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木贼镰孢和马铃薯对培养基中不同浓度的油和重金属的反应研究

# STUDY OF THE REACTION OF FUSARIUM EQUISETI AND CYLINDROCARPON MAGNUSIANUM TO DIFFERENT CONCENTRATIONS OF OIL AND HEAVY METALS IN THE MEDIUM

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注解。根内生菌能够限制化学物质对植物的影响并增强其对胁迫条件的抵抗力。研究中使用了从长期生长在城市土壤中的植物根部分离出的内生微型真菌木贼镰刀菌和马格努西亚努斯的培养物（分离物）。同时，这些真菌物种不是专性内生菌。在实验室实验中，对培养这些微菌的培养基中不同浓度的油和重金属进行了建模，并建立了污染物对菌丝体菌落生长参数和菌丝体中丙二醛含量的影响。发现圆柱果和木贼镰刀菌培养物具有高度金属抗性，对铬和铅的抗性比对营养素（锌和铜）的抗性最大。培养基中铜浓度为150 mg/l时，对木贼的毒性最大，但不是阈值。对蘑菇菌丝体中丙二醛含量动态的研究表明了与重金属相关的物种特异性：C. magnusianum 对高浓度的铜具有更强的抵抗力，F. equiseti 对铬具有更强的抵抗力。真菌培养物还表现出对基质中油含量的抵抗力。适应人类环境的内生真菌可以用作植物抗性管理剂，用于创建人工抗性种植园和恢复受干扰的土地。

关键词：微菌、重金属、金属抗性、耐油污染

***Annotation.*** *Root endophytes are able to limit the impact of chemical substances on plants and increase their resistance to stress conditions. Cultures (isolates) of endophytic micro-mycetes Fusarium equiseti and Cylindrocarpon magnusianum isolated from the roots of plants growing for a long time in urban soils were used in the studies. At the same time, these fungal species are not obligate endophytes.*

*In laboratory experiments, different concentrations of oil and heavy metals in the media on which these species of micromycetes were cultured were modelled, and the effect of pollutants on the growth parameters of mycelium colonies and the content of malondialdehyde in mycelium was established. Cylindrocarpon magnusianum and Fusarium equiseti cultures were found to be highly metal-resistant, with the greatest resistance to chromium and lead than to nutrients (zinc and copper). The concentration of copper in the medium of 150 mg/l was the most toxic for F. equiseti, but not threshold. The study of the dynamics of malondialdehyde content in mushroom mycelium indicated species specificity in relation to heavy metals: C. magnusianum is more resistant to high concentrations of copper, F. equiseti - to chromium. Fungal cultures also showed resistance to oil content in the substrate. Endophytic fungi adapted to the anthropogenic environment can be used as plant resistance management agents in the creation of artificial resistant plantations and restoration of disturbed lands.*

***Keywords:*** *micromycetes, heavy metals, metal resistance, resistance to oil pollution*

Deterioration of soil quality caused by heavy metal and petroleum product pollution is a growing global problem resulting from human industrial activities, which in turn also leads to plant mortality [1, 2]. However, plants in association with root micro-mycetes have wider limits of resistance to external factors [3, 4]. Among such micromycetes, root endophytes, including endophytic fungi, are of the greatest interest: they are morphologically diverse in their structure, have a wide distribution area and are viable under stress conditions. The origin and source of the inoculum is an important factor. Those isolates that were obtained from soils contaminated with heavy metals were the most effective in increasing plant resistance to pollutants [5-7], indicating the ability of endomycorrhiza fungi to adapt to high stress conditions. Hence, the study of the resistance of endophytic fungi to the action of various pollutants, as well as their influence on plant stress tolerance is highly relevant. In this regard, *Fusarium equiseti* and *Cylindrocarpon magnusianum* are of considerable interest. *Fusarium equiseti* is a widely occurring root endophyte in nature and has the ability to colonise the roots of non-host plants. The fungus has long been considered pathogenic, but has recently attracted attention for its ability to act as a biocontroller in controlling root pathogens [8, 9]. *Cylindrocarpon magnusianum* also has a wide range of habitats, in addition, it is referred to the group of "oil and gas-bearing fungi", which may be in demand in the restoration of oil-contaminated land [10, 11].

**The aim** of the study was to establish the limits of resistance of representatives of endophytic fungi to the content of oil and heavy metals in the substrate.

**Objects and methods of research**. The endophytic fungi *Fusarium equiseti* and *Cylindrocarpon magnusianum* were used as research objects. The fungi were isolated from the root system of plants growing in technogenic territories of Izhevsk city (Udmurt Republic). The species affiliation of fungi was established by DNA molecular analysis in the laboratory of the Leibniz Institute of Vegetable and Ornamental Crops (Berlin) [12, 13].

Fungal cultures were grown on nutrient agar medium (PDA) made of dextrose broth and agar-agar with the addition of different concentrations of heavy metals and oil. The diameter and growth rate of fungal colonies were measured every three days after sowing. The experiments were carried out in triplicate. Heavy metals were applied in the following concentrations: Zn - 100, 200, 300 mg/l; Cu - 50, 100, 150 mg/l; Cr - 2.5, 5, 10 mg/l; Pb - 25, 50, 100 mg/l, oil - in concentrations of 1, 2.5, 5, 7.5 and 10%. Heavy metals were introduced into the substrate in the form of salts $ZnSO_4 \times 7H_2O$, $CuSO_4 \times 5H_2O$, $K_2Cr_2O_7$ and $PbSO_4$ with recalculation to modelled concentrations. The peculiarities of fungi response to stress conditions were evaluated by malondialdehyde (MDA) content. MDA content in mushroom mycelium was estimated by the degree of accumulation of the product of its reaction with thiobarbituric acid (TBA), determining the optical density of the solution on a spectrophotometer at a wavelength of 532 nm [14]. For this purpose, 2 ml of distilled water and 3 ml of 10% TCA were added to a test tube with mushroom biomass. A 2 ml sample was taken from the resulting homogenate and 0.5% TBA was added. Mathematical processing of the results was carried out using the package "Statistica 6.0" using descriptive statistics methods. Reliability of the differences between the variants of the experiment was established at $p<0.05$.

**Results and discussion**. The study of growth of *Cylindrocarpon magnusianum* and *Fusarium equiseti* cultures on media with heavy metals showed high limits of fungi resistance to the pollutant (Figures 1-8).

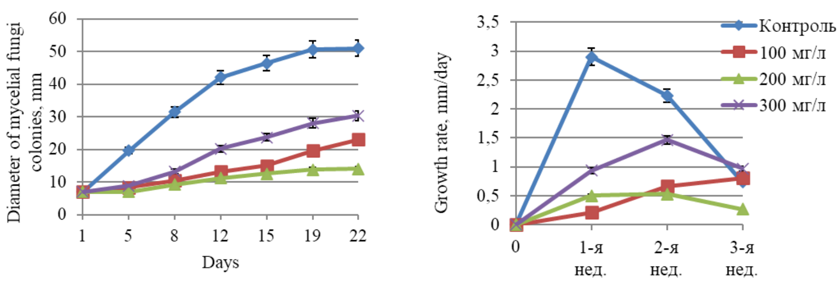


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***Figure 1.** Size dynamics (A) and growth rate (B) of Cylindrocarpon magnusianum colonies on media with different zinc concentrations*

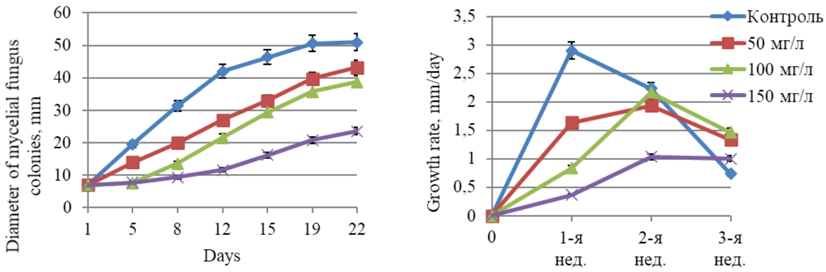


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***Figure 2.*** *Size dynamics (A) and growth rate (B) of Cylindrocarpon magnusianum colonies on media with different concentrations of copper*

The content of copper and zinc in the medium influenced the growth of *Cylindrocarpon magnusianum*: the size of the fungus colonies at all concentrations of metals was significantly smaller than in the control. In this case, the size of the colonies decreased in accordance with the increase in the concentration of metals (except for Zn 300 mg/l). However, threshold concentrations of copper and zinc for *Cylindrocarpon magnusianum* have not yet been found. The growth rate of the fungus, as well as the size of its colonies, at all concentrations of copper and zinc significantly decreased compared to the control, especially in the first week of the experiment.

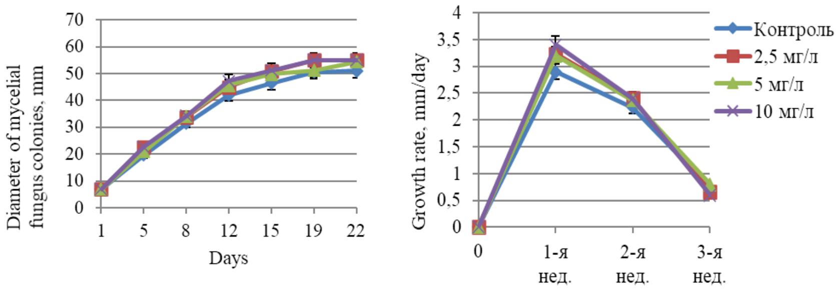


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***Figure 3.*** *Size dynamics (A) and growth rate (B) of Cylindrocarpon magnusianum colonies on media with different chromium concentrations*

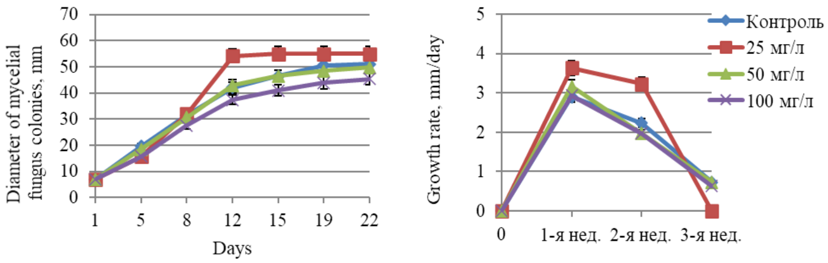


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***Figure 4.*** *Size dynamics (A) and growth rate (B) of Cylindrocarpon magnusianum colonies on media with different lead concentrations*

Chromium and lead were less toxic for *C. magnusianum*. The chromium content in the medium in all concentrations studied had no effect on the fungus growth: the size of its colonies had no significant differences from the control. Variants with lead in concentrations of 50 and 100 mg/l also did not differ significantly from the control variant. However, the content of lead in the medium at a concentration of 25 mg/l stimulated the growth of fungus colonies. No significant difference in the growth rate of fungal colonies between the other experimental variants and the control, as in the experiment with chromium, was observed until the end of the experiment.

In the experiment with *F. equiseti*, the results were similar to the experiment with *C. magnusianum* (Figures 5-8).

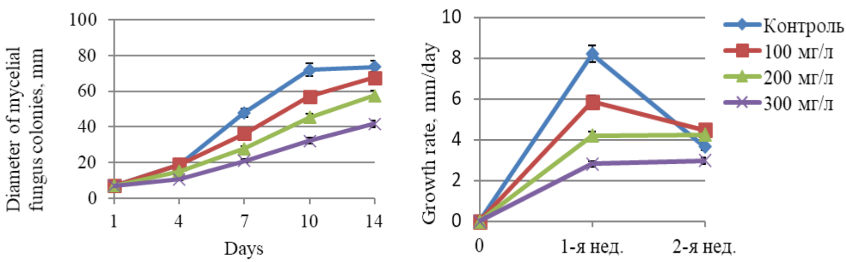


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***Figure 5.*** *Size dynamics (A) and growth rate (B) of Fusarium equiseti colonies on media with different zinc concentrations*

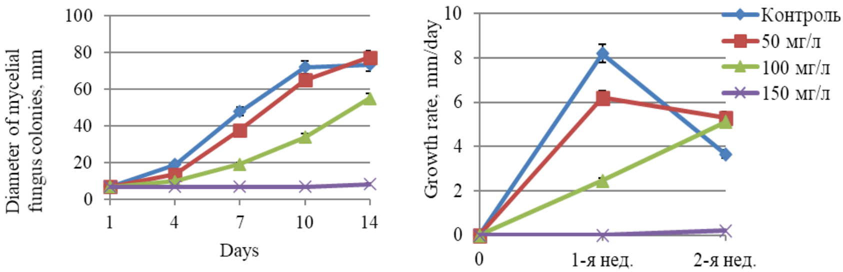


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***Figure 6.*** *Dynamics of size (A) and growth rate (B) of Fusarium equiseti colonies on media with different copper concentrations*

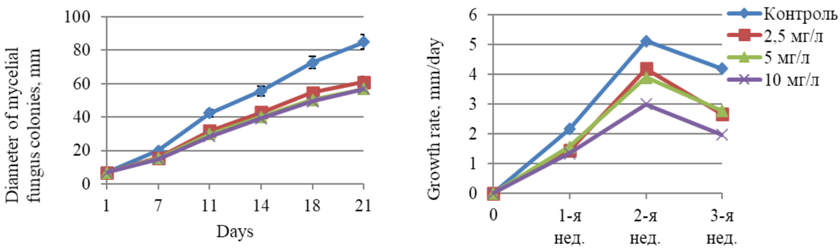


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***Figure 7.*** *Size dynamics (A) and growth rate (B) of Fusarium equiseti colonies on media with different chromium concentrations*

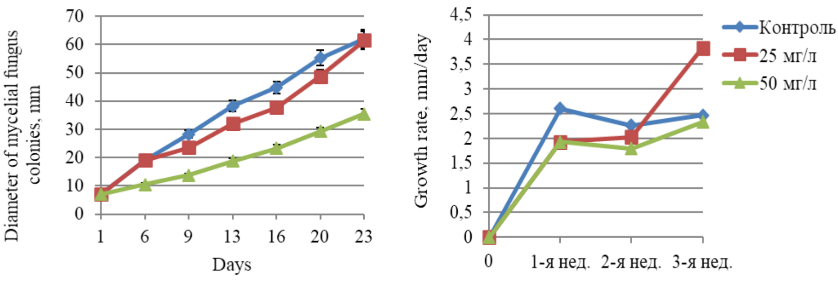


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***Figure 8.*** *Dynamics of size (A) and growth rate (B) of Fusarium equiseti colonies on media with different concentrations of lead*

The greatest inhibition of *F. equiseti* culture growth was caused by zinc and copper content in the medium. With increasing concentration of these elements, the diameter of fungal colonies decreased. The most toxic was the concentration of copper 150 mg/l, but even at this concentration of metal the growth of fungus did not stop.

The content of chromium and lead in the medium, as in the experiment with *C. magnusianum*, had no significant effect on the growth of *F. equiseti*. Nevertheless, the diameter of the fungus colonies at all studied concentrations of chromium in the medium was significantly smaller compared to the control, while the variants Cr 2.5, Cr 5 and Cr 10 mg/l had no significant difference between them. The growth rate of *F. equiseti* when chromium and lead were added to the medium in different concentrations was significantly lower than the control variant, but the difference was insignificant.

Thus, the cultures of the micromycetes *F. equiseti* and *C. magnusianum* showed high resistance to heavy metal (HM) salts in the medium, especially chromium and lead, which confirms the studies of other authors [15, 16]. *C. magnusianum* showed greater tolerance to zinc and copper salts in the medium compared to *F. equiseti*.

To assess the influence of stress factor in the form of heavy metals on the organism state, the content of malondialdehyde in mycelium of *F. equiseti* and *C. magnusianum* was determined during their cultivation on substrates with copper and chromium (Table 1). MDA is a product of lipid oxidation and can serve as an indicator of the degree of damage to membrane structures in the cells of the organism [17, 18]. A number of studies confirm the dependence of MDA concentration in fungal mycelium on the HM content in the medium. Thus, MDA concentration in *Acrocalymma vagum* first increased and then decreased with increasing Cd concentration in the medium [7]. Studies with the pigment-synthesising yeast *Rhodotorula mucilaginosa* showed that with the zinc content in the medium of 100, 150 and 200 mg/l the MDA content in the mycelium of the fungus significantly decreased, and with increasing zinc concentration in the medium (300, 350 mg/l) - significantly increased. On the contrary, on media with chromium addition, at a concentration of 10 mg/l, the MDA content in mycelium increases, and at further increase of chromium content in the medium (20 and 30 mg/l) it decreases [19].

***Table 1***

*Content of malondialdehyde in mycelia of Fusarium equiseti and Cylindrocarpon magnusianum, µmol/1 g crude weight*

| **TM content in the substrate** | **MDA content in mycelium *Cylindrocarpon magnusianum*** | **MDA content in mycelium *Fusarium equiseti*** |
|---|---|---|
| Without HM (control) | 2,034 ± 0,047<br>1,959…2,109 | 1,290 ± 0,071[1]<br>1,064…1,516 |
| Cr 2,5 мг/л | 6,092 ± 0,585↑<br>5,161…7,024 | 1,743 ± 0,071↑[2]<br>1,519…1,968 |
| Cr 5 мг/л | 7,276 ± 1,115↑<br>5,502…9,050 | 1,760 ± 0,070↑<br>1,536…1,983 |
| Cr 10 мг/л | 3,881 ± 0,054↑<br>3,795…3,966 | 1,142 ± 0,014<br>1,098…1,185 |
| Cu 50 мг/л | 2,031 ± 0,021<br>1,997…2,064 | 2,227 ± 0,230<br>1,495…2,958 |
| Cu 100 мг/л | 3,571 ± 0,431↑<br>2,885…4,257 | 6,368 ± 0,775↑<br>3,902…8,833 |
| Cu 150 мг/л | 4,117 ± 0,291↑<br>3,654…4,580 | 8,076 ± 0,311↑<br>7,087…9,066 |

[1]Average value of the indicator ± standard deviation.
[2] Significant difference from the control: increase ↑ or decrease ↓ of the index (p <0.05).

Analysis of MDA content in mycelia of *F. equiset*i and *C. magnusianum* showed the dependence of the increase of this index on the concentration of chemical elements in the medium. The greatest response to chromium content in the medium was observed in *C. magnusianum*: MDA content in all variants was significantly higher than in the control. However, at the highest chromium concentration of 10 mg/l, the MDA content in the mycelium of the fungus was significantly lower than in other variants with chromium. *F. equiseti* was more influenced by the copper content in the medium: with increasing copper concentration, the MDA content in the mycelium increased significantly in relation to the control. In the mycelium of *C. magnusianum*, when copper was added to the medium, the MDA content was also significantly higher compared to the control (except Cu 50mg/l), but the difference was not as significant as in the experiment with *F. equiseti*.

Thus, it can be concluded that MDA synthesis plays a role in the system of adaptive responses of fungi. In the conducted study, the analysis of MDA content in fungal mycelium showed that C. magnusianum showed greater resistance to copper content in the medium, *F. equiseti* - to chromium content. It is also worth noting that when chromium was added to the medium at concentrations of 2.5 and 5 mg/l, the MDA content in the mycelium of *C. magnusianum* and *F. equiseti* increased, but when the metal concentration was increased to the maximum (10 mg/l), the value of this index, on the contrary, decreased. A similar result was observed in studies with the endophytic fungi *Acrocalymma vagum* and *Scytalidium lignicola* when grown on media with cadmium [7] and the yeast fungus *Rhodotorula mucilaginosa* when grown on media with chromium (VI) [19].

The results of the experiment on the growth of fungi on media with different concentrations of oil are presented in Tables 2-4.

***Table 2***

*Growth rate of Fusarium equiseti colonies (mm/day)*

| Observation period | Control | Oil content in the medium | | | | |
|---|---|---|---|---|---|---|
| | | **1%** | **2.5%** | **5%** | **7.5%** | **10%** |
| 1 week | 6,5 | 6,8 | 7,2 | 6,8 | 2,5 | 2 |
| 2 weeks | 2,3 | 2,7 | 1,9 | 2,3 | 3,4 | 2,5 |
| 3 weeks | 2 | 0,9 | 0,3 | 0 | 1,6 | 0 |

***Table 3***

*Growth rate of Cylindrocarpon magnusianum colonies (mm/day)*

| Observation period | Control | Oil content in the medium | | | | |
|---|---|---|---|---|---|---|
| | | **1%** | **2.5%** | **5%** | **7.5%** | **10%** |
| 1 week | 2 | 1,4 | 1,3 | 1,1 | 0,9 | 0,7 |
| 2 weeks | 4,5 | 4 | 2,9 | 3,4 | 2,1 | 2,3 |
| 3 weeks | 2,9 | 3,7 | 0,3 | 2 | 1,9 | 3,2 |

Growth of *F. equiseti* and *C. magnusianum* was observed at all oil concentrations tested. In the experiment with *F. equiseti*, the highest inhibition of growth was caused by the oil concentration of 10 %, but it was not the threshold concentration. At oil concentrations of 1, 2.5 and 5 % in the medium, the growth rate of fungal colonies during the first week was higher than in the control variant. By the end of the experiment, the growth rate of colonies decreased and was lower than in the control in all experimental variants with oil. *C. magnusianum*, on the contrary, at the beginning of the experiment was not characterised by active growth (adaptation period), but from the second week of the experiment showed high colony growth rates.

The following conclusions can be drawn from the results.

1. Cultures of *Cylindrocarpon magnusianum* and *Fusarium equiseti* have generally high metal resistance, with greater resistance to chromium and lead than to nutrients (zinc and copper). The copper concentration of 150 mg/l for *F. equiseti* was the most toxic, but not threshold.

2 The study of the dynamics of malondialdehyde formation in mycelia of *Cylindrocarpon magnusianum* and *Fusarium equiseti* under the action of heavy

metals showed that fungi have species specificity in relation to heavy metals: *C. magnusianum* is more resistant to the action of high concentrations of copper, *F. equiseti* - to chromium.

3. The studied fungal cultures showed resistance to oil content in the medium, but species-specific strategies of species adaptation to the oil pollution factor were observed.

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