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Original Article

Biodegradation and reduction of toxicity of Azo Trypan Blue dye by Amazonian strains of gasteroid fungi (Basidiomycota)

Biodegradação e redução da toxicidade do corante Azo Trypan Blue por Cepas amazônicas de fungos gasteroides (Basidiomycota)

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Abstract

Amazonian strains of *Cyathus* spp. and *Geastrum* spp. were studied for the ability to discolor the trypan blue azo dye and reduce its toxicity. Discoloration of trypan blue dye (0.05%) was evaluated in solid and aqueous medium over different periods. The reduction of dye toxicity after treatment was assessed by seed germination and the development of lettuce seedlings (*Lactuca sativa* L.) and toxicity test in *Artemia salina* (L.) larvae. All evaluated strains showed the potential to reduce the color intensity of trypan blue dye. *Cyathus* strains reached 96% discoloration, and *C. albinus* and *C. limbatus* also reduced dye toxicity. *Geastrum* strains showed a high efficiency degree in color reduction, reaching 98% discoloration, however, the by-products generated during the process presented toxicity and require further investigation. For the first time, Amazonian strains of gasteroid fungi degrading trypan blue are reported, some even reducing its toxicity. Thus, making them promising sources of enzymes of interest to bioremediation scenarios involving synthetic dyes.

Keywords: bird's nest fungi, *Cyathus*, dye discoloration, earthstars fungi, *Geastrum*.

Resumo

Cepas amazônicas de *Cyathus* spp. e *Geastrum* spp. foram estudadas quanto a capacidade de descolorir o corante azo Azul de tripano e reduzir sua toxicidade. Foi avaliada a descoloração do corante Azul de tripano (0,05%) em meio sólido e aquoso sob diferentes períodos de tempo. A redução da toxicidade do corante após o tratamento foi avaliada através da germinação de sementes e do desenvolvimento de plântulas de alface *(Lactuca sativa* L.), além do teste de toxicidade em larvas de *Artemia salina* (L.). Todas as cepas avaliadas apresentaram potencial de reduzir a intensidade da coloração do corante azul de tripano. As cepas de *Cyathus* alcançaram 96% de descoloração, sendo que *C. albinus* e *C. limbatus* também reduziram a toxicidade do corante. As cepas de Geastrum apresentaram alto grau de eficiência na redução de cor, alcançando 98% de descoloração, porém, os subprodutos gerados durante o processo apresentaram toxicidade e necessitam de maior atenção. Pela primeira vez se relata cepas amazônicas de fungos gasteroides degradando o Azul de tripano, algumas ainda reduzindo sua toxicidade, tornando-as fontes promissoras de enzimas de interesse em cenários de biorremediação envolvendo corantes sintéticos.

Palavras-chave: fungos ninho-de-pássaro, *Cyathus*, descoloração de corante, fungos estrela-da-terra, *Geastrum*.

1. Introduction

The disposal of contaminated textile effluents in ecosystems is one of the critical environmental issues (Ceretta et al., 2021; Wang et al., 2021). Most of these effluents are from azo dyes, the largest and most versatile class of synthetic dyes (Lee et al., 2017), which represents approximately 70% of the weight of all dyes used worldwide (Kanagaraj et al., 2015). These dyes cause environmental impacts (Hassaan and Nemr, 2017; Mourid et al., 2017) and are considered recalcitrant, non-biodegradable, persistent (Saratale et al., 2009a, b), stable in light and difficult to remove from water by conventional wastewater treatment methods (Islam et al., 2011).

Among these dyes, we highlight the trypan blue ($C_{34}H_{28}N_6O_{14}S_4$), widely used in medical histology (study of biological tissues) to analyze the viability of cell death (Keogh et al., 1980; Cooksey, 2014), in yeast viability tests (Liesche et al., 2015), in the detection of dead plant tissue and in the staining technique of arbuscular

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mycorrhizal fungi (Phillips and Hayman, 1970). Although versatile in application, this dye has been widely questioned because it is harmful to human health with teratogenic, oncogenic, carcinogenic and mutagenic effects (Weisburger & Weisburger, 1966; Chung, 2016; Field et al., 1977; Brown et al., 1978; Robertson et al., 1982; Nadaroglu et al., 2017; Tang et al., 2021), in addition to chronic cytotoxic effects (Kodjikian et al., 2005).

In an attempt to reduce its use, some substitutes were proposed (Santana et al., 2020a; Silva et al., 2015; Vierheilig et al., 2005), but when indispensable, it is necessary to think about alternatives for the treatment of effluents to reduce the impacts on the environment. One option is biodegradation performed by basidiomycete fungi and, even if other trypan blue discoloration techniques have been proposed (Nadaroglu et al., 2017; Ghime and Ghosh, 2020; Aljadaani et al., 2021), biological discoloration by fungi has many advantages in view of the enormous enzymatic potential proven useful in the treatment of synthetic azo dyes (Nozaki et al., 2008; Ali et al., 2010; Gregorio et al., 2010).

A portion of the basidiomycetes corresponds to gasteroid fungi, widely distributed in the tropics (Mueller et al., 2007), mainly in Brazil, where the diversity of the group increases every year (Accioly et al., 2019; Assis et al., 2022; Ferreira-Sá et al., 2021; Freitas et al., 2023; Santana & Couceiro, 2023). Studies with this group revealed the production of important substances with proven biological activity (Liu & Zhang, 2004; Dore et al., 2007; Coetze and Van Wyk, 2009), but there is a study gap regarding the discoloration of synthetic dyes. The genera *Cyathus* Haller and *Geastrum* Pers., common taxa in forests are known to be producers of bioactive compounds useful for bioremediation and discoloration of some synthetic dyes (Vasdev and Kuhad, 1994; Vasdev et al., 1995; Mishra and Bisaria, 2006; Santana et al., 2016), but there is no information on these genera regarding the discoloration of trypan blue.

Thus, given the need to study other fungal species and increase treatment options for synthetic dyes and encourage enzymatic studies of gasteroid fungi, the aims of this study were to investigate the ability of Amazonian strains of *Cyathus* and *Geastrum* species to discolor the synthetic dye trypan blue and to analyze the toxicity of the dye after the fungal treatment.

2. Materials and Methods

2.1. Sampling and identification of fungi

Fresh and mature specimens of *Cyathus* and *Geastrum* were collected manually (Lodge et al., 2004) in a fragment of the Amazon rain forest (dense rainforest) in the vicinity of the Sílvio Braga Hydroelectric Power Plant (2°48'44.45"S, 54°17'56.23"W), Western Pará, Brazil. The identification of the material was performed based on morphological characters following the descriptions in the specialized literature (Sunhede, 1989; Calonge et al., 2005; Silva et al., 2013; Sousa et al., 2014; Accioly et al., 2018, 2019; Góis et al., 2021). Part of the samples was used to obtain the strains and the other part was assembled in vouchers in the fungi collection of the Herbarium HSTM (HSTM-Fungos) (JBRJ, 2023) of the Federal University of Western Pará (Table 1).

2.2. Fungal strains

The strains of *Geastrum* were obtained from sections removed from the pseudoparenchyma layer of fresh basidiome exoperidium and inoculated in a 90 mm diameter Petri dish containing 15 mL of potato dextrose agar (PDA, Difco®) culture medium®. The strains of *Cyathus* were obtained from sections of the peridioles inoculated in a 90 mm diameter Petri dish containing 15 mL PDA culture medium. After growth, fragments of 3 × 3 mm from the edge of the cultures were inoculated in the center of new Petri dishes with equal volume and content. Then they were incubated under the conditions suggested by Santana et al. (2020b) to obtain the pure culture. Thus, each species was considered a treatment, totaling six treatments.

2.3. Trypan blue dye discoloration in solid culture medium

For the discoloration test of the blue trypan dye in solid medium, a 3 × 3 mm block was used, containing mycelium removed from the edge of the pure strain of each species and transferred to the centre of a Petri dish (90 mm in diameter) containing 15 mL of the homogeneous mixture of PDA plus trypan blue (0.05%). Petri dishes were incubated according to the indications of Santana et al. (2020b), and each plate was considered a replicate. Every three days, from the beginning of the experiment, for a period of 21 d of incubation, the dye discoloration halo was evaluated by measuring the diameter with a ruler (orthogonal directions). The PDA culture medium containing the dye, but in the absence of fungi was used as the control treatment of the experiment.

Table 1. Species used to obtain Amazonian strains of gasteroid fungi.

Family	Species	Voucher
Agaricaceae	*Cyathus albinus* Accioly, R. Cruz & Baseia	14790
	C. limbatus Tul. & C. Tul.	14883
	C. setosus H.J. Brodie	14957
Geastraceae	*G. echinulatum* T.S. Cabral, B.D.B. Silva & Baseia	14901
	Geastrum hirsutum Baseia & Calonge	14865
	G. schweinitzii (Berk. & M.A. Curtis) Zeller	14896

2.4. Trypan blue dye discoloration in aqueous culture medium

For the dye degradation test in aqueous medium, three blocks containing 3 × 3 mm mycelium were removed from the edge of each pure strain and transferred to 250 mL Erlenmeyer bottles with 50 mL of dextrose potato medium (DP) plus trypan blue (0.05%). The mixture was homogenized and sterilized in autoclave at 121 °C for 15 min. The vials were incubated at 25 ± 2 °C according to the indications for cultivation of Santana et al. (2020b), and each bottle was considered a replicate. Dye discoloration was analyzed every 20 days from the beginning of the experiment, for a period of 60 days. The mycelium was separated from the culture medium by filtration. The DP medium containing the dye, but without fungi, was used as control of the experiment.

The trypan blue dye discoloration in the aqueous medium was monitored recording the changes of absorbance of the filtrate at different times. The dye discoloration supernatant in aqueous medium was obtained by centrifugation at 10,000 rpm for 5 min, and the record was taken in the maximum spectral range of 590 nm using a visible UV spectrophotometer. The same procedure was performed for the control treatment. The supernatant resulting from this experiment was used in dye toxicity tests.

Discoloration of trypan blue dye was evaluated according to the Formula 1 below:

$$D(\%) = \left[\left(Af - Ai \right) / Ai \right) * 100] \quad (1)$$

Where Af is the absorbance of the dye treated by the strains of the gasteroid fungi and Ai is the absorbance of the untreated dye.

Culture medium supplementation (DP) was used because it made discoloration more efficient (Chang et al., 2000; Bardi and Marzona, 2010), since azo dyes, in general, have carbon and nitrogen deficiency (Stolz, 2001), elements of great relevance in the development of fungal cultures. Furthermore, supplementation has positive results in the development of *Geastrum* strains (Santana et al., 2020b).

2.5. Dye phytotoxicity to lettuce after discoloration

The phytotoxicity of the dye trypan blue after the discoloration process by the strains of gasteroid fungi was evaluated in lettuce (*Lactuca sativa* L.) from the percentage of seed germination and the early development of seedlings. All seeds were sterilized by immersion in 98.2% alcohol, sodium hypochlorite 2% and rinsed abundantly with sterile water five times. Subsequently, four replicates of 25 seeds, totaling 100 seeds per treatment, were packed on a layer of Whatman filter paper in Petri dishes (90 mm diameter) and kept at 25 ± 2 °C.

The seeds received daily waterings of 1 mL of the dye treated by the strains of gasteroid fungi for seven days, when observations were made to calculate the germination percentage and measure the length of the hypocotyl and radicle with a digital caliper. Seeds watered with sterilized distilled water were used as positive control and seeds watered with untreated dye were used as negative control.

2.6. Dye toxicity to Artemia salina after discoloration

Dye toxicity after treatment with gasteroid fungal strains was tested on *Artemia salina* L larvae. Thus, 0.1 g of *A. salina* eggs were hatched in 100 mL artificial marine water solution (NaCl 77.23%, MgSO4 9.62%, MgCl 7.13%, CaCl23.32%, KCl 2.11% and NaHCO3 0.59%) in a 500 mL Erlenmayer at 27 ± 2 °C in static mode and under constant illumination.

The test was performed in 1 mL of *A. salina* larvae of (about 100 individuals per treatment) transferred to test tubes (10 × 200 mm) containing 300 µL of the dye treated by fungal strains. In the positive control, the larvae were submitted only to artificial seawater solution without dye and in the negative control, the larvae were submitted to artificial seawater solution with 300 µL of pure dye. The tubes were kept at 25 ± 2 °C for 24 hours and under constant illumination. After this period, the live larvae were counted with a stereomicroscope to determine the degree of toxicity (GT) of the dye (Harwig and Scott 1971) using the Formula 2 below:

$$GT(\%) = \left[\left(NIM - TI \right) / TI \right) * 100] \quad (2)$$

Where NIM is the number of individuals killed and TI is the total of individuals in the tube.

The toxicity classification of trypan blue treated by the fungal strains was based on the mortality percentage of the *A. salina* larvae (Teixeira et al. 2011). Thus, between 0 and 9% mortality, the dye is not considered toxic; between 10 and 49% mortality, the dye is considered slightly toxic; between 50 and 89% mortality, the dye is considered toxic; between 90 and 100% mortality, the dye is considered highly toxic.

2.7. Statistical analysis

The experiments were conducted in a completely randomized design. For the experiments in solid culture medium, the experimental unit consisted of a Petri dish, with five replicates per treatment and for the experiments in aqueous culture medium, the experimental unit was an Erlenmeyer bottle, also with five replicates per treatment. To evaluate the discoloration of the trypan blue and its toxicity after treatment by fungal strains, the means of the experiments were submitted to an analysis of variance (ANOVA) and compared by the Tukey test at the level of 5% significance using the software BioEstat 5.0.

3. Results

The strains of gasteroid fungi developed in the presence of trypan blue surviving toits toxicity and causing its discoloration, even at different intensities. In solid culture medium, after 21 days of observation, *Cyathus* strains showed the highest discoloration halos of the dye, especially *C. albinus* strains that reached the edge of the Petri dish in nine days and presented the highest contrast in discoloration (Figure 1A). Among the *Geastrum* strains, none reached the edge of the Petri dish within the observed period, although the *G. hirsutum* strains presented the highest halo of discoloration (Figure 1D).

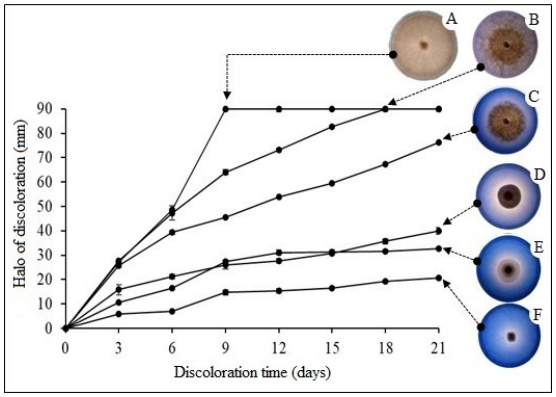

Figure 1. Discoloration halo of trypan blue dye by Amazonian gasteroid fungi after 21 days of observation. A: *Cyathus albinus*; B: *C. limbatus*; *C. setosus*; D: *Geastrum hirsutum*; E: *G. schweinitzii*; F: *G. echinulatum*.

The visual result of dye discoloration by the strains of gasteroid fungi in aqueous medium over time can be seen in Figure 2.

Figure 3 shows the performance of gasteroid fungi strains in the discoloration of trypan blue dye in aqueous culture medium over time. The percentage of discoloration varied between the two genera and *Cyathus* strains presented dye discoloration higher than 90% in the first 20 days of incubation, reaching 96 ± 0.06% (mean ± standard deviation) at the end of 60 days. Likewise, the *Geastru*m strains differed at 20 days, with the best result observed for *G. schweinitzii* with 84.7 ± 0.04% (mean ± standard deviation) dye discoloration and the lowest percentage for *G. hirsutum* with 41.9 ± 0.41% (mean ± standard deviation). At 40 days, the results of *G. schweinitzii* and *G. echinulatum* strains were equal to *Cyathus* strains for dye discoloration. At the end of the 60 days of incubation, all strains of gasteroid fungi showed results above 96% discoloration, especially *G. echinulatum* which almost completely discolored the dye (98.6 ± 0.08%, mean ± standard deviation).

Table 2 presents the dye toxicity after treatment by strains of gasteroid fungi tested on the germination of lettuce seeds, in the early development of seedlings and in the mortality of *Artemia salina* larvae. At 20 days of incubation, the dye treated by *Cyathus* strains maintained toxicity similar to untreated dye on seed germination. However, regarding the early development of seedlings, those watered with the dye treated by *C. albinus* and *C. limbatus* strains had a higher development when compared to the negative control and were equal to the positive control. Larvae mortality remained the same as negative control for all strains. In the same period, the dye treated by *Geastrum* strains did not overcome negative control in any toxicity analysis.

At 40 days, the germination rate of seeds watered with the dye treated by *Cyathus* strains remained the same as the negative control. However, seedlings watered with dye treated by *C. albinus* and *C. limbatus* strains were twice as effective as positive control and the mortality rate of *A. salina* larvae was reduced by 29 and 23% respectively.

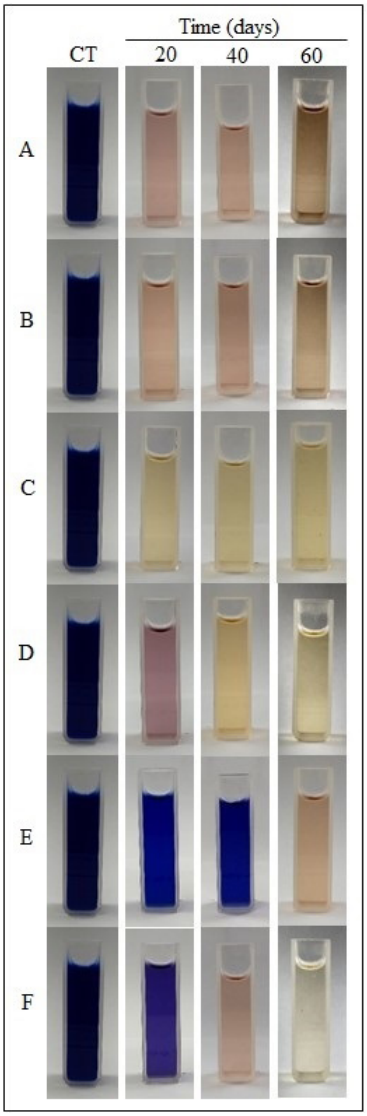

Figure 2. Discoloration aspect of trypan blue dye performed by Amazonian strains of gasteroid fungi in different time periods. CT: Control; A: *Cyathus albinus*; B: *C. setosus*; C: *C. limbatus*; D: *Geastrum schweinitzii*; E: *G. hirsutum*; F: *G. echinulatum*.

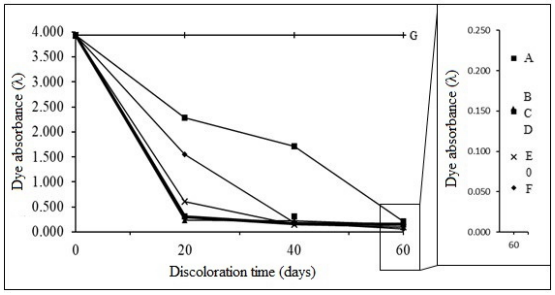

Figure 3. Discoloration of trypan blue dye in aqueous culture medium by Amazonian gasteroid fungi over time. ■ A: *Geastrum hirsutum*; ▲ B: *Cyathus limbatus*; ● C: *C. setosus*; ■ D: *C. albinus*; × E: *G. schweinitzii*; ♦ F: *G. echinulatum*; + G: Control.

Table 2. Trypan blue dye toxicity for seed germination rate (SGR), lettuce seedling development (HL = hypocotyl length and RL = radicle length) and mortality rate of *Artemia salina* (MRA) larvae after the discoloration treatment by Amazonian strains of gasteroid fungi over time. Data are shown as mean ± standard deviation.

Species	20 days				40 days				60 days			
	SGR (%)	HL (mm)	RL (mm)	MRA (%)	SGR (%)	HL (mm)	RL (mm)	MRA (%)	SGR (%)	HL (mm)	RL (mm)	MRA (%)
Positive control[1]	89[a]	10.7 ± 3.2[b]	4.7 ± 2.1[b]	0[a]	90[a]	10.5 ± 3.1[c]	4.6 ± 2.1[c]	0[a]	89[a]	10.8 ± 3.5[c]	4.5 ± 2.4[c]	0[a]
Negative control[2]	41[b]	7.9 ± 4.3[c]	1,9 ± 0.9[d]	100[b]	40[b]	7.2 ± 3.3[d]	1,2 ± 0.7[f]	100[d]	40[d]	7.0 ± 4.1[d]	1.7 ± 0.8[e]	100[d]
Cyathus albinus	41[b]	10.3 ± 1.7[b]	4.8 ± 0.7[b]	100[b]	42[b]	20.3 ± 2.5[a]	9.2 ± 1.5[a]	71.8[c]	79[b]	20.1 ± 3.4[a]	9.5 ± 2.3[a]	54.8[c]
C. setosus	40[b]	3.7 ± 0.5[d]	3.6 ± 0.5[c]	100[b]	40[b]	7.4 ± 1.9[d]	4.5 ± 1.1[c]	100[d]	42[d]	18.1 ± 2.2[b]	8.8 ± 0.7[ab]	100[d]
C. limbatus	40[b]	18.7 ± 0.8[a]	8.6 ± 3.0[a]	100[b]	41[b]	13.1 ± 2.0[b]	7.5 ± 1.4[b]	77[b]	64[c]	18.7 ± 0.8[b]	8.6 ± 3.0[b]	65[b]
Geastrum schweinitzii	36[c]	*	*	100[b]	37[c]	6.2 ± 0.9[e]	3.5 ± 0.5[d]	100[d]	41[d]	7.6 ± 0.7[d]	3.9 ± 0.6[cd]	100[d]
G. hirsutum	25[e]	*	*	100[b]	29[d]	*	*	100[d]	36[e]	6.2 ± 1.1[d]	3.3 ± 0.8[d]	100[d]
G. echinulatum	33[d]	*	*	100[b]	40[b]	5.3 ± 0.5[f]	2.3 ± 0.5[e]	100[d]	36[e]	6.1 ± 2.8[d]	3.4 ± 1.1[d]	100[d]
CV (%)	5.25	22.41	21.84	0.76	5.13	13.12	20.12	1.96	5.13	17.41	21.14	2.54

[1]Watered with distilled water; [2]Watered with untreated trypan blue dye; *Germinated seed without seedling development. CV: Coefficient of Variation; Different letters indicate statistical difference between treatments at 5% significance according to Tukey test.

Geastrum strains were not efficient in decreasing the toxicity of the treated dye, since they provided toxicity data similar to the negative control.

At 60 days, the germination rate watered with the dye treated by *Cyathus* strains was higher than the negative control, except for those watered with the dye treated by *C. setosus*, which remained equal to the negative control. However, regarding the early development of seedlings, all strains presented higher values than the positive control and the mortality rate of *A. salina* larvae submitted to the dye treated by *C. albinus* and *C. limbatus* was reduced by 46 and 35% respectively. For *Geastrum* strains, the results of the treated dye toxicity were not satisfactory when compared to the positive control.

4. Discussion

The search for fungi capable of biodegrading synthetic dyes has attracted more attention, especially by the extracellular enzymes that lignin degrading species produce (Ambrosio et al., 2012). Fungi that produce these enzymes can degrade recalcitrant compounds, such as synthetic dyes (Sen et al., 2016), making the species of this study, degrading lignocellulosic substrates, advantageous targets in bioremediation studies. Furthermore, they are widespread, which facilitates the availability of material for future studies. This is the first report on these species with proven activity in the discoloration of trypan blue.

Cyathus strains were the most efficient time-wise, reaching above 90% in the discoloration first analysis, possibly due to the high rates of extracellular enzymes they produced, such as lacase (Pointing, 2001), one of the most widely used in bioprospecting studies involving *Cyathus*. The production of this enzyme is related to proven activity of dye degradation in *C. striatus* (Huds.) Willd. (Vasdev et al., 1995), *C. stercoreus* (Schwein.) De Toni and *C. bulleri* (Vasdev and Kuhad, 1994; Vasdev et al., 1995; Mishra and Bisaria, 2006) and in some species its production is directly related to mycelial growth (Vasdev and Kuhad, 1994; Sethuraman et al., 1999), similar to other Basidiomycota (Wood, 1980; Rehman and Thurston, 1992).

The performance of *Geastrum* strains in dye discoloration, although low in the first analyses, increased to the detriment of incubation time and strain development, reaching 98% discoloration at the end of the analyses. This result also suggests that mycelial growth provides greater availability of enzymes capable of degrading dyes. Among these enzymes, Kuhar et al. (2016) observed that the lacaseis produced in greater quantity in *Geastrum* cultures and its production varies among the species. Santana et al. (2016) reported that the intensity of phenoloxidase production, a group comprising the lacases and other enzymes, in strains of *G. lloydianum* and *G. subculosum* was directly related to the type of substrate they degraded, and the higher the enzyme expression, the greater the discoloration halo of the textile dye Remazol Brilliant Blue R (RBBR).

The difference between the two genera regarding time and intensity of discoloration is due to the ecophysiological differences of the species, the preference for substrates (Wicklow et al., 1984), and the conditions of mycelial growth *in vitro*. For this study *Cyathus* strains were obtained from basidiomes decomcomposing trunks and branches, which stimulates higher production of lignocellulosic enzymes (Sen et al., 2016), while the *Geastrum* strains were isolated from basidiomes that grew on leaf litter (personal observations), where the need for enzyme production that degrades lignin is reduced (Wesenberg et al., 2003). *In vitro*, *Cyathus* strains showed rapid growth compared to those of *Geastrum*, as described in the literature (Sunhede, 1989; Zamora et al., 2014).

The difference between the species regarding dye discoloration also reflected in the final result of toxicity.

The strains of *C. albinus* and *C. limbatus* were efficient in reducing toxicity, proven by increased seed germination and lettuce seedling development. Furthermore, there was a decrease in mortality of *A. salina* larvae over the analyzed period. For *Geastrum* strains, the post-treatment result showed that the toxicity remained similar to the untreated dye, both for seed germination and seedling development, although they stimulated the length of the radicle and the mortality of *A. salina* larvae. This difference is related to the products derived from the degradation process that each species performs (Singh, 2006) and to the incomplete mineralization of the dye (Wang and Hu, 2008).

Strains of *Penicillium simplicissimum* (Oudem.) have the ability to discolor textile dyes and reduce their toxicity (Bergsten-Torralba et al., 2009), as observed for other fungal species (Martins et al., 2002; Anastasi et al., 2010; Ashrafi et al., 2013). However, *Aspergillus niger* Tiegh., *A. terreus* Thom and *Rhizopus oligosporus* Saito, although quite efficient in discoloration of dyes, produce by-products with high toxicological potential (Almeida and Corso, 2019). These species may produce similar enzymes that act on the discoloration of dyes, like lacase, but other products are also generated during this process (Novotny et al., 2004) in response to the complexity of industrial effluents that, in addition to the dye, contain salts, very high ionic forces or extreme pH values, which may be precursors of toxic by-products for some species (Wesenberg et al., 2003).

Although the lacase enzyme is likely responsible for degrading trypan blue dye, as it was for other synthetic dyes, studies should consider that fungi secrete several other enzymes that together can be even more efficient in the degradation of this dye than purified enzymes (Camarero et al., 2005). The combined effect of the enzymatic system of gasteroid fungi, combined with the cultivation conditions to which the strains were submitted (Santana et al., 2020b), may have occurred in this study, resulting in the different outcomes of the degradation and toxicity of the trypan blue dye. It is possible that other variables, such as supplementation of culture medium, pH, temperature and agitation, may increase or accelerate the enzymatic activity of these species, as reported for other fungi (Asgher et al., 2008; Anastasi et al., 2010; Bibi and Bhatti, 2012).

5. Conclusion

The discovery of new species capable of degrading dyes is relevant as it fosters new perspectives to develop efficient, accessible and safe biotechnologies. Amazonian species of gasteroid fungi, including litter colonizers, have great potential for degradation of azo dye, such as trypan blue. Among them, *Cyathus albinus* and *C. limbatus* are promising due to rapid mycelial growth, reduced time to start dye degradation, and the ability to decrease dye toxicity. The *Geastrum* strains also showed a high degree of efficiency regarding dye discoloration, but require greater attention due to the toxic by-products they produced. Hence, endorsing the need for toxicological tests associated with synthetic dye discoloration experiments. Therefore, there is a need to biotechnologically explore the enzymatic activity of these and other species of gasteroid fungi.

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