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RESEARCH ARTICLE
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Evaluation and Identification of Paper-destructive Micromycetes and Paper Resistance

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ABSTRACT

Historical and cultural documents housed in museums and libraries under inappropriate conditions are vulnerable to fungal contamination. Among the fungi posing a threat to these valuable collections, micromycetes can be noted. Molecular methods offer a means to identify these fungal contaminants, thus protecting these deposits. Antifungal disinfectants can avoid fungal growth on such a paper substrate. The aim of the present study was to detect micromycete fungal contamination in paper samples and the air within library book depositories. The findings led to the identification of 24 micromycetes using biochemical tests. Sterile 50×50 mm filter papers were inoculated with fungal spores, whereupon the fungi used the paper as the only source of carbon and energy. Micromycete growth activity on paper was determined using silica gel saturated with water. The quantification of living cells present on the paper surface was determined using intracellular ATP levels. In addition, the antifungal activity of Sanatex Universal and Rocima was measured as a solution for the problem of fungal paper deterioration. The antifungal activity of these two biocides was determined using the disk diffusion method. A total of 24 micromycetes strains indicating cellulase activity were selected. The results showed that micromycetes grew on paper, utilizing it as the only source of carbon and energy. Ten most active micromycetes were selected based on the fungal activity growth, fouling area, cellulase activity, and the amount of ATP formed during growth. These micromycetes could be used as paper and cardboard fungal resistance standard test cultures. This study revealed the two fungicidal compounds of Sanatex Universal and Rocima may be used to treat cultural heritage sites affected by cellulose-destroying fungi. A comparative analysis of 24 standardized reference strains of micromycetes revealed their ability to develop on paper and utilize it as their sole carbon and energy source.

Keywords: Bio-damage, Cellulose, Cellulase activity, Fungicides, Micromycetes.

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INTRODUCTION

Collections of historical and cultural documents stored in museums, libraries and archives under unfavorable storage conditions are vulnerable to microscopic fungi. Therefore, it is of significant importance to identify micromycetes endangering cultural objects and personnel and to conduct comprehensive studies on their properties to prevent damage to library materials. Such endeavors will yield crucial insights into the presence of opportunistic species in the air of storage facilities. Paper bio-destructor fungi are found in all storage facilities. These include the genera *Alternaria*, *Asergillus*, *Chaetomium*, *Cladosporium*, *Mucor*, *Penicillium*, *Rhizopus*, *Stachybotris*, *Stemphylium*, and *Trichoderma* (Chadeganipour et al., 2013; García et al., 2014; Zerek, 2014; Popikhina and Velikova, 2016). Particular attention is paid to cellulolytic species isolated from paper, namely *Penicillium chrysogenum*, *Aspergillus niger*, *A. oryzae*, *Mucor racemosus*, *P. aurantiogroseum*, *P. commune*, *P. citrinum*, *P. chrysogenum*, *P. decumbens* and *Penicillium implicatum* (Borrego et al., 2010; El Bergadi et al., 2014).

In instances where evident damage is observed on books, and traditional sowing methods fail to detect viable fungi, the simultaneous application of molecular methods and light microscopy is effective (Michaelsen et al., 2010). Employing this methodology, the following fungal species were identified: *Bjerkandera adusta*, *Cordyceps sinensis*, *Fusarium lateritium*, *Gloeotinia temulenta*, *P. miniolueum*, *Polyporus brumalis*, *Saccharicola bicolor*, *Trichoderma citrinoviride*, and *Ulocladium cucurbitae* (Rakotonirainy et al., 2007). Additionally, rare species of *Chromelosporium carneum, Phlebiopsis gigantea,* and *Toxicocladosporium irritans* were identified (Mesquita et al., 2009). Fungi often found on documents included *Alternaria alternata, Cladosporium cladosporioides, Epicoccum nigrum, P. glabrum, P. spinulosum* (Paiva de Carvalho et al., 2016). For the first time, *Chalastospora gossypii* and *Trametes ochracea* were identified on paper, which caused biological damage to historical manuscripts (Karakasidou et al., 2018).

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In case of significant damage to documents, employing disinfection methods with water-soluble fungicides can effectively prevent further fungal growth in conditions of high humidity, thus ensuring the safety of restorers and researchers. This leads to complete inhibition of fungal growth (Golovina and Kuznetsova, 2008; Trepova and Velikova, 2009). Therefore, there is a need to conduct a comprehensive study of each fungicide to assess its efficacy and safety. In case positive results are obtained confirming that these drugs do not harm library materials, they can be recommended for document processing (Velikova et al., 2011). In addition to chemicals, physical treatment methods can be used. For disinfection, high-frequency cold plasma is used in a nitrogen atmosphere with a temperature not exceeding 50°C, after which the most resistant fungi, such as *Penicillium* and *Alternaria*, die within 60 seconds (Dunca et al., 2014).

The purpose of the current study was to identify the most active cellulose-degrading micromycetes using various biochemical methods and to assess their growth ability on paper substrates. This investigation aims to facilitate the utilization of these fungi in evaluating the fungal resistance of library materials. Additionally, the study intends to provide recommendations for the use of fungicides to prevent bio-damage to documents in libraries and archives.

MATERIALS & METHODS

Cellulase activity was studied in 197 strains, 157 of which were isolated from the surface of documents (paper) and 40 from the air of library book depositories. The assessment of cellulase activity was conducted using the reaction between the Congo Red dye with the polysaccharide. This reaction resulted in the enlightenment zone around the fungal colonies. That are producing cellulases that diffuse into the agar (Trepova et al., 2023).

The micromycetes activity from a set of test cultures was assessed by the parameters below:

- by the area fouling by micromycetes on the paper,
- by cellulase activity, and
- by the amount of ATP during the micromycetes fouling on the paper.

Quantifying Micromycetes' Cellulase Activity using the Mendels-Weber Method

The Mendels-Weber method is based on the quantitative determination of reducing sugars (RS) formed due to the hydrolysis of filter paper cellulose under the action of the cellulase complex enzymes in micromycetes. These enzymes act on the β-1,4-glycosidic bond of sucrose, which is determined under standard conditions at 50°C.

The amount of RS formed as a result of the enzymatic reaction was determined by a colorimetric method based on the interaction of sugars with the Somogyi-Nelson reagent. Somogyi-Nelson method relies on the interaction of carbonyl groups of reducing sugars with divalent copper ions and an Arsene-molybdenum reagent (Nelson reagent) to form a blue color. The intensity of this blue color is determined colorimetrically at a wavelength of 610 nm. One unit of integral cellulase activity (1 unit of CA) is the number of enzymes that catalyze the hydrolysis of 50 grams of filter paper cellulose with the formation of 1 µmol (0.00018g) of reducing sugars in glucose equivalent in one hour at a temperature of 50°C and pH of 5.0. Cellulase activity was determined using following formula:

$$CA = 5{,}56 \times RS$$

Where, CA denotes cellulase active and RS is the amount of reduced sugar.

Sterile filter papers sized 50×50 mm were used as a food source for the micromycetes. This paper was inoculated with an aqueous suspension of fungal spores at a concentration of 1-2 million/cm^3. The number of micromycete spores in the inoculated suspension was counted in a Goryaev chamber. The samples were kept in conditions optimal for fungal development (at a relative air humidity of 90-99%) for 21 days. Each micromycete was grown on paper and placed in Petri dishes over substrates made of a layer of sterile silica gel saturated with sterile distilled water. This allowed the relative air humidity inside the Petri dishes to be maintained at 90-95%.

Fungi develop solely due to nutrients in the paper. Micromycetes' growth rate was determined visually by paper fouling area percentage. The experiment was carried out in five replicates for each strain. Sterile paper samples were used as controls. A preliminary experiment was carried out to confirm the ability to maintain relative air humidity at the required level. Sterile-moistened silica gel and a mini-thermohygrometer were placed in Petri dishes to continuously monitor air parameters for three weeks. During the entire experiment, the average relative air humidity was 85-99%. Once a week, the silica gel was moistened with sterile distilled water under sterile conditions to avoid drying out.

Determination of ATP Level

The level of intracellular adenosine triphosphate (ATP) of living cells located on the paper surface was determined by the amount of luminescence generated by the biochemical reaction of luciferin (substrate) oxidation with atmospheric oxygen under the action of luciferase (enzyme) only in the presence of ATP. Luminescence was measured on a Lumitester PD 10 device using the LuciPac W reagent. The degree of contamination of the sample was determined using following formula:

$$\Delta RLU = RLU - RLU_{sterile}$$

Where, $RLU_{sterile}$ is the luminescence of a sample of the sterile test material, RLU signifies the luminescence of a contaminated sample, ΔRLU determines the absolute value of luminescence, characterizing the contamination of the material (Trepova and Velikova, 2016).

Determination of Fungicidal Efficacy

The present study analyzed the biocidal effects of drugs on cellulose-degrading micromycetes. Sanatex Universal (Tikkurila LLC) and Rocima GT (The Dow Chemical Company) were aqueous drug solutions. These two drugs have been comprehensively studied for their harmlessness to documents, with no adverse effect on paper whiteness and strength. Moreover, they have been endorsed for use in libraries and archives to treat documents affected by fungi (Velikova et al., 2011). The paper was treated with Sanatex Universal at 1% wt. and a solution of Rocima GT at 2% wt. These concentrations were previously defined as minimum inhibitory concentrations on paper (Trepova and Velikova 2019). As test cultures were used, 26 strains of the most active micromycetes species destroyed the library samples (Trepova et al., 2023).

To determine the resistance of microscopic fungi to biocides, the disk diffusion method was used to determine the fungicidal effects (Disk diffusion method EUCAST, 2020; 2021 was used for assessing sensitivity to antimicrobial drugs). The technique is based on assessing the diameter of the growth inhibition zone around a paper

disk with a fungicide applied to a culture of the microorganism growing on a solid nutrient medium. A growth inhibition zone occurred due to drug diffusion from the disk into the nutrient medium. A larger diameter of the micromycete inhibition zone indicates greater fungicidal activity of the drug (El Bergadi et al., 2014).

To prepare a spore suspension, fungal cultures were used for 14 days, counting from reseeding the museum cultures onto the Czapek-Dox medium with agar. A suspension of micromycete spores was prepared under GOST 9.048-89 (Slepukhina et al., 2019). When determining the fungicidal properties of biocidal preparations, the concentration of micromycete spores in water was adjusted using the Goryaev chamber to 1.0-2.0 million/cm^3.

In the next step, 0.5 ml of an aqueous suspension of micromycete spores was applied to the surface of the Czapek-Dox solid nutrient medium in Petri dishes. Filter paper disks with a diameter of 25 mm, impregnated with fungicides in the appropriate concentration (1% or 2% w/w), were placed on the surface of the inoculated medium. The dishes were kept at a temperature of 29 ±2 °C. After 7 and 14 days, the zone of fungal growth inhibition was measured. The inhibition zone was determined as the average distance from the edge of the sample to the growth boundary of micromycetes with a measurement error of 0.5 mm. Paper disks not treated with drugs were used as controls. The tests were performed in triplicate.

Statistical Analysis

The fungal communities were analyzed considering the abundance and frequency of occurrence of isolated species using the ANOVA one-way procedure. The abundance of a particular species was defined as the percentage composition of the CFU per gram of a particular species relative to the total CFU per gram of all species obtained. The frequency of occurrence of a particular species was defined as the percentage composition of the number of samples in which a particular species occurred relative to the total number of all obtained samples. Than compared using Sørensen-Czekanowski similarity coefficient (Ks') using formula:

$$K_s' = \frac{2\sum_{i=1}^{p} \min(x_{Ai}, x_{Bi})}{\sum x_{Ai} + \sum x_{Bi}}$$

Where, p is the number of taxa, x_{Ai} determines tge abundance of species i in samples A (plaster samples), and x_{Bi} denotes the abundance of species i in samples B.

Furthermore, fungal communities from building materials were evaluated by the Simpson diversity index (D) which was calculated according to the formula:

$$D = 1 - \sum_{i=1}^{S} x_i^2$$

Where, x_i signifies the relative abundance of the ith species, and S refers to species richness. Simpson diversity index close to one indicates high diversity (Kiel and Gaylarde, 2007).

RESULTS AND DISCUSSION

The fouling areas on paper samples were determined for each micromycete strain (as a percentage of the sample area (Table 1).

Fouling of paper, which serves as the only source of carbon and energy, exceeded 60% of the surface in five species of micromycetes, namely *Aspergillus niger*, *A. sydowii*, *A. ustus*, *Penicillium jenseni*, and *P. canescens.* In another five species of *Chaetomium globosum*, *P. aurantiogriseum*, *P. lanosum*, *Talaromyces funiculosus*, and *Trichoderma aureoviride* fouling ranged from 22% to 44%. Three fungi, namely *A. niger*, *A. sydowii*, and *P. jenseni*, showed active growth already on day 7 and the fouling surface reached 25-48%. Notably, the extent of fouling varied significantly among different strains of the same species, for instance, fouling reached 92% in some strains of *P. aurantiogriseum* and *P. canescens* and 65% in *Trichoderma koningii* strains. Some fungal species detected in the present study are comparable to the species reported in the previous studies (Ljaljević-Grbić et al., 2013; Borrego et al., 2015).

Table 1: Fouling area of paper due to the micromycetes growth

Micromycetes	Strain	Fouling area, %		
		7 days	14 days	21 days
Aspergillus clavatus	F-1738	0	9.6	13.4
Aspergillus niger	F-1742	48	62	78
Aspergillus sydowii	F-1688	40.4	42.4	73
Aspergillus ustus	F-1745	2.6	65	84
Chaetomium globosum	F-1695	1	8.6	22.4
Cladosporium cladosporioides	F-1697	2.2	4.6	4.6
Cladosporium sphaerospermum	F-1663	0.4	3.2	7
Didymella glomerata	F-1672	2.6	4.4	5.4
Dipodascus geotrichum	F-1717	0.8	1.6	2.0
Penicillium brevicompactum	F-1703	0	0	11.6
Penicillium aurantiogriseum	F-1747	1.0	1.4	2.0
Penicillium aurantiogriseum	F-1748	2.2	5.0	26.4
Penicillium canescens	F-1750	0.8	3.4	5.4
Penicillium canescens	F-1752	5.4	13.4	60.0
Penicillium chrysogenum	F-1754	0.8	6.4	11.0
Penicillium glabrum	F-1758	9.4	24.0	15.0
Penicillium jenseni	F-1761	25.0	84.0	88.0
Penicillium lanosum	F-1762	5.0	31.0	44.0
Penicillium simplicissimum	F-1767	0	5.4	14.0
Talaromyces funiculosus	F-1771	0.4	14.0	34.0
Trichoderma aureoviride	F-1772	1.0	13.0	24.0
Trichoderma koningii	F-1715	4.0	14.0	14.0
Trichoderma koningii	F-1774	0	16.0	41.0

Table 2: Integral activity of the cellulase complex components of micromycetes and ATP

All-Russian collection industrial microorganism	Strain	Micromycetes	QR *	M-V**	ATP, ΔRLU
F-1738	M3	*Aspergillus clavatus*	3+	0.03503	4143
F-1742	B7	*Aspergillus niger* van	0	0.04682	2658
F-1688	X8	*Aspergillus sydowii*	3+	0.05310	1203
F-1745	K17	*Aspergillus ustus*	2+	0.00967	7337
F-1695	X18	*Chaetomium globosum*	3+	0.03342	550
F-1697	X21	*Cladosporium cladosporioides*	1+	0.02619	436
F-1663	M5	*Cladosporium sphaerospermum*	3+	0.03102	114
F-1672	M14	*Didymella glomerata*	2+	0.02157	379
F-1717	K53	*Dipodascus geotrichum*	2+	0.10525	2323
F-1703	K28	*Penicillium brevicompactum*	2+	0.03342	3865
F-1747	X26	*Penicillium aurantiogriseum*	3+	0.02068	550
F-1748	X30	*Penicillium aurantiogriseum*	3+	0.05671	1143
F-1750	B24	*Penicillium canescens*	2+	0.02430	367
F-1752	X31	*Penicillium canescens*	3+	0.02569	620
F-1754	D7	*Penicillium chrysogenum*	2+	0.03436	154
F-1758	D8	*Penicillium glabrum*	2+	0.02335	4881
F-1761	M10	*Penicillium jensenii*	2+	0.02930	1641
F-1762	B27	*Penicillium lanosum*	1+	0.02318	1846
F-1767	X43	*Penicillium simplicissimum*	2+	0.11070	1065
F-1771	K44	*Talaromyces funiculosus*	3+	0.02035	28541
F-1772	K48	*Trichoderma aureoviride*	0	0.07228	28699
F-1715	K49	*Trichoderma koningii*	0	0.12488	37517
F-1774	K50	*Trichoderma koningii*	0	0.172527	66700
F-1716	K51	*Trichoderma viride*	0	0.13755	28541

*QR – determination of a qualitative indicator of cellulase activity. **M-V – determination of a quantitative indicator of cellulase activity using the Mendels-Weber method.

Table 3: Micromycetes growth inhibition zone and fouling of the paper surface treated with fungicides on the seventh day of cultivation.

Micromycetes	Strain	Strain All-Russian collection industrial microorganisms	Inhibition zone, mm		Surface fouling, %	
			Sanatex Universal (1%)	Rocima GT (2 %)	Sanatex Universal (1%)	Rocima GT (2 %)
Aspergillus clavatus	M3	F-1738	20±2	25±2	4±1	0
Aspergillus niger	B7	F-1742	0,0	0,0	56±3	30 ±1
Aspergillus sydowii	X8	F-1688	34±3	27±2	0	0
Aspergillus ustus	K17	F-1745	22±1	24±3	3 ±0	2±0
Chaetomium globosum	X18	F-1695	35±2	27±,8	0	0
Cladosporium cladosporiodes	X21	F-1697	33±3	25±0	0	0
Cladosporium sphaerosperum	M5	F-1663	45±3	24±0	0	1±0
Didymella glomaerata	M14	F-1672	24±1	29±2	0	0
Dipodascus geotrichum	K53	F-1717	45±2	24±0	0	0
Microascus brevicaulis	M-6	F-1664	24±2	24±1	0	0
Penicillium aurantiogriseum	X26	F-1748	26 ±1	0,0	0	0
Penicillium brevicompactum	K28	F-1703	35±2	27±0	0	0
Penicillium canescens	X31	F-1752	38±2	27±1	0	0
Penicillium chrysogenum	D7	F-1754	34±0	23±1±	0	±
Penicillium expansum	M9	F-1757	45±3	24±0	±	±
Penicillium glabrum	D8	F-1758	33±2	25±1	0	0
Penicillium jensenii	M10	F-1761	27±1	30±2	0	0
Penicillium lanosum	B27	F-1762	40±2	25±1	0	0
Penicillium ochrochloron	B31	F-1763	30 ±2	26±1	0	0
Penicillium simplicissimum	X43	F-1767	35±0	30±0	0	0
Talaromyces funiculosus	K44	F-1771	29±0	24 ±1	0	0
Trichoderma aureoviride	K48	F-1772	0	0	83 ±3	10±1
Trichoderma koningii	K49	F-1715	0	0	100±5	56 ±2
Trichoderma viride	K50	F-1774	0	30 ±2	1±0	0

All fungi of the genus *Trichoderma*, *Penicillium simplicicicium*, *Penicillium aurantiogriseum*, and *Dipodascus* showed the greatest activity, and *Rhizopus stolonifera* indicated the least activity. Eight micromycetes showed fairly high cellulase activity of 0.05-0.17 (Table 2). The high activity of *Penicillium* species observed in the present study was compatible with the results of a similar study (El Bergadi et al., 2013). However, the differences in the species detected could be due to parameters, such as different experimental conditions.

Penicillium simplicissium and fungi of the genus *Trichoderma*, in which no cellulase activity was detected in the clearing zone, showed very high activity. The effects of two fungicides on 24 fungal cultures were studied. Through cultivating micromycetes on a medium with paper disks treated with fungicides, it was observed that both Sanatex Universal at a concentration of 1% and Rocima GT, had a fungicidal effect on almost all studied types of micromycetes by day 7 (Table 3).

Table 4: Growth inhibition zone of micromycetes and fouling of the paper surface treated with fungicides on the 14th day of cultivation

Micromycetes	Strain	Strain All-Russian collection industrial microorganisms	Inhibition zone, mm		Surface fouling, %	
			Sanatex Universal (1%)	Rocima GT (2 %)	Sanatex Universal (1%)	Rocima GT (2 %)
Aspergillus clavatus	M3	F-1738	0	21±1	6±0	0
Aspergillus niger	B7	F-1742	0	0	0	0
Aspergillus sydowii	X8	F-1688	29±2	26 ±2	0	0
Aspergillus ustus	K17	F-1745	20±1	24±0	10±1	5±1
Chaetomium globosum	X18	F-1695	0	19±1	0	0
Cladosporium cladosporiodes	K21	F-1697	29±1	24±1	4 ±0	3±0
Cladosporium sphaerosperum	M5	F-1663	20±0	22±0	14±1	3±0
Didymella glomaerata	M14	F-1672	21±2	29 ±2	4±1	0
Dipodascus geotrichum	K53	F-1717	42±3	20 ±1	0	14±1
Microascus brevicaulis	M6	F-1664	0	24±0	0	0
Penicillium aurantiogriseum	X30	F-1748	25±1	0	0	0
Penicillium brevicompactum	K28	F-1703	28±1	27±1	30±0	0
Penicillium canescens	X31	F-1752	33±2	26±1	0,0	0
Penicillium chrysogenum	D7	F-1754	9±1	23±1	62±3	4±1
Penicillium expansum	M8	F-1757	30 ±0	23±0	2 ±0	0
Penicillium glabrum	D8	F-1758	27±1	25±0	1±0	0
Penicillium jensenii	M10	F-1761	28±3	29±2	2±0	0
Penicillium lanosum	B27	F-1762	28±2	21±1	0	0
Penicillium ochrochloron	B31	F-1763	28±1	26±1	1±0	0
Penicillium simplicissimum	X43	F-1767	29±2	27±2	0	0
Talaromyces funiculosus	K44	F-1771	22±2	24±1	0	0
Trichoderma aureoviride	K48	F-1772	0	0	10±2	12±1
Trichoderma koningii	K49	F-1715	0	0	10±2	20±2
Trichoderma viride	K51	F-1774	0	0	15±1	17±1

Table 5: Micromycete strains for determining paper and cardboard fungal resistance and their characteristics (average values)

No. Microcet		Strain		*I_{CA}	CA	ATP, ΔRLU	$S_{AofF.}$,%
		Russian National Library	Strain All-Russian collection industrial microorganisms				
1	*Aspergillus clavatus*	M-3	F-1738	+++	0.04	2 294	12
2	*Aspergillus niger*	B-7	F-1742	—	0.05	737	78
3	*Aspergillus sydowii*	X-8	F-1688	++	0.05	823	69
4	*Aspergillus ustus*	K-17	F-1745	++	0.01	1 849	85
5	*Chaetomium globosum*	X-18	F-1695	+++	0.03	341	22
6	*Dipodascus geotrichum*	K-53	F-1717	++	0.11	747	2
7	*Penicillium aurantiogriseum*	X-30	F-1748	+++	0.06	885	26
8	*Penicillium canescens*	X-31	F-1752	+++	0.03	550	60
9	*Talaromyces funiculosus*	K-44	F-1771	+++	0.02	18 231	34
10	*Trichoderma koningii*	K-50	F-1774	—	0.17	20 823	41

*I_{CA} cellulase activity index; determination of cellulase activity by the lysis zone during growth on NaCMC: «++» I_{CA} ranges from 6 to 10; «+++» – I_{CA} is above 10; No lysis zone observed. CA: Determination of cellulase activity by the amount of reducing sugars formed during micromycetes growth on paper. Using the Somogyi-Nelson method (Zeltina et al., 1991). ATP: Determination of micromycete growth activity on paper based on ATP value. SA_{ofF}: The area of paper fouling when cultivating micromycetes for 21 days.

With Sanatex, the maximum inhibition zone on day 14 was 42 mm for *Dipodascus geotrichum*. It was 29 mm for *Didymella glomaerata* and *Penicillium jensenii* under Rocima GT treatment. On day 14, the control samples had no inhibition zone. The surfaces of the samples were covered with spore-bearing mycelium on average by 97% (Table 4). The fungicidal effects observed for the agents used in the present investigation are in accordance with the results of some studies reporting the use of fungicides as promising agents for the conservation of paper artifacts (Meng et al., 2023).

Of the investigated micromycetes species, Sanatex Universal indicated a greater fungicidal effect, compared to Rocima GT on 15 species. Specifically, Sanatex Universal demonstrated superior effectiveness over Rocima GT for three types of fungi, namely *Penicillium jensenii*, *Aspergillus ustus*, and *Didymella glomaerata*.

No fungicidal effect was observed with Sanatex Universal at a concentration of 1% for several micromycetes, including *P. aurantiogriseum*, *Trichoderma viride*, *T. koningii*, *T. aureoviride*, and *Aspergillus niger*. Similarly, with Rocima GT at 2% concentration, there was no fungicidal effect was observed for *T. koningii*, *T. aureoviride*, and *A. niger*. Consequently, higher concentrations (2% and 3%, respectively) were used to achieve fungicidal effects for these micromycetes. Both Sanatex Universal and Rocima GT effectively affect micromycetes with cellulase activity and can be used to treat cultural heritage sites.

For comparative assessment, Table 5 presents the average values of all parameters (lysis index, amount of reducing sugars, ATP value, and degree of paper fouling). Ten most active micromycetes were selected based on the obtained data. These strains can be used as test cultures to determine paper fungal resistance.

Conclusion

As a result of a comparative analysis of 24 standardized reference strains of micromycetes, it was found that most micromycetes can develop on paper and consume it as the only source of carbon and energy. Based on a comparative analysis of fungal growth activity on paper using four methods (determination of cellulase activity by the lysis zone when growing on NaCMC, cellulase activity by the amount of reducing sugars, by measuring the amount of ATP, and by the area of fouling of paper when growing on moist silica gel), 10 most active micromycetes were selected. They can be used as test culture standards for determining the fungal resistance of paper and cardboard. Sanatex Universal and Rocima GT preparations can be utilized to treat cultural heritage sites affected by active cellulose-destroying fungi.

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