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The effect of metal-containing biocomposites of fungal origin on potato plants *in vitro*

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Abstract: *The effect of metal (II)-containing composites based on extracellular metabolites of basidiomycetes Pleurotus ostreatus, Ganoderma lucidum, Grifola umbellata and Laetiporus sulphureus on the viability and response of potato plants in vitro has been investigated. The Lukyanovsky variety of potato, which is susceptible to ring rot, caused by the bacterium Clavibacter sepedonicus, was studied. The parameters investigated included biofilm formation by Clavibacter sepedonicus, various morphometric parameters of plants and the phytotoxicity of substances of fungal origin. The greatest anti-biofilm-forming effect was observed in metal-containing biocomposites based on G. lucidum; Fe- and Co-containing biopreparations inhibited the formation of Clavibacter sepedonicus biofilms by 40–50%. The plant height was adversely affected by composites, in the absence of metal (II), derived from L. sulphureus and P. ostreatus, as well as by a Co-containing composite derived from P. ostreatus. The decrease in plant growth, in comparison with the control, can be associated with the pronounced antibiotic properties of these basidiomycetes and cobalt. The remaining biocomposites studied did not have an adverse effect on the growth of potatoes in vitro. A number of morphometric parameters (length of internodes, number of leaves) remained virtually unchanged when exposed to biocomposites of fungal origin. In contrast to the vegetative part of plants, the biomass and length of the roots increased by 10–20% under the influence of biocomposites. Copper-containing composites derived from G. lucidum had no phytotoxic effect on plants and enhanced potato resistance to Clavibacter sepedonicus. The beneficial properties of biocomosites may be judged by the degree of stimulation of the physiological processes underlying the formation of the underground part of the plants, which is a prerequisite for increasing yields. The biocomposites are environmentally friendly because of their natural origin and being effective at very low doses. The results obtained using metal-containing biocomposites derived from G. lucidum and Gr. umbellata demonstrate the safety and possible improvement in health of potato plants by using biocomposites derived from cultures of higher fungi.*

Keywords: *Solanum tuberosum L., higher fungi, phytopathogenic bacteria, Clavibacter sepedonicus, metals (II), biocomposites*

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Влияние металлсодержащих биокомпозитов грибного происхождения на растения картофеля *in vitro*

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Резюме: *Изучено влияние содержащих металлы(II) композитов, полученных на основе внеклеточных метаболитов базидиомицетов Pleurotus ostreatus, Ganoderma lucidum, Grifola umbellata и Laetiporus sulphureus, на жизнеспособность и ответные реакции растений картофеля in vitro. Исследования проводили на пробирочных растениях картофеля in vitro сорта Лукьяновский, восприимчивого к возбудителю кольцевой гнили – бактерии Clavibacter sepedonicus. Изучали биопленкообразование Clavibacter sepedonicus, морфометрические показатели растений, фитотоксичность субстанций грибного происхождения. Наибольший антибиопленкообразующий эффект наблюдался у металлсодержащих биокомпозитов на основе G. lucidum; Fe- и Co-содержащие биопрепараты подавляли образование биопленок Clavibacter sepedonicus на 40–50%. На высоту растений негативно воздействовали только не содержащие металлы(II) композиты на основе L. sulphureus и P. ostreatus, а также Co-содержащий препарат из P. ostreatus. Снижение прироста растений по сравнению с контролем можно связать с выраженными антибиотическими свойствами этих базидиомицетов и кобальта. Остальные исследуемые биокомпозиты не оказывали негативного влияния на рост картофеля in vitro. Ряд морфометрических показателей растений (длина междоузлий, количество листьев) оставался практически неизменным при воздействии биокомпозитов грибного происхождения. В отличие от вегетативной части растений биомасса корней и их длина увеличивалась на 10–20% под влиянием биокомпозитов. Медьсодержащие препараты из G. lucidum не проявляли фитотоксического действия в отношении растений и обладали эффектом усиления устойчивости картофеля к Clavibacter sepedonicus. Стимуляция физиологических процессов формирования подземной части растений как предпосылка повышения урожайности позволяет судить о полезных свойствах предложенных биокомпозитов, экологически чистых благодаря природному происхождению и эффективных в очень малых дозах. Полученные результаты свидетельствуют о безопасности биокомпозитов на основе G. lucidum и Gr. umbellata для растений картофеля и возможных перспективах оздоровления картофеля с применением металлсодержащих биокомпозитов, полученных с использованием культур высших грибов.*

Ключевые слова: *Solanum tuberosum L., высшие грибы, фитопатогенные бактерии, Clavibacter sepedonicus, металлы(II), биокомпозиты*

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INTRODUCTION

Bacteria of the genus *Clavibacter* infect a wide range of cultivated and weed plants [1], including plants of the *Solanaceae* family, which are the most important agricultural crops [2]. The causative agent of ring rot in potatoes [3] belonging to the genus *Clavibacter* is *Clavibacter michiganensis* ssp. *sepedonicus* (*Cms*) (Spiekermann and Kotthoff, 1914; Davis et al., 1984), designated *Clavibacter sepedonicus* in the new classification [4]. To date, there are no effective means to regulate the population of this pathogen [5, 6]. Moreover, there are no drugs of chemical or biological origin capable of limiting the spread of bacterial potato diseases, and the use of biocontrol agents is highly problematic [1]. Hence, there is an opportunity to develop substances that are effective in combating bacteria, safe to use with the plants and possibly beneficial for the health of the potato when affected by the phytopathogenic gram-positive bacterium *Cms*. Biocomposites of fungal origin avoid both the environmental risks associated with modern chemical pesticides and the increasing resistance of phytopathogens to chemical pesticides [7]; phytopathogens decrease both the yield and quality of these strategically important crops [8]. Therefore, the most satisfactory solution would be to identify preparations based on natural compounds.

The use of biometals in conjugates of biopolymers and edible fungi represents a more environmentally friendly option for plant protection compared to the use of inorganic chemical forms of metals, such as oxides and salts. For instance, one of the most phytotoxic types of nanomaterials is zinc oxide particles, which are capable of inhibiting the growth of roots of radish, rape, ryegrass, lettuce, corn, cucumber [9]. Inorganic nanostructures were shown to inhibit seed germination, based on indicators such as the length of the seedlings and the rate of plant growth, when analysing the degree of bioavailability and toxicity of both copper nanoparticles with *Phaseolus radiatus* and *Triticum aestivum* [10] and magnetite nanoparticles Fe_3O_4 and ZnO with *Arabidopsis thaliana* [11]. The use of other metals that do not belong to microelements and are known for their antimicrobial properties, primarily noble metals, is not economically feasible for combating bacterial phytopathogens. In addition, Ag readily leaks into wastewater during rinsing thereby adversely affecting the beneficial bacteria used in wastewater treatment. Similarly, the ingress of silver into water bodies is dangerous for aquatic organisms [12].

A wide range of biologically active metabolites of higher fungi are represented by high molecular weight protein, carbohydrate and lipid substances, by low molecular weight compounds such as amino acids, monosaccharides and fatty acid substances, and finally by groups of compounds dominant in the chemical composition of a mushroom culture. Basidiomycetes, which produce a unique complex of biologically active substances, are being investigated as a source of substances with antibacterial properties, for example we previously studied [13] the generation of potentially antimicrobial inorganic components in extracellular metabolites of basidiomycetes in hybrid biocomposites.

The purpose of this study is to evaluate a treatment for improving the health of agricultural plants. The treatment is created from composites containing metal (II) compounds in macromycete metabolites and is evaluated by determining the viability and the response of potato plants *in vitro*.

EXPERIMENTAL

Research subjects and cultivation conditions. The strain *Clavibacter sepedonicus* Ac-1405 was obtained from the All-Russian collection of microorganisms (Pushchino-Na-Oke, Moscow Oblast). This species is not listed in the classification of microorganisms by pathogenic groups in the Sanitary and Epidemiological Regulations SP 1.3.2322-08. Bacteria *Cms* were grown on a medium with the composition, g/l: glucose – 5.0; peptone – 10.0; yeast extract – 5.0; $CaCO_3$ – 5.0. The fungal cultures were *Ganoderma lucidum* (Curtis) P. Karst., strain 1315 (reishi mushroom); *Grifola umbellata* (Pers.) Pilát, strain 1622 (umbrella polypore) from the collection of the Department of Mycology and Algology, Moscow State University (Moscow); *Laetiporus sulphureus* (Bull.) Murrill, strain 120707 (sulphur polypore) from the collection of the Department of Botany of Irkutsk State University (Irkutsk); *Pleurotus ostreatus* (Jacq.) Kumm., strain NK352 (oyster mushroom) from the collection of Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (Saratov). Mushroom cultures were maintained on agarized beer wort (4 degrees Balling).

Cultivation of plants in vitro. The studies were carried out *in vitro* on potato plants *Solanum tuberosum* L. of variety Lukyanovsky (A.G. Lorkh All-Russian Research Institute of Potato Farming), which is susceptible to ring rot [14, 15]. Microclonal propagation of test tube plants was carried out using cuttings. The cuttings were planted at the internode depth in an agar nutrient Murashige and Skoog medium (MS) containing vitamins and hormones, at pH = 5.8–6.0; they were cultivated for 2 weeks and maintained at a constant temperature in the range of 24–25 °C, illumination of 5–6 klx, and photoperiod duration of 16 h; and then transferred into MS liquid medium of the same composition, but without agar.

Evaluation of bactericidal activity. Metal-containing biosamples of fungal origin were obtained as described in [16]. Determination of the sensitivity of the phytopathogen to fungal bioagents was carried out by diffusion in agar.

Methods for studying biofilm formation in bacteria. To study the effect of metal-containing preparations on biofilm formation by *Cms*, the bacterial culture was grown for 1 day in a liquid nutrient medium, then the test agent was added to the suspension, and cultured under aerated conditions for another day. Next, the optical density of the bacterial suspension was measured at a wavelength of 595 nm (A_{595}) and the suspension was titrated to 96-well polystyrene plates. After 48 h of incubation, the plates were stained with 1% (w/v) gentian violet solution at room temperature for 45 min. Subsequently, the wells were washed three times with distilled water to remove unabsorbed cells, the dye from bacterial cells was extracted with ethanol, and the A_{595} value was measured on a BIO-RAD model 680 (USA) microplate reader [17].

Determination of biometric parameters of plants. Metal-containing biocomposites were added to the liquid potato growth medium. Then the plants were incubated for 25 days monitoring the following biometric indicators: growth, number of leaves, length of internodes. The fresh biomass of the plant material was assessed gravimetrically.

Determination of the phytotoxicity of a biological product. Potato plants, after removing the roots, were placed in containers with mediums of the following compositions (Table 1).

Table 1. Composition of media for determining the phytotoxicity of a biological product
Таблица 1. Состав сред для определения фитотоксичности биопрепарата

Experiment number	Volume of medium components, ml		
	MS	*Cms* suspension	Biological product
1	25	25	0,5
2	50	–	0,5
3	25	25	–
4	50	–	–

Note. "–" – denotes the absence of the component.

Each experiment was independently replicated 3 times; in each biological variant of the experiment, 3–5 plants were analysed. The results were recorded every hour for a total of 6 hours.

Statistical processing of experimental data was performed using the Excel software package. The arithmetic mean and standard deviation were assessed. The reliability of the data was checked using the Mann-Whitney *U*-test.

RESULTS AND DISCUSSION

A combination of environmentally friendly biopesticides and beneficial microorganisms constitutes an alternative to chemically manufactured pesticides [18]. We studied biopesticides containing biometal (II), in a form generated by fungal action in a nutrient medium, by measuring both bacteriostatic and bactericidal activity of fungal substances against the phytopathogen *Cms*. The total pool of extracellular metabolites was isolated from submerged cultures of *Pleurotus ostreatus, Ganoderma lucidum, Grifola umbellate and Laetiporus sulphureus,* and found to contain products derived from the introduced metal (II) salts which were produced by fungal biotransformation (Table 2).

The substances derived by fungal action were used in comparative studies of biological activity. Earlier, we showed the species-specific features of the antibacterial effect against *Cms* of metal-containing biocomposites obtained from cultures of different genera and species of fungi [16]. In the present work studying metal-containing preparations of fungal origin applied to plant specimens, we selected biocomposites from those studied previously, which were characterized by a noticeable antibacterial effect against *Cms*. The control treatment consisted of biopolymer substances obtained from the same fungus but which did not contain any metal (see Table 2). In studies of the effects of metal(II) cations, inorganic metal salts were added to the growth medium.

Trace-metal elements are essential for well-known biological processes and are therefore vital to living systems, but become toxic when their concentration exceeds certain limits. It is argued that the biocomposites studied are safe because they are composed of trace elements at concentrations of the order of 10^{-4} mol/l. The concentration is further reduced when the treatment dose is diluted in the nutrient media. The content of microelements in plants is 1 10^{-3}–1 10^{-5}% [19], and on average, the molar mass of the microelements studied is 60 g/mol. In nature by comparison, plants can contain up to 0.17 mmol/kg mass, which is the equivalent of more than 0.1 mmol/l in the prepared biocomposites before dosing.

One of the antimicrobial effects of drugs developed to combat bacterial plant pathogens is their ability to counteract the formation of biofilms by phytopathogens [20]. The formation of biofilms by *Cms* in the vascular system of the plant is the main cause of the wilting of potato leaves [21]. Therefore, it was necessary to study the effect of metal-containing biocomposites on *Cms* biofilm formation. The greatest anti-biofilm-forming effect was observed in preparations based on *G. lucidum* (Fig. 1).

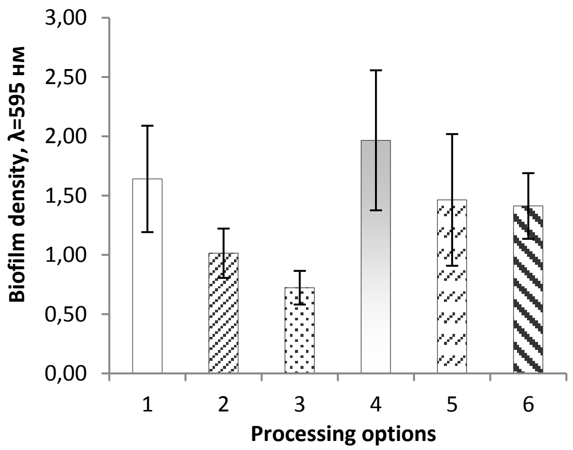

Fig. 1. Anti-biofilm-forming efficiency of biocomposites based on Ganoderma lucidum (4), containing Fe (2), Co (3), Cu (5), Zn (6), compared to Cms (1)

Рис. 1. Антибиопленкообразующая эффективность биокомпозитов на основе Ganoderma lucidum (4), содержащих Fe (2), Co (3), Cu (5), Zn (6), в отношении Cms (1)

Table 2. Biocomposites of fungal origin used in the work
Таблица 2. Биокомпозиты грибного происхождения, использованные в работе

Laboratory sample code	Sample characteristic	Addition of the compound to the liquid medium upon obtaining the sample	Concentration of metal (II)s in the sample, mol/L
1K	Composite based on *Pleurotus ostreatus* HK352	none	0
1Co	Composite based on *Pleurotus ostreatus* HK352	$CoCl_2 \cdot 6H_2O$	$1 \cdot 10^{-4}$
2K	Composite based on *Ganoderma lucidum* 1315	none	0
2Cu	Composite based on *Ganoderma lucidum* 1315	$CuSO_4 \cdot 5H_2O$	$2 \cdot 10^{-4}$
4K	Composite based on *Grifola umbellata* 1622	none	0
4Zn	Composite based on *Grifola umbellata* 1622	$ZnSO_4 \cdot 7H_2O$	$2 \cdot 10^{-4}$
5K	Composite based on *Laetiporus sulphureus* 120707	none	0
5Fe	Composite based on *Laetiporus sulphureus* 120707	$FeSO_4 \cdot 7H_2O$	$2 \cdot 10^{-4}$

Iron-containing and especially cobalt-containing biocomposites inhibited the formation of *Cms* biofilms by 40–50% (see Fig. 1). The fungal preparations incorporating metal(II) cause a significant reduction in biofilm-forming ability, whereas the fungal preparation in the absence of metal(II) (Fig. 1, option 4) enhance biofilm-forming ability (by 15%). The biochemical action of fungus *G. lucidum* made a significant contribution to the anti-biofilm-forming efficiency of the biocomposites. The growing interest in the creation of nutraceuticals and functional products was the impetus to study the species *Ganoderma* from various geographical origins to facilitate the identification of natural compounds with pronounced antibacterial, antioxidant, medical and other useful properties [22]. A distinctive feature of the pool of metabolites from fungi of this genus are triterpines, including ganoderic acids [23], which have antimicrobial properties and are important in biotechnology. Due to their ability to form complexes of Co (II), which adversely affect *Cms* biofilm formation, these low molecular weight compounds formed by *G. lucidum* are considered to be promising substances.

Various growth parameters of potato plants provided quantitative measures of the strength of action of these substances of fungal origin (Fig. 2).

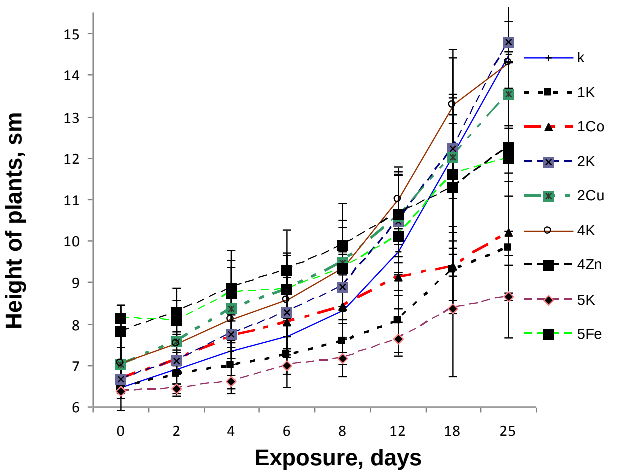

Fig. 2. Dependence of the height of potato plants *In vitro* (k) on the presence of biocomposites derived from Pleurotus ostreatus (1K), Ganoderma lucidum (2K), Grifola umbellata (4K), Laetiporus sulphureus (5K) containing Co (1Co), Cu (2Cu), Zn (4Zn), Fe (5Fe)

Рис. 2. Зависимость высоты растений картофеля *In vitro* (k) от присутствия биокомпозитов на основе Pleurotus ostreatus (1K), Ganoderma lucidum (2K), Grifola umbellata (4K), Laetiporus sulphureus (5K), содержащих Co (1Co), Cu (2Cu), Zn (4Zn), Fe (5Fe)

Plant growth was reduced by 36 and 50% by metabolites produced by *P. ostreatus* (Fig. 2, 1K, 1Co) and *L. sulphureus* (Fig. 2, 5K), respectively, compared to the control substances. It is known that oyster mushroom and sulphur polypore are basidiomycetes with pronounced antimicrobial properties [24, 25]. Therefore, it is likely that the effect of metabolites from these fungi had an antibiotic effect on potato plants with subsequent growth inhibition. There was no observed adverse effect on the growth of potatoes *in vitro* during the entire observation period (25 days) in the presence of the remaining biocomposites investigated from cultures of *L. sulphureus* (Fig. 2, 5Fe), *Gr. umbellata* (Fig. 2, 4K, 4Zn) and *G. lucidum* (Fig. 2, 2K, 2Cu).

When considering a choice of suitable biometal (II)s to study in conjunction with the funghi, it should be noted that only cobalt has an adverse effect on the height of potato plants (Fig. 2, 1Co). In fungi, cobalt is also relatively toxic [26]. This sensitivity to Co-containing substances is not unexpected because only at relatively low concentrations is this trace element necessary for growth, development and the activity of various enzymes in both potato plants and basidiomycetes [27]. The funghal cultures used to form biocomposities respond to the presence of Co (II) by activating various biochemical reactions producing metabolites, which in turn, contribute to the inhibition of plant growth.

A number of morphometric parameters of plants (length of internodes, number of leaves) remained practically unchanged when exposed to biocomposites of fungal origin. The length of internodes in plants was not affected by any of the investigated agents; therefore "stretching" of the plants was not observed. Treatment of potatoes with preparations based on *P. ostreatus* or *L. sulphureus* contributed to the shortening of internodes in plants. None of the observed effects was considered to be adverse.

None of the composites in the study had a significant effect on the number of new leaves in the potato plants during the study (Table 3).

Preparations of fungal origin were shown to affect the colour of the leaves of potato plants. In the presence of Cu-containing composite based on *G. lucidum*, the colour of the leaves became more saturated (Fig. 3, 2Cu). This is probably due to the inclusion of copper ions, in the form of a coenzyme, in the photosynthetic processes of the plant [19].

Pale green leaves and a thinner collet of potato stems were observed with composites, both containing iron and in the absence of iron, based on *L. sulphureus* (Fig. 3, 5K, 5Fe). This is probably the result of both an excess of iron in the plant growth medium and the contribution of the biological properties of *L. sulphureus*, in particular, the high antioxidant activity of the sulphur polypore [28].

It is well known that the coordinated action of reactive oxygen species (ROS) and phytohormones regulates plant growth, development and stress tolerance [29]. ROS are multifunctional plant signalling molecules contributing to the adaptive capacity of any plant species [30]. It was also shown that antioxidant compounds exhibiting a pronounced antioxidant effect might impede the development of oxidative stress in potato plants [31]. Agents with antioxidant properties, for example biocomposites

Table 3. Effect of biocomposites on the number of leaves in potato plants in vitro
Таблица 3. Влияние биокомпозитов на количество листьев у растений картофеля in vitro

Laboratory sample code	Duration of cultivation, days					
	0	2	6	12	18	25
K	12,3±1,2	12,7±1,5	14,3±2,1	16,0±1,0	17,0±0,4	19,0±1,0
1K	12,0±2,7	12,0±2,7	11,7±1,5	14,7±2,3	18,0±3,6	21,0±4,6
1Co	12,7±2,5	12,7±2,5	13,0±3,0	15,7±1,5	17,3±2,1	20,7±2,5
2K	11,0±2,7	11,3±2,3	12,7±2,9	15,0±1,0	16,7±2,5	21,3±4,0
2Cu	13,3±3,1	13,3±3,1	15,3±2,5	14,3±1,5	15,7±2,3	17,3±2,9
4K	11,3±0,6	11,7±0,6	13,3±0,6	14,0±0,4	16,0±1,0	19,0±1,0
4Zn	12,0±1,7	12,3±1,2	13,0±1,0	15,0±2,7	15,7±2,3	18,0±2,7
5K	13,7±1,5	13,7±1,5	13,0±1,7	14,0±1,7	14,3±4,9	16,7±1,5
5Fe	15,3±5,1	15,3±5,1	16,7±4,0	17,7±3,8	20,0±2,7	20,0±1,7

Note. K – control potato plants (without treatment with biocomposites).

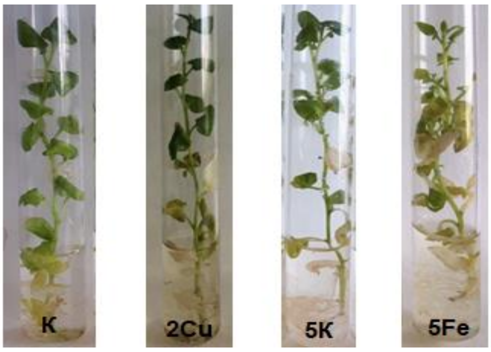

Fig. 3. Change in colour of leaves of potato plants *in vitro* (K) under the influence of biocomposites based on Ganoderma lucidum (2Cu), Laetiporus sulphureus (5K, 5Fe)

Рис. 3. Изменение окраски листьев растений картофеля *in vitro* (К) под влиянием биокомпозитов на основе Ganoderma lucidum (2Cu), Laetiporus sulphureus (5K, 5Fe)

derived from *L. sulphureus,* counteract the action of ROS thereby having an effect on the biochemical processes of potato plants (see Fig. 3). This could serve as one of the reasons for the decrease in growth rates (see Fig. 2) by obviating the need for the plant's stress-dependent activation of certain antioxidant enzymes. The elevated level of exogenous antioxidant substances from another basidiomycete, oyster mushroom (Fig. 2, 1K), had the same effect on the reduction of the growth of potato plants, but this was less pronounced than that caused by the sulphur polypore (Fig. 2, 5K).

The biochemical properties of the funghi from which the biocomposites were derived, including the antioxidant activity of fungi, were not considered to be the predominant source of action on potato plants, despite the ability for enhanced bioproduction of antioxidant compounds [28] commonly found in *P. ostreatus* and *L. sulphureus.* In particular, in an experiment to determine the mass of roots of potato plants using the biocomposite based on *P. ostreatus* (Fig. 4, 1K), no significant increase in mass was observed compared to the control of untreated plants, although it was noted that a positive effect due to a compound derived from *L. sulphureus* was one of the most pronounced (Fig. 4, 5K).

By contrast, the cobalt-containing agent produced a noticeable increase in the biomass of the roots of potato plants (Fig. 4, 1Co). This increase, when taken into account with the suppression of the formation of *Cms* biofilms discussed above (see Fig. 1), forms the rationale for the recommendation to use Co-containing precursors for biocomposites of fungal origin.

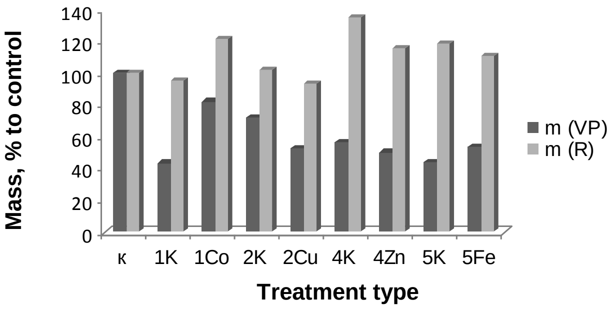

Fig. 4. Mass of vegetative part (VP) and roots (R) of potato plants *in vitro* (K) under the influence of biocomposites based on Pleurotus ostreatus (1K), Ganoderma lucidum (2K), Grifola umbellata (4K), Laetiporus sulphureus (5K), containing Co (1Co), Cu (2Cu), Zn (4Zn), Fe (5Fe)

Рис. 4. Масса вегетативной части (VP) и корней (R) растений картофеля in vitro (К) под влиянием биокомпозитов на основе Pleurotus ostreatus (1К), Ganoderma lucidum (2К), Grifola umbellata (4К), Laetiporus sulphureus (5К), содержащих Co (1Co), Cu (2Cu), Zn (4Zn), Fe (5Fe)

As a general observation of the results of the experiments, the biomass and length of roots either did not change compared to those of the control plants or increased in the range 10–20%. This result is important because the tubers are the most valuable part of the potato which is an important agricultural crop, although it was noted that the mass of the vegetative part of the plants decreased (see Fig. 4). A prerequisite for increasing the yield and quality of cultivated product is the stimulation of the physiological processes of the underground part of the plant which is formed of complex features that consist of many structural elements; the yield is not solely dependent on the morphometric parameters of the aboveground part of plants. Additionally it is of great importance to increase the resistance of plants to unfavourable actors and boost their immunity.

Although the 2Cu biocomposite experiment showed no effect on the increase in mass of plant roots (see Table 2), this preparation based on *G. lucidum* had a moderate bactericidal and bacteriostatic effect on the causative agent of potato ring rot and demonstrated anti-biofilm-forming efficacy against *Cms* (see Fig. 1), without a negative effect on the biometric characteristics of the plants (Fig. 2, *2Cu*; 3, *2Cu*; Table 3). A study of the phytotoxicity of this biocomposite in the potato plant was carried out prior to recommending its use in biotechnology (Fig. 5).

In the absence of bacteria (Fig. 5, *2*) or in the presence of both *Cms* and the biological product under investigation (Fig. 5, *1*), the plants looked healthy after the first hour. The first symptoms of the disease (withering of leaves, wilt) in MS medium were noted after the first hour of incubation in the presence of *Cms* in specimum number 3 (Fig. 5). A slight wilting of plants was noted in all specimens 4 h from the start of the experiment. After 6 hours of observation, almost all leaves of plants in the experiment "*Cms* without biocomposite" had wilted (Fig. 5, *3*), whereas in the specimen "*Cms* with biocomposite" (Fig. 5, *1*) no more than 40% of the leaves had wilted. This effect continued to be observed after 21 h. The results demonstrate that the biological product being studied had a clear positive effect on the plant's ability to fight infection. It is known that plant growth effectors can induce resistance to various phytopathogens [32, 33]. Resistance to infectious diseases is “turned on” in plants in response not only to their local infection, but also to treatment with substances from a large group of structurally diverse organic and inorganic compounds, which do not induce pathogen resistance. This immunity is a result of changes in plant metabolism, which have an adverse effect on the nutrition, growth, development and reproduction of phytopathogenic organisms. Substances derived from the biocomposites proposed in this work potentially effect similar changes in plant metabolism but may be considered environmentally friendly due to their natural origin and effectiveness at low doses.

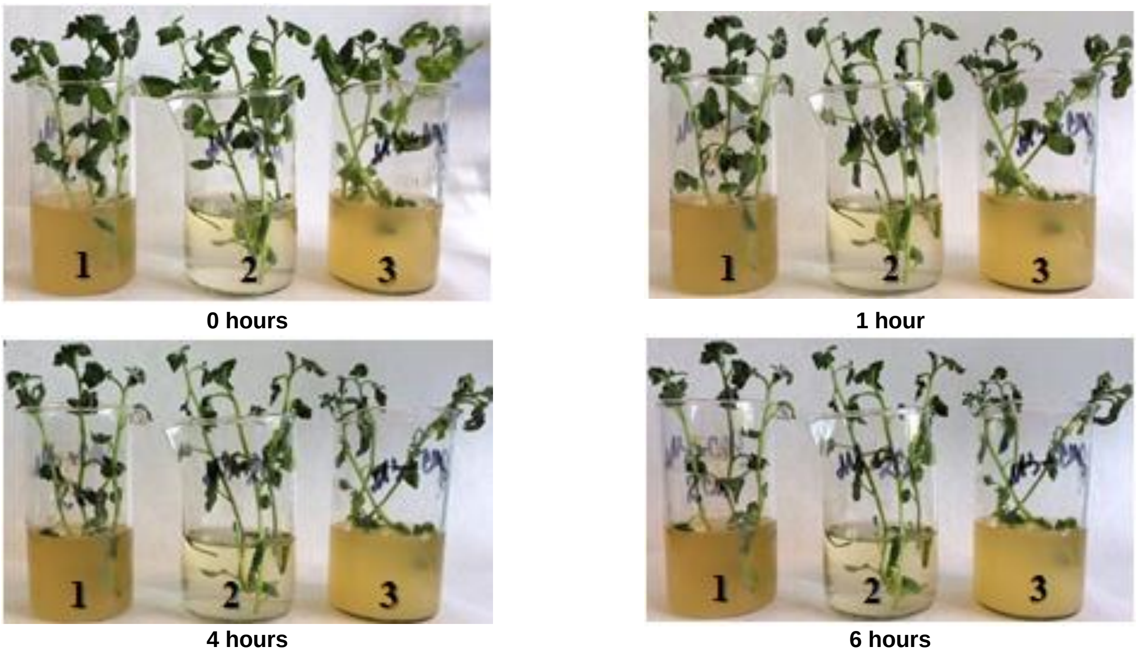

Fig. 5. Study of phytotoxicity of Cu-containing biocomposite derived from *Ganoderma lucidum* in relation to potato plants, grown on MS medium (1, 2, 3) with *Cms* suspension (1, 3) and biocomposite (1, 2)

Рис. 5. Исследование фитотоксичности Cu-содержащего биокомпозита на основе *Ganoderma lucidum* по отношению к растениям картофеля, выращенным на среде MS (1, 2, 3) с суспензией *Cms* (1, 3) и биокомпозитом (1, 2)

CONCLUSIONS

The following conclusions can be deduced from the results of this study of the viability and response of potato plants *in vitro to* the presence of metal (II) containing composites synthesized as extracellular metabolites of basidiomycetes *Pleurotus ostreatus, Ganoderma lucidum, Grifola umbellata* and *Laetiporus sulphureus*.

1. Metal-containing biocomposites based on *G. lucidum* have the highest anti-biofilm-forming efficacy against the *Cms* bacteria, the pathogen of potato ring rot. Fe- and Co-containing biopreparations inhibit the formation of *Cms* biofilms by 40–50%.

2. When exposed to biocomposites of fungal origin, virtually no change was observed in a num-

ber of morphometric parameters in the Lukyanovsky variety potato plants, which are susceptible to *Cms*. The composites studied do not have a significant effect on the number of leaves, do not lead to excessive lengthening of the plant stem, and in most cases, do not have a negative effect on the growth of potatoes *in vitro*. The biomass and length of roots either do not change or increase by 10–20% under the influence of these biocomposites.

3. Plant height is adversely affected not only by composites derived from *L. sulphureus* and *P. ostreatus* in the absence of metal (II), but also by one Co-containing preparation derived from *P. ostreatus*. This effect may be associated with the increased antimicrobial and antioxidant activity of exometabolites from these basidiomycetes, as well as with the antibiotic properties of cobalt.

4. It is recommended to use cobalt compounds to obtain biocomposites based on basidiomycetes because the Co-containing agent is associated with a noticeable increase in the biomass of the roots of potato plants combined with the suppression of the formation of biofilms *Cms*.

5. A copper-containing preparation derived from *G. lucidum* is associated with a moderate bactericidal and bacteriostatic effect on the causative agent of potato ring rot, demonstrates anti-biofilm-forming efficacy against *Cms*, does not have an adverse effect on any of the plant biometric parameters studied, does not exhibit phytotoxic action against plants and enhances potato resistance to *Cms*.

6. It is recommended to study the effect of treatment with biocomposites from metabolites of higher fungi on the whole plant and tubers of potatoes in the field. This study is a prerequisite to their introduction as an environmentally safe treatment against bacterial infection in potatoes. This would be a valuable new treatment given increasing resistance of phytopathogens to pesticides of chemical origin.

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Contribution

Olga M. Tsivileva, Alla I. Perfileva, Antonina G. Pavlova carried out the experimental work, on the basis of the results summarized the material and wrote the manuscript. Autors have equal author's rights and bear equal responsibility for plagiarism.

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