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Effects of Ultraviolet Exposure on the Tropical Fungi *Aspergillus carbonarius* and *Aspergillus japonicus*: Survival, Amylase Production, and Thermostability

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Abstract

Background and Research Aims: Although fundamental to tropical forest biodiversity, fungi have been largely neglected in conservation research. To examine the fungal response to increased ultraviolet C (UVC) radiation, we analyzed UVC radiation effects on the survival, growth, and amylase activity of *Aspergillus carbonarius* and *Aspergillus japonicus*.

Methods: *A. carbonarius* (strain URM 7305) and *A. japonicus* (URM 7270) were exposed to UVC (254 nm) for different periods, and morphological changes were compared to the control.

Results: Survival capacity and growth decreased after 10 min of exposure in *A. carbonarius* and after 25 min in *A. japonicus*. After 40 min, amylase activity decreased (*A. carbonarius*: 35.8%; *A. japonicus*: 30.3%). Amylase thermostability at 60°C was lower in UVC-exposed strains (T50 15 min) compared to controls (*A. japonicus*, 45 min; *A. carbonarius*, 30 min). However, the protein amount remained stable in all UVC-treated strains. Contamination by other fungi was observed in the UVC-exposed strains, confirming competitive strength loss in both species. This was not observed in the controls due to secondary metabolite production, which increased their competitive fitness.

Conclusion: We provide new information about UVC's adverse effects on the survival and enzyme production of *A. carbonarius* and *A. japonicus*, which could mean a loss of species essential for proper soil functioning and biodiversity.

Implications for conservation: Experimental manipulation of biochemical and physiological reactions advances fungal conservation beyond distributional data. The experimental evidence supports previous studies, suggesting that the increased UV radiation caused by climate change may drastically affect fungal biochemistry and physiology.

Keywords

amylase activity, biodiversity maintenance, fungal conservation, tropical forest, ultraviolet C radiation

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Highlights

- *A. carbonarius* showed a gradual decrease of sporulation and growth after 10 minutes at UVC;
- *A. japonicus* showed a gradual decrease in sporulation and growth after 25 minutes at UVC;
- *A. carbonarius* had decreased amylase production with 5 minutes of UVC radiation;
- *A. japonicus* kept 69% of its amylase production after 40 minutes in UVC radiation;
- After 40 minutes of underexposure in UVC, the amylase presented a thermostability above 80% relative activity.

Introduction

Brazil boasts several biological diversity and conservation hotspots (Molotoks et al., 2018). The ecologically complex Atlantic Forest (Barbosa et al., 2020), one of the world's most important biomes, shelters thousands of endemic species and is internationally recognized as a critical biodiversity hotspot (Pasin et al., 2020).

Fungi often serve as models for studies, especially those on climate change. However, they are often not considered in conservation. For example, fungi have as yet to be included in the recently released IUCN Green Status of Species, which assesses the impact of conservation actions (Grace et al., 2021).

Aspergillus is a phenotypically diverse filamentous genus encompassing species important in the natural environment and several economic sectors, including biotechnology and medicine (Tsang et al., 2018). *Aspergillus carbonarius* and *Aspergillus japonicus* belong to the section *Nigri* and are found in tropical environments such as the Atlantic Forest. Both species produce enzymes, notably amylases, that are of biotechnological interest.

Ultraviolet (UV) radiation has been an important driver of natural selection throughout evolution (Karam, 2003). However, due to global warming, UV radiation has been increasing (Bornman et al., 2015). Fungi are susceptible to UV action, and it is important to evaluate their behavior and enzyme production under stressful conditions because they are essential for biodiversity.

Ultraviolet (UV) radiation is divided into three categories: UVA (320–400 nm), UVB (280–320 nm), and UVC (10–280 nm) (Santos et al., 2013). UVC radiation is well-known for its germicidal potential and is commonly used in laboratory experiments (King et al., 2011). Microorganisms are susceptible to UVC because of their small size, which creates a large surface area-to-volume ratio (Goldman & Travisano, 2011). Despite interest in UVC's impact on microbial evolution and mortality, few studies have evaluated UVC effects on tropical microbial fungi (Weigand & Sundin, 2009). Thus, we aimed to evaluate the impact of different durations of UVC exposure on survival, amylase production, and stability, and consequently, the conservation of *A. carbonarius* and *A. japonicus*.

Methods

The fungi were collected from the Atlantic Forest and incubated in 1% starch solid medium, pH 6.0 (*A. carbonarius*) or pH 5.5 (*A. japonicus*), at 30°C for 4 days. To test the UVC radiation effects, a spore solution of each fungus was distributed on Petri dishes positioned 16 cm from a UV lamp. Aliquots were collected every 5 min for up to 40 min. The strains subjected to UVC were then incubated in 1% starch liquid medium for 4 days at 30°C. Thermostability was assessed at 5°C increments from 50°C to 60°C; aliquots were collected every 15 min for up to 60 min of incubation, and amylase activity was evaluated by 3,5-dinitrosalicylic acid methodology. The materials and methods are described in detail in Supplemental Materials.

Results

UVC effects on A. carbonarius and A. japonicus morphology

Aspergillus carbonarius exhibited a gradual decrease in sporulation and growth after 10 min of UVC exposure (Figure 1). The number of contaminating colonies increased significantly after 25 min, demonstrating that UVC exposure rendered the fungus more vulnerable due to its loss of competitive capacity.

Aspergillus japonicus exhibited a gradual decrease in sporulation and growth after 25 min of UVC exposure. After 35 min, the number of contaminating colonies increased progressively, covering almost the entire plate area within 40 min. The spore color slightly changed before maturation, suggesting pigment formation changes that may have led to greater environmental vulnerability. Thus, *A. japonicus* became more vulnerable due to the decreased resistance caused by UVC exposure (Figure 2). However, both fungi continued growing after 40 min of UVC exposure, demonstrating a high tolerance to UVC sterilization.

Effects of high UVC radiation on amylase production

Aspergillus carbonarius showed a significant gradual decrease in amylase production within 10 min of UVC exposure ($p \leq 0.05$, $n = 3$, Tukey's test), presenting 64.2% of the relative amylase production after 40 min of exposure (Table 1). Amylase production by *A. japonicus* demonstrated high resistance to UVC radiation for up to 5 min ($p \leq 0.05$, $n = 3$, Tukey's test), but it was significantly affected within 10 min of exposure. After 40 min, the relative amylase production was only 69.7% that of the control. The protein production of both fungi did not show any significant ($p > 0.05$) differences at all experimental periods of exposure compared with the control (Table 1).

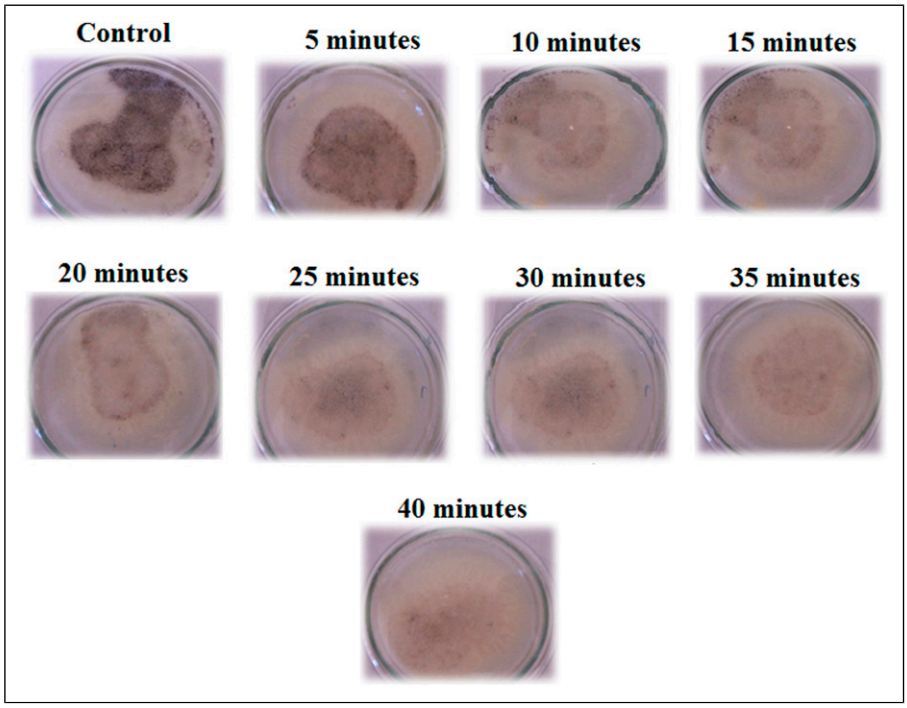

Figure 1. Morphological changes of *Aspergillus carbonarius* URM 7305 after exposure to UV radiation for different periods.

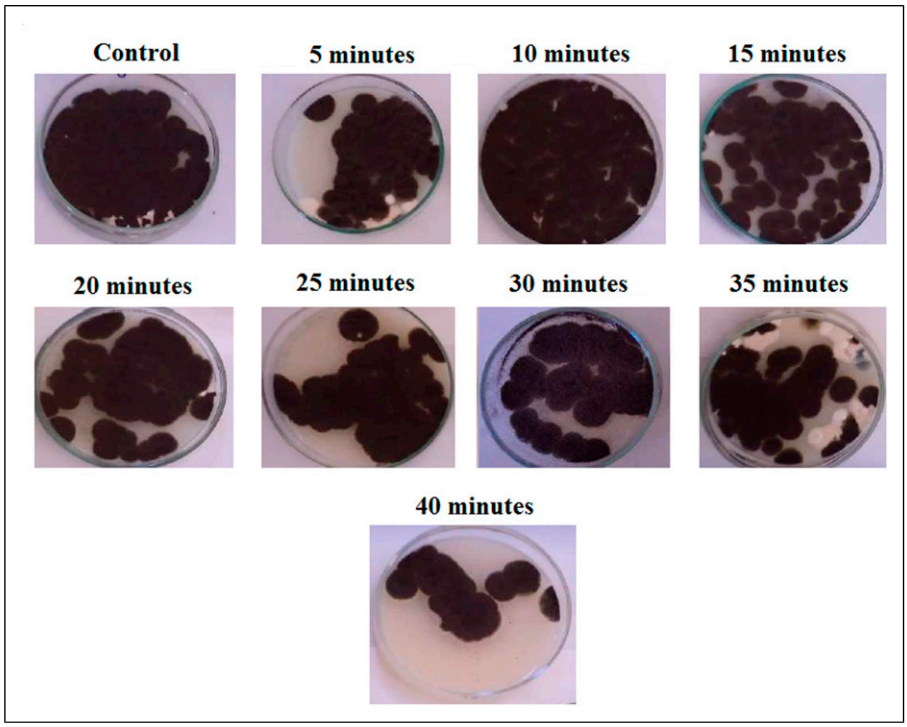

Figure 2. Morphological changes of *Aspergillus japonicus* URM 7270 after exposure to UV radiation for different periods.

Table 1. Enzymatic activity and the measurement of protein produced by fungi exposed to UVC at different periods. The values in the columns of each treatment for each fungus that are followed by different letters are significantly different ($p < 0.05$).

Time exposure (minutes)	Fungi	Enzymatic activity (U/mL)	Protein (mg/mL)
Control	*A. carbonarius*	39.01 (±0.56)^{a}	0,03 (±0,01)^{a}
	A. japonicus	49.61 (±0.69)^{a}	0.10 (±0.01)^{b}
5	*A. carbonarius*	37.45 (±0.38)^{a}	0.04 (±0.01)^{a}
	A. japonicus	48.39 (±0.32)^{ab}	0.09 (±0.02)^{b}
10	*A. carbonarius*	34.23 (±0.18)^{b}	0.05 (±0.02)^{a}
	A. japonicus	45.82 (±0.23)^{bc}	0.11 (±0.08)^{ab}
15	*A. carbonarius*	31.67 (±0.52)^{bc}	0.02 (±0.01)^{a}
	A. japonicus	43.55 (±0.12)^{cd}	0.15 (±0.05)^{ab}
20	*A. carbonarius*	29.38 (±0.15)^{cd}	0.03 (±0.01)^{a}
	A. japonicus	41.87 (±0.87)^{de}	0.13 (±0.09)^{ab}
25	*A. carbonarius*	28.12 (±0.35)^{d}	0.03 (±0.02)^{a}
	A. japonicus	40.91 (±0.56)^{de}	0.15 (±0.04)^{ab}
30	*A. carbonarius*	27.26 (±0.52)^{de}	0.03 (±0.01)^{a}
	A. japonicus	39.43 (±0.90)^{ef}	0.12 (±0.07)^{ab}
35	*A. carbonarius*	26.92 (±0.22)^{de}	0.02 (±0.01)^{a}
	A. japonicus	37.52 (±0.21)^{f}	0.17 (±0.07)^{a}
40	*A. carbonarius*	25.08 (±0.73)^{e}	0.04 (±0.02)^{a}
	A. japonicus	34.59 (±0.43)^{g}	0.13 (±0.05)^{ab}

*Values with different letters are significantly different ($p < 0.05$).

Thermostability of the amylases in fungi exposed to UVC

Amylase produced by *A. carbonarius* not exposed to UV radiation showed a relative activity of 98.8% over 60 min at 50°C. This result was not significantly different from that of the control ($p \leq 0.05$, $n = 3$, Tukey's test). The enzyme presented 88.7% of the relative activity within 45 min at 55°C, but at 60°C, the amylase activity remained at 90.4% of the relative activity after 30 min. These results were not significantly different from those obtained for the control ($p > 0.05$) (Figure 3A). However, *A. carbonarius* exposed to 40 min of UVC radiation produced an amylase with significantly less thermostability ($p \leq 0.05$, $n = 3$, Tukey's test), which maintained 70.9% of the relative activity at 50°C for 60 min. This amylase presented a significant activity ($p \leq 0.05$, $n = 3$, Tukey's test) of 68.7% of the relative activity during 45 min at 55°C and maintained 56.3% of the relative activity after 30 min at 60°C ($p \leq 0.05$, $n = 3$, Tukey's test) (Figure 3B).

Relative activity of the amylase produced by *A. japonicus* not exposed to UV radiation was 99.43% for 60 min at 50°C and not significantly different from the control ($p > 0.05$). Similarly, the amylase activity at 55°C (86.31% of the relative activity) for up to 45 min and at 60°C (81.2% of its relative activity) for 30 min was not significantly different from that of the control ($p > 0.05$) (Figure 3C). The amylase produced by *A. japonicus* exposed to 40 min of UVC radiation presented significant differences in thermostability compared to the strain not exposed to UVC ($p \leq 0.05$, $n = 3$, Tukey's test). At 50°C, the relative activity (96.6%) was not significantly different from that of the control ($p > 0.05$); however, after 60 min at 55°C, the enzyme stability decreased significantly ($p \leq 0.05$, $n = 3$, Tukey's test), retaining 40.6% of the relative activity. At 60°C, there was a greater decrease in stability ($p \leq 0.05$, $n = 3$, Tukey's test), maintaining 26% of the relative activity for 60 min (Figure 3D).

Therefore, the amylases produced by *A. carbonarius* and *A. japonicus* strains subjected to UV presented lower thermostabilities than those of the control strains ($p \leq 0.05$, $n = 3$, Tukey's test), showing that increasing UV exposure affects microorganisms and enzymes essential for metabolism, soil ecology, and sustainability.

Discussion

Our results are compatible with those of Brancini et al. (2016), who obtained a modified *Metarhizium acridum* strain with 60% relative survival after UVB exposure. The high number of contaminating colonies, modification in spore coloration, and growth delay show that UVC light adversely affected the metabolisms of *A. carbonarius* and *A. japonicus*. However, the strains remained alive despite impaired melanization, which serves many biological purposes (Cordero & Casadevall, 2017), and loss of their competitive fitness.

Yadav & Siddalingeshwara (2017) obtained a mutant *A. japonicus* after 70 min of exposure to UVC irradiation with a relative fibrinolytic activity of 107% compared to the control. However, *A. carbonarius* and *A. japonicus* in our study showed lower amylolytic production after 40 min of UVC exposure. In contrast, our findings corroborated the study by Hu et al. (2017) on an *Aspergillus niger* mutant strain with

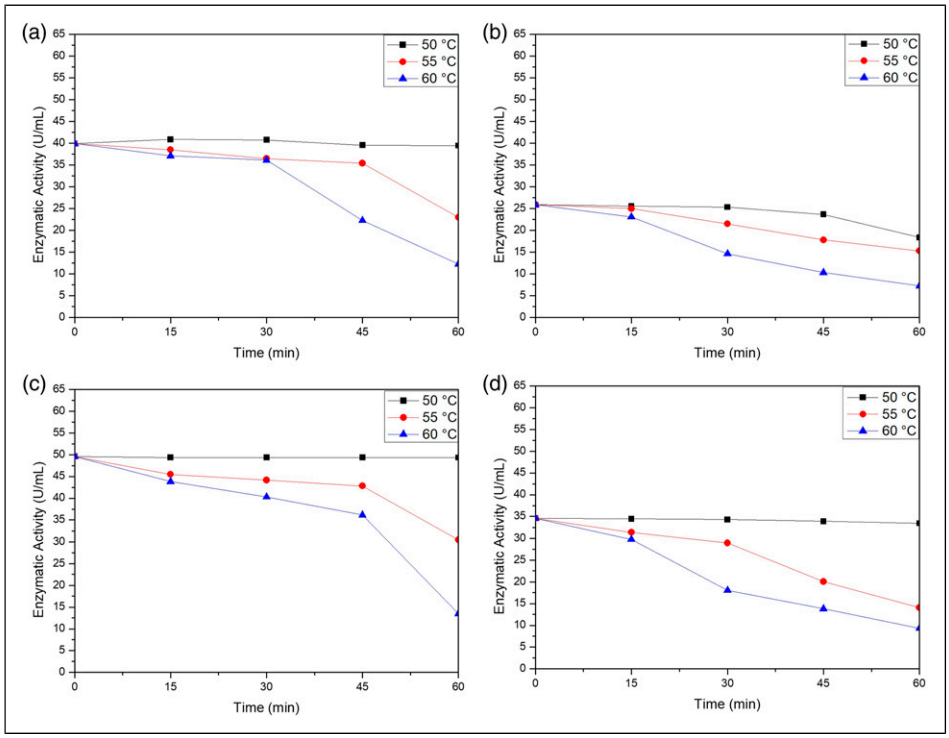

Figure 3. (A) Stability of the enzyme produced by *Aspergillus carbonarius* without exposure to UV radiation, at different temperatures and various times. (B) Stability of the enzyme produced by *A. carbonarius*, exposed to UVC radiation, at different temperatures and different times. The mean standard deviation was ±0.73 for enzymatic activity. (C) Stability of the enzyme produced by *A. japonicus* without exposure to UV radiation at different temperatures and various times. (D) Stability of the enzyme produced by *A. japonicus* exposed to UVC radiation, at different temperatures and different times. The mean standard deviation was ±1.25 for the enzymatic activity. Statistically significant differences were assumed at $p \leq 0.05$, $n = 3$, Tukey's test. All values are presented as mean ± standard error ($n = 3$).

lower relative activity (±50%) than the control. Moreover, the relative amylase activity of *A. carbonarius* and *A. japonicus* remained more stable than that of the *A. niger* mutant strain. The amylase levels remained stable in all exposure periods, differing from the report of Shahbazi et al. (2014) who obtained a mutant *Trichoderma reesei* with a significant increase in protein quantity under 250 Gy gamma radiation.

Amylase produced by *A. carbonarius* showed good relative thermostability at different times. Our results contradict those of Agrawal et al. (2013) who reported 90% relative stability at 60°C for 15 min for a purified β-glucosidase obtained from a *Bacillus subtilis* strain exposed to UV radiation for 30 min. However, amylase in *A. japonicus* not exposed to UV showed a relative thermostability at 60°C, whereas the amylase produced by the strain exposed to UV for 40 min maintained 52.1% of its relative activity for 30 min at the same temperature.

Our results are important for future research on the factors behind the weakening effects of UV on these fungi. In addition, the mechanistic enzymology of the amylases produced by these mutant fungi is important for soil health. These results show the impact of UV radiation increase (due to climate change) on microorganisms that are essential for life, forest maintenance, and biodiversity.

Implications for conservation

Occurring widely in forests and other environments, members of the *Aspergillus* genus merit further study, with a particular focus on their morphology and physiology. This work provides additional information about *A. carbonarius* and *A. japonicus*, which can act as models for studies on the impacts of global warming and UV increase on forest microbial diversity, particularly in tropical environments. Fungal conservation policies and programs have previously relied on distributional records (Dahlberg, Genney, & Heilmann-Clausen, 2010). By contrast, our study provides a basis for further assessment of fungal reactions in anthropogenically altered environments, preservation of the original fungal species, and restoration of the environmental balance.

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Author contribution

T.M.P., E.A.M., V.M.B, and N.T.A.P developed the theory and analyzed the data obtained. M.C revised and edited the original draft. M.L.T.M.P supervised this work, contributed to final writing, and

was responsible for financial funds. All authors discussed the results and contributed to the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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