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Scopulariopsis sp. and *Fusarium* sp. in the Documentary Heritage: Evaluation of Their Biodeterioration Ability and Antifungal Effect of Two Essential Oils

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Abstract Fungi produce pigments and acids, generating particular local conditions which modify the physicochemical properties of materials. The aims of this work are (i) to investigate bioadhesion, foxing production and biofilm formation by *Scopulariopsis* sp. and *Fusarium* sp. isolated from document collections under laboratory conditions; (ii) to verify attack on cellulose fibres and (iii) to study the possibility of reducing fungal growth using natural products. Biofilm formation and extracellular polymeric substance (EPS) production by fungi were demonstrated in laboratory assays and by scanning electron microscopy (SEM) observations. The biocidal activity of two essential oils of *Origanum vulgare* L. and *Thymus vulgaris* L. was evaluated using the microatmosphere method. SEM observations showed that these strains were able to attach to paper and form biofilms, causing damage on them, which demonstrates the biodeterioration ability of these microorganisms. *Scopulariopsis* sp. and *Fusarium* sp. isolated from paper books showed the formation of fox-like reddish-brown colour spots, attack to the paper structure and pigment production on aged paper samples. The strains tested produced a decrease in the pH of one unit. This would substantiate the effect of the strains in paper biodeterioration. The microatmosphere method showed that volatile compounds of the essential oils have antifungal activity.

Keywords Biodeterioration · Fungi · Microatmosphere method · Paper

Introduction

Materials deposited in archives, libraries and museums, e.g. papers, textiles, ceramics, metals and paintings, are exposed to the effects of physical, chemical and biological factors [1–6]. Paper is frequently affected by the microbial excretion of pigments and acids, causing spots of different colours which have been associated with a process called foxing [7–9]. This term only vaguely describes the size, shape and colour of certain stains, of which interpretation may depend on each viewer's subjectivity [10]. Although their origin is debatable, it has been found that microorganisms begin to adhere to the substrate in those areas and attack the paper by releasing and excreting pigments and acids, causing the appearance of reddish-brown spots [11].

Microorganisms are the cause of serious forms of damage, such as weakening of paper, loss of structure and discoloration on the surface [7–9, 12–14]. The identification and the use of microscopy to investigate materials and fungi involved in this process are useful techniques for the understanding of the effects on materials of heritage significance [15–17]. Similarly, it is important to elucidate the functional properties of these microorganisms and their role in biodeterioration processes [18]. The microorganisms begin to grow in these areas, developing into a biofilm and producing biodeterioration. A recent article [19] has demonstrated that microbiological attack by *Bacillus* sp. results in the

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formation of micropits (2–3 μm in diameter), while *Scopulariopsis* sp. degrades cellulose fibres. It refers to a chemical-physical attack by biological activity, which in this case is of fungal origin. It also involves physical pressure from hyphae on cellulose fibres. This leads to a complete disintegration of the fibre.

When biofilms attach to materials of documentary heritage (e.g. paper, photographs and magnetic and optical media), social and cultural damage is irreparable and there is a loss of valuable information. The deterioration is a function of the physicochemical composition of the support and the environmental conditions associated with microorganisms. The moisture content of a material, also called water activity (A_w), is one of the most important factors in microbial growth. Many species of fungi and bacteria begin developing as a function of the moisture content on the surface of an object. Fungi are the most harmful microorganisms to paper as they have the ability to grow at lower A_w than bacteria. It has been reported that most microorganisms can grow in the A_w range of 0.6–0.98 [20]. Poulsen and Lindelow [21] indicated that a value of 0.75 allowed fungal growth. According to Florian [22], bacteria require A_w above 0.95, while fungi need a lower A_w (commonly 0.70–0.85). However, bacteria are able to develop on this type of substrate when environmental conditions are not suitable for the conservation of materials [11].

Methods used to prevent biodeterioration should consider the growth inhibition of organisms or their metabolic activity and the modification of the environmental characteristics. In recent years, there has been a renewed interest by scientists in the use of natural products (natural extracts and essential oils) for their antibacterial and antifungal properties. However, of several reported studies, only a few mention their possible use in the field of conservation of cultural properties [23–26]. Natural products are a viable alternative to the use of toxic chemicals and other environmental pollutants, with advantages from the environmental, economic and ecological standpoints. The antimicrobial properties of essential oils have been known for many centuries, and a great variety of methods have been used to study them. In agar diffusion methods, some compounds of essential oils do not demonstrate antimicrobial activity because of their volatility. The so-called microatmosphere method was developed for the assessment of essential oil activity in the vapour phase [27].

The aims of this work are (i) to investigate bioadhesion, foxing production and biofilm formation by *Scopulariopsis* sp. and *Fusarium* sp. isolated from document collections under laboratory conditions; (ii) to verify attack on cellulose fibres; (iii) to study a possible reduction in environmental conditions of two fungal strains using volatile compounds of natural products.

Materials and Methods

Materials deposited in the Archive of Historical and Cartographic Research Department from the Geodesy Direction, the Ministry of Infrastructure Buenos Aires Province (AHCR) and the Historical Archive of the Museum of La Plata (HAMLP), located in La Plata City, Buenos Aires Province, Argentina, were investigated. Environmental microbiological sampling and isolation of microorganisms in these archives have been recently reported [11, 19]. The temperature (T) and relative humidity (RH%) were measured in the repositories. Standard reference points were T maximum and minimum 22 and 15 °C, respectively, and RH% 65 and 45, respectively [28].

Documentary Heritage Analysed

Deteriorated nineteenth-century photographs, books and maps were examined with a stereoscopic microscope (Nikon SWZ-10). On the basis of aesthetic/structural damage on the surface, the following documents were chosen for further studies: three photographs: two photographic papers (P1 and P2) and one on a glass slide (P3) from HAMLP and two books (B1 and B2) and one map (M1) made of paper from AHCR.

Microbiological Sampling of Documentary Heritage and Isolation of Microorganisms

Samples (1 cm^2) from the surface of the documents were taken using sterile cotton swabs [29, 30]. The cotton swabs were submerged in 1 mL of sterile distilled water and vortexed, and decimal dilutions were performed. Suitable dilutions of each sample were inoculated onto YGC agar (Yeast Extract Glucose Chloramphenicol Agar, Merck®, Germany) for the isolation of moulds and yeasts, and incubated at 28±2 °C for 7 days [31]. The number of colony-forming units (CFU) was determined after 7 days of incubation.

Identification of Fungi

Cultural and morphological characteristics of fungal colonies were observed by optical microscopy (Olympus BX51) and genera identified according to manuals and taxonomic keys [32, 33].

Laboratory Assays: Bioadhesion, Biofilm Formation and Foxing

Scopulariopsis sp. and *Fusarium* sp. were selected for laboratory studies because of their ability for bioadhesion, pigment production and viability and growth on cellulose fibres. Prior to laboratory assays, isolated fungal strains from documentary heritage were seeded on a solid medium containing sodium

Table 1 Measurements of environmental parameters in AHCR and HAMLP

Archive	Temperature	RH%
AHCR	18.7±1.6	50.1±5.2
HAMLP	19.7±1.4	50.6±5.1

nitrate 2 g; dipotassium phosphate 1 g; magnesium sulphate 0.5 g; potassium chloride 0.5 g; yeast extract 0.5 g; ferrous sulphate 0.01 g and agar 20 g per 1 L; pH=5.5. A strip of aged sterilized filter paper 4.8×1 cm, corresponding to 25 years of aging (72 h at 105 °C), was used as sole carbon source in a slant containing solid medium. The control was the same medium with the addition of 1 % glucose and without the filter paper strip. The inocula were 10^7 conidia mL^{-1} in saline solution counted with a Petroff-Hausser camera. Cultures were incubated at 28±2 °C for 21 days.

Selected strains were seeded in a slant containing solid mineral medium (pH 5.5) and incubated for 7 days, with a strip of aged sterilized filter paper as the sole carbon source. The strip was removed and sonicated (5 min) in a mineral liquid medium (pH 5.5). After sonication, the pH of the mineral medium was measured (Hanna Instrument pH Meter HI 9321).

Both strains were also deposited in pure culture collection at the Biodeterioration Group of the Research Institute of Theoretical and Applied Physical Chemistry (INIFTA).

Monitoring of Bioadhesion and Biofilm Formation by Scanning Electron Microscopy

Samples from original documents were observed by SEM (Jeol 6360LV). Paper strips from the laboratory bioadhesion assays with isolates of *Scopulariopsis* sp. and *Fusarium* sp. from documents were also monitored to observe bioadhesion to paper and attack on cellulose fibres. Samples were kept in a closed chamber with pure ethanol for 24 h and metalized with Au/Pd prior to observation.

Table 2 Fungal count in documents

Document	Material	Location	Fungi (CFU cm^{-2})
Photograph 1 (P1)	Photographic paper	AHMP	$1.03\times10^2\pm15$
Photograph 2 (P2)	Photographic paper	AHMP	-
Photograph 3 (P3)	Glass slide	AHMP	$9.37\times10^2\pm65$
Book 1 (B1)	Paper	AHCR	2±1
Book 2 (B2)	Paper	AHCR	8±3
Map 1 (M1)	Paper	AHCR	4±1

- below the detection limit

Table 3 Fungal genera isolated in different samples

Microorganism	B1	B2	M1	P1	P2	P3
Aspergillus niger	X		X	X		
Aspergillus flavus	X		X	X		
Penicillium sp.				X		X
Fusarium sp.		X				
Scopulariopsis sp.		X	X			
Cladosporium sp.		X				
Alternaria sp.	X	X	X			
Non-sporing isolated (mycelia sterilia)						X

X presence of fungal species

Evaluation of Antifungal Activity: Microatmosphere Method

The essential oils were provided by the Food Industry Research, Havana Cuba. All were extracted by hydrodistillation using Clevenger-type apparatus. The antifungal activity of *Origanum vulgare* L. and *Thymus vulgaris* L. against *Scopulariopsis* sp., *Fusarium* sp. was assayed by the microatmosphere method under laboratory conditions. These strains were seeded in Petri dishes on agar medium and incubated upside down. Essential oils were placed on a commercial disc of 0.5 cm diameter (Britania Lab® s.a.) in the middle of the cover. Dishes were sealed with Petrifilm®. The assay was performed in triplicate. The volatile phase of essential oils exerts its inhibitory effect on the tested microorganisms [27, 34, 35]. Some of the volatile compounds (terpenes, sesquiterpenes) have poor water solubility and cannot be evaluated by agar diffusion methods [27, 35]. A commercial sterile disc moistened with 10 μL of pure essential oil was

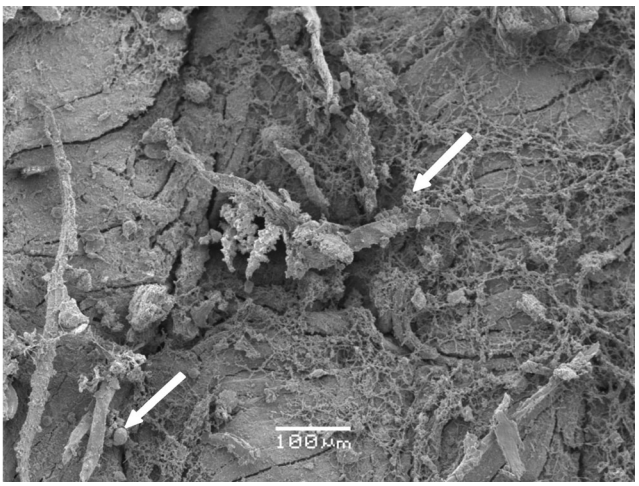

Fig. 1 SEM image of photograph (P1) with biofilm formation. *Arrows* showing fungal structures (hyphae and conidia) and particulate matter (dirt)

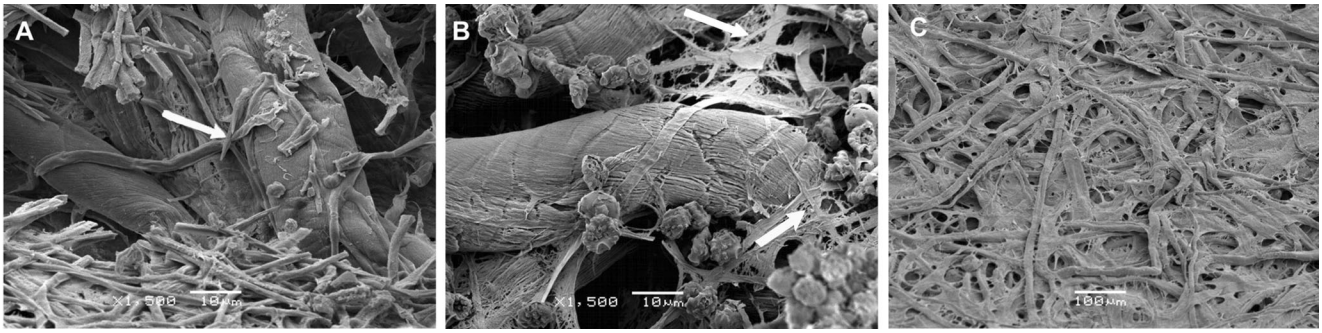

Fig. 2 SEM images of biofilm formation after 7 days of incubation: **a** *Fusarium* sp., *arrow* showing hypha attached to cellulose fibres, and **b** *Scopulariopsis* sp., *arrows* showing conidia interspersed with deteriorated cellulose fibres. **c** Control paper

attached to the lid of the Petri dish. The base, containing the culture medium seeded uniformly with the fungal strain (the inoculum was 10^7 conidia mL^{-1} in saline solution), was inverted over the lid containing the commercial disc. Petri dishes were incubated at 28±2 °C for 15 days. Dishes were controlled after 3, 7 and 15 days of incubation. Control plates with commercial discs moistened with ethanol (10 μL) were used.

Result and Discussion

The environmental parameters of AHCR and HAMLP are similar and can be observed in Table 1. Fungal adhesion to documentary substrata showed viable fungi isolated from the documents. Counts showed in Table 2 depended on the chemical composition of the substrate being analysed (photographs, paper, glass slide). It is known that the majority of the fungal genera isolated from archives, libraries and museums exhibit cellulolytic, proteolytic and/or amylolytic activities; produce acids; excrete different pigments and contribute to the formation of biofilms, which accelerate the deterioration of the different documentary substrata [36–38].

Seven different genera were isolated from the documents (Table 3). The *Aspergillus* genus was predominant and *Aspergillus niger* and *Aspergillus flavus* were isolated from documents B1, M1 and P1. Colonies of *Penicillium* sp., mycelia sterilia (non-sporing isolated), *Cladosporium* sp., *Scopulariopsis* sp., *Fusarium* sp. and *Alternaria* sp. were isolated in the different substrata.

Regarding the original documents, particulate matter (dirt) and fungal conidia interspersed with the cellulose fibres of the material were observed in the photograph (P1) from AHMLP (Fig. 1).

S*copulariopsis* sp. and *Fusarium* sp. were able to adhere to paper, using it as the sole carbon source. *Scopulariopsis* sp. has remained viable for more than 36 months without renewal of the culture medium. Also, *Scopulariopsis* and *Fusarium* genera are common etiological agents causing onychomycosis by filamentous fungi, which would be a risk for personnel that work in archives, libraries and museums [39].

Production of pigments [40] by the fungi, with consequent aesthetic damage, and a marked difference in colour of the pigments between the two strains were demonstrated on filter paper strips in laboratory cultures. *Fusarium* sp. and *Scopulariopsis* sp. were able to attach to aged paper after incubation, develop a biofilm and cause a generalized attack on the paper. Figure 2 shows degraded cellulose fibres and extracellular polymeric substance (EPS) on the paper. An SEM image demonstrates the biodeterioration ability of these microorganisms, but the biodeterioration was unlike the micro-pitting in cellulose fibres caused by *Bacillus* sp., recently reported by Lavin et al. [19]. The generalized attack and micro-pitting [15, 17, 41] should be measured in real time using advanced techniques such as those applied in the field of biodeterioration of cultural heritage [42–44]. The tested strains produced a decrease in the pH of one unit (from 5.5 to 4.5) which would constitute a hazard for the cellulose fibres from paper. Organic acids (acetic, citric, lactic, gluconic, glucuronic,

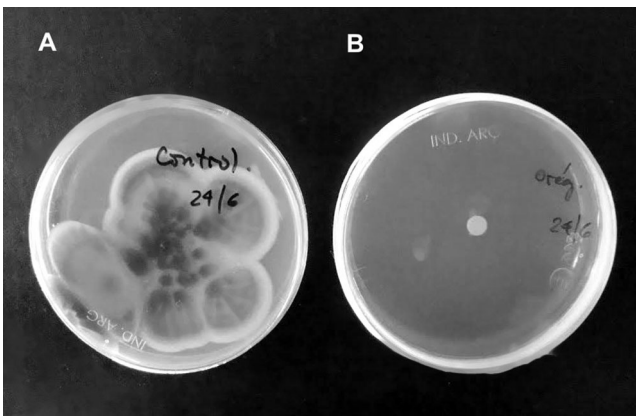

Fig. 3 Microatmosphere method testing: **a** *Scopulariopsis* sp. control and **b** antifungal activity of *Origanum vulgare* L

fumaric, oxalic) interfere with the support components, modifying their chemical and physical properties [40, 45].

In all cases, the microatmosphere method showed that volatile compounds of the two essential oils tested had antifungal activity, inhibiting the total growth of both tested strains. The results showed clearly that the vapours of the compounds under study reduced or stopped temporarily the growth of *Scopulariopsis* sp. and *Fusarium* sp. Figure 3 shows the antifungal activity of volatile compounds of *O. vulgare* L. against *Scopulariopsis* sp. after 7 days of incubation.

Although there are many publications on the study of volatile products extracted from plants, there are only a few scientific publications on their possible exploitation in the field of the preservation of cultural properties [46]. Our investigations would contribute to technological applications of the essential oils to reduce environmental fungal load and minimize biodeterioration risks.

Conclusions

- *Scopulariopsis* sp. and *Fusarium* sp. isolated from documentary heritage are able to form biofilms, produce pigments, cause foxing (corresponding to reddish-brown spots) and decrease pH in one unit, with consequent aesthetic and structural damage, constituting a hazard for the documentary heritage.
- The microatmosphere method showed that volatile compounds of *O. vulgare* L. and *T. vulgaris* L. had antifungal activity against both tested fungal isolates.
- This research presents alternative antifungal products that offer a high level of activity and low environmental impact and are safe for both human health and museum, archive and library collections.

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