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Article

Treatment of seeds with cyanobacterial biofilm to increase plant resistance to methylphosphonate pollution

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Abstract. The effect of biofilms dominated by the cyanobacterium *Nostoc commune*, methylphosphonic acid and their combined action on the biochemical and physiological parameters of the vital activity of barley plants was studied. Methylphosphonic acid at a concentration of 0.5 and 1 mM was shown to promote the activation of lipid peroxidation processes in leaves by 20 and 60%, respectively, as well as a decrease in the amount of chlorophyll and the length of seedlings by 20%. Inoculation of seeds with natural multispecies biofilms dominated by the cyanobacterium *Nostoc commune* reduced the phytotoxic effects of methylphosphonic acid, which manifested itself in the inhibition of the intensity of lipid oxidation processes, the accumulation of chlorophyll (under the action of acid at a concentration of 0.5 mM), antioxidant carotenoids, and also in the stimulation of growth processes. In response to the treatment of seeds with biofilms, the accumulation of anthocyanins in the leaves decreased, while the pigment content did not exceed 70% of the level of control plants. It has been established that biofilms dominated by the cyanobacterium *Nostoc commune* contribute to an increase in plant resistance to contamination with methylphosphonic acid.

Keywords: methylphosphonic acid, cyanobacteria, lipid peroxidation, pigments, growth, anthocyanins, *Nostoc commune*, *Hordeum distichon*

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Научная статья

Обработка семян биопленками цианобактерий для повышения устойчивости растений в условиях химического загрязнения метилфосфонатами

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Аннотация. В ходе работы было исследовано влияние биопленок с доминированием цианобактерии *Nostoc commune,* метилфосфоновой кислоты и их совместного действия на биохимические и физиологические показатели жизнедеятельности растений ячменя. Показано, что метилфосфоновая кислота в концентрации 0.5 и 1 мМ способствовала активации процессов перекисного окисления липидов в листьях ячменя на 20 и 60% соответственно, а также снижению количества хлорофиллов и длины проростков на 20%. Инокуляция семян ячменя природными многовидовыми биопленками с доминированием цианобактерии *Nostoc commune* снижала фитотоксические эффекты метилфосфоновой кислоты, что проявилось в ингибировании интенсивности процессов окисления липидов, накоплении хлорофиллов (при действии кислоты в концентрации 0.5 мМ), антиоксидантов – каротиноидов, а также в стимуляции ростовых процессов. Отмечено, что в ответ на обработку семян биопленками снижается накопление антоцианов в листьях: содержание пигмента не превышало 70% от уровня контрольных растений. Таким образом, биопленки с доминированием цианобактерии *Nostoc commune* стимулируют механизмы устойчивости растений при загрязнении среды метилфосфоновой кислотой.

Ключевые слова: метилфосфоновая кислота, перекисное окисление липидов, пластидные пигменты, антоцианы, рост, *Nostoc commune*, *Hordeum distichon*

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Introduction

Agroecosystems currently occupy more than 10% of the land (Sheugen et al., 2018). At the same time, as the population grows, the agriculture will grow accordingly to sustain its needs, which, in addition to transforming natural landscapes, is associated with other environmental problems, such as environmental pollution and depletion of soil cover. Active agro-chemicalization contributes to pollution with pesticides and their degradation products, which include methylphosphonates.

Rapid increases in crop production require the use of numerous chemicals, including organophosphorus pesticides. Glyphosate (Roundup, Hurricane, Glyfor, etc.), the active ingredient of which is a derivative of methylphosphonic acid, N-phosphonomethyl glycine is one of the best-selling herbicides in the world (Baylis, 2000). Methylphosphonates in the environment degrade to methylphosphonic acid (MPA) (Bryzgalina et al., 2014). The rate of decomposition of some methylphosphonates and their biodegradation products in the soil depends on the climate and the chemical characteristics of the soil and varies from 55 days to 3 years or more (Savelieva et al., 2002). MPA is resistant to photolysis, as well as to chemical and thermal effects (Kononova and Nesmeyanova, 2002). This persistence is ensured by the presence of a chemically stable carbon–phosphorus (C–P) bond in the MPA molecule. It MPA is known to interfere with activity of phototrophs and to affect soil microbiota (Ashikhmina et al., 2007; Koval et al., 2013; Ogorodnikova et al., 2004).

The soil environment is inhabited by many microorganisms: bacteria, including actinomycetes, which participate in the cycle of nitrogen, phosphorus, sulfur, iron, carbon and other elements, organic-decomposing fungi, soil algae, which enrich the soil with oxygen and serve as nutrient for other food chains participants. Metabolism of microorganisms helps to saturate the soil with humic acids, available macro- and microelements, enzymes, growth stimulants, vitamins, and hormones. All this improves soil structure and, in general, soil fertility (Bat'kaev, 2013). Hence, microbial biopreparations, which are already being used in agriculture and forestry, are of great interest for increasing soil productivity, improving the well-being of plants, stimulating their growth and increasing immunity (Domracheva et al., 2020).

The cyanobacterium (CB) *Nostoc commune* Vaucher ex Bornet & Flahault is one of the most active widespread colonizers (Domracheva et al., 2007), even in polluted environments (Kireeva et al., 2003). Possibly, the unique adaptive and ecological abilities of this CB are related to the fact that the species is an edificator of multispecies algocyanobacterial cenoses with a rich spectrum of associated heterotrophs (Domracheva et al., 2007). Often, various groups of microorganisms (including *N. commune*) form soil biofilms (SB), i.e., structured colonies with a high cell density of various organisms. Studies of the bioremediation capabilities of such biofilms with the prospect of using them to increase the resistance of plants to chemical pollution are relevant.

The purpose of this study was to study the effect of inoculation of barley seeds with multispecies biofilms dominated by the CB *N. commune* on the biochemical and growth parameters of plants affected by MPA pollution.

Material and methods

The objects of the study were barley plants of the biserial variety Novichok (*Hordeum distichon* L.) and SB dominated by the CB *N. commune*, collected by L.V. Kondakova on a sand and gravel embankment along the railway in the Nizhny Novgorod region. The barley variety Novichok is characterized by resistance to black and covered smuts, medium susceptibility to root rot pathogens, which may include phytopathogenic fungi of the genera *Fusarium, Bipolaris*, *Typhula, Rhizoctonia*, etc., stable yield, as well as aluminum tolerance (Golovko et al., 2004; Rodina et al., 2007).

In the course of the work, natural SB were preliminarily grown under 12 hours of illumination (3000 lux) and at a temperature of +25 °C on a nitrogen-free Gromov no. 6 culture fluid. Immediately prior to use in the experiment, SB was broken up to obtain a cell suspension. In addition to the dominant species, the biofilms included homocystic and heterocystic cyanobacteria (eight species), as well as unicellular green algae (*Chlorella mirabilis* V.M. Andreeva). Among other CB, SB included *N. punctiforme* (Kütz.) Har.*, Phormidium autumnale* (C. Agardh) Gomont*, Ph. mole* (Kütz.) Gomont*, Leptolyngbya foveolarum* (Mont. Ex Gomont)*, Plectonema nostocorum* Gomont*, Leptolyngbya fragilis* (Gomont) Anagnostidis & Komárek, *Phormidium uncinatum* Gomont ex Gomont, and *Borzia trilocularis* Cohn ex Gomont. The abundance of the dominant species *N. commune* in the cell culture was 1.61 (± 0.21) 10^9 cells/g (83.12%) (Gornostaeva et al., 2013). The species composition of associated bacteria has not been studied.

For the experiment, a 7-week-old SB culture was used (the biofilm age was counted from the beginning of dry film cultivation in Gromov's culture fluid). The cell titer was $1.6 \cdot 10^8$ cells/ml (determined by direct counting under a microscope in the Goryaev chamber).

Within a week, barley seeds were germinated in distilled water in Petri dishes (15–20 seeds per dish) with and without SB suspension. The experiments were run on an aqueous medium, in particular, on the Knop nutrient solution (control variant) and MPA solutions of various concentrations (0.5 mM and 1 mM), where week-old barley seedlings were transplanted, 20 pieces per container.

The effect of SB on the viability of barley seedlings, including those under conditions of MPA contamination, was assessed by physiological (changes in seedling growth) and biochemical parameters: the activity of lipid peroxidation processes (LPO) in the roots and leaves of experimental plants, the content of anthocyanin and photosynthetic pigments.

Growth of organs (roots and shoots) was studied using direct measurement, which involved 20 plants from all experimental variants. The intensity of LPO processes in plant cells was analyzed by the color reaction of thiobarbituric acid with malondialdehyde (MDA), one of the LPO products (wavelength 532 nm) (Lukatkin, 2002). The content of anthocyanin pigments in barley leaves was determined spectrophotometrically using Muravieva's method at wavelengths of 510, 657 nm (Muravieva et al., 1987). The abundance of plastid pigments was estimated using spectrophotometric measurement of the level of chlorophyll a and b, as well as carotenoids in acetone extract (wavelength 662, 644 (chlorophylls), 440.5 nm (carotenoids)) (Shlyk, 1971). All experiments were duplicated three times.

Results and discussion

MPA had a toxic effect on barley seedlings shown by activation of oxidative processes in cells assessed by the accumulation of MDA (Fig. 1). In experiments with contamination of the growing culture with MPA (0.5 mM and 1 mM), the content of MDA in leaves was 1.2 and 1.6 times higher than in the control, respectively. The roots were more resistant to MPA, no significant differences with the control in the content of MDA were found.

In studying the effect of cyanobacterial inoculation on the intensity of LPO processes in barley roots and leaves, it was found to that the addition of *N. commune*-dominated SB during germination contributed to a decrease in the amount of MDA in plant cells (Fig. 1, K + SB). Leaves reacted most sensitively to SB treatment, where the accumulation of LPO products decreased by 10% from the control.

The protective effect of SB for plants was noted on media contaminated with MPA. In the experimental variants with SB treatment and growing plants in the presence of MPA, the intensity of LPO processes in plant cells decreased to the level of the control plants, which is significantly less than in experiments with the action of MPA without SB (Fig. 1).

The content of plastid pigments is an important indicator reflecting the physiological state of plants. The study showed that priming the seeds with SB during germination subsequently inhibited accumulation of the total amount of chlorophylls *a* and *b* in the leaves of experimental plants (Table 1). MPA at a low concentration (0.5 mM) also caused a significant decrease in the level of green pigments in plant tissues by 20% compared to control plants ($p \leq 0.5$). When exposed to 1 mM MPA, the amount of chlorophylls remained within the control. Seed inoculation with SB stimulated the accumulation of chlorophylls under conditions of MPA contamination (0.5 mM) to the level of control plants. When exposed to MPA at an increased concentration, no significant changes

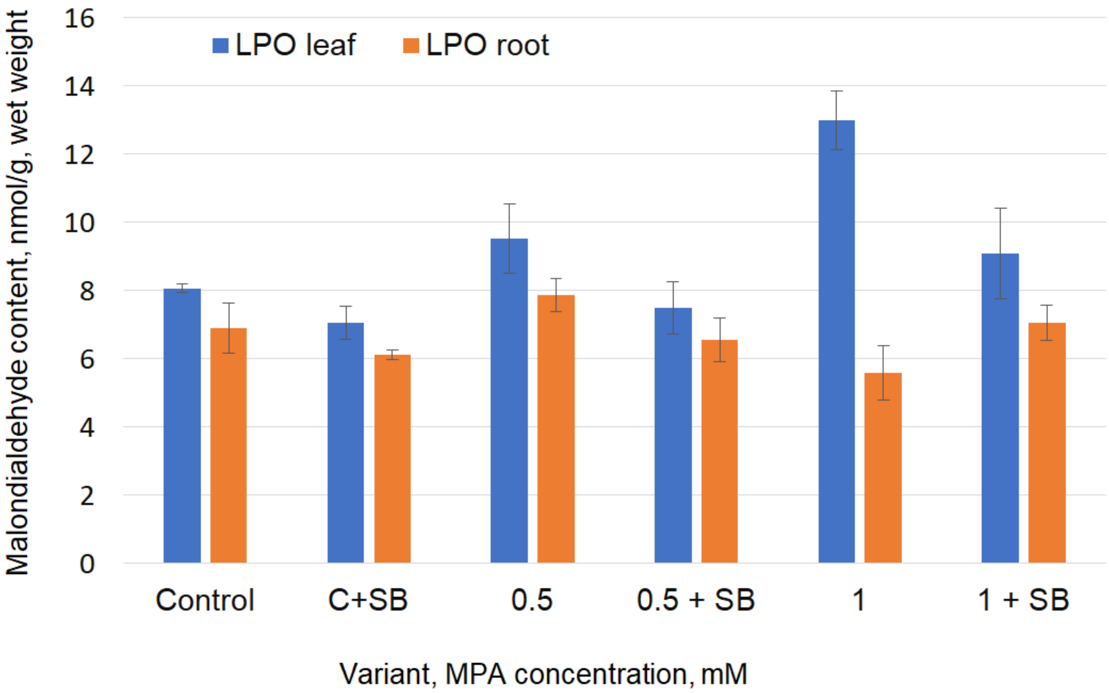

Fig. 1. Changes in the activity of lipid peroxidation processes in barley organs under the influence of methylphosphonic acid (MPA) and *Nostoc commune*-dominated natural biofilms (SB).

were found in the amount of chlorophyll in plants of which the seeds had been treated with SB during germination.

In response to MPA, substances with antioxidant properties, carotenoids and anthocyanins, accumulated in plant cells, which is a nonspecific response to stressful growing conditions (Chupakhina et al., 2011). Both anthocyanins and carotenoids are low molecular weight antioxidants in the plant defence system. Thus, 0.5 mM MPA contributed to a decrease in the level of anthocyanins, and MPA at a higher concentration (1 mM), on the contrary, activated the accumulation of anthocyanins on average 1.2 times higher than the control (Fig. 2). Plants grown with the addition of SB, both in the presence of MPA and in the control variant, were distinguished by a reduced content of anthocyanins (on average, 40% lower than the control). The intensity of LPO processes and the content of anthocyanin pigments closely correlated with each other (r = 0.79).

Under the combined action of MPA and SB on seedlings, the leading role in antioxidant protection was played by carotenoids, the level of which increased compared to their amount in the variants with the action of pure MPA. Significant differences in the content of carotenoids were revealed in the variant with 0.5 mM MPA: the abundance of yellow pigments decreased by 20% from the control level (Table 1). At the same time, MPA at a higher concentration (1 mM), on the contrary, stimulated an increase in the abundance of pigments. In seedlings inoculated during germination with SB and grown on a solution of 1 mM MPA, this pattern was preserved, and the increase in the abundance of carotenoids was 27% of the control.

Plant growth is also an integral indicator of adaptation to environmental conditions. Seed treatment with SB had a growth-activating effect on barley seedlings: the length of shoots and roots was significantly higher than in the control variant (Fig. 3, K + SB). In experiments with MPA, on the contrary, the linear growth of shoots was inhibited. At the same time, the roots were less sensitive to contamination of the growing culture; their length did not differ significantly from the roots of control plants. Seed inoculation with SB during germination reduced the growth inhibitory effect of MPA. The length of shoots and roots of barley in experiments with the action of SB and MPA was significantly longer compared to plants that were not treated with SB. Probably, the growth-stimulating effect is associated with the presence of auxin- and gibberellin-like substances in CB cells (Domracheva et al., 2007).

Conclusions

1. MPA has a phytotoxic effect on barley plants. It manifests itself in the activation of LPO processes, a decrease in the amount of chlorophylls (at 0.5 mM MPA), and inhibition of shoot growth. MPA at the studied concentrations inhibits the linear growth of the terrestrial part of seedlings, while the pollutant at a high dose (1 mM) significantly activates LPO processes in leaves, which is accompanied by an increase in the concentration of antioxidant substances in cells (anthocyanins and carotenoids).

2. Seed inoculation with multispecies biofilms dominated by *N. commune* has a protective effect on barley grown on MPA-contaminated substrate. The phytoprotective properties of SB are realized by inhibiting the intensity of lipid oxidation processes in the leaves of experimental plants and increasing the amount of carotenoids that play the role of antioxidants in plant cells.

3. Pre-sowing inoculation of seeds with *N. commune*-dominated biofilms induces significant growth of barley organs grown in the presence of MPA. This shows the phytoprotective effect of the organisms that make up the biofilms.

Table. 1. The content of plastid pigments in barley leaves under the action of methylphosphonic acid and biofilms (SB) dominated by *Nostoc commune*.

Variant, mM	Pigment content, mg/g dry weight	
	Chlorophyll *a+b*	Carotenoids
Control (0)	8.34 ± 1.24	1.17 ± 0.14
Control + SB	6.42 ± 0.15	1.00 ± 0.15
0.5 MPA	6.76 ± 0.30	0.96 ± 0.02
0.5 MPA + SB	7.89 ± 0.40	1.10 ± 0.03
1 MPA	9.01 ± 0.17	1.35 ± 0.03
1 MPA + SB	8.93 ± 0.10	1.49 ± 0.11

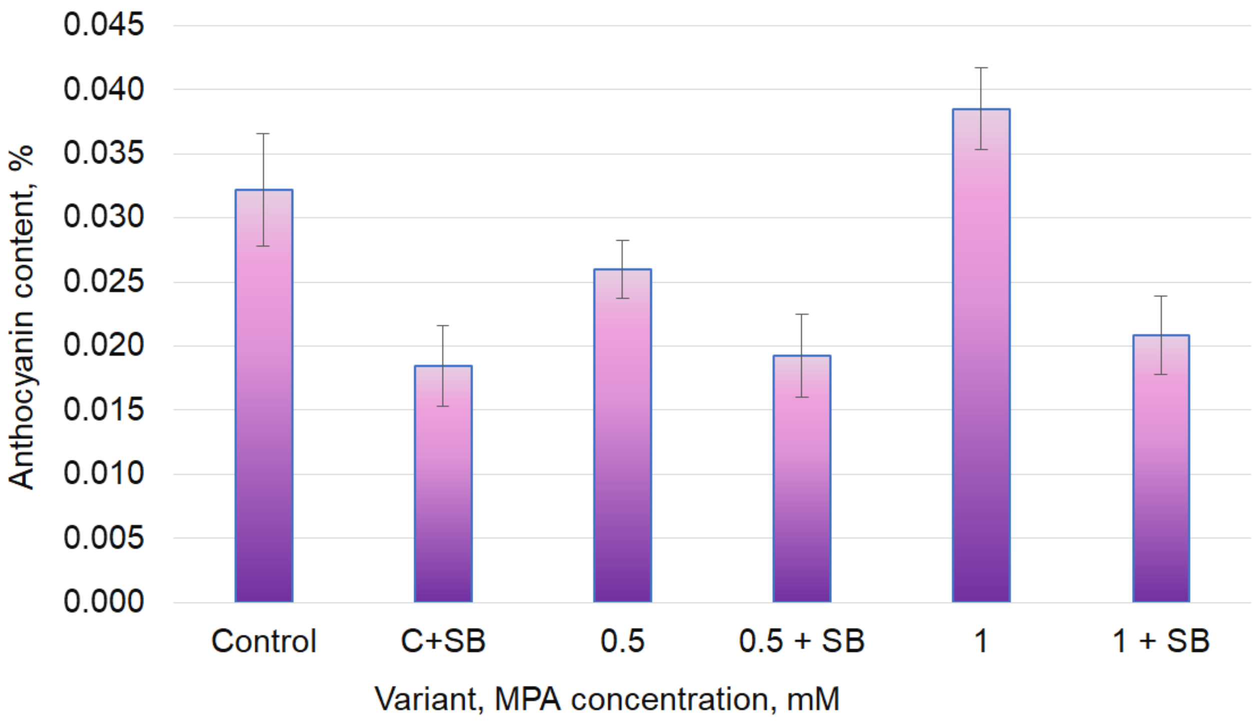

Fig. 2. The effect of methylphosphonic acid and *Nostoc commune*-dominated natural biofilms on the accumulation of anthocyanins in barley leaves.

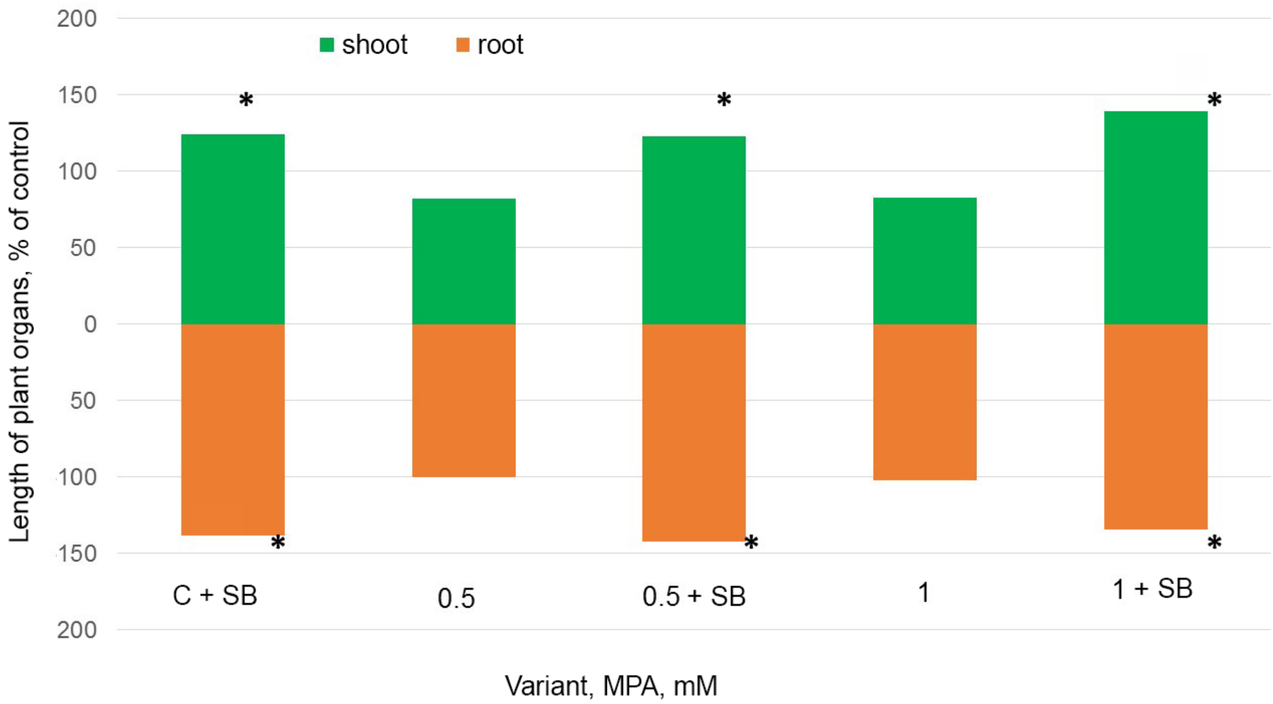

Fig. 3. Influence of methylphosphonic acid and *Nostoc commune*-dominated biofilms on the growth of barley plants. * – differences with control are significant at $p \geq 0.05$.

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