PLEUROTUS OSTREATUS (JACQ.) P. KUMM. E P*LEUROTUS ERYNGII* (F.) QUÉL. COMO POTENCIAIS AGENTES MICORREMEDIADORES DE EFLUENTES CONTAMINADOS COM BENTAZONA E 2,4-D

Pleurotus ostreatus (Jacq.) P. Kumm. and Pleurotus eryngii (F.) Quél. as potential mycoremediator agents of wastewater contaminated with bentazon and 2,4-D

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Resumo: Micorremediação tem sido estudada como alternativa para remoção de pesticidas de efluentes. Foi avaliada a capacidade in vitro dos fungos basidiomicetos Pleurotus ostreatus e Pleurotus eryngii como potenciais agentes de remediação de efluentes que contêm herbicidas. P. ostreatus e P. eryngiiforam cultivados em meios líquidos contendo, respectivamente, bentazona (4,5 g L-1) e 2,4-D (5,4 g L-1), analisando a capacidade de crescimento dos fungos (biomassa seca), remoção dos compostos (CG/MS) e toxicidade do meio após tratamento com os fungos (testes Allium cepa). P. ostreatus e P. eryngii se mostraram tolerantes à bentazona e 2,4-D, respectivamente, uma vez que cresceram nos meios líquidos. A análise CG/MS mostrou que os fungos não foram eficientes na remoção da bentazona e do 2,4-D após 7 e 21 dias de cultivo, respectivamente. Em ambos os casos os efluentes finais resultaram tóxicos para A. cepa. Apesar de não ter sido comprovada a remoção de pesticidas, os resultados do trabalho mostram que P. ostreatus e P. eryngiitêm o potencial de tolerar a bentazona e o 2,4-D em meios líquidos. Portanto, algumas condições de cultivo diferentes devem ser testadas para explorar o potencial destes fungos para a remoção de pesticidas.

Abstract: Mycoremediation has been studied as an alternative to removing pesticides from wastewater. Here, we evaluated the *in vitro* capability of the basidiomycetes *Pleurotus ostreatus* and *Pleurotus eryngii* as potential remediation agents of effluents containing the pesticides bentazon and 2,4- D, respectively. *P. ostreatus* and *P. eryngii* were cultivated in liquid media containing bentazon (4.5 g L^{-1}) and 2,4-D (5.4 g L^{-1}), respectively. Biomass dry weight and CG/MS analysis were performed to evaluate the fungi' capability to grow in the contaminated media as well as to degrade the studied pesticides. Toevaluate the toxicity of the wastewater after the cultivation of the fungi, tests with*Allium cepa* were conducted. *P. ostreatus* and *P. eryngii* presented tolerance tothe bentazon and 2,4-D, respectively. CG/MS analysis showed that the fungi were not efficient in the removal of bentazon and 2,4-D after seven and twenty-one days of cultivation, respectively. Finally, in both cases, the final effluents were toxic to *A. cepa*. Despite the non-removal of pesticides, *P. ostreatus* and *P. eryngii* have the potential to tolerate bentazon and 2,4-D in liquid media. Therefore, some different cultivation conditions must be tested to explore the potential of these fungi for pesticide removal.

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1 INTRODUCTION

Pesticides are chemical compounds widely used to protect crops from undesirable organisms, such as weeds, fungi, insects, and pathogens (SJERPS et al., 2019). Nevertheless, several of these pesticides remain in the effluents of conventional crops, which are normally discharged untreated into waterways (YADAV et al., 2015). Consequently, the use of these substances worldwide has led to the contamination of environmental matrices as ground and surface water, including drinking water (SJERPS et al., 2019; QUINTANA et al., 2019; CHEN et al., 2018; XIE et al., 2019; XU et al., 2019; CALDAS et al., 2013; POSTIGO et al., 2010; KÖCK-SCHULMEYER et al., 2014; VIEIRA et al., 2016). It is a major environmental and public health problem since most of these compounds offer a high-level risk to aquatic organisms. Therefore, the occurrence of pesticides in the environment has been extensively studied in the last decades (CHEN et al., 2018; XIE et al., 2019).

Among the pesticides that may remain in wastewater, there are the 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one2,2-dioxide (Bentazon) and the 2,4-dichloro phenoxy acetic acid (2,4-D). Bentazon is a post-emerging herbicide largely applied in cereal crops and it is one of the most widely used herbicides in conventional agriculture and gardening (SALMAN; HAMEED, 2010). Besides, the systemic herbicide 2,4-D is used globally in the selective control of nest plants in agriculture and forestry (ZUANAZZI et al., 2020). Several studies have related the occurrence of bentazon in the environment (CALDAS et al., 2013; POSTIGO et al., 2010; VIEIRA et al., 2016) and recent studies have shown the contamination of surface and underground water bodies by 2,4-D (ECHEVERRI GONZÁLEZ et al., 2019; CASTRO LIMA et al., 2020).

The findings of the occurrence and risks of pesticides have heightened the need for advanced treatments to remove the contaminants from the environment since conventional treatment is not capable to remove such compounds. In this context, mycoremediation has been studied as an economical, eco-friendly, and effective biological tool that recruits fungi to degrade, transform or immobilize contaminants from the environment (PURNOMO et al., 2010; COELHO-MOREIRA et al., 2018; PEREIRA et al., 2013; CUPUL et al., 2014; FREITAS et al., 2017; GANASH et al., 2016; SERBENT et al., 2019; 2020; KAPAHI; SACHDEVA, 2017; BOSCO; MOLLEA, 2019; AKHTAR; MANNAN, 2020).

Fungi tend to degrade a vast number of contaminants (HARMS et al., 2011; SINGH, 2006; VALDEZ-VAZQUEZ et al., 2020; ZAPANA-HUARACHE et al., 2020) and the white-rot fungi (WRF) are one of the major ecological categories of wood-decaying fungi (RILEY et al., 2014) responsible for complex ecosystem processes going on during wood decay and nutrient cycling (LEONHARDT et al., 2019). WRF can be promising candidates for the treatment of contaminated wastes (AKHTAR; MANNAN, 2020), but their potential in bioremediation processes regarding chlorinated compounds is still far from being fully explored (SERBENT et al., 2020). Furthermore, basidiomycetes, such as the *Pleurotus* genre, are known for producing enzymes including lignin peroxidase, manganese peroxidase, and laccase, which promote the degradation of substances (PURNOMO et al., 2010; SINGH, 2006; ZAPANA-HUARACHE et al., 2020).

In this context, this study aims to evaluate the *in vitro* tolerance and growth of *Pleurotus ostreatus* (Jacq.) P. Kumm. and *Pleurotus eryngii* (Fr.) Quél. in liquid medium contaminated with bentazon and 2,4-D, respectively, as well as the capability of these fungi to remove the corresponding herbicide from spiked water and evaluation of its toxicity by *Allium cepa* test after treatments.

2 MATERIAL AND METHODS

2.1 Source of pesticides and fungi

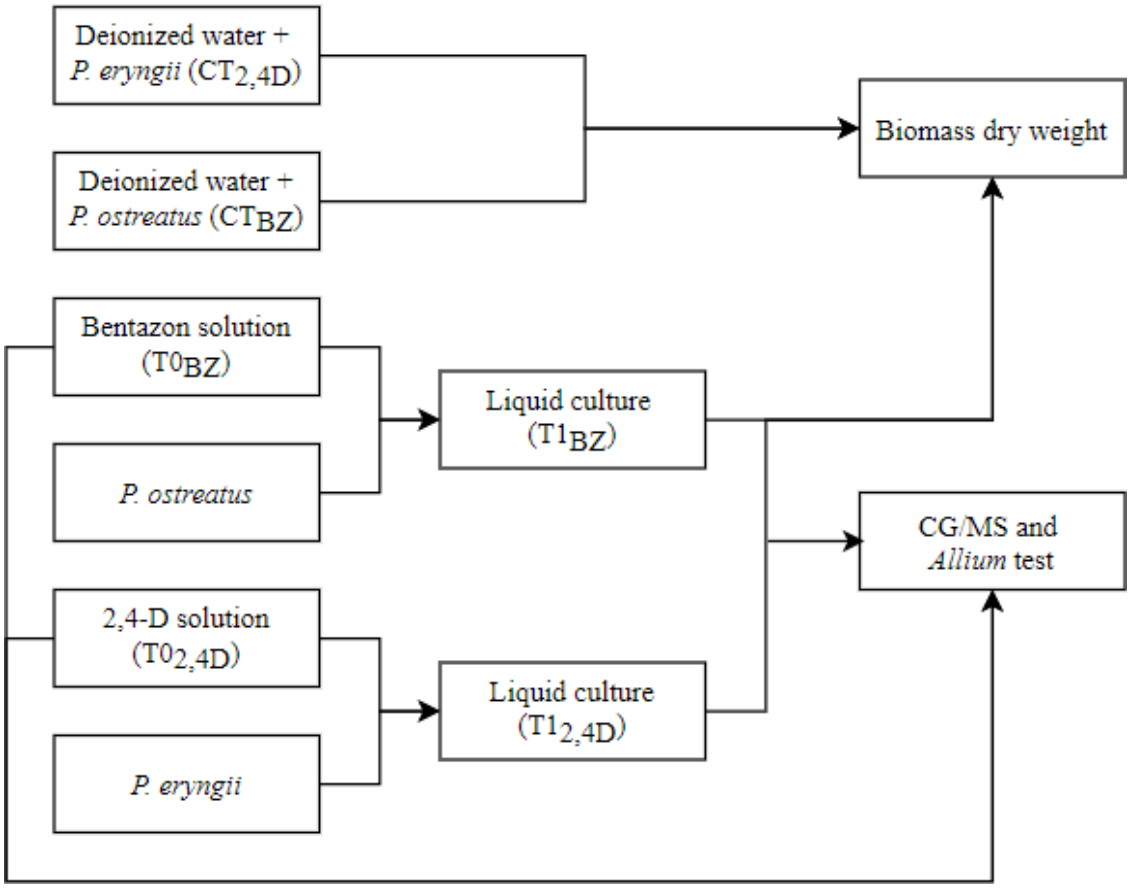
Bentazon was obtained from its commercial form, Basagram®, containing 600 g L^{-1} of the active ingredient, and 2,4-D was obtained from its commercial form, Aminol®, containing 670 g L^{-1} of the active ingredient. In both cases, the spiked water was prepared with sterilized and deionized water. *P. ostreatus* and *P. eryngii* were provided by the culture collection of the laboratory of microorganisms and biotechnological processes at the Federal University of Santa Catarina.

2.2 In vitro evaluation of pesticides tolerance and removal by strains of *Pleurotus*

P. ostreatus and *P. eryngii* were cultivated in Petri dishes containing potato dextrose agar (PDA) at 27 ± 2 °C before inoculation in the liquid medium and its subsequent analyses.

Figure 1 summarizes the studied treatments and each analysis for the *in vitro* evaluation of pesticide tolerance and removal by *Pleurotus* fungi.

Figure 1. Studied treatments and each analysis for the in vitro evaluation of pesticide tolerance and removal by Pleurotus fungi.



2.3 Fungal growth in liquid culture media

To establish the tolerance and removal of bentazon by *P. ostreatus* and 2,4-D by *P. eryngii*, experiments of liquid cultures were conducted in a stationary state.

To represent real concentrations of the pesticides in wastewater (CUPUL et al., 2014; FREITAS et al., 2017; GANASH et al., 2016; SERBENT et al., 2019; 2020), the bentazon and 2,4-D were diluted in deionized water separately, resulting in 4.5 g L^{-1} ($T0_{BZ}$) and 5.4 g L^{-1} ($T0_{2,4D}$) initial solutions, respectively. Deionized water was used as a control treatment for both pesticide experiments. Each sample was prepared in a glass flask previously sterilized.

For bentazon experiments, five mycelial disks (diameter: 8.0 mm) of *P. ostreatus* were inoculated in $T0_{BZ}$ ($T1_{BZ}$) and control treatment (CT_{BZ}). The samples ($T0_{BZ}$, $T1_{BZ}$, and CT_{BZ}) were incubated at 27 ± 1 °C for 7 days (SANTOS et al., 2019; KIST et al., 2020).

For 2,4-D experiments, five mycelial disks (diameter: 8.0 mm) of *P. eryngii* were inoculated in $T0_{2,4D}$ ($T1_{2,4D}$) and control treatment ($CT_{2,4D}$). The samples ($T0_{2,4D}$, $T1_{2,4D}$, and $CT_{2,4D}$) were incubated at 27 ± 1 °C for 21 days.

2.4 Biomass dry weight of fungi and pesticides concentration

After the incubation period, the biomass dry weight of the *P. ostreatus* and *P. eryngii* in liquid cultures were performed according to the procedure proposed by Souza et al. (2006).

Bentazon and 2,4-D concentration in the samples was analyzed by gas chromatography/mass spectrometry (GC/MS) as determined by the EPA 8270D method (US EPA, 2014), in which the quantification limit is 0.1 µg L^{-1}.

2.5 Toxicity evaluation of treated water by *Allium* test

The toxicity test was conducted with *Allium cepa* L. (onion) samples as the adapted method used by Marinho et al. (2017). First, 20 onions with similar shapes and sizes were selected, peeled, and their roots were carefully removed. Then, the onions were placed in plastic cups with tap water in the dark for 72 hours. After this period, the roots were observed and the ones with similar sizes were selected for the toxicity test. These roots were removed, and the bulbs were placed in plastic cups with the samples $T0_{BZ}$, $T1_{BZ}$, $T0_{2,4D}$, and $T1_{2,4D}$ in the dark for 72 hours. Additionally, a control sample (tap water) was tested. After 3 days, the roots were measured.

2.6 Data analysis

Statistical analysis of all data obtained in the liquid cultures assays was performed through the t-test and the significance levels were set at 95% ($p<0.05$).

3 RESULTS AND DISCUSSION

3.1 In vitro evaluation of pesticides tolerance and removal by *Pleurotus* fungi

After the incubation period, it was observed mycelial growth of *P. ostreatus* and *P. eryngii* for the treatments of liquid cultures containing bentazon and 2,4-D ($T1_{BZ}$ and $T1_{2,4D}$). Therefore, the studied fungi may tolerate the pesticides, growing in liquid environments that are similar to the real conditions

of pesticide concentrations.

The biomass dry weight of fungi and the pesticide concentrations after the incubation period for all the studied treatments is presented in Table 1.

Table 1 - Biomass dry weight of fungi and the pesticide concentrations of each treatment after the incubation period.

Treatment	Biomass dry weight (g)	Pesticide concentration (g/L)
CT_{BZ}	0.001 ± 0.000 a	0
$T0_{BZ}$	0	0.56 ± 0.03 a
$T1_{BZ}$	1.585 ± 0.247 b	0.76 ± 0.01 b
$CT_{2,4D}$	0.001 ± 0.000 a	0
$T0_{2,4D}$	0	0.03 ± 3.72 c
$T1_{2,4D}$	0.012 ± 0.002 c	0.03 ± 3.29 c

* vertical lowercase letters indicate the results of comparisons between treatments for each measured variable. Equal letters indicate means that did not statistically differ from each other (t-test, p ≤ 0, 05).

Treatments $T1_{BZ}$ and $T1_{2,4D}$ presented biomass dry weight statistically different. The fungal biomass of *P. ostreatus* cultivated in a liquid medium containing bentazon was higher than the fungal biomass of *P. eryngii* cultivated in a liquid medium containing 2,4-D, though the cultivation time (7 and 21 days, respectively) as indicated in Table 1. The biomass dry weight of control treatments (CT_{BZ} and $CT_{2,4D}$) represented just the mycelial disks inoculated in each one due to the absence of an energy source for fungi growth. No biomass dry weight was found for initial treatments ($T0_{BZ}$ and $T0_{2,4D}$) because of the absence of fungi.

The pesticide concentration of the initial solutions ($T0_{BZ}$ and $T0_{2,4D}$) was lower than the treatments with fungi growth ($T1_{BZ}$ and $T1_{2,4D}$). It was observed that bentazon and 2,4-D concentration after the incubation period decreased when compared to the concentration added at the beginning of the experiment (4.5 g L^{-1} and 5.4 g L^{-1}) independently of the fungi inoculation. This indicates that the herbicide may have been degraded or volatilized during the incubation time.

The results of GC/MS analysis obtained for 2,4-D treatments have no significant statistical difference between them. However, the bentazon concentration was statistically different between the treatments $T0_{BZ}$ and $T1_{BZ}$ as well as about 2,4-D treatments.

In the *in vitro* evaluation of bentazon (4.5 g L^{-1}) and 2,4-D (5.4 g L^{-1}) tolerance by *P. ostreatus* and *P. eryngii*, respectively, the results showed that the fungi can grow up in aqueous media containing these pesticides and, consequently, can tolerate them. The obtained results match those observed in different studies (COELHO-MOREIRA et al., 2018; PEREIRA et al., 2013; FREITAS et al., 2017; GANASH et al., 2016; MARINHO et al., 2017) that reported the fungi tolerance and their potential as agent degradation of pesticides. The biomass dry weight of *P. ostreatus* and *P. eryngii* suggests that the fungi can use bentazon and 2,4-D, respectively, as a source of nutrients. Several reports have shown the ability of *P. ostreatus* to grow in liquid medium with pesticides, such as chlorotoluron, isoproturon, diuron, linuron, propanil, malathion, lindane, and 2,4-dichlorophenol (GANASH et al., 2016; SILVA et al., 2009; RIGAS et al., 2005; KHADRANI et al., 1999; TORRES-DUARTE et al., 2009). Although, some of these experiments highlighted that *P. ostreatus* mycelial growth was inhibited by increasing the concentration of the pesticide (malathion, lindane, and 2,4-dichlorophenol).

Previous studies showed that other basidiomycetes can grow in the presence of bentazon. Coelho-Moreira et al. (2018) studied bentazon removal by *Ganoderma lucidum* in a solid culture medium, using

corn cob as substrate. Despite the authors observed that the herbicide had a negative effect on the mycelial growth of *G. lucidum,* the fungus could grow up in bentazon concentrations below 14.4 g L^{-1}. Castillo et al. (2001) observed the development of *Phanerochaete chrysosporium* in the presence of bentazon (0.22 g L^{-1}) in bioreactors with unsterile wheat straw. In both studies, the fungi could degrade the herbicide.

Silva et al. (2009) observed similar behavior of compound removal by *P. ostreatus* in an experiment where the 2,4-D concentration was 23% lower than the added amount. The degradation of 2,4-D in different concentrations by *T. versicolor* (Hernández Mendieta et al., 2013) and *P. chrysosporium* (DONNELLY et al., 1993) was also reported, though some high concentrations can inhibit their performance (HERNÁNDEZ MENDIETA et al., 2013).

In liquid cultures, the inability of *P. ostreatus* to remove bentazon and *P. eryngii* to remove 2,4-D was observed in this study. Despite the fungal tolerance to pesticides, the cultivation conditions, such as exposure time and pesticide concentration, may not favor the removal of these substances. The same behavior was observed by Donnelly et al. (1993) in an experiment using atrazine as a pollutant, where all fungi grow up in the medium containing the pesticide, but none removed it. In addition, the role of *P. eryngii* and its enzymes have been explored in degrading aromatic hydrocarbon as DDT (PURNOMO; MAULIANAWATI; KAMEI, 2019), fluorene (HADIBARATA; KRISTANTI, 2014), naphthalene (HADIBARATA et al., 2013), and bisphenolic compounds (CHANG; CHANG, 2016).

The degradation of pollutants by fungi is influenced by several factors, including the tested strain, enzyme production, pesticide initial concentration, exposure time, and cultivation conditions (SERBENT et al., 2019). In some organisms, when nutritional sources are present in the medium, the production of enzymes used to metabolize other carbon sources is limited to save energy (MARINHO et al., 2017), which may slow or decrease pesticide removal. Moreover, the mechanisms in the microbial conversion of a pesticide involve secondary metabolism (VALLI; GOLD, 1991), which strongly depends on the cultivation time.

Additionally, the need for supplementation of the culture medium using carbon and nitrogen sources depends on the fungus species. Ganash et al. (2016) observed that the degradation of malathion by *P. ostreatus* was increased when the mineral liquid medium was supplemented with lignin at 0,2%. In that study, the removal of the pesticide reached 76% after 25 days of culture. However, in the presence of *P. ostreatus*, the degradation of the herbicide 2,4-DCP in a liquid medium with wheat straw was better in the absence of glucose, reaching 54% of removal in 96 hours of cultivation (SILVA et al., 2009). According to Donnelly et al. (1993), some fungi needed nitrogen supplementation in the culture media for the complete degradation of 2,4-D. Coelho-Moreira et al. (2018) reported that after 5 days of *G. lucidum* cultivation in a solid and a liquid culture medium with 7.2 g L^{-1} and 0.6 g L^{-1} of bentazon respectively, there was no significant removal of the compound. However, after 10 days of treatment, it was detected a removal of 88% of bentazon in the solid medium and 53% in the liquid medium. In experiments in a sucrose liquid medium, Ferreira-Guedes et al. (2012) observed no degradation of 2,4-D by *Penicillium chrysogenum* fungus after the first week of the experiment. However, after 25 days of culture, 18% of this compound was removed. According to these findings, it can be inferred that longer detention time could positively influence the mycoremediation of this herbicide.

Under certain cultivation conditions, basidiomycete fungi can produce nonspecific extracellular enzymes that promote the degradation of pollutant molecules such as laccases, manganese peroxidases, and lignin peroxidases. *P. eryngii* and *P. ostreatus* produce versatile peroxidase that is capable to oxidate aromatic compounds (BANSAL; KANWAR, 2013), such as the ones present in organochloride pesticides. Additionally, Mougin et al. (2000) analyzed the degradation of the herbicide isoxaflutole by the fungi *Phanerochaete chrysosporium* and *Trametes versicolor* in a liquid medium. The authors noted that the metabolization of the herbicide occurred mainly at the end of the growth phase, after 15

days of cultivation of both fungi. This may be related to the production of oxidative enzymes such as laccases. Coelho-Moreira et al. (2018) reported that the herbicides bentazon (20 mM) and diuron (80 µM) stimulated laccase production above 140 and 90 U g^{-1}, respectively, at 10 days of cultivation.

3. 2 Toxicity evaluation of treated water by *Allium test*

The results obtained in the *Allium* test for both bentazon and 2,4-D pesticides with *P. ostreatus* and *P. eryngii*, respectively, are shown in Table 2.

Table 2 - Root growth in each studied treatment in the *Allium* test.

Treatment	Root growth (cm)
CT_{BZ}	4.30 ± 0.30 a
$T0_{BZ}$	0.45 ± 0.15 b
$T1_{BZ}$	0.55 ± 0.05 b
$CT_{2,4D}$	3.78 ± 0.96 a
$T0_{2,4D}$	0.33 ± 0.03 b
$T1_{2,4D}$	0.45 ± 0.13 b

* Vertical lowercase letters indicate the results of comparisons between treatments for each measured variable. Equal letters indicate means that did not statistically differ from each other (t-test, p ≤ 0, 05).

The samples $T0_{BZ}$, $T1_{BZ}$, $T0_{2,4D}$, and $T1_{2,4D}$ presented low root growth when compared to tap water (CT_{BZ} and $CT_{2,4D}$) (Table 2). The tap water samples presented a significant difference compared to the samples in that the roots were exposed to treatments containing bentazon and 2,4-D pesticides. All the treatments containing pesticides present no significant statistical difference in root growth.

Many tests have been developed to identify the harmful effects of chemicals on animals and plants, with emphasis on plant-based assays for being short-term and low-cost alternatives (YILMAZ et al., 2018). To verify the toxicity of samples, some assays can be performed, such as the *Allium* test. This is an interesting tool for conducting toxicity and mutagenicity research using onions (FREITAS et al., 2017; MARINHO et al., 2017) once they are cultivated during all seasons and have a low economic cost. Its roots are activated when the bulb contacts water, however, the presence of toxic compounds in the water reduces the root growth due to the delay in division cellular (MARINHO et al., 2017).

A. cepa test is a useful bioindicator of cytotoxicity and genotoxicity and serves as an alert for the population that uses pesticides indiscriminately (DATTA et al., 2018). The results obtained in the *Allium cepa* test highlighted the toxicity of the liquid culture with bentazon and 2,4-D due to the low root growth of onions observed in the samples containing the pesticides when compared to tap water. The toxicity of the samples remains after the treatments with *P. ostreatus* and *P. eryngii* because of the non-removal of pesticide compounds in the studied cultivation conditions, as demonstrated in GC/MS analysis.

No data was found on toxicity tests with bentazon in the literature. However, other studies demonstrate the detoxification of pesticides by basidiomycete fungi. In *Lactuca sativa* bioassays, Coelho-Moreira et al. (2018) observed a reduction in toxicity after fungal treatment compared to untreated samples. In another study, the *Allium cepa* test showed an inhibition decrease from 100% to 54% after atrazine treatment with *Aspergillus niger* in liquid culture after the incubation period of 7 days (MARINHO et al., 2017). Additionally, these authors obtained favorable results in the removal of pesticides methyl parathion and atrazine. After seven days of treatment with the fungus *Aspergillus niger*, the *Allium cepa* test demonstrated that the toxicity of the treated sample had decreased, as there was a growth of the

roots of the onions.

Fatima and Ahmad (2006) analyzed the EROD (o ethoxy resorufin O-demethylase) activity in *Allium cepa* exposed to 2,4-D, as a response of the organism to the presence of pollutants in the aquatic environment. The 2,4-D caused about a 40-fold rise in EROD at a dose of 1.2 ppb. The authors concluded that the strategy allows the detection of this pesticide in water monitoring studies before using analytical techniques like HPLC.

Özkul et al. (2016) investigated the cytogenetic effects of different concentrations of 2,4-D (0.67, 1.34, 2.01, 2.68, 3.35, and 4.02 mg/L) on *Allium cepa* bulblets' root tips treated for 24 and 48 h. The results showed that the herbicide affected mitotic index (MI), whose value increased significantly at three lower concentrations (0.67, 1.34, 2.01 mg/L) after treatment with 2,4-D for 24 h and decreased significantly at higher concentrations (2.68, 3.35, and 4.02 mg/L). Also, the authors showed that roots were very sensitive to the herbicide if they had been in touch with 2,4-D (4.02 mg/L) for a longer time (48 h).

The 2,4-D incidence and the cytotoxic and genotoxic potential of sediment elutriate of rivers from southern Brazil, by *Allium cepa* test, as evidenced by Rambo et al. (2017) while Martins and Campos Pereira (2018) reported that the herbicide Tordon®, which has in its formulation the compound 4-amino-3,5,6-trichloro picolinic acid (Picloram - 103,6 g/L) and 2,4-D (402 g/L) inhibited (concentrations of 0.1%, 1.0%, 10%, and 100% of the herbicide) or significantly decreased the length of the onion roots at concentrations of 0.01%, 0.001%, and 0.0001%.

Besides the non-removal of bentazon and 2,4-D, the toxicity results obtained in our study may be explained by the possible occurrence of pesticide metabolites that are not metabolized by the fungi and inhibit the onions-root growth.

There are different mechanisms involved in pesticide conversion by microorganisms. Depending on the environmental conditions, each mechanism can produce metabolites that are different from the initial compound (SERBENT et al., 2019). Consequently, even the pesticide is susceptible to degradation by fungi, in some cases, its degradation process can produce compounds that are more toxic than the pesticide itself (LURQUIN, 2016). On the other hand, some metabolites may be more accessible to fungal degradation compared to the initial compound, as proved by Vroumsia et al. (2005). The authors showed that different fungal strains more efficiently degraded the 2,4-DCP than the 2,4-D, suggesting that this behavior can occur because of the compounds' bindings and free phenolic radicals. Besides, other fungal strains better-degraded 2,4-D than 2,4-DCP, leading to hypothesizing that the conversion of 2,4-D and 2,4-DCP are catalyzed by different enzymes (VROUMSIA et al. 2005). Similarly, the metabolites of bentazon on surface water were identified as more toxic than the initial substance for fish and invertebrates while the opposite occurs for other aquatic organisms (EFSA, 2015).

Considering the obtained data on this study it may be hypothesized that the studied fungi are capable to tolerate 2,4-D and bentazon, but the cultivation conditions were not favorable to the degradation of the compounds. This can be explained by the fact that the degradation ability depends on the fungi species, the contaminant and its concentration, and the nitrogen concentration in the culture media (DONNELLY et al., 1993). In addition, these authors also concluded that it may have an increase in degradation rate with the biomass increase and the enzymatic activity when there is a high amount of carbohydrate in the culture media. Finally, the basidiomycete fungi are considered potential accumulators (BOSCO; MOLLEA, 2019). The aromatic herbicides' degradation appears to be through the compounds' incorporation into the fungus tissue and not through the complete degradation of the contaminant (mineralization) (DONNELLY et al., 1993).

Thus, the use of *P. ostreatus* and *P. eryngii* for mycoremediation of bentazon and 2,4-D in contaminated effluents needs to be further studied. To investigate the role of this organism in the treatment of bentazon and 2,4-D contaminated effluents, new experimental conditions must be tested. In addition, the tests

should include the monitoring and quantification of enzymatic activity as well as the pesticide degradation metabolites. As a result, it could be possible to know the moment that *P. ostreatus* and *P. eryngii* start to release oxidative enzymes and if it contributes to bentazon and 2,4-D metabolization and its metabolites.

Also, the fungi tolerance to herbicides should investigate the mycelium characteristics after herbicide exposition regarding reproductive structures, hyphae feature, and exposition time–structure-property relationships, which are relevant to a variety of applications of mycelium as is the case of a bioreactor for the treatment of contaminated effluents (SERBENT et al., 2020).

4 CONCLUSION

Due to the characteristics of WRF, the purpose of the present study was to evaluate the potential use of Basidiomycota fungi as degradation agents of bentazon and 2,4-D by assessing their tolerance, growth, and removal capacity of pesticides in liquid cultures. In summary, the fungus *P. ostreatus* and *P. eryngii*, grew up in liquid culture mediums with 4.5 g L^{-1} of bentazon and 5.4 g L^{-1} of 2,4-D, respectively, and tolerate this environment. Despite that, the fungi were not efficient in removing the pesticides after seven and twenty-one days of cultivation. The *Allium cepa* test evidenced that samples containing pesticides were still toxic after the treatments with fungi, in the studied conditions. The results represent the starting point of mycoremediation processes for future applications on a pilot and full scale.

Disclosure statement

There are no relevant financial or non-financial competing interests to report. No potential competing interest was reported by the authors.

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