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ANTAGONISTIC ACTIVITY OF WOOD-INHABITING *XYLARIA* SPECIES AGAINST OTHER FUNGI IN DUAL CULTURE EXPERIMENTS

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ABSTRACT

In this survey, two wood-inhabiting species – *Xylaria polymorpha* and *Xylaria longipes* (10 strains each*)* were evaluated in dual culture assay against 6 fungi: *Aspergillus niger*, *Candida albicans*, *Fusarium solani*, *Mucor plumbeus*, *Penicillium polonicum*, and *Trichoderma viride*. Detailed descriptions of the interspecific interactions, morphological changes and comparison of *X. longipes* and *X polymorpha* reaction types with each fungus are provided. The results indicate significant inhibitory activity of both *X. longipes* and *X. polymorpha* against *A. niger*, *M. plumbeus*, *P. polonicum*, *F. solani*, *C. albicans* with differences in reaction types on a strain specific level. Most commonly, co-cultivating resulted in *Xylaria* species replacing fungi (60.8% of all interactions), with a partial replacement after initial deadlock with mycelial contact as the most frequent type of interaction. Inhibiting activity at a distance occurred in the majority of dual cultures of *X. polymorpha* with *A. niger and P. polonicum (*deadlock at a distance), and *X. longipes* with *M. plumbeus* (deadlock at a distance followed by a partial replacement). Among all tested cultures, *T. viride* turned out to be the only fungus suppressing *Xylaria* (except for *X. polymorpha* strains that formed a deadlock at mycelial contact). Based on the listed reaction types, for each studied *Xylaria* strain antagonism index was calculated, revealing that *X polymorpha* IBK 2720 had the highest value among all screened strains amounted to 20. The results indicate the effectiveness of *Xylaria* against tested fungi via contact antagonism and provide valuable insights for further investigations of novel biocontrol agents.

Keywords: fungal antagonism, dual culture, *Xylaria*, Ascomycota, antagonism index

INTRODUCTION

Xylaria Hill ex Schrank is the largest genus of the family *Xylariaceae* Tul. & C. Tul. (Xylariales, Sordariomycetes) which currently includes over 800 accepted name records listed in *Index Fungorum*. *Xylaria* species are generally endophytes or saprophytes (**Petrini and Petrini, 1985; Ju and Hsieh, 2007**) and the vast majority of the stromatic *Xylaria* spp. colonise decaying wood (**Fournier *et al.*, 2011**). Among them *Xylaria polymorpha* (Pers.) Grev and *Xylaria longipes* Nitschke are common representatives predominant in almost all forested habitats ranging from temperate to tropical regions (**Chareprasert *et al.*, 2011**). These are closely related species, which are hardly distinguishable as both exhibit similar highly variable stromatal shapes and are mainly separated based on certain micro-morphological features. Yet, much more research has been devoted to one of these species – *X. polymorpha*. This well-known fungus is used in laboratory spalting due to its ability to produce zone lines in monoculture (**Robinson and Laks, 2010**). While there are evidences of antagonistic activity of endophytic *Xylaria* spp. against pathogenic fungi (**Hamzah *et al.*, 2018; Kinamot and Monotilla, 2022**), information on wood-inhabiting *Xylaria* species is limited. However, as pioneer colonizers of the wood substrate, these fungi possess metabolic and enzymatic mechanisms for competing with other fungi, making them interesting objects for studying their antagonistic potential. Understanding the antagonistic capabilities of *Xylaria* spp. marks an initial step toward harnessing the full potential of these species for specific applications.

To assess the antagonistic potential, a comprehensive range of mechanisms must be taken into account. These include both aggressive and protective strategies, the outcome of which is either deadlock and inability to displace a competitor, full compatibility between organisms, or replacement, when one mycelium displaces another, along with various sub-types of these interactions. Such antagonistic reactions occur either at a distance or during/after physical contact between individual hyphae or mycelial colonies (**Falconer *et al.*, 2007**). Competing mycelia change in morphology, accumulation of reactive oxygen species, and pigment deposition (**Watkinson *et al.*, 2016**). Changes in morphology during interaction are certain to be correlated with differences in physiology, enzyme and toxin production and require specific metabolic abilities of fungi (**Griffith *et al.*, 1994; Rayner *et al.*, 1994**). Notably, during confrontations between different fungal competitors, and between wood-inhabiting fungi particularly, production of secondary metabolites becomes heightened (**Humphris *et al.*, 2001; Wheatley, 2002; Peiris *et al.*, 2008**). Many of these metabolic compounds are not typically required during normal development of fungi but are synthesized in response to antagonist action (**Chan-Cupul *et al.*, 2019; Dullah *et al.*, 2021a, b**). For instance, four new alkyl aromatics were isolated from a mixed culture of *Penicillium crustosum* and endophytic *Xylaria* sp. Two of the substances were produced by a collaboration of the fungi, while other compounds were produced by *Xylaria* alone, but in noticeably increased quantities during co-cultivation (**Yu *et al.*, 2019**). Therefore, hidden metabolites responsible for fungal protective mechanisms could be explored through co-cultivation *in vitro* studies. Ultimately it could lead to the discovery of new antifungal compounds (**Woodward *et al.*, 1993**). For this reason, interspecific fungal interactions are among the subjects of biotechnological research.

The present study concentrates on mycelial interactions of two related wood-inhabiting *Xylaria* species with 6 fungi, including filamentous and yeasts.

As test cultures, we have chosen fungi against which the search for new biocontrol agents is relevant as they are causative agents of infections and/or spoilage of agricultural products. Representatives of the *Fusarium solani* (Mart.) Sacc. species complex include phytopathogens of agricultural importance (**Coleman, 2016**) that can also cause onychomycosis and systemic infections in immunocompromised individuals (**Guilhermetti *et al.*, 2007; Lodato *et al.*, 2006**). *Aspergillus niger* Tiegh., *Penicillium polonicum* K.W. Zaleski, and *Mucor plumbeus* Bonord *are associated* with the spoilage of diverse foods and feeds (**Khalil *et al.*, 2019; Koka *et al.*, 2021**). Moreover, these species are recognized as opportunistic pathogens contributing to allergic disorders and mycotoxin production (**Gugnani, 2003; Rubio-Portillo *et al.*, 2020**). One of the chosen opportunistic pathogens causing severe mucosal infections and life-threatening systemic infections was *Candida albicans* (C.P. Robin) Berkhout (**Mayer *et al.*, 2013**). *Trichoderma viride* Pers. was selected for this study because it has received attention as an acclaimed biological control agent against fungi (including some of the listed above) due to its ability to parasitize (mycoparasitism), among other mechanisms of action (**Rajendiran *et al.*, 2010; Poveda, 2021; Yassin *et al.*, 2021**).

The aim of performed *in vitro* screening was to investigate and compare the antagonistic activity of *X. longipes* and *X. polymorpha* (ten strains each) against listed fungi and to identify competitive strains that can be further extensively studied as potential biocontrol agents. Screening and evaluation of the antifungal potential were conducted using a rating scale and calculation of an antagonism index (AI) – a qualitative measure defined as the ability of a studied species (*X. polymorpha, X. longipes*) to dominate and compete with test culture.

MATERIALS AND METHODS

Ten strains of each *X. polymorpha* and *X. longipes* were studied against *Aspergillus niger* VURV-F 822, *Candida albicans* N-023, *Fusarium solani* 1P.2II.3, *Mucor plumbeus* N-018, *Penicillium polonicum* VURV-F 823, *Trichoderma viride* N-022. All *Xylaria* strains used in this study were from the Mushroom Culture Collection (*IBK*) of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (**Bisko *et al*., 2016, 2022**). *Aspergillus niger* VURV-F 822 and *Penicillium polonicum* VURV-F 823 from the Culture collection of microorganisms of Crop Research Institute (Prague, Czech Republic). Other fungi were from the Collection of microorganisms of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine.
Competitive interactions between fungi were evaluated in dual culture experiments on 90 mm diameter Petri dishes, containing 20 ml glucose-yeast-peptone agar (g/l: glucose – 25, peptone – 3, yeast extract – 3, $MgSO_4$ – 0.25; KH_2PO_4 – 1; K_2HPO_4 – 1; agar–agar – 21; pH – 6.0). GPYA medium was chosen as optimal for the growth and development of *Xylaria* species based on previous cultural and morphological studies (**Atamanchuk and Bisko, 2022**) and proved to be suitable for growing all tested fungi. To obtain mycelial colonies all fungi were pre-cultivated on GPYA at 26±1ºC in darkness.

Table 1 Types of fungal interactions and corresponding values. Modified from **Badalyan *et al*. (2002, 2004**).

Categories	Interaction	Score
A	Mutual inhibition, in which neither test culture nor *Xylaria* spp. was able to overgrow the other (deadlock) at mycelial contact	1
B	Deadlock at a distance	2
C	Replacement of test culture by *Xylaria* spp., overgrowth without initial deadlock	3
C_{A1}	Partial replacement of test culture by *Xylaria* spp. after initial deadlock with mycelial contact	3.5
C_{B1}	Partial replacement of test culture by *Xylaria* spp. after initial deadlock at a distance	4
C_{A2}	Complete replacement of test culture by *Xylaria* spp. after initial deadlock with mycelial contact	4.5
C_{B2}	Complete replacement of test culture by *Xylaria* spp. after initial deadlock at a distance	5
D*	Suppression of *Xylaria* spp. by the test culture	0

Legend: *type D was introduced by us for the proper calculation of the antagonistic index for *Xylaria*, with the resulting numerical value accurately indicating its competitiveness.
Each Petri dish was inoculated with 5-mm diameter mycelial disks from *Xylaria* spp. colonies on one side and tested fungi with an inoculation needle or loop on the other. Immediately after inoculation, the plates were sealed with parafilm and incubated in darkness at 26±1ºC for 30 days. Colony growth and the type of interaction were examined daily. Three replicates were prepared for each pairing. The type of interaction was evaluated on the 30th day of the experiment.
The antagonism index (AI) was calculated by assigning one of the categories listed in Table 1 to each interaction; the resulting numerical scores of all assays were then added to achieve a final score per *Xylaria* species. The antagonistic ability of each fungal organism was determined based on the AI.

The antagonism index (AI) was calculated as follows:

$$AI=A(n\times1)+B(n\times2)+C(n\times3)+C_{A1}(n\times3.5)+C_{B1}(n\times4)+C_{A2}(n\times4.5)+C_{B2}(n\times5)+D(n\times0),$$

where n – frequency of each type or sub-type of reaction with a given category or categories (A, B...D), resulting in the corresponding score; greater value was given to the categories representing inhibitory responses.
During co-cultivation, morphological changes like the coloration of mycelia and reverse, delay in coloration and sporulation, changes in density, formation of stromata and exudate droplets, etc., were noted. The controls to compare morphological changes consisted of each fungus growing individually under the same conditions.

RESULTS AND DISCUSSION

According to **Woodward and Boddy (2008)** interactions between fungi can occur through three main mechanisms: at a distance, facilitated by volatile and diffusible chemicals such as enzymes, toxins, and other antifungal metabolites; following contact at the hyphal level, and following contact at the mycelial level, which likely involves various mechanisms like the release of enzymes, toxins, and other antifungal compounds. Regardless of the antagonistic mechanism, these interactions can lead to different outcomes, particularly: a deadlock, where neither species progresses, a substitution where one species replaces another, a partial substitution involving one species capturing some but not the entire territory of the antagonist, or a mutual substitution where each species takes the place formerly occupied by the other and vice versa **(Watkinson *et al*., 2016**). In general, the ability of fungi to interact with other fungi by any type of reaction is inherent to the fungal genome, and can be activated under certain cultivation conditions.
Results of this study revealed that the interspecific mycelial interactions among the examined fungi were competitive, typically resulting in either a deadlock (at mycelial contact or at a distance) or the complete or partial replacement of tested fungi by *Xylaria*. Performed screening of the antagonistic ability of *X. polymorpha* and *X. longipes* (10 strains each) allowed us to evaluate the antifungal potential of these common wood-inhabiting species and to determine the most active strains with high antagonism indexes (AI). *Xylaria polymorpha* IBK 2720 had the highest AI value among all studied strains – 20. This fungus completely replaced *M. plumbeus*, *F. solani* and *C. albicans*, and partially replaced *A. niger* and *P. polonicum* (Table 2). The highest AI among *X. longipes* strains did not differ much from the above-mentioned and amounted to 19 for strain IBK 2718 (Table 3). *Xylaria longipes* IBK 2730 showed the lowest AI rate – 9.5, while the analogical value among *X. polymorpha* strains amounted to 14 for strain IBK 2729.

Table 2 Interaction types of *X. polymorpha* strains and their antagonism index (AI)

IBK strain	*A. niger*	*M. plumbeus*	*P. polonicum*	*F. solani*	*T. viride*	*C. albicans*	AI
2382	B	C_{A2}	C_{B1}	C_{A1}	A	C	18
2430	B	C_{B1}	C_{A1}	C_{A1}	A	C_{A2}	18.5
2719	B	C_{A2}	B	C_{A1}	A	C	16
2720	C_{A1}	C_{A2}	C_{A1}	C_{A2}	A	C	20
2721	C_{B1}	C_{B1}	C_{A1}	C_{A1}	D	C	18
2723	C_{A1}	C_{A2}	B	C_{A1}	A	C	17.5
2727	C_{A1}	C_{A1}	B	C_{A1}	D	C	15.5
2729	B	C_{A1}	B	C_{A1}	D	C	14
2736	C_{A1}	C_{A2}	C_{A1}	C_{A1}	A	C_{A1}	19.5
2737	B	C_{A2}	B	C_{A1}	D	C_{A2}	16.5

Table 3 Interaction types of *X. longipes* strains and their antagonism index (AI)

IBK strain	*A. niger*	*M. plumbeus*	*P. polonicum*	*F. solani*	*T. viride*	*C. albicans*	AI
2715	B	C_{B1}	A	A	D	C_{A1}	11.5
2716	C_{A1}	C_{A1}	A	C_{A1}	D	C_{A2}	16
2717	C_{A1}	C_{B1}	A	A	D	C_{A1}	13
2718	C_{A1}	C_{B1}	C_{A1}	C_{A1}	D	C_{A2}	19
2722	B	C_{B1}	A	A	D	C_{A1}	11.5
2726	A	C_{A2}	C_{A1}	C_{A1}	D	C_{A2}	17
2730	A	C_{B1}	A	A	D	C_{A1}	9.5
2733	C_{A1}	C_{B1}	C_{A1}	B	D	C_{A1}	16.5
2738	A	C_{B1}	C_{A1}	C_{A1}	D	C_{A1}	15.5
2739	B	B	C_{A1}	C_{A1}	D	C_{A1}	14.5

All types of interactions described in Table 1, except complete replacement after an initial deadlock at a distance (sub-type C_{B2}) were noted during the survey. The interaction of *X. polymorpha* and *X. longipes* with the same species differed depending on the studied strain (Tables 2–3). We compared reaction types of *X. longipes* and *X. polymorpha* against test cultures. As can be seen in Figure 1, studied *X. longipes* strains formed an initial deadlock at mycelial contact with tested fungi twice as frequently and also interacted via types C_{A1} and C_{B1} slightly more frequently. However, the percentage of *X. polymorpha* strains that formed a deadlock at a distance was almost twice the number of such for *X. longipes*. In addition, *X. polymorpha* strains were twice as likely to completely overgrow tested fungal colonies after an initial deadlock at mycelial contact. At the same time, 11.7% of *X. polymorpha* strains completely overgrew colonies of *C. albicans* without any initial deadlocks (type C), while strains of *X. longipes* did not form this type of reaction at all. Type D was observed by us only for interaction with *T. viride*. All strains of *X. longipes* and half of *X. polymorpha* were suppressed via type D, which amounted to 16.7 and 6.7 percent of tested pairs of each species, respectively.

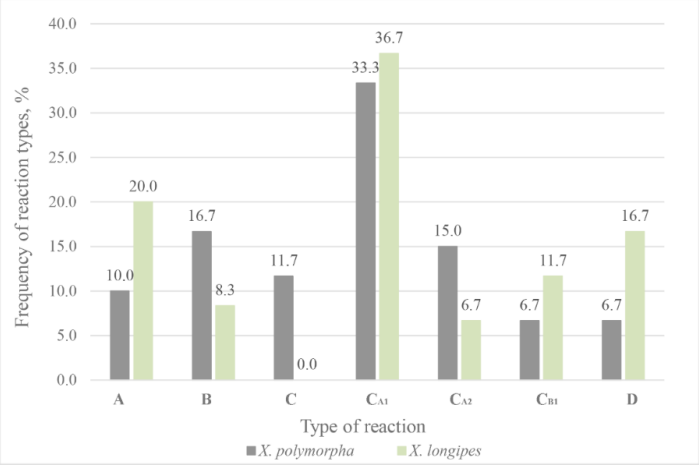

Figure 1 Comparison of the frequency of reaction types between the studied species and the test cultures among *X. longipes* and *X. polymorpha* (percentage calculated on the number of interactions noted for each species separately).

Talking about all tested strains of both *Xylaria* species, the most frequent type of interaction was a partial replacement after initial deadlock with mycelial contact (35%). Overall, domination of replacement of tested fungi by *Xylaria* species (60.8 %) was established (Table 4). These results indicate the effectiveness of *Xylaria* species against tested fungi via contact antagonism. Further detailed descriptions of the interactions with each fungus specifically are provided below.

Table 4 Frequency of interactions between *Xylaria* species and tested fungi in dual culture.

Deadlock		Replacement of test culture by studied species		Replacement of studied species by test culture	
A	15.0%	C	5.8%	D	11.7%
B	12.5%	C_{A1}	35.0%		
		C_{A2}	10.8%		
		C_{B1}	9.2%		
Total	27.5%	Total	60.8%	Total	11.7%

Legend: percentage calculated from the total number of observed interactions among all strains of both species.

Antagonism against filamentous opportunistic pathogenic fungi

In our survey, both *X. longipes* and *X. polymorpha* showed significant inhibitory activity against *A. niger*, *M. plumbeus*, *P. polonicum*, *F. solani*, although there were differences in reaction types. For instance, all *X. polymorpha* strains, except IBK 2720, partially replaced *F. solani*. Strain IBK 2720 fully overgrew the surface of the pathogenic colony (Table 2). Meanwhile, strains of *X. longipes* interacted with *F. solani* not so unambiguously. Only half of strains overgrew the pathogen via type C_{A1}, while the other part formed deadlocks with *F. solani* colonies (Table 3).

Replacement of fungi was accompanied by some noticeable changes in the morphology of both *Xylaria* species. The formation of an aerial mycelium at the site of initial contact was most pronounced among morphological changes (Figure 3B). Additionally, certain *X. polymorpha* strains exhibited sectorization, characterized by the formation of aerial hyphae and disproportionate growth towards the pathogenic colony (Figure 2E). Moreover, the interaction of all *X. polymorpha* strains with *F. solani* resulted in the color changes of the reverse visible as pink pigmentation at the site of initial mycelial contact. From ten *X. longipes* strains, only IBK 2738 formed this pigmented interaction zone (Figure 3H).

These observations correlate with data obtained by **Hamzah *et al*. (2018)**, who studied the antagonistic activity of endophytic *Xylaria* sp, although by a different method. Authors showed that mycelium development of *F. solani* in dual culture plates was significantly inhibited by *Xylaria* sp. with an inhibition percentage (I%) at 65.11% (which was the second highest I% among fungal isolates tested in the assay). They also reported yellowish colouration at the border of two colonies, which turned into rust-like pigmentation later.

Besides interaction with *F. solani*, the pigmentation of colonies reverse was observed by us while co-cultivating both *Xylaria* spp. with *P. polonicum.* The medium around *P. polonicum* colonies turned yellow *i*n all dual culture plates with *X. polymorpha* strains (Figures 2B, 3A). It is noteworthy that co-culturing with *X. longipes* strains did not show such a pronounced change in coloration of the medium, except for a slight darkening. A coloration of the medium could be caused by secondary metabolites of *Penicillium* spp., which often can be visible as diffusible colors, colony reverse and exudate colors. Moreover, *Penicillium* spp. can produce yellow pigments (**Samson and Pitt., 2003**). Many species of *Penicillium* genus produce large amounts of acid as secondary or excess primary metabolites, and acidity may be visualized as a yellow halo around a colony (**Frisvad *et al*., 2008**). Production of acids followed by changes in the pH may influence the interaction between *Xylaria* spp. and *P polonicum observed by us in this assay.*

Figure 2 Competitive interactions between colonies on the 20th day of cultivation (unless otherwise noted): deadlock at mycelial contact of *X. longipes* IBK 2726 and *A. niger (*A); deadlock at a distance between *X. polymorpha* IBK 2727 and *P. polonicum* (B); initial deadlock between *X. polymorpha* IBK 2382 and *M. plumbeus* 7 days after inoculation (C), followed by complete replacement of *Mucor* by mycelium of *Xylaria* on the 20th day of cultivation (F); overgrowth without initial deadlock, the interaction of *X. polymorpha* IBK 2723 and *C. albicans* (D and G – front and reverse sides of Petri dishes, respectively); partial replacement of *F. solani* after an initial deadlock with mycelial contact by *X. polymorpha* IBK 2382 (E); partial replacement of *M. plumbeus* after initial deadlock at a distance by of *X. longipes* IBK 2733 (H); suppression and overgrowth of *X. polymorpha* IBK 2737 by *T. viride* (I). Note: *Xylaria* on the left, test culture on the right.

The antagonistic activity against another opportunistic pathogen – *A. niger* was similar for *X. polymorpha* and *X. longipes*. Half of *X. polymorpha* strains interacted with *A. niger* via deadlocks, as well as 6 of 10 of *X. longipes* strains. The other part of the studied strains partially replaced *A. niger* after initial deadlock with mycelial contact or at a distance (strain IBK 2721) (Tables 2–3). Notably, our data on the antagonistic activity of *Xylaria* species against *A. niger* and *P. polonicum* are consistent with similar studies conducted for medicinal fungi **(Krupodorova *et al*., 2023)**.

In co-cultivation with all *X. longipes* and some *X. polymorpha* strains, *A. niger* showed reduced asexual sporulation, which appeared in colonies sectorization with yellow non–sporulation sectors (Figure 3F). The formation of hetero-morphological sectors is a general phenomenon observed in molds and is not restricted to certain species or strains. However, sector formation, observed in

some productive strains, resulted in a reduced yield of secondary metabolites and enzymes (**Chun *et al*., 2019**).

After 15 days of co-cultivation with *A. niger*, the appearance of fungal metabolites on the colony of *X. longipes* IBK 2739 in the form of exudate of clear yellowish drops was observed (Figure 3D). Generally, exudates producing, as well as color changes, could be an indicator of significant production of constitutively present compounds while co-cultivation. It could also indicate an accumulation of compounds with antifungal or fungistatic activity that are not detected in axenic cultures (**Marmann *et al*., 2014**).

Similar but smaller liquid droplets appeared on the surface of *X. longipes* IBK 2739 colonies in dual cultures with *M. plumbeus*. During interspecific interactions with *M. plumbeus* both *Xylaria* spp. showed significant activity against this fungus partially or completely replacing its colonies (Tables 2–3). Only strain IBK 2739 stopped growing before reaching the colony *M. plumbeus*, forming a deadlock at a distance. However, a delay in growth was observed for the colony of *M. plumbeus* as well, which besides had a delay in sporulation (Figure 3I). Such antagonism between fungi without physical mycelial contact is a focus of heightened interest because it is ensured by production of volatile and/or diffusible antifungal metabolites, enzymes, toxins, and alters the pH of the environment (**Boddy, 2016**). Overall, the antagonism displayed by *Xylaria* species was aggressive as it advanced and further colonized the surface of most of the test cultures. This could be attributed to the production of lytic enzymes, which degrade the pathogen's cell wall and allow to colonize the surface of the pathogen's colony.

Figure 3 Changes in morphological characteristics of the colonies on the 20th day of cultivation (unless otherwise noted): coloration of the medium around *P. polonicum* (A); air hyphae at the initial deadlock of *X. polymorpha* IBK 2721 co-cultivating with *F. solani* (B); abundant aerial mycelium of *X. polymorpha* IBK 2721 overgrowing *C. albicans* (C); excudate on the *X. longipes* IBK 2739 colony co-cultivating with *A. niger* (D); complete absence of spore production of *T. viride* co-cultivating with *X. longipes* IBK 2722 (E); sectorization of *A. niger* co-cultivating with *X. polymorpha* IBK 2430 (F); stromata production of *X. longipes* IBK 2738 co-cultivating with *F. solani* (G) and pigmentation in the initial deadlock (H); delay in growth of *X. longipes* IBK 2739 and in sporulation of *M. plumbeus* (I). Note: *Xylaria* on the left, test culture on the right.

Nearly all *X. polymorpha* strains exhibited stromata production when co-cultured with *A. niger* and *P. polonicum*. However, strain IBK 2736 failed to produce stromata, whether co-cultured with these opportunistic pathogens or in dual cultures with *M. plumbeus*. With the latter, only half of the *X. polymorpha* strains (IBK 2719, IBK 2721, IBK 2727, IBK 2729, IBK 2737) displayed stromal formation. In the meantime, among *X. longipes* strains, only strain IBK 2738 formed stromata specifically when co-cultivated with *F. solani* (Figure 3G). Notably, there was no observed relation between stromata formation and specific types of fungal interactions. Stromata were observed during deadlocks at a distance as well as various types of replacements.

Antagonism against *T. viride*

Among the test cultures, only *Trichoderma viride* demonstrated the ability to suppress *Xylaria* species. The mycelium of *T. viride* completely overgrew colonies of all studied *X. longipes* strains, which were covered in green sporulation all over the surface on the 20th day of co-cultivation (Figure 4C). Such strong antagonistic activity of *Trichoderma* may be explained by the production of a variety of secondary metabolites and volatile compounds potentially inhibiting other organisms (**Hermosa *et al*., 2014, Keswani *et al*., 2014**). *Trichoderma* species are cosmopolitan on decaying wood due to their aggressive competitive nature. They can colonize wood by using non-structural carbohydrates, such as sugar and starch, and then limit colonization by secondary invaders such as wood-decaying mushrooms (**Badalyan *et al*., 2004**). However, when co-cultivating with *X. longipes* IBK 2722 and IBK 2738, no sporulation of *T. viride* was observed at all, although its mycelium overgrew *Xylaria* colonies completely (Figure 3E.). Such absence, as well as delay in the sporulation of *T. viride* which was observed at the site of initial deadlock with mycelial contact (Figure 4B), indicates that *X. longipes* shows some activity against *T. viride*. This activity was more pronounced for *X. polymorpha*, for which half of the strains formed deadlock at mycelial contact with *T. viride* (Table 2). In contrast to *X. longipes*, which in initial stages grew proportionally to the tested colony (Figure 4A), *X. polymorpha* possessed some visible activity against *T. viride*, which was revealed in the retardation of its colony growth towards *Xylaria* (Figure 5A).

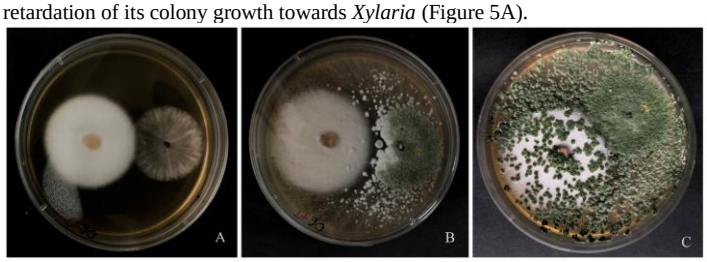

Figure 4 *X. longipes* IBK 2717 (on the left) and *T. viride* (right) in dual culture on GPYA Petri dishes: initial deadlock with mycelial contact on the 5th day of co-cultivation (A); delay in sporulation of *T. viride* on 14th day (B); complete replacement of *Xylaria* by *Trichoderma* on 20th day (C).

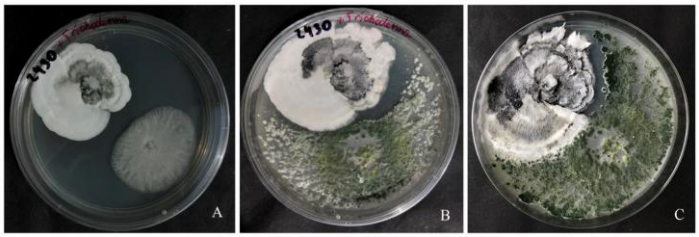

Figure 5 *X. polymorpha* IBK 2430 (top left) and *T. viride* (bottom right) in dual culture on GPYA Petri dishes: retardation of *T. viride* growth towards *X. polymorpha* colony on the 5th day of co-cultivation (A); inhibition zone between two colonies on 14th day (B); deadlock at mycelial contact on 20th day (C).

Rajendiran *et al*. (2010) showed that *T. viride* restricted the growth of some pathogens on Czapek Dox Agar medium in the dual culture study, including *A. niger* (percentage inhibition 55%), *Fusarium sp*. (64%) and *Penicillium sp*. (54%), growing over pathogenic colonies. Such activity was explained by fast-growing nature, secretions of harmful extra-cellular compounds, cell wall degrading enzymes (gluconases, endochitinases and chitinases). In our survey *X. polymorpha* displayed activity against those fungi and *T. viride*, forming a mutual deadlock at mycelial contact with the last or by affecting its sporulation. This indicates significant activity of *X. polymorpha* against mycoparasitic fungus and its potential as a biological control agent.

Antagonism against *C. albicans*

Mycelium of all strains of both *Xylaria* species in our study partially or completely overgrew *C. albicans* colonies, indicating aggressiveness towards this pathogen. All strains of *X. polymorpha* except for IBK 2723 showed a change in colony morphology, characterised by a significant increase in the production of an aerial mycelium (Figures 2D, 3C). Notably, air hyphae in abundance were also observed for 7 of 10 strains of *X. polymorpha* when co-cultured with *M. plumbeus* (Figure 2F) and 4 of 10 with *F. solani* (Figure 3B).

As far as the current literature considered, there were only reported results regarding the activity of other macromycetes against *C. albicans* in dual culture assays (**Krupodorova *et al*., 2021**). Studies concerning *Xylaria* spp. mainly focused on antifungal activity of extracts. For instance, **Hacıoğlu *et al*. (2011)** showed that an ethanolic extract obtained from the fruiting bodies of *X. polymorpha* revealed antimicrobial activity against *C. albicans* in a disk diffusion test. Applying that same agar disc diffusion assay, **Canli *et al*. (2016)** showed that an ethanolic extract of another common wood-inhabiting species *X. hypoxylon* was active against *C. albicans*. Of particular interest are studies regarding the antimicrobal effect of certain secondary metabolites produced by species of the genus *Xylaria*. For instance, the study of **Xu *et al*. (2017)** concerning

two cyclic pentapeptides with moderate antibacterial and antifungal effects against *C. albicans.*

CONCLUSIONS

This study contributes to the knowledge in the field of interspecific fungal interactions. The dual culture method used in this work allowed us to evaluate and compare the antifungal potential of *X. polymorpha* and *X. longipes* strains against harmful fungi. Inhibiting activity of the studied *Xylaria* strains comprised a deadlock, consisting in mutual inhibition at a distance or at mycelial contact, and replacement, consisting in the initial deadlock followed by partial or complete overgrowth. The predominance of replacement of *A. niger*, *M. plumbeus* and *C. albicans* colonies by *Xylaria* spp. was established. Interaction at a distance was noted when co-cultivating *X. polymorpha* with *A. niger* and *P. polonicum*, while *X. longipes* interacted with these species at contact. In contrast, *M. plumbeus* was partially replaced after an initial deadlock at a distance by *X. longipes* strains, while at contact by *X. polymorpha. Trichoderma viride* was the only fungus from tested cultures, that inhibited the mycelial growth of studied *Xylaria* species. All *X. longipes* strains were suppressed, whereas six of the ten *X. polymorpha* strains studied formed a mutual deadlock at mycelial contact with *T. viride*.

Co-cultivation resulted in morphological changes in certain fungal colonies. The most pronounced were the formation of abundant aerial mycelia of *X. polymorpha* while co-cultivating with *M. plumbeus* and *C. albicans* and stromata with *A. niger* and *P. polonicum*, colony sectorization of *A. niger* in dual cultures with *X. longipes*, and also pigment diffusions, formation of mycelial barrages and exudate droplets. Given the multitude of reaction types and morphological changes observed, it is likely that a wide variety of antagonistic mechanisms are involved

The screening allowed us to identify the most active strains with a high antagonism index (AI), which was 20 for *X. polymorpha* IBK 2720 and 19 for *X. longipes* IBK 2718. Not to mention strains of both *Xylaria* species that interacted with fungi at a distance, emphasizing them as producers of potential diffusible or volatile bioactive compounds. These strains, with considerable antagonistic activity against *A. niger*, *M. plumbeus*, *C. albicans*, *P. polonicum* and *F. solani* are promising for further investigations with the purpose of studying and isolation of potential antifungal metabolites.

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