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Research Article

Antifungal Activity of Vinegar on Different Types of Artistic Rear Canvases

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Abstract

In the present research the antifungal activity of Vinegar in comparison to the commercial biocide Preventol® RI80, liquid formulation of quaternary ammonium salts, on different types of artistic rear canvases was assessed. Indeed, these surfaces as well as other artistic surfaces, are colonized by fungi and the growth on rear canvases can lead to their diffusion in the front side. As first, the minimum inhibitory concentration and the minimum fungicidal concentration (MIC and MFC) of the compounds against *Aspergillus niger* ATCC 9642, *Penicillium citrinum* LS1 and *Cladosporium cladosporioides* ATCC 16022 was determined and, successively, the protecting effect of biocide and Vinegar was assessed applying them on the surface of different rear canvases pieces experimentally contemned with the selected fungal strains. MIC values of Vinegar ranging from 0.75% to 1.5% (*A. niger* ATCC 9642) and those of Preventol® RI80 from 0.25% to 0.5% (*A. niger* ATCC 9642); the fungicidal activity of Vinegar ranged from 1.5% to 3% (*A. niger* ATCC 9642), while Preventol® RI80% showed lower MFC value (1 - 1.5%). The observation after 7 days of incubation of the test samples of the canvases treated with the Vinegar (3%) and Preventol® RI80 (2%) solutions, indicated that the application of the Vinegar limited the growth of the tested microorganisms around the rear canvases pieces similarly (growth delay rating between 1 and 2), and in some cases, more efficiently than the biocide (growth delay rating between 1 and 2). The observed differences between the canvases can be attributed to the specific features of the textile that could also have affected the growth of the selected fungi, with the prevalent growth of *P. citrinum* LS1, a mold ubiquitous in the environment. Overall, the presented results indicate that Vinegar was able to limit the growth of the selected fungi as well as the commonly used biocide Preventol® RI80. For this, the Vinegar can be suggested as a natural antifungal agent on particular and limited areas of substrate materials, such as the considered rear canvases, to prevent the growth of filamentous fungi and their following diffusion on the front side of canvas itself. The possible association of natural Vinegar with a commercial biocide to obtain a "green detergent solution" final product could be further investigated.

Keywords: *Rear Canvases; Vinegar; Antifungal Activity; Growth Delay*

Introduction

The organic component of the textile support of canvas paintings provides useful material for fungal and bacterial growth, supporting microbiological alteration processes under different environmental conditions [1]. Microorganisms act autonomous activities to metabolize artworks and biodeterioration can occur in different ways depending upon the type of metabolic activity, mainly either heterotrophic or autotrophic. In addition, the nature and composition of the organic materials of canvas paintings, the composition of the textile support, the agglutinants, pigments and protection layers of the substrate play an important role in this context.

The action of bacteria and fungi is recognized by the appearance of color spots and texture changes that significantly modify the pictorial layers [2]. Particularly, the pigmentation of mycelia, such as in the case of *Dematiaceae*, is responsible to remarkable aesthetic superficial damage than other species [1]. The heterogeneity of the observed alteration is one of the most important characteristics of canvas painting biodeterioration, due to the diversity of substances and materials employed in the artwork. Indeed, some filamentous fungi can dissolve cellulose fibers through the action of cellulolytic enzymes, decolor supports, and degrade glue, pigments and binders. In addition, these microorganisms can hydrolyze collagen fibers and other proteinaceous materials, modifying inorganic components and causing pigmentation and organic acid production [3].

Nowadays there aren't specific treatments to protect canvas paintings from biodeterioration, thus it's necessary to refer to studies performed in other fields of artistic heritage. Generally, the restoration has been carried out with organic materials that were added to the original constitution of the piece, as occurs in the treatment of textile support reinforced with flour glue [2]. As methodological approach, two methods to prevent the microbial growth can be proposed, the maintaining of the artwork in controlled environmental conditions (i.e. low humidity and adequate temperature) or the treatment with fungicide or bactericidal agents.

Biocides, chemical compounds able to kill undesirable organisms, still remain the most used practical solution [4,5], even if commercial and traditional biocides may be dangerous for human health and the environment. Indeed, some biocides such as hydrogen peroxide, can oxidize metal ions, leading to corrosion of minerals and causing rust or black stains and chlorine-containing compounds are avoided in this field for the recognized interactions with different materials [6].

Alternative solutions to address biodeterioration issues are focused on natural biocides, such as essential oils [7,8] or substances derived from plants or other organisms [9], considered safer and eco-friendly. In this direction, our attention was attracted by vinegar, an old and traditional compound widely used in human activities. Vinegar is mostly composed by acetic acid (AA), that have demonstrated effective antimicrobial properties, limiting bacterial and fungal contamination in fresh and post-harvested products [10,11] and recently on surfaces [12].

Aim of the Study

The aim of the present research was to compare the antifungal activity of the commercial biocide Preventol® RI80, based on quaternary ammonium salts, and Vinegar on different types of artistic rear canvases. The experimental design was subdivided in two distinct phases; in the first, the minimum inhibitory concentration (MIC) of the compounds against different fungal strains was firstly determined by micro-dilution method; in the second phase, a comparison of the protecting effect of biocide and vinegar was assessed applying them on the surface of different rear canvases test samples experimentally contemned with selected fungal strains.

Materials and Methods

Vinegar and canvases features

Vinegar was purchased from a local store and contained 6% of acetic acid. Four types of lining rear canvas, routinely used in the restoration of painting canvases, were considered; the samples (herein named as A, B, C and D), kindly furnished by the Restoration Laboratory (University of Urbino Carlo Bo), presented different wefts and consistency (Figure 1) and were preventively subjected to a sanitizing treatment by ultraviolet rays. As control, the biocide Preventol® RI80 used as a disinfectant solution for the elimination of bacteria, mould, algae and lichens from different materials, was used at a concentration of 2% (water-diluted), as indicated for restoration activity.

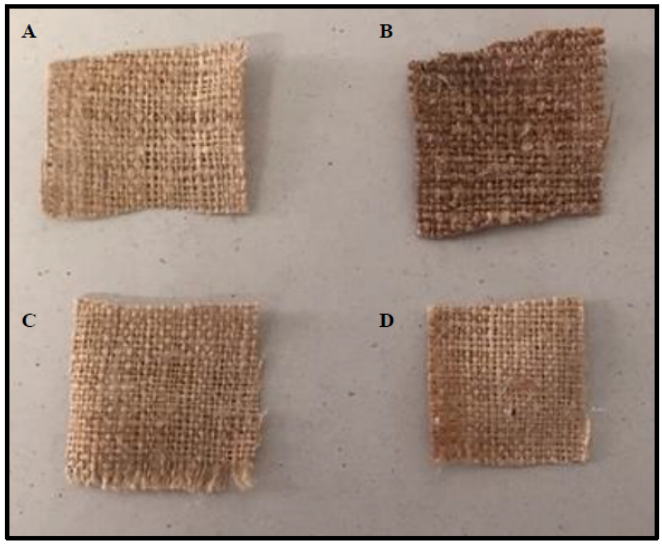

Figure 1: *Pieces of the different rear canvases (A, B, C and D) used in this study.*

Fungal strains and culture conditions

The antifungal activity was performed against three filamentous fungi, belonging to our culture collection, including *Aspergillus niger* ATCC 9642, *Penicillium citrinum* LS1 and *Cladosporium cladosporioides* ATCC 16022. The fungal strains were grown on Potato Dextrose Agar (PDA, Liofilchem, Italy) at 25°C for 7 days.

Inoculum preparation

Fungal suspensions were prepared according to National Committee for Clinical Laboratory Standards [13]. For each strain, the spores were harvested from PDA plate adding 2 ml of sterile 0.85% saline solution; the surface was then scraped with a sterile loop or spatula. The suspension was transferred in a sterile tube and left at room temperature for 5 minutes to allow the sedimentation of hyphal fragments. The upper homogeneous suspension was vortexed for 15 seconds and adjusted to an optical density at 530 nm corresponding to about 10^6 spores/ml. The quantification of each inoculum was verified with the agar plate count method on PDA.

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) determination

MICs were determined following the standard micro-dilution method [13]. Briefly, 100 µl of each fungal suspension, prepared as above described, were diluted 1:50 in standard RPMI 1640 medium (Sigma, Milan, Italy) and inoculated into 96-well plates together with the appropriate volumes of the test solutions (the products were 1:2 serially diluted for 10 times from the initial concentration). Two rows were left for positive control growth and negative controls (no fungi). Plates were incubated at 25°C and examined after 72h of incubation. MIC is defined as the lowest drug concentration that inhibits the visible growth in comparison with the control (untreated sample).

To determine the minimum fungicidal concentration (MFC), from each well showing complete growth inhibition (clear well), the last positive well (growth similar to that of the growth control), and the growth control respectively, 20 µl were removed and streaked on PDA

(Liofilchem). The plates were incubated at 25°C for 7 days; for each microorganism, MFC is defined as the lowest drug concentration that showed either no growth or fewer than three colonies (approximately 99 to 99.5% killing activity).

Assessment of antifungal activity on canvases

The fungal strains, *A. niger* ATCC 9642, *P. citrinum* LS1 and *C. cladosporioides* ATCC 16022, were grown in PDA at 28°C for 5 - 7 days; afterward, the inoculums were prepared as described above to obtain the final concentration of about 10^6 spores/ml. Nutrient Salt Agar (NSA) was prepared with KH_2PO_4 0.7 g/L, $MgSO_4 \cdot 7H_2O$ 0.7 g/L, NH_4NO_3 1 g/L, NaCl 0.005 g/L, $FeSO_4 \cdot 7H_2O$ 0.002 g/L, $ZnSO_4 \cdot 7H_2O$ 0.002 g/L, $MnSO_4 \cdot H_2O$ 0.001 g/L, K_2HPO_4 0.7 g/L, agar 15 g/L (final pH 6.0 - 6.5). The agar was then sterilized by autoclaving at 121°C for 15 minutes, cooled at 50°C and then distributed into plates (90 mm Ø) (VWR). The lining canvases (A, B, C, D) were cut in specimen pieces (2 x 2 cm), kindly posed on the surface of NSA plates and sprayed with 100 µl of Vinegar (3%) or Preventol® RI80 (2%) (Figure 2) and air-dried for 60 minutes. At this point, four plates (A, B, C or D specimen) were inoculated with 75 µl of mixed fungal strains suspension (viability controls); four plates (A, B, C or D specimen) were treated Preventol® RI80 or Vinegar water solution and inoculated with 75 µl of mixed fungal strains suspension and four plates (A, B, C or D specimen) were not inoculated (negative controls). All the plates were incubated at 28°C and after 7 days, the fungal growth was visually evaluated by the naked eye in accordance with ASTM G21-96 [14] standard. The fungicidal activity was then estimated as fungal growth delay on the test specimen surface, using the following visually determined rating (R): R = 0, no visible growth; R = 1, trace of growth (less than 10%); R = 2, light growth (ranging from 10 to 30%); R = 3, medium growth (ranging from 30 to 60%); and R = 4, heavy growth (ranging from 60% to complete coverage) as described in Campana., *et al* [15]. The experiments were performed two times in triplicate.

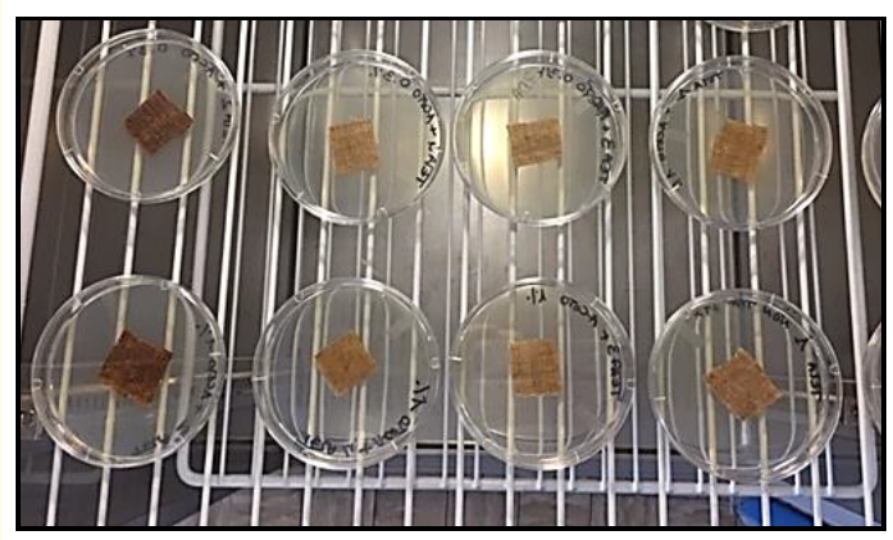

Figure 2: *Samples of the rear canvases (A, B, C and D) placed on NSA plates surface, treated with Vinegar or Preventol® RI80 and incubated at 28°C for 7 days.*

Results and Discussion

There is a great diversity of fungi capable of growing on paint coatings and artistic substrates and, among these, the most frequently detected were *Penicillium* spp., *Aspergillus* spp. and *Cladosporium* spp. [16]. These microorganisms were thus used in the experimental

design of the presented research since it's of great importance to know the surface-associated microbial communities for an effective conservation program of the selected substrate. Indeed, the cellulosic content of canvases may be a favoring factor to its microbial deterioration under particular environmental conditions, such as lack of aeration and humidity fluctuation. Given the lack of specific protocols to protect the artistic canvases from biodeterioration, we decide to evaluate the efficacy of an ancient natural compound, Vinegar, on several types of normally used rear canvases in the artistic field. As first, the MIC and MBC concentrations of Vinegar as well as those of the control solution (Preventol® RI80) on the selected fungi were assessed (Table 1). As shown, MIC values of Vinegar ranging from 0.75% (*P. citrinum* LS1 and *C. cladosporioides* ATCC 16022) to 1.5% (*A. niger* ATCC 9642) and those of Preventol® RI80 from 0.25% (*P. citrinum* LS1 and *C. cladosporioides* ATCC 16022) to 0.5% (*A. niger* ATCC 9642). In term of fungicidal activity, the MFCs values of Vinegar resulted to be 1.5% for *P. citrinum* LS1 and *C. cladosporioides* ATCC 16022, reaching 3% in the case of *A. niger* ATCC 9642. As expected, the commonly used biocide Preventol® RI80 showed lower MFC values (1 - 1.5%) for all the examined fungi. Based on these preliminary data, the artificially contaminated rear canvases pieces were treated with 3% of Vinegar solution or 2% of Preventol® RI80. The observation of the samples after 7 days of incubation evidenced that the application of Vinegar was able to limit the growth and the diffusion of the tested microorganisms around the canvases pieces (Figure 3), even if some differences appeared. Indeed, in the samples A, a very lower number of colonies (R = 1), mostly of *P. citrinum* LS1, were visible compared to the untreated control sample (R = 4) (Figure 3A); moreover, this antimicrobial effect was similar to that exerted by the standard biocide Preventol® RI80 for all the examined fungi (R = 1). This feature is also observed in the samples B and C, with a reduction of fungal growth (only few colonies of P*. citrinum* LS1) induced by Vinegar (R = 1). Instead, the growth reduction by Preventol ® RI80 in test samples B and C corresponded to R = 3 and R = 1 respectively (Figure 3B and 3C). In the last analysed samples (D), the effect of Vinegar (R = 2) resulted quite similar to that of Preventol® RI80 (R=1), compared to the untreated control sample (R = 4) (Figure 3D).

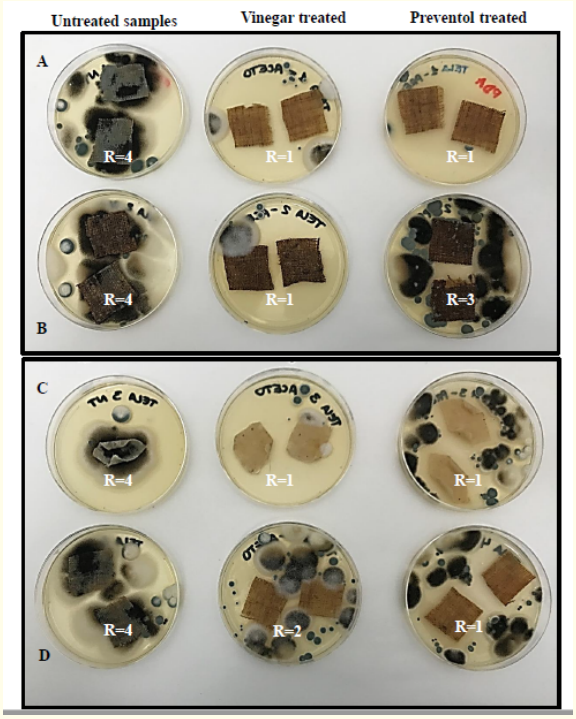

Figure 3: *Visual observations of the Vinegar inhibitory growth effect against fungal strains on the surface of the different rear canvases (A, B, C and D) after 7 days of incubation, in comparison to the Preventol® RI80 treated samples and the untreated controls.*

Strains	Vinegar (6%)	Preventol® RI8(2%)
	MIC/MFC	MIC/MFC
A. niger ATCC 9642	1.5/3	0.5/1.5
P. citrinum LS1	0.75/1.5	0.25/1
C. cladosporioides ATCC 16022	0.75/1.5	0.25/1

Table 1: *Antifungal activity of vinegar in comparison to Preventol® RI80. Data are expressed as MIC and MFC (%, v/v).*

The application of aqueous solutions and water-based systems is generally discouraged on water-sensitive substrates because the swelling phenomena induced by water absorption in these hydrophilic materials can lead to mechanical stress. However, the application of aqueous systems in a limited area is successfully used for the cleaning of water-sensitive artistic manufacture [17] and in the case of wood materials, complete inhibition of *A. niger* growth was observed using Vinegar diluted 1:1 up to 14 days of incubation [18]. Considering the limitations of using aqueous solution on water-sensitive substrates, in the present research we have decided to proceed applying the two selected solutions (Vinegar and commercial biocide) on the rear canvases (instead of the front side canvases) and, to avoid problems related the penetration within the treated substrate, the samples were air-dried before the experimentally contamination with fungi. In addition, it can be observed that the application of Vinegar was intended to exert a protective effect rather than a restoration of fungi damaged surface. In this direction, the efficacy of the Vinegar, expressed as fungal growth delay, is quite similar and, in some cases, lower (R = 1) compared to that obtained using Preventol® RI80. The differences observed between the four examined canvases (A, B, C and D), can be related to the features of each rear canvas or to the diverse penetration of the applied solution in the textile, factors that could also have affected the growth of the selected fungi. Indeed, among these*, P. citrinum* was the most frequently detected, being able to grow in almost any environment, due to its undemanding nutritional requirements [16,19], characteristic that led to consider these microorganisms as the primary colonizers of artistic objects. The potential negative effects of *Penicillium* spp., as well as other genera belonging to the Deuteromycetes, is related to the production of pigments and enzymes [20], responsible of mainly colouring changes and stains mostly observed in the artworks. Moreover, their hyphae can penetrate the painted layer, degrading some of its components (such as glues and binders) and leading to exfoliations, cracking and final loss of the paint [21].

Conclusion

In conclusion, the use of natural substances and the application of plant extracts to artistic objects [22,23] have been proved the antifungal properties of these compounds, as an alternative to strong chemical treatments. In this context, the Vinegar can be considered for its possible use in particular and limited areas of substrate materials, such as the herein considered rear canvases, to prevent the growth of filamentous fungi and their following diffusion on the front side of the canvas itself. In the future, the association of natural compounds (such as Vinegar) with a commercial biocide to obtain a final product (a so-called "green detergent solution") with a reduced environmental impact and able not only to clean a specific substrate but also to defend it from microbial attack, could be explored.

Conflict of Interest

The authors declare no conflict of interest.

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