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Antagonistic activity of selected macromycetes against two harmful micromycetes

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Competition between 31 macromycete species and two harmful micromycetes *Aspergillus niger* and *Penicillium polonicum* was evaluated using dual-culture plate assay. All investigated fungi, except for *Inonotus obliquu*s and *Lepista luscina*, possessed different levels of antagonistic activity against the tested micromycetes. *Hypsizygus marmoreus* and *Lyophyllum shimeji* were inactive against *A. niger*. *Morchella esculenta* and *Oxyporus obducens* were passive in the case of co-growth with *P. polonicum*. The study of fungal interactions showed variability in types of reactions and level of their visualisation. Co-cultivation of the studied species resulted in the following reactions: deadlock after mycelial contact and at a distance, partial or complete replacement after initial deadlock on contact. In general, the studied macromycetes showed moderate activity against the two micromycetes, as 11 species showed active and 7 species moderate antagonism. Results revealed higher antagonistic activity of macromycetes against *P. polonicum* than in *A. niger* co-cultures. Wood-decaying species *Ganoderma lucidum* and *Trametes versicolor* were the most active fungi against the two tested micromycetes with a maximal antagonism index. These findings provide valuable insights which can be further explored by means of in vivo assays to find a suitable agent for the biocontrol of diseases or spoilage caused by *A. niger* and *P. polonicum*.

Key words: ascomycetes, *Aspergillus niger*, basidiomycetes, dual-culture, *Penicillium polonicum*.

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Kompetice mezi 31 druhy makromycetů a dvěma potenciálně škodlivými mikromycety *Aspergillus niger* a *Penicillium polonicum* byla hodnocena s využitím duálních kultur. Různou úroveň antagonistické activity vykazovaly všechny zkoumané houby s výjimkou *Inonotus obliquu*s a *Lepista luscina*. Proti *A. niger* se neprosadily *Hypsizygus marmoreus* a *Lyophyllum shimeji*, zatímco *Morchella esculenta* a *Oxyporus obducens* mají pasivní bilanci s *P. polonicum*. Studie interakcí vybraných hub ukázala značnou variabilitu na úrovni jejich vizualizace, kokultivace konkrétních druhů vedly k těmto

typům reakcí: uzamčení po kontaktu hyf i na dálku, částečné či úplné nahrazení po prvotním kontaktu. Jedenáct druhů makromycetů vykazuje silnou antagonositickou aktivitu, sedm dalších pak průměrný antagonismus proti uvedeným dvěma mikromycetům. Celkově lze říci, že silnější antagonismus se uplatňuje proti *P. polonicum* než proti *A. niger*. Nejsilnějšími antagonisty (s nejvyšším antagonistickým indexem) vůči testovaným mikromycetům se ukázaly být dřevožijné druhy *Ganoderma lucidum* a *Trametes versicolor*. Výsledky testů poskytují cenné poznatky, které lze dále využít v pokusech in vivo s cílem nalézt vhodné agens pro biokontrolu zdravotních rizik nebo škod působených houbami, jako jsou *A. niger* a *P. polonicum*.

INTRODUCTION

Fungi, as a fairly diverse group, are an important component of most ecosystems and an essential part of their functioning. At the same time, some fungal species are common pathogens, causing plant diseases and animal and human infections (Gnat et al. 2021, Seagle et al. 2021). Opportunistic microorganisms (including fungi) exhibit their potentially pathogenic properties when a human or animal body's defences are weakened and then can cause various diseases, mainly those affecting the respiratory and digestive tracts.

Food spoilage remains an enormous problem throughout the world and filamentous fungi, commonly referred to as moulds, represent the most important group of spoilage microbes responsible for significant economic losses. Fungal spoilage affects the visual and organoleptic properties of food but can also lead to the production of toxins. At the same time, these micromycetes are often opportunistic human pathogens. *Aspergillus niger* can cause rotting of a large number of fruit and vegetables, causing substantial economic loss. Onions, mangoes, grapes and tomatoes are the most cited fruit and vegetables for which *A. niger* is the major cause of rot plant disease. This species has also been described for humans and animals as an opportunistic pathogen causing allergic disorders or mycotoxin production (Gautam et al. 2011). The fungus *A. niger* is a type of mould, which is sometimes attributed to the cause of some cases of pneumonia. *Penicillium polonicum* is a psychrotolerant xerophilic fungus associated with spoilage of many foods such as onions, cereals, dried meats, peanuts, yam tubers and citrus fruits (Khalil et al. 2019). This species was part of a specific fungal population accounting for the incidence of seborrheic dermatitis in humans (Mahmoudi et Rezaie 2020). In recent years, *P. polonicum* has been found in the lung microbiome of patients with chronic inflammatory respiratory diseases. The development of this opportunistic infection is attributed to a decrease in immune system efficiency (Rubio-Portillo et al. 2020). The common negative impact of two potential opportunistic fungi, *A. niger* and *P. polonicum*, led us to our choice of test microorganisms in this study.

The integration of fungi into the environment at broad spatial scales highlights the need for a better understanding of the growth, morphology and innate defence characteristics of fungi against microbial competitors, which is very important in terms of basic and applied research. Competition may lead to significant change in the growth rate of the studied fungi (Schmit 2001). Fungal interactions can also induce changes in hyphal morphology (Rayner et al. 1994, Mali et al. 2017, Dullah et al. 2021a) and, particularly, in extracellular enzyme secretion (Freitag et Morrell 1992, White et Boddy 1992, Savoie et al. 1998, Hatvani et al. 2002, Mali et al. 2017, Dullah et al. 2021a). In general, fungi can produce, when grown on natural or artificial media, a variety of metabolites which have one or another physiological activity. The ability of fungi to synthesise many of these compounds, which are not needed during normal development, can only be activated in response to the action of an antagonist, as well as the presence of significant resources for their synthesis (Kempken et Rohlfs 2010, Künzler 2015, 2018, Ma et Ruan 2015, Luo et al. 2017, Shi et Wang 2019, Chan-Cupul et al. 2019, Dullah et al. 2021b, Yu et al. 2021). Studies of such unique natural phenomena during fungal antagonistic interactions can be successfully carried out in laboratory conditions (Peiris et al. 2008, Zhu et al. 2014, Serrano et al. 2017, Owaid 2017, Chan-Cupul et al. 2019, Krupodorova et al. 2021).

Experimental data regarding specific protective reactions during fungi competition shows that research on co-cultivation of plant and human pathogenic, or opportunistic pathogenic micromycetes with macromycetes is promising: isolates of *Trichoderma*, *Penicillium* and *Aspergillus* with *Trametes versicolor* and *Neolentinus lepideus* (Bruce et Highley 1991); *Trichoderma harzianum* with *T. versicolor* (Freitag et Morrell 1992); *Botrytis cinerea* and *Cladosporium cucumerinum* with 11 basidiomycete species and the ascomycete fungus *Rhizina undulata* (Woodward et al. 1993); *Bipolaris sorokiniana*, *Fusarium culmorum*, *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis* with 17 species of xylotrophic basidiomycetes (Badalyan et al. 2002); *Trichoderma* species and *Clonostachys rosea* with 17 species of xylotrophic basidiomycetes (Badalyan et al. 2004); *Trichoderma* strains with *Lentinula edodes* (Shulga 2005); *Trichoderma* species with basidiomycetes *Ganoderma adspersum*, *G. lipsiense*, *Inonotus hispidus*, *Polyporus squamosus* and the ascomycete *Kretzschmaria deusta* (Schubert et al. 2008); *B. sorokiniana*, *Chrysosporium keratinophilum*, *Fusarium culmorum*, *Microsporum gypseum*, *Trichoderma harzianum* and *T. asperellum* with *Pleurotus tuber-regium* (Badalyan et al. 2008); *Trichoderma* strains with *L. edodes* (Menolli et Paccola-Meirelles 2010); *B. sorokiniana*, *Ophiostoma ulmi*, *Pestalotiopsis funerea*, *F. culmorum* and *F. oxysporum* with *Flammulina velutipes* (Borhani et al. 2011); *C. keratinophilum*, *Trichophyton terrestre* and *M. gypseum* with *Ganoderma adspersum*, *G. lucidum*, *G. resinaceum* and *Ganoderma* sp. (Badalyan et al. 2012); *B. cinerea* with *Pycnoporus*

coccineus (Arfi et al. 2013); *Aspergillus* spp. with *Agaricus campestris* (Raipuria et Sobin 2015); *Alternaria alternata*, *Colletotrichum* sp., *Phyllosticta citricarpa* and *Moniliophthora perniciosa* with *Schizophyllum commune* (Orlandelli et al. 2015); *Trichoderma reesei* with *Coprinus comatus* (Ge et al. 2009, Ma et Ruan 2015); *Verticillium* sp. and *Pythium* sp. with *Coriolus versicolor*, *Hericium erinaceus* and *L. edodes* (Owaid 2017); *Aspergillus* sp., *Fusarium* sp. and *Pythium* sp. with unidentified species of *Agaricaceae*, *Agrocybe pediades*, *Clitopilus scyphoides*, *Irpex lacteus* and *Trametes ochracea* (Chaudhary et Tripathi 2016); *Trichoderma viride* and *Aspergillus flavus* with *Pleurotus ostreatus* (Erwin et al. 2018); *Aspergillus* sp., *Beauveria brongniartii*, *Metarhizium anisopliae*, *Paecilomyces carneus*, *Penicillium hispanicum*, *Purpureocillium lilacinum* and *Trichoderma* sp. with *Pycnoporus sanguineus* and *Trametes maxima* (Chan-Cupul et al. 2019).

All these in vitro studies indicate significant antagonistic potential of macromycetes from different ecological and systematic groups. From a practical point of view, a deeper understanding of such intensive studies may help to explore the hidden resources of fungal protective processes, which will contribute to the further discovery of new antifungal metabolites (Weber et Gloer 1988, Woodward et al. 1993, Caudal et al. 2022). The present study aimed to evaluate the competition between selected macromycetes and two micromycetes which may be harmful for humans, *Aspergillus niger* and *Penicillium polonicum*.

MATERIAL AND METHODS

Fungal strains. Thirty-one macromycetes (Tab. 1) from different ecophysiological and taxonomic groups were kindly provided by the Mushroom Culture Collection of the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine (IBK) (Bisko et al. 2020).

Micromycete species, namely *Aspergillus niger* Tiegh. IFBG 134 and *Penicillium polonicum* K.W. Zaleski IFBG 138, were kindly obtained from the Collection of Strains of Microorganisms and Plant Lines of the Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine. The stock culture on beer-wort agar slants was stored in a refrigerator at 4 °C before use.

The names of the fungal species are given according to the nomenclature database Index Fungorum (www.indexfungorum.org/Names/Names.asp), except for *Mensularia radiata* and *Pseudospongipellis litschaueri*, for which the currently accepted taxonomic concept (Wu et al. 2022 and Wang et Dai 2022, respectively) was adopted.

Cultivation conditions. Potato dextrose agar (PDA; Difco, Leeuwarden, the Netherlands) was used for fungus cultivation. The prepared media were autoclaved at 121 °C for 15 min. Each fungal species was transferred from stored slants onto Petri dishes with PDA and incubated at 26 ± 1 °C in the dark to obtain fully colonised Petri dishes. PDA was used as a culture medium for fungus growth on Petri dishes, and evaluation of antagonistic activity as well.

Dual-culture experiments. Fungal competition between the studied macromycete and micromycete species was evaluated according to their ability to suppress the mycelial growth in dual-culture experiments on Petri dishes with PDA medium, using a rating scale for three primary types of reaction (A, B, C) and four sub-types (C_{A1}, C_{B1}, C_{A2}, and C_{B2}) and the antagonism index (AI) was calculated by means of the method described by Badalyan et al. (2002, 2004).

For macromycetes demonstrating growth reactions of types A and B, the percentage of growth inhibition of contact cultures was calculated by applying the formula by Singh et Tripathi (1999):

$$PI = \frac{d_c - d_t}{d_c} \times 100$$

where PI = percent inhibition of micromycete growth, d_c = growth of micromycete on control plate (mm), and d_t = growth of micromycete on treated plate (mm).

The studied micromycetes together with the macromycetes were incubated in a thermostat with forced air circulation in a chamber (TC-80; Medlan, Kyiv, Ukraine) at 26 °C in the dark at 75% relative humidity. The growth of fungi was monitored daily for a month, and compared with mycelia taken from the controls. Morphological changes (colouration of mycelia and agar, visualisation of the demarcation line, production of exudate drops, formation of dense zones of mycelium, fruitbody primordia, etc.) in interacting colonies were monitored. The mycelial growth pattern towards the micromycete species was observed periodically (every three days). The percent inhibition of mycelial growth was recorded on the 9th day, and the type of interaction was noted every three days for 30 days.

Statistical analysis. Experiments were performed in triplicate. The PI data was analysed as colony diameter values and expressed as a percentage in the figures 3 and 4. Pairwise comparison of means was performed with the Fisher Least Significant Difference (LSD) test at $p \leq 0.05$.

RESULTS

The types of fungus interaction depended on the studied macromycete species and the corresponding one of the two species of micromycete (Tab. 1). However, 8 species (*Coprinus comatus*, *Cyclocybe aegerita*, *Flammulina velutipes*, *Ganoderma lucidum*, *Grifola frondosa*, *Mensularia radiata*, *Ophiocordyceps sinensis*, *Phellinus igniarius*) had the same type of interaction with competing micromycetes. Co-cultivation of the studied fungi species based on their relative combative ability resulted in the following reactions: deadlock after mycelial contact (Fig. 1A), deadlock at a distance (Fig. 1B), partial (Fig. 1D, E) or complete (Fig. 1G, H) replacement after initial deadlock on contact.

Strong antagonism of the tested fungi (both macromycetes and, in a smaller number of cases, micromycetes) was expressed by reactions C_{A1} and C_{A2}, and approximately equal combative capabilities of competing fungi were expressed by reactions A and B (Tab. 1). Considering each type or subtype of interaction of macromycetes with *Aspergillus niger* separately, a significant prevalence of deadlock after mycelial contact (42%) was found, while the total frequency of replacement (partial or complete) was slightly higher than half (58%, Fig. 2a).

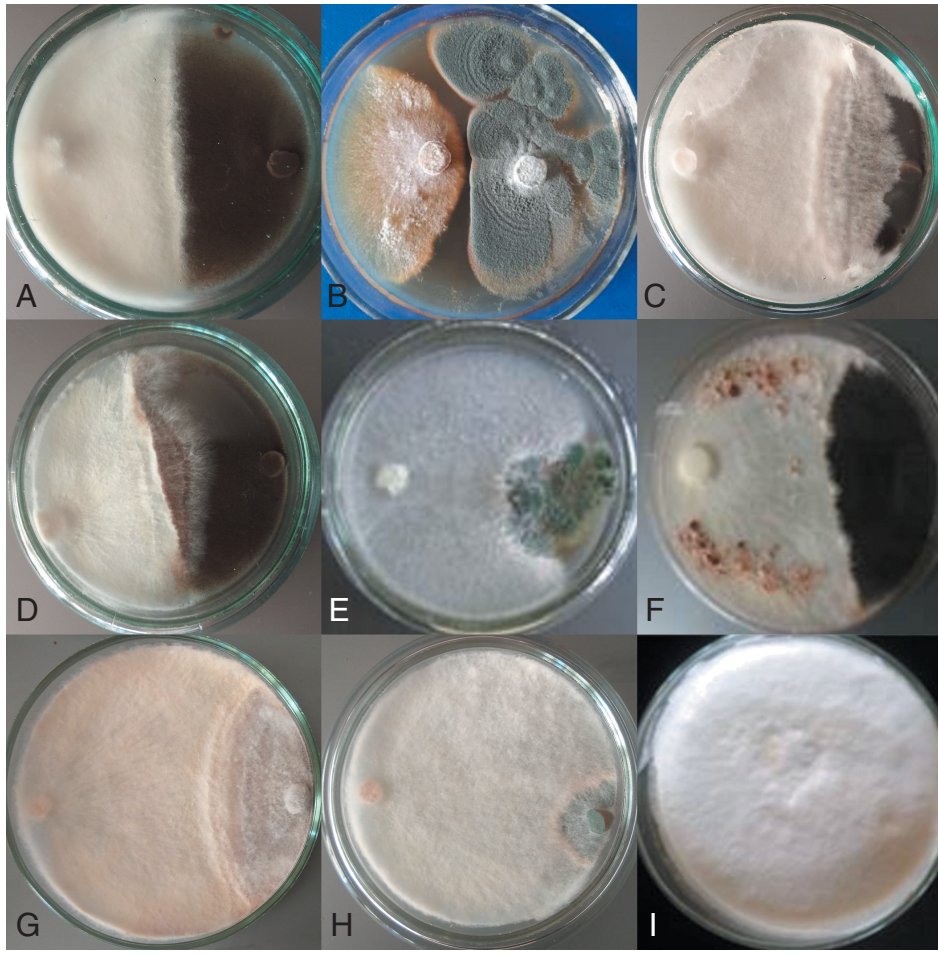

Fig. 1. Interspecific interactions between myceliums of some of the studied fungi (left – macromycete fungus, right – micromycete fungus).
A – *Schizophyllum commune* and *Aspergillus niger*, deadlock on mycelial contact, **B** – *Hericium erinaceus* and *Penicillium polonicum*, deadlock at a distance, **C** – localised aerial mycelia of *Crinipellis schevczenkoi* in pairings with *A. niger*, **D** – *Pleurotus ostreatus* and *A. niger*, **E** – *Pleurotus eryngii* and *P. polonicum*, both partial replacement after initial deadlock on contact, **F** – formation of primordia on colony of *Fomes fomentarius*, **G** – *Trametes versicolor* and *A. niger*, **H** – *T. versicolor* and *P. polonicum*, both complete replacement after initial deadlock on contact, **I** – 30 day old monoculture of *T. versicolor*. Photos by T. Krupodorova.

In experiments with *Penicillium polonicum*, complete replacement after initial deadlock on contact (39%) slightly prevailed over other interaction types. The high total frequency (68%) of replacement reactions (partial or complete) in co-growth of macromycetes and *P. polonicum* (Fig. 2b) is notable.

Tab. 1. Types of interaction between macromycetes and micromycetes and their antagonism index (AI). Interaction types: A – deadlock after mycelial contact, B – deadlock at a distance, C_{A1} – partial replacement after initial deadlock on contact, C_{A2} – complete replacement after initial deadlock on contact. * Micromycete species replacing the macromycete.

Macromycetes		Micromycetes		AI
Species	**Strain**	***A. niger***	***P. polonicum***	
Auriporia aurea (Peck) Ryvarden	5048	C_{A1}	C_{A2}	8.0
Coprinus comatus (O.F. Müll.) Pers.	137	A	A	2.0
Cordyceps militaris (L.) Fr.	1862	A	C_{A1}	4.5
Crinipellis schevczenkoi Bukhalo	31	C_{A2}	C_{A1}	8.0
Cyclocybe aegerita (V. Brig.) Vizzini	1853	C_{A1}	C_{A1}	7.0
Flammulina velutipes (Curtis) Singer	1878	A	A	2.0
Fomes fomentarius (L.) Fr.	355	C_{A1}	C_{A2}	8.0
Fomitopsis betulina (Bull.) B.K. Cui, M.L. Han et Y.C. Dai	327	C_{A1}	C_{A2}	8.0
Fomitopsis pinicola (Sw.) P. Karst.	1523	C_{A1}	A	4.5
Ganoderma applanatum (Pers.) Pat.	1701	C_{A1}	C_{A2}	8.0
Ganoderma lucidum (Curtis) P. Karst.	1900	C_{A2}	C_{A2}	9.0
Grifola frondosa (Dicks.) Gray	976	A	A	2.0
Hericium erinaceus (Bull.) Pers.	970	A	B	3.0
Hohenbuehelia myxotricha (Lév.) Singer	1599	C_{A2}	C_{A1}	8.0
Hypsizygus marmoreus (Peck) H.E. Bigelow	2006	C_{A2}*	A	1.0
Inonotus obliquus (Fr.) Pilát	1877	C_{A2}*	C_{A2}*	0
Laetiporus sulphureus (Bull.) Murrill	352	C_{A2}	C_{A1}	8.0
Lentinula edodes (Berk.) Pegler	502	C_{A1}	C_{A2}	8.0
Lepista luscina (Fr.) Singer	64	C_{A1}*	C_{A2}*	0
Lyophyllum shimeji (Kawam.) Hongo	1662	C_{A2}*	A	1.0
Morchella esculenta (L.) Pers.	1953	A	C_{A2}*	1.0
Mensularia radiata (Sowerby) Lázaro Ibiza	2454	A	A	2.0
Ophiocordyceps sinensis (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones et Spatafora	1928	A	A	2.0
Oxyporus obducens (Pers.) Donk	5085	C_{A1}	C_{A2}*	3.5
Phellinus igniarius (L.) Quél.	1578	A	A	2.0
Pleurotus djamor (Rumph. ex Fr.) Boedijn	1526	A	C_{A1}	4.5
Pleurotus eryngii (DC.) Quél.	2015	A	C_{A1}	4.5
Pleurotus ostreatus (Jacq.) P. Kumm.	551	C_{A1}	C_{A2}	8.0
Pseudospongipellis litschaueri (Lohwag) Y.C. Dai et Chao G. Wang	5312	A	C_{A1}	4.5
Schizophyllum commune Fr.	1768	A	C_{A1}	4.5
Trametes versicolor (L.) Lloyd	353	C_{A2}	C_{A2}	9.0

In terms of growth, macromycetes with deadlock reactions demonstrated inhibition of micromycete growth at varying level (Figs 3, 4). Stronger macromycete growth inhibition was recorded in co-cultures with *A. niger* (25.0–43.75%, Fig. 3) compared to co-cultures with *P. polonicum* (10.0–35.0%, Fig. 4).

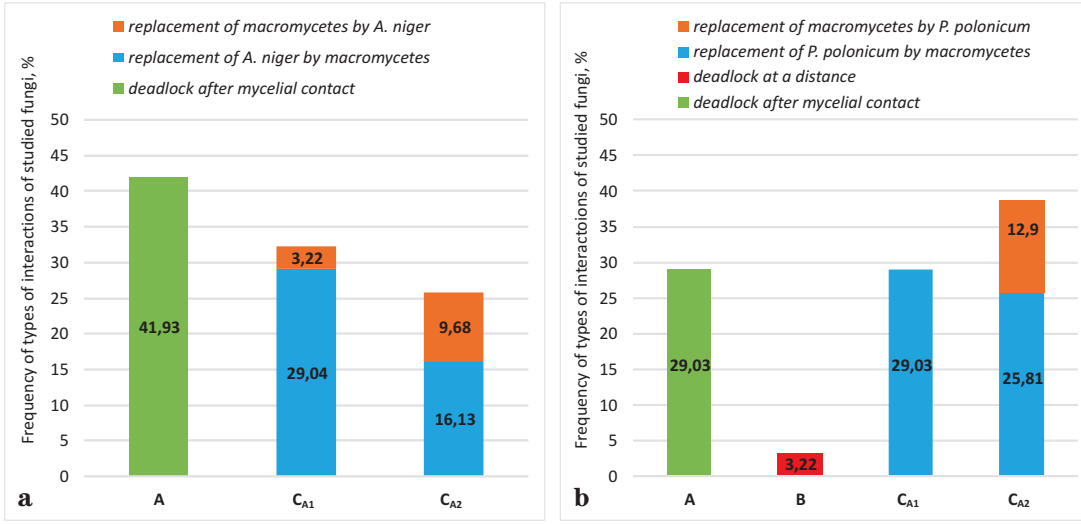

Fig. 2. Frequency of types of interaction between colonies of macromycetes and micromycetes: *Aspergillus niger* (**a**), *Penicillium polonicum* (**b**), expressed as percentage of the total number (93) of pairings tested.
A – deadlock after mycelial contact, B – deadlock at a distance, C_{A1} – partial replacement after initial deadlock with contact, C_{A2} – complete replacement after initial deadlock with contact.

All investigated higher fungi, except for *Inonotus obliquus* and *Lepista luscina*, possessed different levels of antagonistic activity against the tested two micromycetes. *Hypsizygus marmoreus* and *Lyophyllum shimeji* were inactive in co-cultivation with *A. niger*. The species *Morchella esculenta* and *Oxyporus obducens* were passive in co-growth with *P. polonicum*. The results of the analysis of interactions between macromycetes and micromycetes showed that antagonism index (AI) levels ranged from 0 to 9.0 (Tab. 1). Determination of the AI allowed us to conditionally distinguish the studied fungi into three groups based on their relative antagonistic ability: active species (AI = 8–9) *Auriporia aurea*, *Crinipellis schevczenkoi*, *Fomes fomentarius*, *Fomitopsis betulina*, *Ganoderma applanatum*, *G. lucidum*, *Hohenbuehelia myxotricha*, *Laetiporus sulphureus*, *Lentinula edodes*, *Pleurotus ostreatus* and *Trametes versicolor*, moderately active species (AI = 4.5–7) *Cordyceps militaris*, *Cyclocybe aegerita*, *Fomitopsis pinicola*, *Pleurotus djamor*, *Pleurotus eryngii*, *Pseudospongipellis litschaueri* and *Schizophyllum commune*, and species with low activity (AI = 1–3.5) *Coprinus comatus*, *Flammulina velutipes*, *Grifola frondosa*, *Hericium erinaceus*, *Hypsizygus marmoreus*, *Lyophyllum shimeji*, *Mensularia radiata*, *Morchella esculenta*, *Ophiocordyceps sinensis*, *Oxyporus obducens* and *Phellinus igniarius*.

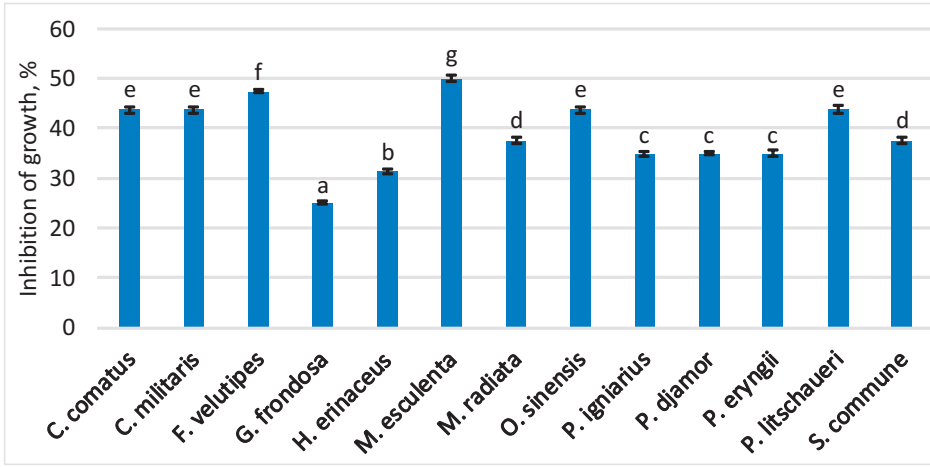

Fig. 3. Percentage of *Aspergillus niger* growth inhibition by macromycetes observed after 9 days of co-cultivation. Values with the same letters are not significantly different ($p \leq 0.05$) according to Fisher's LSD test.

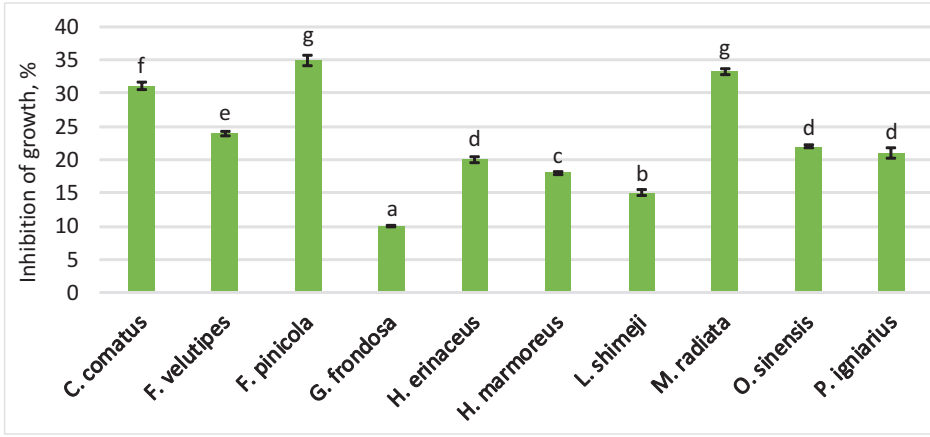

Fig. 4. Percentage of *Penicillium polonicum* growth inhibition by macromycetes observed after 9 days of co-cultivation. Values with the same letters are not significantly different ($p \leq 0.05$) according to Fisher's LSD test.

One of the possible life-history strategies used by fungi in response to different environmental biotic and abiotic stresses is the ability to change their morphology. Changes in mycelial morphology occurred in areas in direct contact with the competitor: the presence of central bands in the co-culture of *S. commune* with *A. niger* (Fig. 1A) and localised aerial mycelia of *C. schevczenkoi* also in

contact with this micromycete (Fig. 1C). A slight colour change of the *T. versicolor* colony (from white to cream) was found in the co-cultures with both tested micromycetes (Fig. 1G–I). The formation of primordia in the *F. fomentarius* colony and the production of fungal metabolites in the form of light and dark brown droplets were observed in co-cultivation with *A. niger* (Fig. 1F).

DISCUSSION

Fungal interactions are very important ecological driving forces linking different levels of biological and ecological interactions between competitors and antagonists to the habitat and functioning of ecosystems as a whole (Bahram et Netherway 2022). Co-culture is a potentially promising way to study fungal interactions under laboratory conditions. This paper reports on the interspecific fungal interactions, with the aim to elucidate patterns observed in experiments with dual cultures. Furthermore, interactions of 31 macromycetes from different ecological (soil- or wood-inhabiting saprotrophs, entomophilous fungi, and leaf-litter decaying fungi) and systematic groups with diverse distribution patterns (species with local or narrow distribution ranges as well as cosmopolitan fungi) with widespread opportunistic pathogenic or toxigenic micromycete species such as *Aspergillus niger* and *Penicillium polonicum* were examined to compare the relative antagonistic abilities of these macromycetes.

Interactions can be carried out at a distance, after contact at the level of individual hyphae, and after contact at the level of the whole mycelium (Woodward et Boddy 2008). Distance antagonism is caused by volatile and diffusible chemicals, including enzymes, toxins, and other antifungal metabolites. Two broad types of antagonism occur at the level of hyphae, i.e. hyphal interference and parasitism (not studied). At the mycelial level, often referred to as the rough mycelial contact, there are probably many mechanisms involving the release of enzymes, toxins and other antifungal compounds. Regardless of the mechanism of antagonism, the ultimate outcome may be a deadlock end where neither species advances, a substitution in which one species takes the place of another, a partial substitution in which one species takes part but not the entire antagonist's territory, or a mutual substitution in which one species takes the place previously occupied by another and vice versa.

The observed interspecific mycelial interactions between the studied fungi are competitive, since their usual result was either a deadlock (after mycelial contact or at a distance) or (complete or partial) replacement of one fungal species by another. In the *A. niger* co-cultures, deadlock interaction on mycelial contact (A) was the prevailing reaction. In *P. polonicum* co-cultures, the main interactions were deadlock on mycelial contact (A) and fungal replacement (partial – C_{A1} or

complete – C_{A2}) without initial deadlock. Intermingling interactions (a neutral interaction leading to colony fusion or spatial mixing for incompatible genotypes; Dullah 2021b) nor mutualistic interactions were observed in the experimental fungal pairs. These reactions observed here are similar to those previously observed for other higher fungi and microscopic test fungi (Badalyan et al. 2002, 2004, 2008, Borhani et al. 2011, Owaid 2017, Erwin et al. 2018, Chan-Cupul et al. 2019, Krupodorova et al. 2021).

In general, the studied macromycetes showed moderate activity against the studied micromycete species, as 11 and 6 of the species showed active and moderate antagonism, respectively. However, results revealed higher antagonistic activity of macromycetes against *P. polonicum* (total AI = 74) than in the case of *A. niger* co-culture (total AI = 67.0). Our findings indicate the potential of certain macromycetes to be used as biological agents to manage opportunistic pathogens. Based on the AI values, wood-destroying fungi of the species *Ganoderma lucidum* and *Trametes versicolor* were identified as the most active. From a practical point of view, these fungal species require further research into the primary and secondary metabolites which can inhibit or suppress the growth of opportunistic micromycete pathogens and can be applied in vivo to achieve effective control of fungal diseases caused by *A. niger* and *P. polonicum*. The white-rot fungus *T. versicolor* is quite an active competitor, showing inhibitory effects on plant pathogenic fungi such as *Verticillium* sp. and *Pythium* sp. (Owaid 2017) and on vexatious wood-decay fungi *Phlebia radiata* (White et Boddy 1992), *Coniophora puteana* and *Laetiporus sulphureus* (Owens et al. 2014). Also, a very strong competitive effect was found for *Ganoderma lucidum* in dual culture with *Clonostachys rosea*, *Trichoderma pseudokoningii*, *T. viride* (Badalyan et al. 2002), *Bipolaris sorokiniana*, *Gaeumannomyces graminis* var. *tritici*, *Fusarium culmorum*, *Rhizoctonia cerealis* (Badalyan et al. 2004).

Active species of the current study *C. schevczenkoi*, *F. fomentarius*, *G. applanatum*, *L. edodes* and *P. ostreatus* have also been found effective against *Issatchenkia orientalis* and *Candida albicans* strains in similar experiments (Krupodorova et al. 2021). In this context, among the 17 species of xylotrophic fungi tested by Badalyan et al. (2002, 2004), *P. ostreatus* exhibited the strongest competitive ability against pathogenic fungi of cereals and mycoparasitic fungi such as *Trichoderma*. Some of our results are in agreement with those showing inhibition of *A. niger* growth under the action of extracts of *P. ostreatus* (Gerasimenya et al. 2002), *L. edodes* (Hearst et al. 2009), *G. lucidum* (Heleno et al. 2013), *T. versicolor* (Okull et al. 2003) and *M. esculenta* (Shameem et al. 2017). The effectiveness of extracts of *G. lucidum* (Heleno et al. 2013) and *T. versicolor* (Pranitha et al. 2014) has been demonstrated in the case of *Penicillium* spp.

Interaction of fungi can lead to various modifications of the organisms involved. Visible signs of interaction between paired fungi can appear after mycelium

contact such as changes in colour and colony morphology, in particular production of aerial hyphae, formation of fungal cords, massed mycelial fronts or zones of mycelial lysis, and pigment production. But in this experiment, except for a couple of cases, no significant changes in the morphology of the studied fungi were observed, in contrast to the results obtained from macromycetes grown in co-culture with *Issatchenkia orientalis* or *Candida albicans* strains (Krupodorova et al. 2021). Formation of different pigments has been described from *L. edodes* colonies in double culture with *Verticillium* sp. and *Pythium* sp. (Owaid 2017), colonies of *P. ostreatus* and *L. shimeji* paired with *C. albicans*, and from cultivation of *F. fomentarius* with *I. orientalis* and *C. albicans* strains (Krupodorova et al. 2021).

In co-cultures, the intricacy of possible interactions (either positive or negative) should be taken into account. Therefore, this study can be a guide to discovering possible fungal species interactions in nature, but these should be further studied in the habitat. It should be borne in mind that modelling conditions may favour a certain type of antagonism, but under the influence of other experimental or environmental conditions, the types of fungal interactions may differ.

In general, the ability of fungi to interact with other fungi is inherent to the fungal genome, and can be activated under certain cultivation conditions. Any morphological changes in the fungal colony as well as exudate formation are a response to the fact that co-cultivation leads to a significant increase in the production of key metabolites and/or accumulation of active compounds which are not found in the axenic cultures of the producing strain (Marmann et al. 2014) and may be the result of antifungal and/or fungistatic activity or a response to stress factors (Luo et al. 2017).

CONCLUSION

Studying fungal interactions by screening a large number of macromycetes (31 species) for antagonistic activity against two potentially harmful micromycetes *Aspergillus niger* and *Penicillium polonicum* remains relevant as the first stage in identifying macromycetes with high activity. The type and strength of the interaction is highly dependent on the species of macro- and micromycetes used. Although the co-cultures in this study were carried out under laboratory conditions whereas in their habitat also different abiotic and biotic factors play a role, this research offers a preliminary, yet rapid way to examine and predict how the tested higher fungi will interact with pathogenic fungi. As a number of fungi were investigated in this study for the first time, the obtained results expand the current knowledge and confirm a certain antagonistic/antifungal potential of the studied macromycetes. It should be noted that the study of antagonistic activity

of strains of macromycetes and micromycetes which have not yet been studied can provide unexpected results concerning strain-dependence of fungus properties, as demonstrated by our work and that of other authors. Wood-decaying species *Ganoderma lucidum* and *Trametes versicolor* were the most active reaching the highest antagonism index. These fungi are promising species for further isolation and study of metabolites which may be applied in the creation of new, safer and more environmentally friendly fungicides or fungistatics capable of fighting *Aspergillus niger* and *Penicillium polonicum*.

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