



Fomes fomentarius Extract Decrease Negative Impact of Cadmium Ions in Barley Seedlings [†]

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Abstract: Cadmium ions are toxic to living organisms and cause oxidative stress. Current research is focused on the search of new antitoxinants with antioxidant, antiradical, and chelating activity. *Fomes fomentarius* is one of the most common wood-destroying fungi in Eurasia; however, its chemical composition and biological effects have not been studied sufficiently. This work aimed to study separate and joint effects of fungal extracts and Cd²⁺ ions (250 µM) on barley growth. Cd²⁺ caused a 95% decrease in root length compared to control (water). Fungal extract (2 mg/mL) decreased it by 25%. Diluted extract (1 mg/mL) stimulated root growth by 12%. The shoot length in the case of Cd²⁺ decreased by 44% compared to control and by 36% in the case of extract (2 mg/mL). At lower extract concentration the length of shoots increased by 20% compared to water. Under the joint action of Cd²⁺ and extract (1 mg/mL) the root length reached 30% of control, which is six times higher than in the case of Cd²⁺. The shoot length was also higher compared to Cd²⁺ and reached 71% of the control. Thus, the addition of tinder fungus extract (1 mg/mL) reduced the negative effect of Cd²⁺ but did not completely block it. We suppose that this effect was associated with the high content of phenolic compounds in the extract (3.5 µg/mL) and their antioxidant activity. The ABTS* test showed that fungal extract inhibited the formation of radicals by 51%, which is comparable to standard antioxidant rutin. We suggested that *Fomes fomentarius* extract could be tested further as a bio-based product, reducing the toxic effects of heavy metals.

Keywords: barley; growth; stress; cadmium; *Fomes fomentarius*



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1. Introduction

Soil pollution is a common risk for plant growth. In many cases human activity leads to heavy metal contamination. One of the most toxic metals for plants is cadmium [1]. A lot of human activities, such as the metallurgy and electroplating industries, as well as the production of phosphate fertilizers, emit cadmium into environment. Therefore, the search for protective substances that could reduce the negative effects of cadmium on plants is important. Natural raw materials as a source of such protectors, for example, plants and fungi, are preferable compared to synthetic ones. It is well known that fungi have diverse secondary metabolic pathways. It makes them promise for finding new products for plant protection. Xylotrophic fungi have a parasitic or saprotrophic lifestyle, and thus decompose wood. They are widely spread in the forests, and form fruiting bodies with a large biomass. Some of them are rich of organic acids, polysaccharides, proteins, phenols, and triterpenes, which are known as biologically active substances with antioxidant, immunomodulatory, anticancer, and other effects [2,3]. Such chemical composition enables their use as bioproducts for plant protection.

Our study deals with the effects of fungi extracts on barley growth under the impact of toxic dose of cadmium ions.

2. Experiments

Barley seeds were germinated on filter paper in Petri dishes at 25 °C, and photoperiod of 16/8 h (day/night). The length of roots and shoots, the content of photosynthetic pigments (80% acetone extracts) in the first leaf were measured on the 5th day of plant growth. The organ size was measured in 30 plants of each variant, chlorophyll content—in 3 average leaf samples and in 3 analytical replications in each of the samples.

For preparation of the extract 10 g of dry mass of *Fomes fomentarius* fruit bodies were extracted 4 times by 40% ethanol (100 mL) at 50 °C for 40 min with ultrasonic treatment. Extract was evaporated up to 100 mL of final volume and then diluted to concentrations of 2 mg/mL and 1 mg/mL with distilled water, which is equivalent to 1 and 2 mg of dry fungi biomass in 1 mL of the extract volume.

The content of phenolic compounds in the extract was determined spectrophotometrically by the reaction with Folin–Ciocalteu reagent; flavonoids concentration—by the aluminum chloride method; and antioxidant activity by the ABTS test [4,5]. The number of replicates in each case was 4.

Water solution of cadmium sulfate (250 mM) was used as a toxicant.

Results are presented as mean and standard error. The significance of differences was evaluated by the Mann–Whitney nonparametric U-test.

3. Results

250 µM cadmium ions caused a significant decrease (about 30%) in the shoot length of barley seedlings, in comparison with the control plants. The length of the roots decreased drastically by more than 20 times (Figure 1). Cadmium ions also caused a significant decline in chlorophyll content (Figure 2).

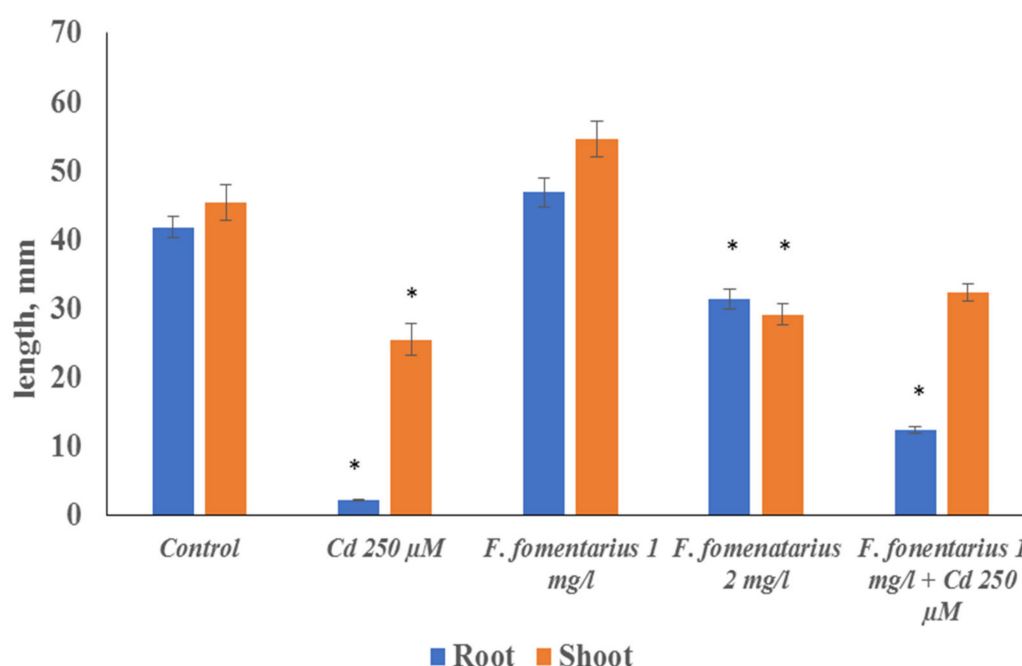


Figure 1. The influence of cadmium ions and extract from *F. fomentarius* on barley seedlings: root and shoot length, *—significantly different from the control at $p < 0.05$.

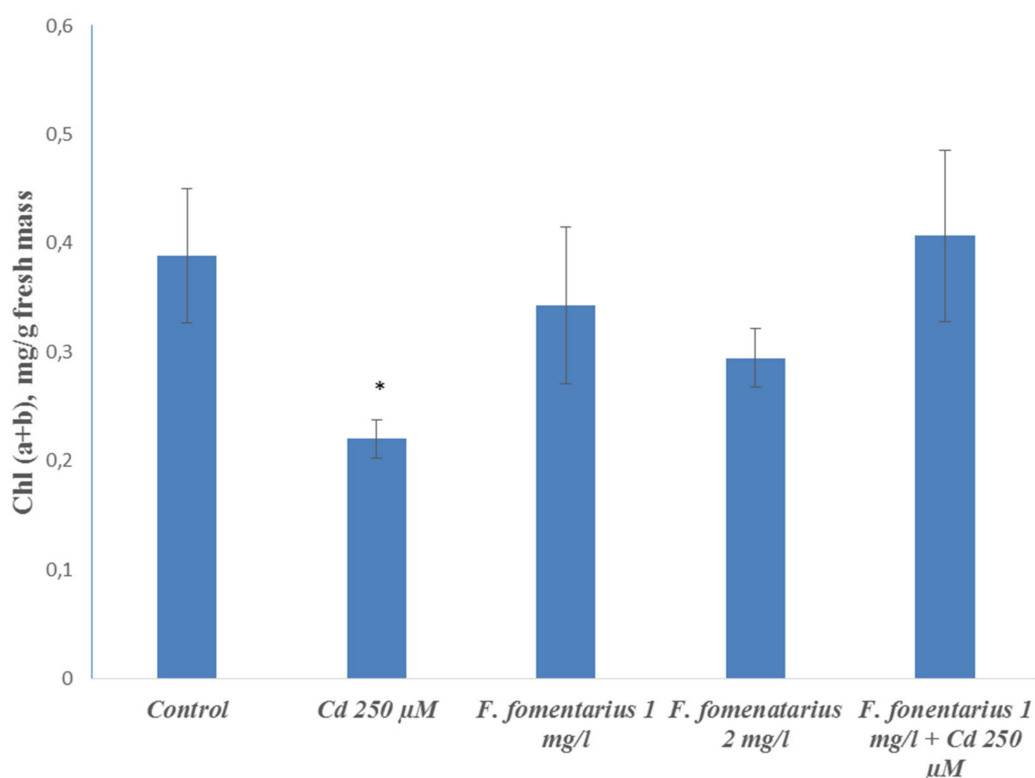


Figure 2. Chlorophyll content in primary barley leaf, *—significantly different from the control at $p < 0.05$.

The application of fungal extracts of different concentrations showed different effects. The treatment by the concentration of 1 mg/mL did not significantly affect the length of barley shoot and roots, compared to the control. In the case of the more concentrated extract (2 mg/mL) a significant reduction in roots and shoot was observed. However, both roots and shoots were significantly bigger than in the case with cadmium treatment (Figure 1).

The chlorophyll content in both the plants treated with cadmium ions and those treated with fungal extracts did not differ from the control (Figure 2).

For the study of the combined effect of cadmium ions and fungal extract we used *F. fomentarius* extract with 1 mg/mL concentration, as it was not toxic to plants. Under the joint treatment the toxic effect of Cd^{2+} was significantly low: the root length was six times higher than under the separate action of cadmium ions. The shoot length increased by 1.5 times compared to the impact of cadmium (Figure 1). The chlorophyll concentration did not significantly differ from the level of the control plants and was higher than under cadmium ions.

The diluted (1 mg/mL) extract from *F. fomentarius* contained 3.5 µg/mL of phenolics (Table 1).

Table 1. Total content of phenols and flavonoids in *F. fomentarius* dry mass.

	Phenols, mg/g	Flavonoids, mg/g
Fungi fruit body	13.6 ± 0.1	0.45 ± 0.1

These compounds are known antioxidants [6], and their high content could provide the positive anti-toxicant effect of fungal extract in joint treatment with cadmium ions.

The antioxidant activity of fungal extract (based on ABTS*-test) also showed its quenching potential: the formation of radicals was inhibited by 51%, which was comparable to the standard antioxidant—rutin (Table 2).

Table 2. Total antioxidant activity of *F. fomentarius* extracts and standards.

Compound	ABTS-Radical Scavenging, %
Fungi extract	51 ± 2.1
Gallic acid, 0.5 mg/mL	93.4 ± 0.1
Rutin, 0.5 mg/mL	61.2 ± 2.3
Control (water)	0 ± 0.0

4. Discussion

Cadmium ions are widely spread in contaminated soils and are highly toxic to plants. To a greater extent, they affect the growth of roots rather than shoots, which may be explained by the barrier function of the root. The application of fungal extracts together with toxic concentrations of cadmium ions improved the growth of barley seedlings compared to the separate action of Cd^{2+} . So, the negative effects of cadmium ions on plant growth were reduced by the fungal extract, but the plants did not reach the control level. The pigment content was also higher under the joint treatment compared to the separate action of cadmium ions and reached the control level. The probable reason for these effects is the antioxidant activity of the found biologically active compounds in the extract—phenolics and flavonoids.

To decrease the negative impact of heavy metals ions on plants, different approaches are practiced, for example the use of plant growth promoting bacteria on the contaminated soils the, introduction of metal chelators into the soil and so on. Heavy metal stress in plants is accompanied by oxidative stress, so the use of fungal extracts, which are the natural sources of antioxidants, may be promising for plant protection from heavy metals pollution.

5. Conclusions

Our study has shown that the use of low concentrations of *Fomis fomentarius* extract (1 mg/mL) did not practically suppress plant growth and also reduced the negative effect of cadmium ions (250 μM) in the case of joint application.

F. fomentarius is a widespread fungus in the forest ecosystems. Its availability as a natural resource, the possibility to cultivate it in vitro, and the low effective concentrations make it possible to recommend this fungus for the production of an ecologically safe plant protective agent, improving plant growth under the heavy metal stress.

Supplementary Materials: The poster presentation is available online at <https://www.mdpi.com/article/10.3390/IECPS2020-08746/s1>.

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Abbreviations

ABTS—2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid).

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