INFLUENCE OF FUNGI OF THE GENUS TRICHODERMA ON PHYTOAVAILABILITY CADMIUM AND PHYSIOLOGICAL CONDITION OF MAIZE (ZEA MAYS)

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ARTICLE INFO

ABSTRACT

The aim of conducted research was to determine in what degree inoculation of soil by fungi Trichoderma modifies process of phytoavailability of cadmium through roots and above ground parts of maize (Zea mays) and how it affects cadmium activity as damaging factor of photosynthetic apparatus of plants. Outline of vase experiment covered 5 objects (control without addition of cadmium and 4 objects with increasing amount of cadmium in amount from 10 to 80 mg.kg-1 d.m. soil), and within each object two variants: soil without (-T) and with (+T) addition of fungi of Trichoderma (+T) in shape of granulated preparation of Traniun-G in amount of 750 g.m-2 soil. Increasing amount of cadmium in surface caused decrease of harvest size of roots and above ground parts of maize, but inoculation of surface by Trichoderma fungi softened those changes. Amount of cadmium in plants was increasing promotionally to increasing amount of that metal in soil, but the presence of fungi of Trichoderma type caused decrease of phytoavailability cadmium by above ground parts of maize. Decrease parameters of chlorophyll fluorescence Fv, Fm, Fv/Fm showed lack of disturbances in photosynthetic apparatus of maize despite decrease the amount of chlorophyll in leaves under the influence of inserted cadmium doses to soil.

Keywords: Maize, cadmium, Trichoderma, chlorophyll fluorescences

INTRODUCTION

Cadmium is a heavy metal, which huge concentration in the environment results in absorption by plant organisms and causes effects of toxicity (Czeczot and Skrzyczny, 2010). Possibilities of taking cadmium by plants, and including it to the trophic chain by that, are different and dependent on chemical shape of its compounds, and from its solubility in liquids at the same time (Maciołek and Zielinska, 2013). Cadmium phytoavailability increases when reaction of soil is acid when content of organic material is low and in presence of fungus in surface (Matkowski et al., 2007). Maize is one of the oldest and commonly cultivated plants in the world. Flour, karsha and corn flake are used for food. Flour made from corn does not have properties of supporting the growth of plants, protection against pests or against environmental changes. Inoculation of surface with fungi Trichoderma modifies processes of phytoavailability of cadmium through roots and above ground parts of sweet corn (Zea mays). It was sought to determine the influence of cadmium on damages of photosynthetic apart of plants and determining influence of fungi of the genus Trichoderma from a view of modifying factor on stressor’s activity.

MATERIAL AND METHODS

Researches were conducted based on potted and lab experiments. Outline of potted experiment covered 5 objects (control without application of Cadmium and 4 objects with increasing cadmium dose in soil), and within each object two variants (Table 1): 1. Soil without application of fungi of the genus Trichoderma (-T) 2. Soil with application of fungi of the genus Trichoderma (+T) in shape of granulated preparation Traniun-G in amount preferred by the producer (Koppert B.V.) that is first application: 750 g.m-2 of soil, and second application: 375 g.m-2 of soil. Tested plant was maize (Zea mays) stock of 3 plants in one vase. Lightly acid soil (pH of 5.57) with granulometric composition of simple dust was used in the experiment. Identical primary fertilization: 0.2 g N; 0.05 g P; 0.2 g K and 0.025 g Mg on kg d.m. soil was used on every object. Fertilizers were applied in forms of water solutions of given salts: NH4NO3, KH2PO4, KCL and MgSO4•7H2O, simulating pollution of soil on four levels. Solutions of fertilizers and salts of cadmium were being mixed with soil for one week before sowing the seeds. Plants were cultivated through 72 days, maintaining temperature 23-25°C with photoperiod 16/8 h (day/night).
Table 1 Scheme of experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soil with addition of fungi of the genus Trichoderma (+T)</th>
<th>Soil without addition of fungi of the genus Trichoderma (-T)</th>
<th>Cadmium dose [mg.kg(^{-1}) d.m. soil]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>III</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>IV</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
</tbody>
</table>

During vegetation plants were watered by distilled water, maintaining soil humidity during first phase of experiment on level of 40%, and next on 50% of capillary water capacity. Disturbances in the process of photosynthesis were monitored through fluorescence measurement and content of chlorophyll (fluorometer IMAGING-PAM Walz company).

The following parameters were measured: \(F_0\) – fluorescence zero of objects adapted to darkness, \(F_{m}\) – maximal fluorescence, \(F_v\) – fluorescence changeable, \(F_v/F_0\) – maximal effectiveness of water fission after donor side PSII and \(F_v/F_m\) – maximal photochemical effectiveness of PSII. After finishing potted experiment plant material was dried in temperature of 75°C, majority of harvest and cadmium amount in both roots and above ground parts were marked with application of spectrophotometer of atomic absorption ASA (Hitachi Z-2000). Roots and above ground parts were analyzed separately.

The significant differences between the treatments were analyzed by analysis of variance (ANOVA) and Tukey’s test at \(p<0.05\) by using the Statistica program, version 10.

RESULTS AND DISCUSSION

Obtained results of maize yield indicate negative influence of cadmium in soil on majority of yield of growing plants without application of fungi of the genus Trichoderma (Figure 1).

**ROOTS**

![Roots Graph]

**ABOVE GROUND PARTS**

![Above Ground Parts Graph]

Yield of roots was in difference between 0.51 - 1.08 g.pot\(^{-1}\) (variant –T) and 0.57 – 1.09 g.pot\(^{-1}\) (variant +T). Improvement of size of root harvest of growing plants can be seen in presence of Trichoderma on soil of second and third pollution level in comparison with analogical but without protective role of fungus.

Yield of above ground parts of maize was at interval from 2.36 – 12.65 g.pot\(^{-1}\) (variant –T) and 4.64 – 9.47 g.pot\(^{-1}\) (variant +T). Inoculation of soil with fungi of the genus Trichoderma caused growth of yield on soils with second, third and fourth level of cadmium pollution.

Cadmium application to soil had an influence on significant growth of content of that metal in plants (Figure 2). In roots of plants, growing without Trichoderma in surface the amount of cadmium was at interval between 1.02 – 539.38 \(\mu g.g^{-1}\) whereas in roots of plants growing in presence of fungi was from 1.20 to 409.22 \(\mu g.g^{-1}\). Differences in amount of cadmium in roots, caused by application of Trichoderma to soil, turned out to be vital statistically only in plants, growing in soil with the highest cadmium dose.

The amount of cadmium in above ground parts of maize reached presented values: 0.95 – 145.42 \(\mu g.g^{-1}\) (variant –T) and 0.98 – 123.93 \(\mu g.g^{-1}\) (variant +T). It was observed that above ground parts of growing plants with presence of fungi of the genus Trichoderma took less cadmium from polluted soils than analogical, deprived from protective activity of Trichoderma.

Kinetics of chlorophyll fluorescence is useful informative tool in studies of influence of environmental stresses on photosynthesis (Kalaji and Loboda, 2010). In optimal conditions for course of photosynthesis solar energy absorbed by chlorophyll is only in small part reemitted in shape of fluorescence. In case of anomaly in function of PSII the stop of photosynthesis takes place with the growth of fluorescent radiation.

Obtained results in parameters of chlorophyll fluorescence in maize leaves present figure 3. Values of starting fluorescence \(F_o\) did not change significantly with growth of cadmium dose in surface. Also from the point of view of presence and lack of fungi of the genus Trichoderma in surface differences in values \(F_v\) were not significant statistically.

The value of maximal fluorescence \(F_m\) belongs to many factors, including type of sucking out light, its parameters and from content of photosynthetic dye in studied tissue. Decrease values of \(F_o\) indicates that studied photosynthetic object is under the influence of stress, for not all acceptors of electrons in PSII can be reduced totally (Kalaji and Loboda, 2010). Decrease of parameter values \(F_v\) is not observed in obtained results during the growth of the intensity of stress factor, which was cadmium. In general higher value of \(F_m\) was observed in plants, growing on soils highly polluted with cadmium in presence of fungi of the genus Trichoderma. Parameter \(F_v\) is a difference of values \(F_m\) and \(F_o\). Values of \(F_v\) depends on maximal quantum productivity of PSII and is lower under influence of environmental stresses (Kalaji and Loboda, 2010). In studied plants were observed higher values of \(F_v\) in plants growing on soils with addition of fungi of Trichoderma type, whereas the stree factor, which was cadmium, did not cause essential changes in values of parameter \(F_v\).

Maximal productivity of water splintering in donated side of PSII determined by parameter \(F_v/F_o\) in optimal conditions is 3–4 (Kalaji and Guo, 2008). Obtained results indicate that values \(F_v/F_o\) are included in range from 3–4 in plants, growing with and without presence of fungi of Trichoderma type.

Parameter \(F_v/F_o\), determining potential productivity of PSII can be used as a reliable indicator of photochemical activity of photosynthetic apparatus, because for the majority of plants in stage of full development and in stress free condition maximal value of that quotient is 0.83 (Björkman and Demmig 1987; He et al., 1996; Basu et al., 1998). Obtained result in the experiment indicate decrease of \(F_v/F_o\) parameter below values of 0.83 in all studied objects.
IV. The cadmium amount in roots and above ground parts of maize, cultivated on soil with increasing cadmium concentration without addition (-T) and with addition (+T) fungi of the genus Trichoderma. Different letters show significant difference (p<0.05).

The amount of photosynthetic dye in given plant depends on species and variety of studied plant, and also from both environmental and anthropogenic factors (Chen and Kreeb, 1990; Kozlowski et al., 2001; Krzesłowska, 2004). In studied objects it was observed not high decrease of indicator of chlorophyll content SPAD in leaves of growing plants on polluted with cadmium soils in comparison to control (Table 2).

Table 2 Index of chlorophyl content [SPAD] in maize leaves cultivated on soil with increasing cadmium concentration (I–IV) without addition (-T) and with addition (+T) fungi of the genus Trichoderma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(+T)</th>
<th>(-T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.80</td>
<td>13.81</td>
</tr>
<tr>
<td>I</td>
<td>13.19</td>
<td>12.04</td>
</tr>
<tr>
<td>II</td>
<td>11.24</td>
<td>11.53</td>
</tr>
<tr>
<td>III</td>
<td>12.17</td>
<td>12.03</td>
</tr>
<tr>
<td>IV</td>
<td>12.05</td>
<td>12.02</td>
</tr>
</tbody>
</table>

Addition of fungi of the genus Trichoderma to soil did not have an influence on amount of chlorophyll in leaves of maize significantly.

Figure 2 The cadmium amount in roots and above ground parts of maize, cultivated on soil with increasing cadmium concentration without addition (-T) and with addition (+T) fungi of the genus Trichoderma.

Figure 3 Radar charts of physiological changes of features in maize leaves cultivated on soil with increasing cadmium concentration without addition (-T) and with addition (+T) fungi of the genus Trichoderma.

DISCUSSION

Tolerance index is a relation of the amount of yield, obtained on soil, polluted by metals, to yield, which was gathered from monitored soil and it can present the following values: Ti<1; Ti=1; Ti>1 (Spiak and Wall, 2000). Values Ti<1 means stop of plant growth or their total death; Ti=1 indicates lack of influence of metal on both growth and development of plants. Presented results of experiments allowed determining the tolerance index in division from 0.19 – 0.68 for yield of roots and from 0.47–1.23 for yield of above ground parts (Table 3). Ti took bigger values for plants growing with participation of fungi of the genus Trichoderma in comparison to the rest. Values of Ti>1 were stated in case of roots of plants, growing on soil with addition of Trichoderma on second and third level of cadmium pollution. The others objects values Ti<1, which indicates, that in this case cadmium was a factor, which limited growth of plants, and its action was in small degree eased by addition of fungi of the genus Trichoderma.

Table 3 Index tolerance of maize cultivated on soil with increasing cadmium concentration (I–IV) without addition (-T) and with addition (+T) fungi of the genus Trichoderma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-T)</td>
<td>0.66</td>
<td>0.47</td>
<td>0.56</td>
<td>0.55</td>
<td>0.48</td>
<td>0.32</td>
<td>0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>(+T)</td>
<td>0.64</td>
<td>1.02</td>
<td>1.23</td>
<td>0.82</td>
<td>0.60</td>
<td>0.68</td>
<td>0.66</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Fungi of the genus Trichoderma are known from having ability of symbiosis with roots of plants - mycorrhiza. Effect of mycorrhizal fungi on taking and accumulation of cadmium depends from species of fungus, species of plant and metal concentration in ground. Matkowski et al. (2007) stated, that introduction of fungi Trichoderma harzianum to polluted by cadmium soil caused increased concentration of that element in above ground parts of sweet corn. According to those authors it was connected with increased amount available for plants forms Cd in soil. On the other hand, Majewska et al. (2012) in their researches on influence of fungi Trichoderma koningii on cadmium accumulation in biomass of grass (Festuca ovina) stated significant statistically decrease of content of that...
metal in roots and above ground parts of plants, growing on polluted by cadmium soil, grafted on spore from fungi of Trichoderma type.

In table 4 there were compared values of coefficients of straight correlation (r) mentioning in numbers relation between amount of cadmium in soil and its accumulation in roots and above ground parts of maize. Counted values r indicate strong, positive correlation between those parameters. That relationship is practically the same on soil with and without fungi of Trichoderma type.

**Table 4 Simple correlation coefficients (r) between the amount of cadmium in soil, and its concentration in roots and above ground parts of maize, cultivated on soil without addition (-T) and with addition (+T) fungi of the genus Trichoderma**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>0.99***</td>
<td>0.99***</td>
<td>0.97***</td>
<td>0.98***</td>
</tr>
</tbody>
</table>

r significant at ***p=0.001

During conducted research technique of parameters measurement of chlorophyll fluorescence was used to assess function of photosynthetic apparatus of plants. Big advantage of that method is the fact, that it does not cause long-lasting damages of plants and it can be conducted during their growth (Baker and Rosenquist, 2004). Counted coefficients of correlation r between cadmium content in soil and values of individual parameters of chlorophyll fluorescence indicate presence of negative relations of F0, Fv, Fv/Fs in case of plants growing without addition of fungi of the genus Trichoderma (Table 5). In other words, as the amount of cadmium in soil increases, the value of beginning, maximal and changeable fluorescence decreases. Also the amount of chlorophyll in leaves decreases. Addition to soil fungi of Trichoderma type weakened that relation and it caused change of its mark (besides values of SPAD).

**Table 5 Simple correlation coefficients (r) between the amount of cadmium in soil and individual fluorescence parameters and chlorophyll amount in above ground parts of maize cultivated on soil without addition (-T) and with addition (+T) fungi of the genus Trichoderma**

<table>
<thead>
<tr>
<th>r</th>
<th>F0</th>
<th>Fv</th>
<th>Fv/Fs</th>
<th>Fv/Fp</th>
<th>SPAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>-0.55*</td>
<td>0.54*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fv</td>
<td>-0.68**</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fv/Fs</td>
<td>-0.74**</td>
<td>0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fv/Fp</td>
<td>0.34</td>
<td>0.51</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPAD</td>
<td>-0.56*</td>
<td>-0.44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

significant at *p=0.05 **p=0.01

**CONCLUSION**

1. Increasing amount of cadmium in soil (from 10 to 80 mg.kg⁻¹) caused decrease in yield size of both roots and above ground parts of maize. Addition of fungi of the genus Trichoderma to soil softened negative cadmium influence on yield of maize.

2. The amount of cadmium in roots and above ground parts of maize increased with growth of that metal in surface both in soils with and without addition of fungi of the genus Trichoderma. Significantly higher cadmium amounts accumulated in roots than in above ground parts.

3. Inoculation of soil by fungi of the genus Trichoderma had an influence on reduction of cadmium phytoavailability by above ground parts of maize.

4. Values parameters of chlorophyll fluorescence F0, Fv, Fv/Fs, Fv/Fp indicate on lack of disturbances in photosynthetic apparatus of maize under the influence of cadmium doses in soil.

5. Inserting cadmium dose to soil caused decrease in amount of chlorophyll in maize leaves, whereas the presence of fungi of the genus Trichoderma did not have any influence on those changes.

**REFERENCES**


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