots (cm); c – average leaf area (cm^2); d – average dry weight (g).

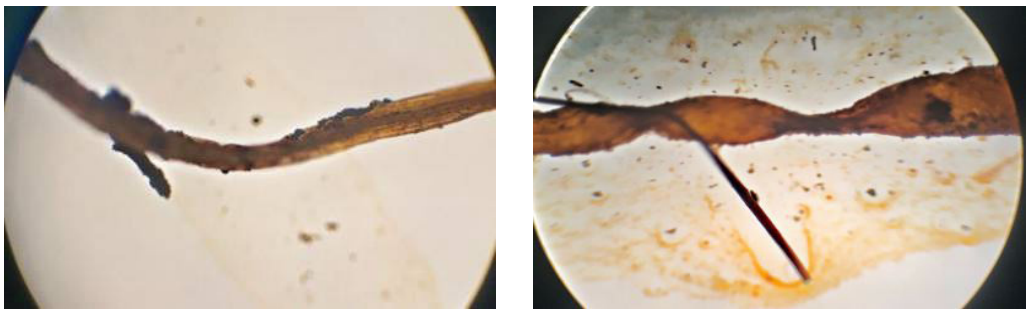
Treatment with a suspension of bacilli increased leaf area by 21,93% (fig. 2, c). Phytostimulation effect of inoculation with bacilli was also observed in case of dry weight of seedlings: it increased by 17.8% as compared with the control (fig. 2, d).

The results of our study coincide with the literature data. Previous studies of Tverdokhlib et al. [18], Teslyuk et al. [10] and Dahmani et al. [5] indicate the significant phytostimulatory effect of *B. megaterium* strains on plant growth, development of roots and vital functions [5; 10; 18]. Literature data indicated that cultural medium LB by itself did not cause the stimulation effect [16] but in majority of cases inhibited plant growth [6]. The results of our previous studies also have shown the inhibitory effect of LB and LB mixed with MRS medium on germination and plants growth [2; 3]. According to this, we could suggest that increasing of some biometric indexes of sunflower seedlings could be explained by the influence of bacteria *B. megaterium* ONU 500 but not the cultural medium.

It could be hypothesized that improvement of biometric indexes of the plants occurred due to ability of *B. megaterium* to synthesize phytohormones [7; 10; 15; 16; 19], but this suggestion needs further investigations.

The influence of *B. megaterium* ONU 500 on formation of biofilms on roots of sunflower seedlings was studied. In the control variant, slightly developed bio-

films on roots were formed. In presence of bacteria *B. megaterium* ONU 500 more developed, mature biofilms with well-formed matrix (++++) were observed in all experimental variants. The level of biofilm formation became higher in plants grown in the soil treated with bacilli of this strain (fig. 3).



Control *B. megaterium* ONU 500 (++++)

Fig. 3. Biofilms on the sunflower seedlings roots in the control (without inoculation) and experimental (after inoculation of soil with *B. megaterium* ONU 500) variants (100x)

Microorganisms, in particular bacilli, are able to form biofilms on plant roots and integrate into natural biofilms of other microorganisms, enhancing their protective properties [20].

The obtained results allow us to suggest that bacteria *B. megaterium* ONU 500 are able to enhance the formation of biofilms by other representatives of plant and soil microbiota. As a result, developed integrated biofilms on the roots of sunflower plants grown in the soil inoculated with *B. megaterium* ONU 500 were formed as compared with the plants from the non inoculated soil.

Thus, the study of *B. megaterium* ONU 500 has shown that these bacteria could be used for stimulation of plant growth. The further investigation of the mechanisms of biofilm formation in presence of *B. megaterium* ONU 500 could elucidate another positive aspect of using bacilli – as stimulators of plant and soil microbiota.

Conclusions

The investigations demonstrated that bacteria of *B. megaterium* ONU 500 strain caused a positive effect on development of sunflower seedlings and formation of biofilms on their roots.

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ВПЛИВ *BACILLUS MEGATERIUM* ОНУ 500 НА ПРОРОСТАННЯ ТА РІСТ СІЯНЦІВ СОНЯШНИКА

Реферат

Мета. *Дослідити вплив B. megaterium ОНУ 500 на проростання насіння та ріст сіянців соняшника.* ***Матеріали і методи.*** *Насіння соняшника висівали у контейнери із грунтом, в який вносили суспензію бактерій штаму B. megaterium ОНУ 500 у концентрації 10^6 КУО/мл. Через 21 день після висіву визначали ростові характеристики паростків: схожість, середню висоту паростків, довжину кореня, площу листової пластинки, суху масу паростків. Методом світлової мікроскопії оцінено вплив даних мікроорганізмів на формування біоплівки на коренях.* ***Результати.*** *За інтродукції бактерій B. megaterium ОНУ 500 у грунт спостерігали збільшення довжини коренів сіянців соняшника на 21%, площі листків – на 22%, сухої маси паростків – на 17,8%. На схожість та висоту рослин суттєвого впливу не виявлено. На коренях рослин, які росли у грунті з інтродукованими B. megaterium ОНУ 500, спостерігали більш розвинені біоплівки з повністю сформованим матриксом, що свідчить про підсилення здатності мікробіоти рослин і грунту утворювати біоплівки під впливом бацил даного штаму.* ***Висновок.*** *Проведені дослідження показали, що бактерії штаму B. megaterium ОНУ 500 чинять позитивний вплив на розвиток сіянців соняшника та формування біоплівок на їх корінні.*

Ключові слова: B. megaterium, соняшник, біометричні показники паростків, біоплівка.

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ВЛИЯНИЕ *BACILLUS MEGATERIUM* ОНУ 500 НА ПРОРОСТАНИЕ И РОСТ СЕЯНЦЕВ ПОДСОЛНЕЧНИКА

Реферат

Цель. *Оценить влияние B. megaterium ОНУ 500 на прорастание семян и рост сеянцев подсолнечника.* ***Материалы и методы.*** *Семена подсолнечника высевали в контейнеры с почвой, в которую вносили суспензию бактерий штамма B. megaterium ОНУ 500 в концентрации 10^6 КОЕ/мл. Через 21 день после посева сравнивали ростовые характеристики ростков: всхожесть, среднюю высоту ростков, длину корня, площадь листовой пластинки, сухую массу проростков. Методом световой микроскопии проведена оценка вли-*

яния этих микроорганизмов на формирование биопленки на корнях. ***Результаты.*** *Внесение в почву B. megaterium ОНУ 500 привело к увеличению длины корней сеянцев на 21%, площади листьев – на 22%, сухой массы ростков – на 17,8%. На всхожесть и высоту растений существенного влияния выявлено не было. На корнях растений, которые росли в почве с интродуцированными B. megaterium ОНУ 500, наблюдались более развитые биопленки с полностью сформированным матриксом, что свидетельствует о повышении способности микробиоты растений и почвы образовывать биопленки под влиянием бацилл данного штамма.* ***Вывод****. Проведенные исследования показали, что бактерии штамма B. megaterium ОНУ 500 оказывают положительное влияние на развитие сеянцев подсолнечника и формирование биопленок на их корнях.*

Ключевые слова: B. megaterium, подсолнечник, биометрические показатели проростков, биопленка.

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