Jan A. Wendt[1]
Liliana Indrie[2]
Paula Dejeu[3]
Adina Albu[2]
Dorina Camelia Ilieş[4]
Monica Costea[5]
Tudor Caciora[4,*]
Gabriela Ilieş[6]
Nicolaie Hodor[7]
Ioana Josan[4]
Zharas Berdenov[8]
Vasile Grama[4]
Bahodir Safarov[9]

Natural Sources in Preventive Conservation of Naturally Aged Textiles

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Abstract
Natural antimicrobials can eliminate fungi and prevent the aging of cotton fabrics. While fungi can cause severe infections to the fabric user etc, natural antimicrobials have the advantage of not being toxic to humans. The present study showed that the essential oils of lemon (Citrus limon), lavender (Lavandula angustifolia) and mint (Mentha piperita) have inhibitory effects on yeast and mould spores on a piece of textile from Romanian cultural heritage, "ie", stored in a space within an ethnographic museum., inhibitory action against Botrytis sp., the inhibitory effect of lemon essential oil on Cladosporium sp. and that of peppermint essential oil on yeast species Rhodotorula mucilaginosa were demonstrated, respectively. Being environmentally friendly, these sources, can be tested on a large scale.

Key words: *essential oils, cotton, antibacterial agents, health, prevention and conservation.*

[1] **Gdansk University, Faculty of Oceanography and Geography, Institute of Geography,**
4 Bażynskiego Street, 80309, Gdansk, Poland, e-mail: jan.wendt@ug.edu.pl

[2] **University of Oradea, Faculty of Energy Engineering and Industrial Management, Department of Textiles, Leather and Industrial Management,**
4 Barbu Stefanescu Delavrancea Street, 410058, Oradea, Bihor County, Romania, e-mail: lindrie@uoradea.ro, aalbu @uoradea.ro

[3] **Medical Laboratory service M.D., Bethany Medical Clinic Oradea,**
12 Menumorut Street, 410004, Oradea, Bihor County, Romania, e-mail: office@betania-centrumedical.ro

[4] **University of Oradea, Faculty of Geography, Tourism and Sport, Department of Geography, Tourism and Territorial Planning,**
1 Universitatii Street, 410087, Oradea, Bihor County, Romania, e-mail: dilies@uoradea.ro, tudor.caciora@yahoo.com, ijosan@uoradea.ro, vgrama@uoradea.ro

[5] **University of Oradea, Faculty of Environmental Protection,**
26 General Magheru Boulevard, 410035, Oradea, Bihor County, Romania, e-mail: mcostea @uoradea.ro

[6] **Babes-Bolyai University, Sighetu Marmației extension,**
6 Avram Iancu Street, 435500, Sighetu Marmației, Maramures County, Romania, e-mail: gabriela.ilies@geografie.ubbcluj.ro

[7] **University "Babes-Bolyai" Cluj Napoca, Faculty of Geography,**
Street Clinicilor no 5-6, Cluj Napoca, Cluj County, Romania, e-mail: nicolaie.hodor@ubbcluj.ro

[8] **L. N. Gumilyov Eurasian National University, Department of Physical and Economic Geography, Faculty of Science,**
2 Satpayev Street, 010008, Nur-Sultan, Republic of Kazakhstan, e-mail: berdenov-z@mail.ru

[9] **Samarkand State University, Faculty of Human Resources Management, Department of Digital Economy,**
Samarkand, Uzbekistan, 40104, University blv. 15, Samarkand, e-mail: saforvb@rambler.ru

Introduction

Among the many elements of tangible cultural heritage specific to the Beius Depression, Romania, the traditional women shirt called 'IA' stands out. It is a part of the cultural heritage with implications for shaping place-specific identity [1-4]. 'IA' is also an indisputable historical landmark which provides information about the people who made and wore such garments, their social status, the level of knowledge and understanding of the universe in which they lived and worked, etc. [5-7].

Museums, especially ethnographic museums with their clothes, fabrics, throws, scarves, etc., classic for the region, play an important role in education and preserving the original cultural heritage of each country and region [8]. Therefore, the preventive conservation of naturally aged textiles is of fundamental importance for the protection and preservation of the material heritage of the region. The high collections presented are also important in shaping regional tourist centers [9] and the potential for cultural heritage marketing [10-12] influencing the choice of destinations preferred by tourists.

Textiles are exposed to many challenges, such as indoor temperature and relative humidity fluctuations, light impact, insects, dust and particulate matter, non-standard storage and display, housekeeping methods, very poor restoration and conservation etc [13].

In this context, the present study aimed at non-invasive analysis in order to biore-mediate an 'ia' clothing element located in the depository of the Beius Municipal Museum (***Figure 1***). The importance of the study lies in the need to preserve and protect such elements of tangible heritage of national and even international importance and of inestimable value. Antiquity together with time and the simultaneous action of some factors related to microclimate, microorganisms and the 'human' represent causes that endanger the integrity of such textile creations. Hence, the need arises to carry out interdisciplinary studies (by conservators, chemists, microbiologists, geographers, etc.), as non-invasively as possible, in order to preserve these artefacts for posterity.

An assessment of contamination and the effects of essential oils on potentially existing fungi on the surface of the material was conducted. The microscopic appearance of threads in the area with the most damaged macroscopic appearance, precisely the inner left fibre, was further evaluated. Microscopic images are shown in ***Figure 1***.

The microscopic images (x 20; x 40) (***Figure 1***) of threads taken from the inner left sleeve respectively show the deterioration of their quality. Due to single broken fibres, the arrangement of fibres visible under the microscope is disordered. Fibres do not form a bundle, indicating their location of mechanical damage (left picture). Visible is a change in its arrangement, probably under the influence of chemical factors [14]. Several cracks oblique to the fibre axis can be observed, and there are cracks propagating from the constriction in both directions of the fibre (***Figure 1***, right down).

Literature review

Hammer et al. [15] tested 52 natural vegetal oils and plant extracts, showing very good antimicrobial and preservation activity. Mimica-Dukic et al. [16] analysed the antimicrobial activities of essential oil from *Mentha* Sp; Bozin et al. [17, 18] displayed the antioxidant and antimicrobial activities of essential oils such as *Ocimum basilicum* L., *Origanum vulgare* L., and *Thymus vulgaris* L., as well as *Rosemary, Sage* (*Rosmarinus officinalis* L. and *Salvia officinalis* L., *Lamiaceae*). Abdel-Kareem [19] tested the prevention/inhibition of biodeterioration and the improvement of textiles properties using polymers in combination with biocides, the results of which being among the best. The effects of cleaning materials containing natural dyes were also tested for silk fabric stored in unsuitable conditions in the Islamic Museum [20]. Mahesh et al. [21] studied antimicrobial textile finish using natural plant products. Four species of Laminaceea: *Pogostemon cablin*, *Lavandula angustifolia*, *Melissa officinalis*, and *Salvia officinalis*, native to Pakistan, were tested by Hussain et al. [22], and showed antioxidant action. Some biotreatments and fungal washing of heavy metals in wastewaters were examined by Gupta et al. [23]. The paper of Pannu [24] analysed and proposed natural solutions for antimicrobial finishes.

Lavender, thyme, and clove essential oils are extracted from natural sources and used in the treatment of cotton fabrics for the attainment of antibacterial characteristics and giving-off of a good smell [25]. Gutarowska et al. [1] mentions in her research paper the short disinfection effect of essential oils; however, most importantly, the tests conducted were environmentally friendly. In Romania, Iordache et al. [26] showed the great potential of *Rosemary* and *Orange* oils for obtaining very good antimicrobial textile effects [27]. Hercules and Papadopoulou [28] analysed the antimicrobial proprieties of *Basil, Oregano*, and *Thyme* essential oils. Nazzaro et al. [29] describes the antifungal properties of essential oils, as well as their importance in the communicative inhibition of the cellular system, in the creation of biofilms, and in the production of mycotoxins. Andra et al. [30] obtained crude extract from *Pangamic pinnate* and evaluated its antibacterial activity against *E. coli*, upon being coated on cotton fibre. Elsayed and Shabana [31] studied the effects of some essential oils on the fungi contamination (*Aspergillus niger* and *Alternaria alternate*) of archaeological heritage objects. Othman et al. [27] concludes in his/her study that the essential oils tested can be successfully used for fungicidal preparations for the disinfection of biodeteriorated linen and papyrus artefacts. Pereira et al. [32] ``demonstrated that the phenolic compounds in olive leaves have antimicrobial action (*Olea europaea L. Cv. Cobrançosa*)", even in lower concentrations. Stan et al. [33] and Thilagavathi and Bala [34] mention the use of the microencapsulation of essential oils in textiles with promising antioxidant and antibacterial results.

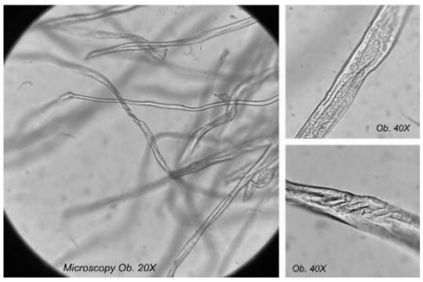


Figure 1. *Microscopic assessment of the quality of twisting fibres in the inner left fibre.*

Material and method

To assess the presence of viable fungi on the surface of an examined area (***Figure 2***) before and after the application of essential oils, the following materials were required: a delimiting frame, sterile swabs, sterile distilled water, three essential oils with antifungal effects [35-40], namely *Lavandula angustifolia* – lavender, *Mentha piperita* – mint and *Citrus limon* – lemon [39],glass containers with a sprayer, Saboruaud and Csapek-Dox sterile culture media, a microbiological hood, an incubator, glass slides and slides, microbiological handles, KOH dissociative solution, an optical microscope, an API® 20C AUX kit for yeast identification, a densitometer, and a 10 volumetric automatic pipette -100μL.

Working technique

Three areas of dimensions of 5/5 cm were examined, as shown in ***Figures 2*** and ***3***: inner left sleeve, medial-upper right face, and left shoulder.

Figure 2. *Traditional Romanian "ie".*

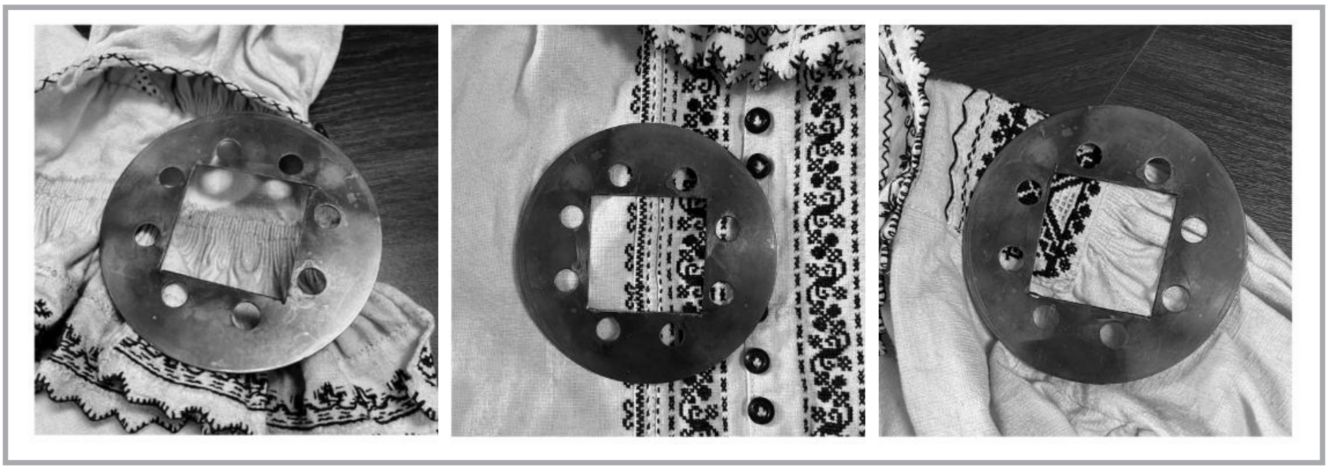


Figure 3. *Areas examined (from left to right: left inner sleeve, right medial-upper front, left shoulder).*

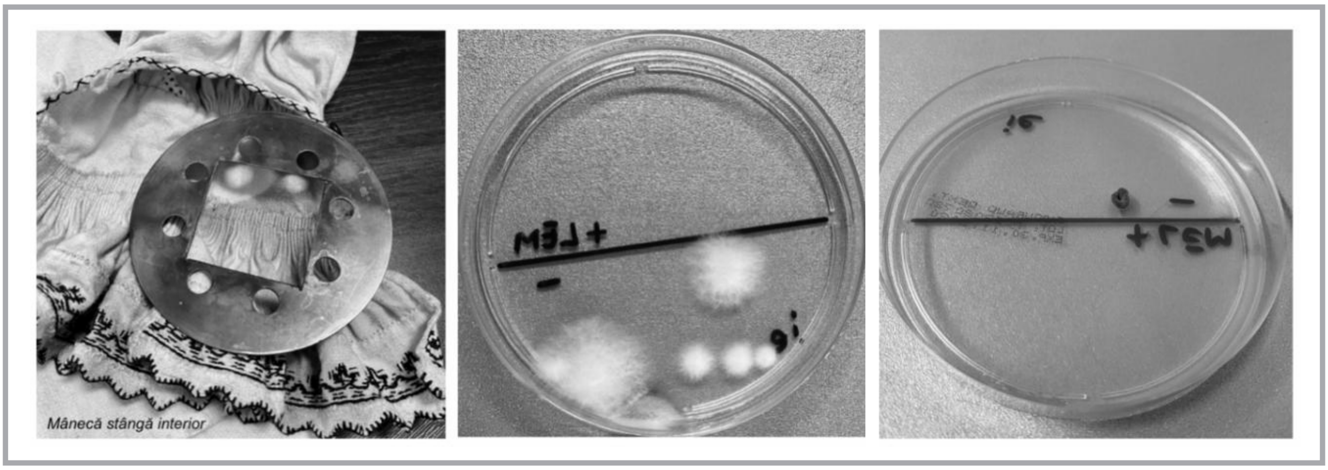


Figure 4. *From left to right: the area examined (inside the left sleeve), the Csapek-Dox environment and Sabouraud environment.*

The sampling step was performed using sterile swabs. The size of the parts examined was 5/5 cm in accordance with the delimiting frame. Samples were taken before and after 15 minutes from the application of essential oils according to ***Table 1***.

5 drops (150 µL) of each essential oil were applied to the corresponding area of the fabric. To make their application as accurate as possible, glass containers with a sprayer were used, in which 50 drops of sterile distilled water, the equivalent of 7.5 mL, were previously added. After application, a break of 15 minutes was made before the second set of samples was taken. The samples were seeded on Sabouraud and Csapek-Dox culture media. Petri dishes with the seeded culture media were incubated for 7 days at 28 °C. After 3 days of incubation, fungal colonies became macroscopically visible. On the seventh day, three different types of colonies could be identified: two as a mould species and one as a yeast species . Identification of the fungi was performed by evaluation of macroscopic and microscopic characteristics in the case of moulds, respectively, and with the use of the API® 20 C AUX6 kit for identification of the yeast species. API® 20 C AUX principly evaluates the biochemical characteristics of yeasts by following 19 assimilation reactions. In the microwells of the API gallery, 100 µL of fresh yeast suspension calibrated at a turbidity of 2 McFarland was pipetted. Incubation was performed at temperatures of 29 °C ± 2 °C, and readings done after 48h and 72h by comparing each with the negative control. On the results sheet, the positive reactions are denoted by +, corresponding to the digit 1, and the negative ones by –, corresponding to the digit 0. The final result obtained at 72 h is a 7-digit numerical profile interpreted using ApiwebTM computer software.

Table 1. *Areas of traditional cloth "ie" examined and essential oils applied.*

Area examined	**Essential oil applied**
Area examined	Essential oil applied
Left inner sleeve	*Citrus limon* (lemon)
Upper medial face	*Lavandula angustifolia* (lavander)
Left shoulder	*Mentha piperita* (mint)

Results and discussions

All fungal colonies were developed from samples before the application of essential oils. ***Figures 4***, ***5*** and ***6*** represent the areas examined and fungal colonies developed on the culture media.

Two different kinds of mould (*Cladosporium* sp. and *Botrytis* sp.) and a yeast species (*Rhodotorula mucilaginosa*) de-

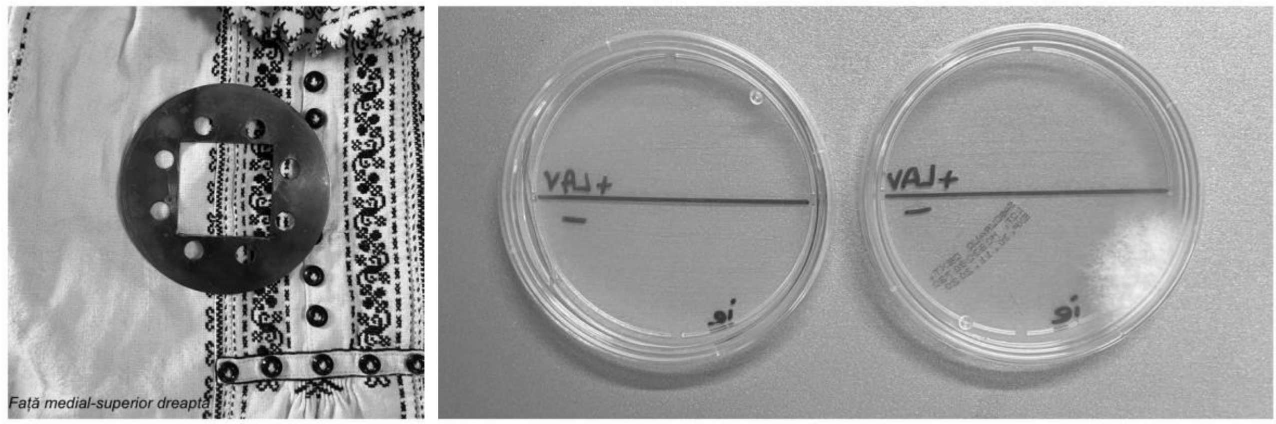


Figure 5. *From left to right: area examined (medial-upper right front), Csapek-Dox environment and Sabouraud environment.*

Figure 6. *From left to right: area examined (left shoulder), the Csapek-Dox environment and Sabouraud environment.*

veloped on the surface of the Petri dishes [41]. The different moulds identified and the effect of the essential oil on them are shown in ***Table 2***.

Since *Cladosporium* belongs to mould fungi, its spores are particularly troublesome for allergy sufferers, and its presence in the external and internal environment makes it difficult to prevent. However, it develops mainly in the external environment, often on dead plants, but also indoors [42-44]. In addition to moisture, its multiplication is favoured by dust and rare airing, typical of museum exhibitions, despite the use of humidity and air conditioning controls. Hence, due to its frequent presence in exhibits and display cases. *Cladosporiums* has a strong allergic effect [45]. The main symptoms of allergic reactions in the case of skin are most often atopic inflammation, with dry and cracked skin and itching. If it enters the respiratory tract, symptoms may include sneezing, itching of the nasal mucosa, larynx, and dry and

Table 2. *Types of moulds identified.*

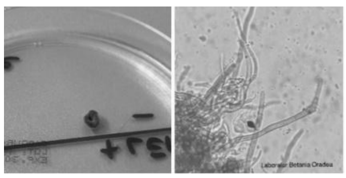
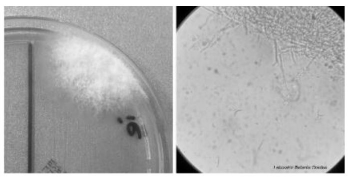
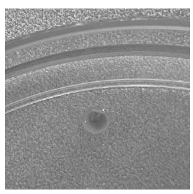
Images	Name	Effect of essential oils
	*Cladosporium* sp.	*Citrus limon* (lemon) – inhibitory effect
	*Botrytis* sp.	*Citrus limon* (lemon) – inhibitory effect *Lavandula angustifolia* (lavander) – inhibitory effect *Mentha piperita* (mint) – inhibitory effect

Table 3. *Yeast species identified*

Images	Name	Effect of essential oil
	*Rhodotorula mucilaginosa* – profil API: 6642272	*Mentha piperita* (mint) – inhibitory effect

irritating cough. Allergic effects on the mucous membranes of the larynx cause a dry cough, and in the case of the mucous membranes of the eyes, the effect is often conjunctivitis on the rim of the eyelids, discharge from the eyes etc.

More than ten years ago *Rhodotorula* fungi, due to the increasing number of immunocompromised patients, was classified by some researchers as pathogens [46]. This is owing to the high resistance to some medicines and the high adaptability of this species. In humans, it primarily causes bloodstream infections, but hypersensitivity pneumonitis is also possible [47]. Risk factors increasing its presence and activity include solid and haematologic malignancies in patients who receive corticosteroids and cytotoxic medicines, the presence of CVC, and the use of broad-spectrum antibiotics [48].

Unlike *Cladosporium* and *Rhodotorula*, fungi of the *Botrytis* species are not reported as pathogenic in humans. However, in the case of people with primary or acquired immunodeficiency and structural lung disease, they may experience systemic fungal infections [49]. This may apply to staff working in museum rooms, at exhibitions, and in places where fungi of the *Botrytis* family have good conditions to thrive. In such cases, systemic symptoms such as weight loss and/or a recurrent cough, shortness of breath etc. appear [50-52]. *Botrytis* in immunocompromised people, such as patients with the hepatitis C virus, AIDS, and organ transplant patients, can cause local infections, causing pathologies of the eye, brain, heart, peritoneum, or onychomycosis. It is highly resistant to conventional and most modern antifungal agents when its concentration is elevated or the body's resistance is low [53, 54].

From the present study, we can state that the essential oils of lemon (*Citrus limon*), lavender (*Lavandula angustifolia*) and mint (*Mentha piperita*) have inhibitory effects on yeasts and mould spores. As well as this, the inhibitory action of lemon, lavender and mint essential oils against *Botrytis* sp that of lemon essential oil against *Cladosporium* sp. and that against the yeast species *Rhododtorula mucilaginosa* were demonstrated.

Conclusions

The research conducted allowed to identify the main species of developing fungi in the material studied: *Cladosporium, Rhodotorula* and *Botrytis*. The first two are definitely pathogenic, while *Botrytis*, which is generally not hazardous to health, may show pathogenic features in selected, specific cases. As museum rooms and exhibitions are characterised by specific climatic conditions, also generated by CVC, they often become a place for fungal growth, especially on plant-based materials, such as cotton. Due to the time of creation of the exhibit (fabrics) as well as the storage and climatic conditions in the exhibition rooms, microorganisms (fungi, yeasts etc) often develop there.

Fungi can easy contaminate clothing exhibits stored in museums and improper exhibition halls. They are one of the factors favouring the process of damage to the fabrics from which they are made.

The fungi detected on the samples, depending on the species, may cause many allergic reactions and serious diseases. They are primarily exposed to museum workers who stay in rooms with fungal spores for several hours a day. Yeasts can also be dangerous to eldery people or those which have a weakened immunological system.

Bearing in mind these risks, and all of the above, to try and prevent them, testing with essential oils should be implemented on a large scale, which, as shown by the results of the research, can be considered natural agents for biodesinfection to preserve museum exhibits and are non-toxic for humans.

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