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ECOLOGY

Effect of Inoculating the Root System of Plants with Endophyte *Cylindrocarpon magnusianum* on Plant Performance When Exposed to Heavy Metal Salts

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Abstract—The effect of inoculation with endotrophic micromycete *Cylindrocarpon magnusianum* on the physiological and biochemical parameters of test tomato plants when exposed to heavy metal salts was studied. The experimental scheme included inoculation with a fungal culture (control population) and with populations of this fungus that were previously adapted to this stress factor. The inoculated plants were then grown under control conditions and on substrates with different concentrations of heavy metal salts (zinc, copper, lead, and chromium). No stimulating effect that increases the resistance of plants to heavy metal salts after inoculation of plants by the control population of the fungus *C. magnusianum* was detected. When using nonbiogenic chemical elements, adaptive plant responses associated with the content of photosynthetic pigments in leaves and the formation of plant biomass were significantly expressed when plants were inoculated with adapted fungal populations of *C. magnusianum* and when they were further cultivated on substrates introduced with chromium and lead salts. Under these conditions, the fungal infection in plant roots was more intense compared to the use of the control population of the fungus. These facts indicate the more efficient partnership of the fungus *C. magnusianum* and the root system of plants under conditions that are extreme for plant life.

Keywords: *Cylindrocarpon magnusianum*, fungi, heavy metals, inoculation, biochemical indicators

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At present, the scientific community has increased interest in the study of the role of consortium relations of plants with root micromycetes. Certain progress has been achieved in studying the role of endomycorrhiza and its most common form, arbuscular mycorrhiza (AM), which is characteristic of most modern phylogenetic groups of plants and is represented in all biomes of the globe [1]. It is formed by fungi belonging to the Glomeromycotina subdivision of the Mucoromycota division [2]. But the use of AM in crop production is limited due to their obligate symbiotrophy [3]. In this regard, it is important to investigate the role of other groups of root micromycetes—endophytes and their individual representatives in the formation of resistance mechanisms in higher plants.

Historically, two groups of endophytes (Clavicipitaceous (C) and Nonclavicipitaceous (NC)) have been distinguished based on phylogeny and life cycle traits [4, 5]. In general, this diverse group of fungi can have a strong effect on plant communities by providing plant resistance to abiotic and biotic stress. Particularly interesting are studies into the role of endophytes in the formation of metal resistance in plants, including agricultural crops [4–8], and especially resistance to chemical elements highly dangerous for plants [9–13]. A number of works were aimed at studying the possibility of using micromycetes as herbicides [14–17].

One of the promising micromycetes is the endophyte *Cylindrocarpon magnusianum* Wollenw. [18–22]. Its metabolites can be used in the fight against nematodes [19], and it is able to grow under conditions of a high content of oil products in the soil [18, 19]. A series of the author's experiments carried out with *C. magnusianum* revealed that the culture of this fungus is able to withstand the action of high osmotic pressure while maintaining the growth of the cultural mycelium. Experiments with plants inoculated with this fungus demonstrated the possibility of its application as an agent for increasing salt tolerance and heat resistance of plants [20–22].

The aim of our work was to investigate the effect of inoculation with the culture of the fungus *C. magnusianum* on the formation of adaptive responses of plants to heavy metal salts in the substrate (on the example of the test tomato (*Solanum lycopersicum*) culture).

MATERIALS AND METHODS

The culture of *C. magnusianum* was isolated from the root system of woody plants (*Acer negundo* L. in good life condition) that have long grown in urban soils with a high content of heavy metal salts (near-highway plantings, the sanitary protection zone of the Izhstal enterprise in Izhevsk, Udmurtia). The fungus was cultivated on a nutrient medium outside the plant root system. Its species was identified by microscopy and molecular DNA analysis in the laboratory of the Leibniz Institute of Vegetable and Ornamental Crops (Berlin) [23].

According to the experimental scheme, fungal populations adapted to substrates with different concentrations of heavy metal salts (mg/L) were prepared: A0, control; A1, on substrate with Zn_{100}; A2, Cu_{50}; A3, Cu_{100}; A4, Cu_{150}; A5, Pb_{10}; A6, Pb_{50}; A7, Cr_{25}; A8, Cr_{10}. Mycelial disks of the fungal culture ($\varnothing = 5$ mm) were transferred to a pentose-dextrose agar (PDA) medium with added, according to the calculated concentrations, salts of heavy metals and incubated for 2 weeks in the a BinderKBWF720 climatic chamber at the 25°C. Suspension cultures of these populations were then prepared (spore content 3 million pcs/mL; mycelium fragments content 200 pcs/mL) and plants were inoculated by spraying the seedlings during transplanting. To prepare suspension cultures of the fungus, mycelial disks of adapted fungal populations were introduced into sterile potato dextrose broth and incubated for 10 days in a thermal shaker-incubator (temperature 5–27°C at 60 rpm) [24].

The experiment included the following variants: 1, inoculated tomato plants (inoculation with control isolate A0) were grown on substrates with different contents of heavy metal salts (mg/L): B0, control, without heavy metals; B1, Zn_{100}; B2, Cu_{50}; B3, Cu_{100}; B4, Cu_{150}; B5, Pb_{10}; B6, Pb_{50}; B7, Cr_{25}; B8, Cr_{10}; 2, tomato plants inoculated with populations of fungi adapted to heavy metals (A1–A8), grown on substrates without salts (B0) and with the addition of heavy metal salts (B1–B8). The variants of the experiment were repeated four times. The substrate was a 1 : 2 mixture of low ash peat and sand. Plants were grown in the BinderKBWF720 climatic chamber under conditions optimal for tomato culture (substrate moisture 75%, illumination 20000 lx (16 h/day), air temperature of 23°C during the daytime and 19°C at night). The dwarf tomato cultivar Balkonnoe Chudo was used. Plants were grown over 4 months before the start of fruiting. Experimental studies were carried out during 2017–2019 in the scientific laboratory of Environmental Biotechnology of Udmurt State University. At the end of the experiment, the development of endophytic fungi in the roots was assessed by light microscopy [25].

Plant resistance was estimated on the basis of the content of nitrates in leaves by the ionometric method (GOST 29270-95); biomass and percentage of dry matter in the aerial part and root system of plants were determined by the weight method (GOST 28561–90); photosynthetic pigments in leaves of the middle layer (chlorophylls *a* and *b*, carotenoids) were detected by spectrophotometry in acetone extracts (absorption at 662, 644, and 440.5 nm, respectively); pigment concentrations were calculated using the Holm–Wettstein equations. Mathematical processing of the material was carried out using the Statistica 6.0 statistical package by methods of descriptive statistics. Significant differences were considered at $p < 0.05$.

RESULTS AND DISCUSSION

In all variants with the addition of zinc, the content of pigments in plant leaves had general patterns: inoculation of plants with the control population (A0) when grown on the substrate with zinc did not affect the content of photosynthetic pigments (Figs. 1–3). Inoculation of plants with adapted populations when grown on the control substrate (B0) caused a significant increase in the content of chlorophylls *a* and *b* and carotenoids, while the content of pigments decreased by almost two times when grown on the substrate with zinc. In addition, inoculation with the control population when growing plants on the substrate with zinc led to a significant decrease in the dry matter content in the root system of plants (Table 1). Inoculation with adapted populations of the fungus caused a significant decrease in the aerial plant biomass (when grown on the control substrate) and did not affect the studied parameters of plants when cultivated on the substrate with zinc.

The control population/Zn_{100} variant showed high rates of *C. magnusianum* infection in the root system of plants (Table 1); when using adapted populations, fungal infection was less developed, especially in the Zn_{100}/Zn_{100} variant.

In variants with Cu_{100}, the content of chlorophylls *a* and *b* increased when using the adapted populations, while inoculation with the control population led to a significant sharp decrease in the content of pigments. Under conditions of maximum copper content (Cu_{150}), no changes in the content of carotenoids were revealed, but the use of the adapted populations increased the content of chlorophylls.

Inoculation of plants with the control fungal population led to an increase in the content of nitrates in leaves in the variants with substrates Cu_{50} and Cu_{100} as well as to an increase in the percentage of dry matter in the root system of plants in variants Cu_{100} and Cu_{150}. This is consistent with the data on the effect of inoculation on plants under the influence of heavy metals, which is associated with changes in the architecture of the root system and accumulation of total nitrogen [9]. The use of adapted populations of the fungus during the cultivation of inoculated plants on the control substrate contributed to a decrease in the biomass of the root system as well as in the content of nitrates in

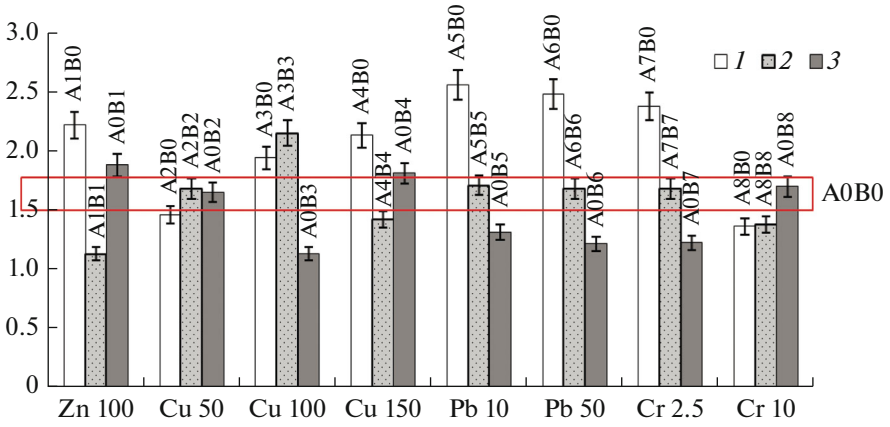

Fig. 1. Content of chlorophyll *a* in leaves of inoculated tomato plants at different concentrations of heavy metals in the substrate: (*1* and *2*), the fungal population (A1, Zn_{100}; A2, Cu_{50}; A3, Cu_{100}; A4, Cu_{150}; A5, Pb_{10}; A6, Pb_{50}; A7, $Cr_{2.5}$; A8, Cr_{10}) + the substrate, respectively, without heavy metals (B0) and with heavy metal salts, mg/L (B1, Zn_{100}; B2, Cu_{50}; B3, Cu_{100}; B4, Cu_{150}; B5, Pb_{10}; B6, Pb_{50}; B7, $Cr_{2.5}$; B8, Cr_{10}); (*3*) the control population (A0) + the substrate with heavy metal salts B1–B8, mg/L; A0B0, the control fungal population on the substrate without heavy metals (the rectangle indicates the confidence interval of the average values of the indicator for this variant).

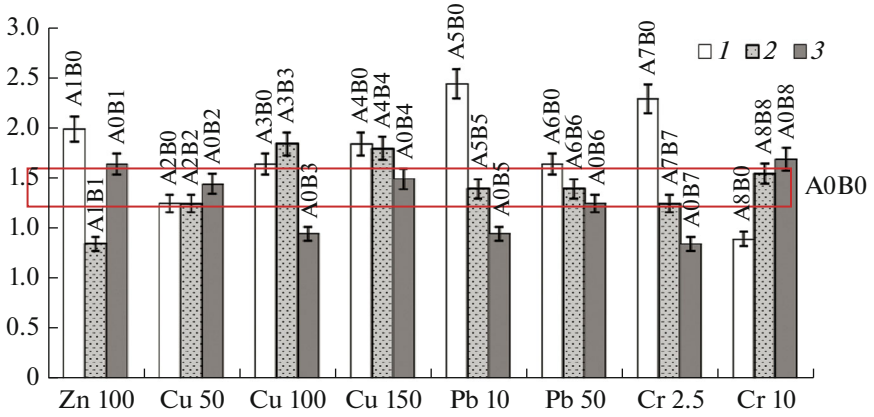

Fig. 2. Content of chlorophyll *b* in leaves of inoculated tomato plants grown at various concentrations of heavy metals in the substrate. For designations, see Fig. 1.

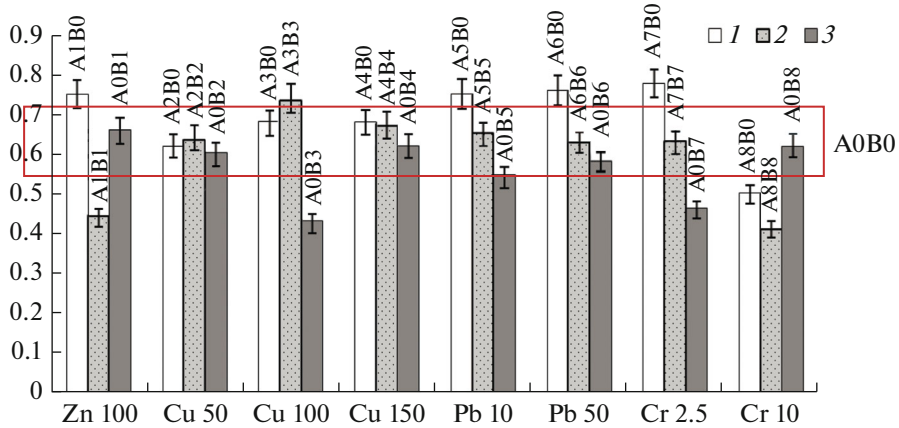

Fig. 3. Content of carotenoids in leaves of inoculated tomato plants grown at various concentrations of heavy metals in the substrate. For designations, see Fig. 1.

Table 1. Biological parameters of inoculated tomato plants under experimental conditions

Variant: population (A)/substrate (B)	Indicator					Development of fungal infection	
	biomass, g		dry matter content, %		nitrate content, mg/100 g	frequency, %	intensity, %
	aerial part	roots	aerial part	roots			
Control/Zn_{100}	29.37 ± 2.23*	4.70 ± 0.28	12.83 ± 0.67	7.85 ± 0.33↓	3890.43 ± 159.98	86.7	4.3
Control/ Cu_{50}	25.54 ± 0.80↓*	3.81 ± 0.24	13.01 ± 1.99	5.32 ± 1.96↓	4327.69 ± 144.6 ↑	80	4
Control/ Cu_{100}	24.31 ± 1.86	4.40 ± 0.30	12.64 ± 0.02	13.79 ± 3.80↑	5326.66 ± 110.4↑	86.7	4.3
Control/ Cu_{150}	27.60 ± 0.70	3.63 ± 0.24	12.14 ± 0.89	13.29 ± 1.13↑	4308.72 ± 298.07	53.6	2.7
Control/Pb_{10}	24.51 ± 1.28	3.88 ± 0.35	8.85 ± 0.50↓	10.58 ± 2.01	4321.20 ± 258.40	93.3	4.7
Control/Pb_{50}	28.81 ± 0.39	3.81 ± 0.07	10.02 ± 0.86↓	8.75 ± 1.38	5014.62 ± 466.07	93.3	4.7
Control/$Cr_{2,5}$	26.87 ± 0.35	3.30 ± 0.14↓	11.74 ± 1.87	8.89 ± 1.79	4415.13 ± 331.23	40	2
Control/Cr_{10}	25.58 ± 0.45↓	4.72 ± 0.28	11.59 ± 0.98	7.75 ± 0.18↓	3213.16 ± 96.82↓	40	2
Zn_{100}/Control	25.58 ± 0.73↓	3.84 ± 0.12	14.92 ± 2.32	9.35 ± 1.41	3476.33 ± 325.75	60	3
Zn_{100}/ Zn_{100}	27.80 ± 0.64	4.72 ± 0.45	14.95 ± 1.23	11.19 ± 2.20	3585.72 ± 606.07	33.3	1.7
Cu_{50}/ Control	23.96 ± 1.63	2.16 ± 0.18 ↓	10.99 ± 1.14	14.78 ± 2.82	3365.41 ± 72.51	100	5
Cu_{50}/ Cu_{50}	29.68 ± 1.05	2.13 ± 0.23	14.10 ± 1.64	15.22 ± 2.97	4638.21 ± 346.8↑	66.7	3.3
Cu_{100}/Control	19.82 ± 0.40↓	2.30 ± 0.15↓	10.91 ± 1.64	13.17 ± 2.43	4837.86 ± 206.82	93.3	4.7
Cu_{100}/$Cu1_{00}$	35.29 ± 0.25↑	2.39 ± 0.69	12.67 ± 0.82	12.68 ± 2.45	3534.60 ± 99.78	100	5
Cu_{150}/ Control	27.99 ± 0.81	1.93 ± 0.04↓	9.44 ± 1.79	16.10 ± 3.80	305 8.14 ± 25.50↓	86.7	4.3
Cu_{150}/$Cu1_{50}$	24.16 ± 1.12	2.23 ± 0.18	12.26 ± 1.21	13.25 ± 2.73	4487.60 ± 103.3↑	86.7	4.3
Pb_{10}/ Control	32.66 ± 2.01	2.98 ± 0.15 ↓	13.67 ± 1.92	10.24 ± 0.65	3356.96 ± 241.51	73.3	3.4
Pb_{10}/Pb_{10}	26.30 ± 0.87	2.36 ± 0.22	11.41 ± 1.09	11.71 ± 1.01	4488.58 ± 102.6↑	66.7	3.3
Pb_{50}/Control	21.88 ± 1.31↓	1.55 ± 0.10↓	12.39 ± 1.36	10.98 ± 1.16	3986.02 ± 82.59	86.7	4.3
Pb_{50}/Pb_{50}	28.16 ± 1.30	2.49 ± 0.36	12.92 ± 1.16	10.01 ± 1.17	4229.96 ± 177.36	86.7	4.3
$Cr_{2.5}$/Control	21.59 ± 2.04↓	1.92 ± 0.08↓	12.24 ± 0.26	11.38 ± 1.85	4384.27 ± 195.22	73.3	3.4
$Cr_{2.5}$/$Cr_{2.5}$	29.54 ± 0.09↑	2.50 ± 0.01↑	13.16 ± 0.61	9.52 ± 1.49	4161.79 ± 494.02	73.3	3.4
Cr_{10}/Control	16.36 ± 0.94↓	1.56 ± 0.15↓	13.12 ± 1.98	14.17 ± 2.00	5188.76 ± 622.04	80	4
Cr_{10}/Cr_{10}	27.30 ± 0.26↑	2.06 ± 0.22	14.23 ± 2.73	11.90 ± 1.12	3583.89 ± 471.03	80	4
Control/ Control	29.30 ± 0.70	5.44 ± 0.63	15.33 ± 2.02	9.46 ± 0.15	3693.55 ± 87.76	60	3

* Average value of the indicator ± standard deviation.
** Significant difference from control: increase ↑ or decrease ↓ in the indicator ($p < 0.05$).
The control is the initial population not adapted to heavy metals (A0 in Figs. 1–3) and the substrate without metals (B0 in Fig. 1–3).
A, adapted fungal populations grown on agar substrates with different concentrations of heavy metal salts (mg/L) (A1–A8 in Figs. 1–3); B, substrates with different content of heavy metal salts (mg/L) (B1–B8 in Figs. 1–3).

leaves in the Cu_{150} variant. Upon inoculation with adapted populations of the fungus on substrates with Cu_{50} and Cu_{150}, the content of nitrates in leaves was significantly higher; on substrates with Cu_{100}, an increase in aerial plant biomass was observed. The most intensive fungal infection was formed when using adapted populations on substrates with Cu_{100} and Cu_{150}. The maximum development of fungal infection was observed in the Cu_{100}/Cu_{100} variant.

We should note the use of nonbiogenic chemical elements (chromium and lead). Plants inoculated with the control fungal population and cultivated on the Pb_{10} substrate showed a significant decrease in chlorophyll *a* and *b* and they showed a significant decrease in chlorophyll *a* when cultivated on the Pb_{50} substrate, while no significant decrease in the content of carotenoids was observed. The use of adapted populations of the fungus when growing plants on B0 caused an increase in the content of all studied pigments, and no significant changes were found compared to the control when grown on substrates with the addition of lead salts.

Inoculation of plants with the control fungal population significantly reduced the percentage of dry matter in the aerial part of the plants. When using adapted

populations and they were cultivated on the control substrate, the plants showed a decrease in the plant root system biomass, while when cultivated on substrates with Pb_{10} and Pb_{50}, the biomass and dry matter content did not change significantly, but the nitrate content in leaves was higher. In all variants with lead, fungal infection in the root system of plants had high development rates, with the highest rates in variants control/Pb_{10}, Pb_{50} and Pb_{10}, and Pb_{50}/control.

In variants with chromium, inoculation of plants with the control population and cultivation on the substrate with Cr_{25} led to a significant decrease in the content of pigments in leaves, which was not observed for the substrate with Cr_{10}. Inoculation of plants with the adapted fungal populations and their cultivation on control substrates showed different results: at Cr_{25}, it caused a significant increase in the content of photosynthetic pigments, while it resulted in a significant decrease in their content at Cr_{10}. When the plants were cultivated on substrates with Cr_{25}, no significant changes were revealed; the content of chlorophyll *a* and carotenoids decreased only with the introduction of Cr_{10}, while no significant differences in the content of chlorophyll *b* compared to the control were observed.

When plants were inoculated with the control fungal population and cultivated on the substrate with Cr_{10}, the aerial biomass and the percentage of dry matter in the root system of plants and nitrates in leaves decreased. We should note inoculation of plants with adapted populations of the fungus: when plants were cultivated on control substrates, the biomass of the aerial part and root system decreased, but the plant biomass was higher when cultivated on substrates with chromium. In variants with chromium, the use of adapted populations of the fungus led to the highest rates of development of the fungal infection in the root of plants, which was at maximum at the highest chromium content in the substrate (variant Cr_{10}/Cr_{10}).

The results of these studies using nonbiogenic chemical elements hazardous to the life of plants are consistent with the data of our works carried out earlier [20–22] and scientific publications of other researchers regarding a peculiar form of partnership of endotrophic fungi with the root system of plants [9–11, 23]: the protective effect of fungi is most effectively manifested in conditions unfavorable for the life of plants. The most sensitive indicator of plants to the effect of inoculation was the content of chlorophylls *a* and *b*. Inoculation with the control fungal population did not contribute to the formation of adaptive responses in plants, which resulted in a decrease in the content of photosynthetic pigments and a number of other studied parameters of plants when they were cultivated on substrates with heavy metal salts.

Inoculation of plants with adapted populations had a positive effect for the variants Cu_{100} and Cu_{150} when grown both on the control and on substrates with copper; for Zn_{100}, this was the case only when plants were cultivated on the control substrate.

In variants with nonbiogenic elements, the adaptive responses of plants were most significantly expressed when plants were inoculated with adapted populations of the fungus and further cultivated on substrates with chromium and lead salts. This fact may indicate the most efficient partnership between the *C. magnusianum* fungus and plants under stress conditions.

Fungal infection in plant roots in all the variants was fairly well developed. The use of *C. magnusianum* isolates for inoculation of plants adapted to the action of chromium salts with their further cultivation on substrates with chromium salts stimulated the development of fungal infection in the root of plants.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interests. This article does not contain any studies involving animals or human participants performed by any of the authors.

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