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Fomes fomentarius Extract Decrease Negative Effects of Cadmium Ions at the Early Stages of Barley Development †

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† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Published: date

Abstract: Cadmium ions are toxic to living organisms and cause oxidative stress. The search of new anti-toxicants with antioxidant, antiradical and chelating activity is actual. *Fomes fomentarius* is one of the most common wood-destroying fungi in Eurasia; however its chemical composition and biological effects are not studied sufficiently. This work was aimed to study separate and joint effects of fungal extracts and Cd^{2+} ions (250 μM) on barley growth. Cd^{2+} caused 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 Cd^{2+} 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 Cd^{2+} and extract (1 mg/mL) the root length reached 30% of control, which is 6 times higher than in the case of Cd^{2+}. The shoot length was also higher compared to Cd^{2+} and reached 71% of the control. Thus, the addition of tinder fungus extract (1 mg/mL) reduced the negative effect of Cd^{2+} 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 has shown 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 toxic effects of heavy metals.

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

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 as metallurgy, electroplating industries as well as the production of phosphate fertilizers emit cadmium into environment. Therefore, the search of protective substances that could reduce negative cadmium effects 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 a diverse secondary metabolic pathways. It makes them promising for finding out new products for plant protection. Xylotrophic fungi perform 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 substancies 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 doze 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% aceton 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 4 times consequently extracted 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 by distilled water, that 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 aluminium chloride method, and antioxidant activity by the ABTS test [4,5]. The number of replicates in each case – 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).

The application of fungal extracts of different concentrations showed different effects. The treatment by the concentration of 1 mg/mL did not significantly affect on 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 of 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 treated both by cadmium ions and fungal extracts plants did not differ from the control (Figure 2).

For the study of the combined effect of cadmium ions and fungal extract we used the fomis 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 6 times higher than under the separate action of cadmium ions. The shoot length increased by 1.5 times compared to cadmium impact (Figure 1). The chlorophyll concentration did not significantly differ from the level of the control plants, and was higher than under cadmium ions.

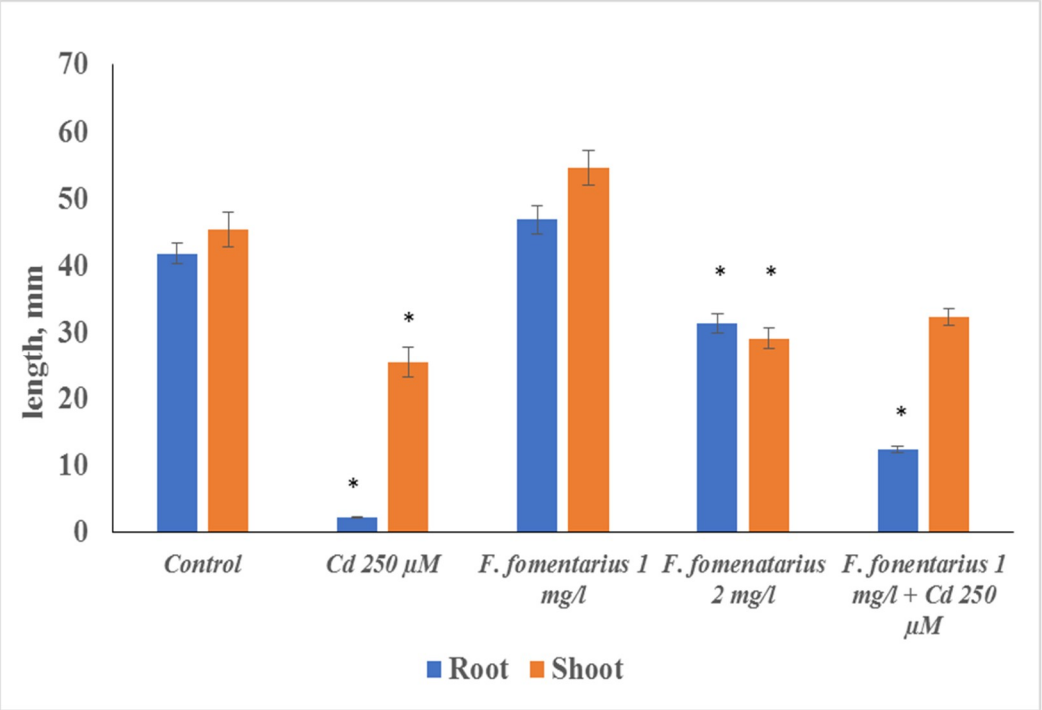

Figure 1. The influence of cadmium ions and extract from *F. fomentaris* on barley seedlings: root and shoot length, * - significantly different from the control at $p < 0.05$.

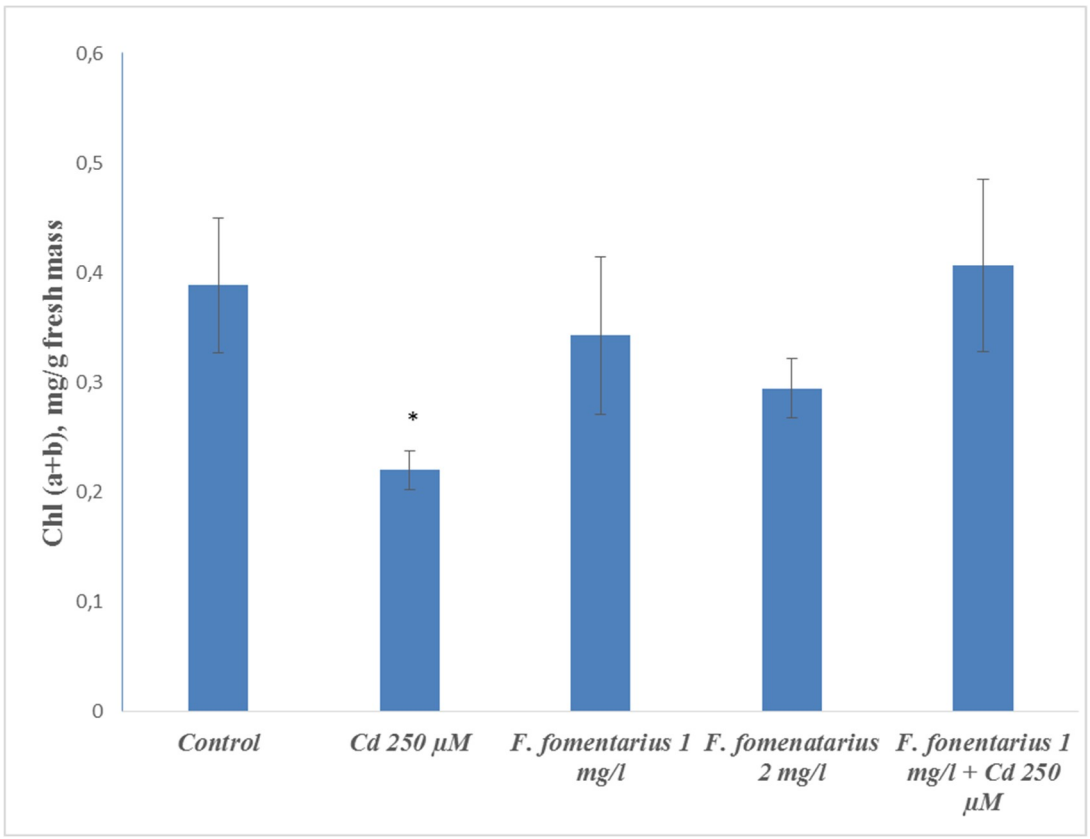

Figure 2. Chlorophyll content in primary barley leaf, * - significantly different from the control at $p < 0.05$.

The 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 as antioxidants [6], and their high content could provide the positive antitoxicant 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 standard antioxidant – rutin.

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 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 leve. 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 of 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 practicised, p.e. the use of plant growth promoting bacteria at the contaminated soils, introduction of metal chelators into the soil and so on. Heavy metal stress in plants is accompanied by the 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 have shown that the use of low concentrations of *Fomis fomentarius* extract (1 mg/mL) practically did not 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 fungi 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 fungi for the production of ecologically safe plant protective agent, improving plant growth under the heavy metal stress.

Author Contributions: A.E. and I.K. conceived and designed the experiments; I.N., V.N., O.S., A.E. and I.K. performed the experiments; A.E. performed the statistical analyses; A.E. and I.K. analyzed the data; A.E. and I.K. wrote the paper.

Acknowledgments: The research was supported by The Ministry of Education and Science of the Russian federation Agreement no.02.A03.21.0006.

Conflicts of Interest: The authors declare no conflict of interest

Abbreviations

ABTS – 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)

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