Biodegradation of atrazine, glyphosate and pendimetaline employing fungal consortia

Biodegradação de atrazina, glifosato e pendimetalina empregando consórcios fúngicos

Biodegradación de atrazina, glifosato y pendimetalina empleando consorcios de hongos

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Abstract

The objective of the present study was to evaluate the bioremediation of soils artificially contaminated with atrazine, glyphosate and pendimethalin by fungal consortia in biodegradation processes in microcosms. Biodegradation was evaluated from microbial respiration over a period of 15 days and genotoxicity analysis in Allium cepa roots exposed to elutriate samples at zero and 50 μg mL⁻¹ concentrations of the herbicides after the biodegradation process. The results were submitted to analysis of variance, the Tukey test and the Fischer test (p<0.05%) for comparison of means. The Aspergillus fumigatus - Penicillium citrinum consortium had a larger capacity to degrade atrazine but metabolism was inhibited in the presence of glyphosate and pendimethalin. There was a delay in the mitotic index in the meristematic cells of the Allium cepa roots exposed to the elutriates in the 50 μg mL⁻¹ atrazine and pendimethalin concentration. There was a cellular alteration in the metaphase phase of the cells exposed to the elutriates at the 50 μg mL⁻¹ concentration of the three herbicides. The changes occurred were low, indicating that there was degradation of part of the herbicides.

Keywords: Degradation; Pesticides; Fungi; Agricultural soils.
Resumo

O objetivo do presente estudo foi avaliar a biorremediação de solos contaminados artificialmente com atrazina, glifosato e pendimetalina por consórcios fúngicos nos processos de biodegradação, em microcosmos. A biodegradação foi avaliada a partir da respiração microbiana no período de 15 dias e análise de genotoxicidade em raízes de *Allium cepa* expostas a amostras de elutriato nas concentrações zero e 50 μg mL\(^{-1}\) dos herbicidas após o processo de biodegradação. Os resultados foram submetidos à análise de variância, teste de Tukey e teste de Fischer (p<0,05%) para comparação de médias. O consórcio *Aspergillus fumigatus - Penicillium citrinum* apresenta maior capacidade de degradar atrazina, porém, há inibição do metabolismo na presença de glifosato e pendimetalina. Há atraso no índice mitótico nas células meristemáticas das raízes *Allium cepa* expostas aos elutriados na concentração 50 μg mL\(^{-1}\) de atrazina e pendimetalina. Há alteração celular na fase metáfase das células expostas aos elutriados na concentração 50 μg mL\(^{-1}\) dos três herbicidas. As alterações ocorridas são baixas, indicando que há a degradação de parte dos herbicidas.

**Palavras-chave:** Degradação; Agrotóxicos; Fungos; Solos agrícolas.

Resumen

El objetivo del presente estudio fue evaluar la biorremediación de suelos contaminados artificialmente con atrazina, glifosato y pendimetalina empleando consorcios de hongos en los procesos de biodegradación, en microcosmos. La biodegradación se evaluó mediante respiración microbiana durante un período de 15 días y análisis de genotoxicidad en raíces de *Allium cepa* expuestas a muestras de elutriados en concentraciones de cero y 50 μg mL\(^{-1}\) de los herbicidas después del proceso de biodegradación. Los resultados fueron sometidos a análisis de varianza, prueba de Tukey y prueba de Fischer (p <0,05%) para comparación de medias. El consorcio *Aspergillus fumigatus - Penicillium citrinum* tiene una mayor capacidad para degradar la atrazina, sin embargo, hay inhibición del metabolismo en presencia de glifosato y pendimetalina. Existe un retraso en el índice mitótico en las células meristemáticas de las raíces de *Allium cepa* expuestas a los elutriados en la concentración 50 μg mL\(^{-1}\) de atrazina y pendimetalina. Existe una alteración celular en la fase de metafase de las células expuestas a elutriados en la concentración de 50 μg mL\(^{-1}\) de los tres herbicidas. Los cambios ocurridos son bajos, lo que indica que hay una degradación de parte de los herbicidas.

**Palabras clave:** Degración; Plaguicidas; Hongos; Suelos agrícolas.
1. Introduction

The continued use of pesticides, although essential for productivity in modern agriculture, results in damage to the environment. The residues of these products persist for long periods of time and cause a reduction in microbiota, biodiversity and loss of essential soil functions, such as nutrient cycling and environmental buffering power (Cheng et al., 2016).

In the soil, pesticides can be transformed into more toxic metabolites than the initial product or be completely mineralized in carbon dioxide (CO$_2$) and water through a variety of processes. Biodegradation is the main process responsible for the natural attenuation of these compounds, but the natural degradation is very slow due to the complex structure, recalcitrant nature of the molecules and the metabolic capacity of the microorganism (Geed et al., 2018; Gupta et al., 2019).

The techniques available to accelerate degradation rates include bioremediation, which consists of an ecological technology to efficiently degrade organic contaminants and their metabolites in water and soil through living organisms, including plants, bacteria and fungi (Quintella et al., 2019; Zhang et al., 2020).

Fungi are efficient in bioremediation because they are able to adapt their metabolism to different carbon sources. This metabolic flexibility occurs due to the production of enzymes that catalyze different steps of the metabolic pathways of different compounds (Kanagaraj et al., 2015).

Microorganisms in general do not have the potential to completely degrade a toxic compound, so the complement of enzymes from different members of stipes can be essential for metabolization. Microorganisms that do not have the potential to completely degrade a molecule can become a substance assimilable to another microorganism (Saez et al., 2015).

Biodegradation by fungal intercropping presents itself as an attractive option to degrade atrazine, glyphosate and pendimethalin, since these herbicides can be metabolized by soil organisms. Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) is a persistent herbicide, with low adsorption and moderate aqueous solubility that contributes to the accumulation of contaminating levels in soils (Coelho & Bernardo, 2017).

Glyphosate (N-(phosphonomethyl) glycine) is a moderately persistent herbicide with a high adsorption capacity and rapid degradation in the soil, generating aminomethylphosphonic acid (AMPA), molecule of long persistence and potential to interfere in environmental quality (Wang et al., 2016). Pendimethalin (N-1-(ethylpropyl)-2,6-dinitro-
3,4-methyl-toluidine) is a moderately persistent herbicide, very dangerous to the environment with low sorption capacity. The degradation products, although not yet identified, are more polar than pendimethalin itself (Kpagh et al., 2016).

The use of a microbial consortium is a viable alternative to remove or reduce pesticides from the soil, so the objective of the present study was to evaluate the bioremediation of soils artificially contaminated with atrazine, glyphosate and pendimethalin by fungal consortia in the biodegradation processes in microcosms.

2. Methodology

The experiments were carried out at the Environmental Biotechnology Laboratory (LABITEC), at the Center for Exact, Natural and Technological Sciences at the State University of the Tocantina Region of Maranhão (UEMASUL), Brazil. The fungi used in the study were Aspergillus fumigatus, Fusarium verticillioides and Penicillium citrinum, isolated from agricultural soils with a history of application of the herbicides atrazine, glyphosate and pendimethalin. The strains belong to the Micoteca of the Laboratory of Environmental Biotechnology - LABITEC, maintained on potato dextrose agar (PDA) and 1% glycerin at 4 ± 0.1°C to maintain viability.

The fungal inoculum was prepared from pure fungi cultures, transferring individual 7 mm diameter mycelium discs to Petri dishes containing sabouraud dextrose 4% solid culture medium and incubated at 28 ± 0.1°C for 20 days. After the incubation period, the spores were removed with 10 mL sterile mineral medium (1 L distilled water, 18.34 g K₂HPO₄,3H₂O; 6.0 g KH₂PO₄; 0.2 g MgSO₄.7H₂O and 4.0 g (NH₄) 2SO₄, pH 7.0). The suspension was transferred to 250 mL Erlenmeyers and stirred for 5 minutes on a horizontal shaker (SPLabor, Model SP-160).

One milliliter of each suspension was transferred to a sterile centrifuge tube (falcon), containing 2 mL of 0.05% Tween 80 solution and stirred for 5 minutes. A spore suspension was prepared by counting in a NeuBauer chamber and microscope (Brand Zeiss Axiostar Plus) at a concentration of 1×10⁶ spores mL⁻¹ (Lira & Orlanda, 2020).

Fungal respiratory activity was evaluated in microcosms, using the principle of Bartha’s respirometric method with modifications (Villaverde et al., 2018). The microcosms consisted of sealed and sterilized 600 mL glass jars containing 30 mL 0.50 mol L⁻¹ KOH solution in a sterile 50 mL beaker; 100 g sterile soil artificially contaminated with atrazine,
glyphosate and pendimethalin at concentrations zero and 50 μg mL⁻¹; 1 mL fungal spore suspension and sterile ultrapure water to maintain moisture.

Thus prepared, the microcosms were closed and incubated at 30 ± 0.1 °C for a period of 15 days. Three titrations were performed, the first at 5 days, the second at 10 days and the third at 15 days. From the volume spent on titrations, it was possible to calculate microbial respiration and relate the results through microbial growth curves (Equation 1).

\[
\text{mg de CO}_2 = (VB - VA) \times C \times f \times 22 \times FA
\]

Where: VB= HCl volume spent on the control titration; VA= HCL volume spent on sample titration; C= HCl concentration; f= correction factor for acid concentration; 22= gram of CO₂; FA= correction factor of the aliquot used in the titration (KOH volume) used as a CO₂ trap/KOH aliquot volume used in the titration.

To test the viability of fungi in the consortia, an experiment was carried out in microcosm in a factorial scheme with three levels of the fungal spore factor associated with three levels of the herbicide factor (atrazine, glyphosate and pendimethalin), a level of herbicide concentration (50 μg mL⁻¹) and the respective controls, in triplicate, resulting in 30 experimental units. The microorganisms that showed the ability to metabolize the herbicides were used to compose the consortia.

After intercropping the fungal spores, tests were carried out in a completely randomized design for each herbicide. For the microcosm artificially contaminated with atrazine, the consortia Fusarium verticillioides - Aspergillus fumigatus (Fv-Af), Fusarium verticillioides - Penicillium citrinum (Fv-Pc) and Aspergillus fumigatus - Penicillium citrinum (Af-Pc) were used at zero and 50 μg mL⁻¹ concentrations, with five replications, resulting in 30 experimental units. In microcosms artificially contaminated with glyphosate and pendimethalin, the consortium used was Aspergillus fumigatus - Penicillium citrinum (Af-Pc) at zero and 50 μg mL⁻¹ concentrations, with five replications, resulting in 10 experimental units for each treatment.

To detect possible residues after herbicide degradation by fungal consortia, genotoxicity analyzes were performed. The bioindicator used was Allium cepa and the technique was described by Freitas et al. (2017) where 12.5 g soil samples treated in microcosms and soil samples free of contamination were placed in 125 mL Erlenmeyers, then 50 mL distilled water was added at the proportion soil 1:4 water (m/v). After stirring for 1
hour and sedimentation for 24 hours under refrigeration, the suspensions were centrifuged at 3000 rpm for 5 minutes to obtain the supernatant, called elutriate.

Previously rooted *Allium cepa* bulbs remained in contact with the treatment elutriate for 24 hours. The roots were collected and fixed in Carnoy 3:1 (3 ethanol: 1 acetic acid) for 24 hours and then kept in test tubes containing 70% alcohol in a refrigerator until the slides were made.

In the preparation of the slides, two root tips per bulb were used, which were hydrolyzed in HCl (mol L\(^{-1}\)) for 5 minutes, washed in distilled water and stained with 2% acetic orcein for 30 minutes. The blades were prepared using the crushing technique according to Santos et al. (2020). Images of 250 cells were analyzed under a microscope (Brand Zeiss Axiostar Plus) for each test treatment, observing the number of meristematic cells in division to determine the mitotic index (MI) (Equation 2) and the occurrence of cellular changes (OCC) (Equation 3).

\[
MI(\%) = \frac{\text{number of dividing cells}}{\text{total cells}} \times 100
\]

\[
OCC(\%) = \frac{\text{number of cells with cellular changes}}{\text{total dividing cells}} \times 100
\]

Simultaneous tests were carried out in a completely randomized design. For the atrazine elutriate at zero and 50 µg mL\(^{-1}\) concentrations after the microcosm biodegradation process, the fungal consortia used were *Fv-Af, Fv-Pc* and *Af-Pc*, with 5 replications, resulting in 30 experimental units.

In glyphosate and pendimethalin elutriates at zero and 50 µg mL\(^{-1}\) concentrations after the microcosm biodegradation process, the consortium used was *Af-Pc* at zero and 50 µg mL\(^{-1}\) concentrations, with 5 replications, resulting in 10 experimental units for each of the treatments. The mitotic indices of the treatments were evaluated separately.

The data were submitted to analysis of variance, the means were compared by the Tukey test and the Fisher test (p<0.05%), with the aid of the Sisvar statistical program (Ferreira, 2019).
3. Results and Discussion

Microbial growth curves show that *Aspergillus fumigatus*, *Fusarium verticillioides* and *Penicillium citrinum* adapted quickly to microcosms artificially contaminated with atrazine (Figure 1A) and *Aspergillus fumigatus* and *Penicillium citrinum* in microcosms artificially contaminated with glyphosate (Figure 1B) and pendimethalin (Figure 1C).

**Figure 1.** Respiratory activity of fungi in microcosm with soil artificially contaminated with 50 µg mL⁻¹ of the herbicides after 5, 10 and 15 days.

(A) Atrazine. (B) Glyphosate. (C) Pendimethalin.
Source: Research Data.

In growth kinetics, the adaptation phase (lag) occurred on the first day for all treatments. Exponential growth (log) occurred from the fifth day, where fungi started the cell division process. The growth rate decreased between 5 and 10 days (stationary phase), in this phase the microbial population became stable, but the metabolic activity decreased. From the tenth day onwards, the decline phase was observed, this phase continues until the population has decreased or disappeared entirely, as there is no more herbicide as a carbon source.

In microcosms artificially contaminated with atrazine, *Penicillium citrinum* did not show a decline in 15 days, which indicates that after that period there was still atrazine or
degradation products in the microcosm (Figure 1A). There was no release of CO₂ by *Fusarium verticillioides* in microcosms artificially contaminated with glyphosate and pendimethalin, indicating that this fungus was not able to metabolize these herbicides as shown in Figure 1B and Figure 1C. The *Fusarium verticillioides* fungus showed a lower CO₂ release rate for atrazine and did not show CO₂ release for glyphosate and pendimethalin.

The microbial growth curves shown in Figure 2 demonstrate that during 5, 10 and 15 days the consortia *Fv-Af*, *Fv-Pc* and *Af-Pc* inoculated in the microcosms artificially contaminated with atrazine showed CO₂ release indicating the biodegradation of this herbicide while *Af-Pc* showed a higher CO₂ rate.

**Figure 2.** Respiratory activity of fungal consortia in microcosm with artificially contaminated soil with 50 µg mL⁻¹ of the herbicides after 5, 10 and 15 days.

In microcosms artificially contaminated with glyphosate and pendimethalin, the *Af-Pc* consortium was not able to degrade the herbicides, as it did not release CO₂ (Figure 2). The growth of these fungi was inhibited, which may have been caused by antagonism between the strains.
Castro-Gutiérrez et al. (2016) state that the microbial consortium cometabolism can be better than isolated strains for the bioremediation of recalcitrant compounds such as the herbicides studied. The microbial degradation of atrazine occurs by N-dealkylation reactions, in which alkyl groups attached to the ring nitrogen atom, in amines, carbamates or amides are removed by oxidation to aldehyde, producing deethylatrazine (DEA), desisopropylatrazine (DIA), didealkylatrazine (DDA), desethylhydroxyatrazine (DEHA) and hydroxyatrazine (HA) (Coelho & Bernardo, 2017).

Bonfleur et al. (2015) demonstrated that the application of atrazine tended to temporarily reduce microbial biomass and increase the metabolic quotient of the microbiota, indicating a stress situation caused by atrazine toxicity. Research by Zhu et al. (2019) pointed out that two microbial strains reached about 98% and 99.6% efficiency of atrazine degradation in seven days and that the degradation was faster when the two strains were used in consortium.

Góngora-Echeverría et al. (2020) in a study on the bioremediation of pesticides from microbial consortia, showed that atrazine and glyphosate could reach 90% degradation in 15 days and the herbicide glyphosate had the lowest residual amount. According to Yu et al. (2015) glyphosate degradation in the soil depends on microorganisms. Microbial activity is an important factor that determines the presence of glyphosate and its by-product, aminomethylphosphonic acid (AMPA), in the soil. The action of enzymes such as oxidoreductases and transaminases and glyoxylic acid cleave the glyphosate molecule in bonds other than carbon and phosphorus, which can occur under aerobic and anaerobic conditions in the soil profile (Wang et al., 2016).

The aerobic degradation pathway of pendimethalin occurs through the dealkylation of the amino group, followed by the reduction of the nitrile group. In anaerobic conditions, there is a sequential reduction of nitro groups (Tobler et al., 2007). Strange-Hansen et al. (2004) stated that glyphosate degradation in the soil environment is a cometabolic process and the rate of decomposition would depend on the general activity of microbial populations, the type of soil and environmental conditions.

Kočárek et al. (2016) in a study on pendimethalin degradation in a haplastic chernosol, under controlled conditions, did not observe any effect of pendimethalin on the quantity or activity of soil microorganisms. But it was observed that the fungi Aspergillus fumigatus and Penicillium citrinum used this herbicide as a carbon source in the present research.
Figure 3 shows the cell divisions and changes that occurred after the genotoxicity test. In this study 250 meristematic cells from *Allium cepa* roots were observed per experimental unit and disorganized cell division only occurred in the metaphase phase.

**Figure 3.** Divisions and changes in meristematic cells from *Allium cepa* roots exposed to atrazine, glyphosate and pendimethalin elutriates.

(A) Normal prophase. (B) Normal metaphase. (C) Normal anaphase. (D) Normal telophase. (E) Disorganized metaphase. (F) Disorganized metaphase.

Source: Research Data.

There were no occurrences of changes in the cell divisions of the cells exposed to the control elutriates (Tables 1, 2 and 3).
Table 1. Cell cycle of meristematic cells from *Allium cepa* roots exposed to atrazine elutriate.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>C Fv-Af</th>
<th>Fv-Af</th>
<th>C Fv-Pc</th>
<th>Fv-Pc</th>
<th>C Af-Pc</th>
<th>Af-Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td>780</td>
<td>898</td>
<td>796</td>
<td>839</td>
<td>795</td>
<td>822</td>
</tr>
<tr>
<td>Prophase</td>
<td>432</td>
<td>319</td>
<td>438</td>
<td>391</td>
<td>430</td>
<td>397</td>
</tr>
<tr>
<td>Metaphase</td>
<td>12</td>
<td>9</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Anaphase</td>
<td>17</td>
<td>11</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Telophase</td>
<td>9</td>
<td>13</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Total cells</td>
<td>1250</td>
<td>1250</td>
<td>1250</td>
<td>1250</td>
<td>1250</td>
<td>1250</td>
</tr>
<tr>
<td>Cellular changes</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>MI (%)</td>
<td>37,6</td>
<td>28,2</td>
<td>36,3</td>
<td>29,1</td>
<td>36,4</td>
<td>34,2</td>
</tr>
<tr>
<td>OCC (%)</td>
<td>0</td>
<td>1,8</td>
<td>0</td>
<td>1,4</td>
<td>0</td>
<td>1,3</td>
</tr>
</tbody>
</table>


Source: Research Data.

Table 2. Cell cycle of meristematic cells from *Allium cepa* roots exposed to glyphosate elutriate.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>C Af-Pc</th>
<th>Af-Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td>805</td>
<td>827</td>
</tr>
<tr>
<td>Prophase</td>
<td>408</td>
<td>396</td>
</tr>
<tr>
<td>Metaphase</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Anaphase</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Telophase</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Total cells</td>
<td>1250</td>
<td>1250</td>
</tr>
<tr>
<td>Cellular changes</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>MI (%)</td>
<td>35,6</td>
<td>33,8</td>
</tr>
<tr>
<td>OCC (%)</td>
<td>0</td>
<td>0,7</td>
</tr>
</tbody>
</table>

C (control). Af-Pc (*Aspergillus fumigatus* + *Penicillium citrinum*).

Source: Research Data.

Table 3. Cell cycle of the meristematic cells of *Allium cepa* roots exposed to pendimethalin elutriate.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>C Af-Pc</th>
<th>Af-Pc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interphase</td>
<td>806</td>
<td>855</td>
</tr>
<tr>
<td>Prophase</td>
<td>415</td>
<td>375</td>
</tr>
<tr>
<td>Metaphase</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Anaphase</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Telophase</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Total cells</td>
<td>1250</td>
<td>1250</td>
</tr>
<tr>
<td>Cellular changes</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>IM (%)</td>
<td>35,5</td>
<td>31,6</td>
</tr>
<tr>
<td>OAC (%)</td>
<td>0</td>
<td>1,8</td>
</tr>
</tbody>
</table>

C (control). Af-Pc (*Aspergillus fumigatus* + *Penicillium citrinum*).

Source: Research Data.

There was a significant difference between the treatments for MI of cells exposed to atrazine elutriate (Figure 4). It is observed that at zero µg mL⁻¹, the MI were higher than in
treatments with 50 µg L\(^{-1}\). Cell division decreased when the concentration was increased, which indicates that this herbicide interfered with cell proliferation.

**Figure 4.** Mitotic index means (%) of cells exposed to atrazine elutriate, at concentrations zero and 50 µg mL\(^{-1}\), after 24 hours.

Mean values followed by the same letter do not differ according to the Fisher test at a 5% significance level.

Source: Research Data.

...The MI of the cells exposed to the glyphosate elutriate treated with the *Af-Pc* consortium in microcosm demonstrated that there was no influence from the herbicide (Figure 5).
Figure 5. Mitotic index means (%) of cells exposed to glyphosate elutriate at concentrations zero and 50 µg mL\(^{-1}\), after 24 hours.

Mean values followed by the same letter do not differ according to the Fisher test at a 5% significance level.
Source: Research Data.

The results for treatments with *Allium cepa* roots exposed to pendimethalin elutriate and treated with the *Af-Pc* consortium in microcosm indicated that this herbicide was able to interfere in cell proliferation, as the test was significant (Figure 6).
Figure 6. Mitotic index means (%) of cells exposed to pendimethalin elutriate at concentrations zero and 50 µg mL⁻¹, after 24 hours.

Mean values followed by the same letter do not differ according to the Fisher test at a 5% significance level.
Source: Research Data.

It was not possible to perform the statistical test for OCC in the roots of *Allium cepa* that were exposed to the herbicide elutriate, considering that there was no OCC at the zero µg mL⁻¹ concentration, as expected, but at the 50 µg mL⁻¹ concentration cellular changes were observed for the three herbicides.

The OCC varied in the treatments for the atrazine elutriate and was lower for the cells exposed to the glyphosate elutriate and treated with the *Af-Pc* consortium, in microcosm. The OCC index for pendimethalin elutriate and treated with *Af-Pc* was equal to the index found in the treatment with the *Fv-Af* consortium exposed to atrazine (Tables 1, 2 and 3).

In a study by Felisbino et al. (2018), atrazine was responsible for producing chromosomal aberrations in *Allium cepa* meristematic cells, such as delayed MI, genotoxic changes and mutagenic changes in relation to control. The MI found in atrazine treatments in the present study also showed a delay compared to the control.

Silveira et al. (2017) stated in a study on the toxic effects of environmental pollutants that there was a high frequency of chromosomal changes and condensed nucleus in *Allium cepa* cells treated with atrazine, but the MI did not differ from the control.
After 24 hours, the *Allium cepa* cells used in the present research also showed cellular alterations, but at a lower rate compared to the quoted study, demonstrating that the fungal consortia were able to degrade atrazine almost completely, as there were still OCC, but at low levels.

According to Agência Nacional de Vigilância Sanitária [Anvisa] (2020), mutagenicity and genotoxicity tests have been negative for glyphosate. However, Dias et al. (2014) found chromosomal aberrations such as decreased MI in *Allium cepa* roots treated with glyphosate. In the present study, the MI in the cells treated with the elutriatro artificially contaminated with glyphosate and treated with the Af-Pc consortium did not present significant differences and the OCC was low.

No studies were found evaluating the cytotoxic effect of pendimethalin. However, Tomlin (2011) stated that this herbicide, when absorbed by roots and leaves, inhibits cell division and cell elongation. OCC for the treatment with the elutriate artificially contaminated with pendimethalin and treated with the Af-Pc consortium was high among the treatments, as there was no mineralization of this compound in the microcosms and since there was no release of CO$_2$ by the consortium, it is assumed that pendimethalin caused changes in the cells analyzed.

4. Conclusions

The *Aspergillus fumigatus - Penicillium citrinum* consortium has a larger capacity to degrade atrazine, but metabolism is inhibited in the presence of glyphosate and pendimethalin. There is a delay in the mitotic index in the meristematic cells of the *Allium cepa* roots exposed to the elutriates at the 50 μg mL$^{-1}$ atrazine and pendimethalin concentration. There is a cellular change in the metaphase phase of cells exposed to elutriates at 50 μg mL$^{-1}$ concentration of the three herbicides. The changes occurred were low, indicating that there was degradation of part of the herbicides.

This work expands the knowledge about biodegradation processes from fungal consortia. The results of the study demonstrate the possibility of using the metabolism of fungal consortia for the degradation of herbicides in agricultural soils.
References


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