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EVALUATION OF THE INHIBITORY EFFECT OF DIMETHYL SULFOXIDE ON FUNGAL DEGRADATED ARCHAEOLOGICAL WOOD

Mohamed El-sayed OSMAN[1], Amany Abo El-nassr EL-SHAPHY[1], Maha Mahmoud AYID[2*]

[1] Department of Botany and Microbiology, Faculty of Science, Helwan University, Egypt.
[2] Microbiology Lab, Conservation Centre, Grand Egyptian Museum, Ministry of Antiquities Affairs, Egypt.

Abstract

Fungi play a very important role in deterioration of ancient wood antiques and therefore must not be neglected due to the increasing aesthetic value of art objects as well as the impact on health of conservators. A number of chemicals have been used for the treatment of museum artefacts. Biocides are the most effective at eradicating spores and mature organisms. Dimethyl sulfoxide (DMSO) is frequently used as a solvent for anti-fungal drugs. This study was carried out to evaluate in vitro and in vivo antifungal efficacy of DMSO against Aspergillus parasiticus. In vitro, fifty percent of DMSO gave complete inhibition of the growth. Also, 25% of DMSO inhibition growth by 60%. On the other hand low concentrations of DMSO were less effective. In vivo studies, treatment with DMSO on biodeteriorated sycamore wood resulted in inhibition of fungal growth. Furthermore, the application of DMSO had no effect on the colour, structure and chemical characteristic of the wood as well as, DMSO removed extraneous wood components that easily dissolve in DMSO.

Keywords: *Biodeterioration; Wood treatment; Biocides; DMSO; Aspergillus parasiticus.*

Introduction

Wood decay is important for ecosystem functioning and recycling of organic matter in the environment, but sometimes this natural process leads to destruction of wooden objects of historic and cultural value. Although wood persists for long periods of time, chemical, physical, and morphological modifications produced by unfavourable environmental conditions along with biodeterioration caused by microbial attack can result in loss of cultural heritage [1-3]. Biodeteriogens are organisms involved in deterioration of artefacts. They are very specific for each type of artefact in accordance with its chemical structure and environment. They also have different nutritional requirements and act directly or indirectly on the substrate.

The preventive strategy is to inhibit or slow down the growth of fungal organisms through modification of both the museum environment and the stored artefacts to make them unavailable for fungal growth [2]. In most cases this strategy is based on decreasing water activity in organic materials comprising museum collections [4]. In museum practice, fungi are traditionally deactivated by physical methods (exposure to X- and gamma-rays, drying and freezing, heating, and creating anoxic atmospheres), and chemical methods (fumigation and treatment with non-volatile biocides) [5-7]. A variety of non-gaseous industrial and agricultural biocides were used for the treatment of museum collections. For application to artefacts, non-

* Corresponding author: Ayidmaha@gmail.com

gaseous biocides are generally diluted in distilled water or organic solvents at low concentrations 0.1-3% [7].

Dimethyl sulfoxide (DMSO) is highly polar, stable substance with exceptional solvent property. It also acts as a penetrant of drugs through skin e.g. it has been shown to increase the effectiveness of idoxuridine in herpes simplex [8]. Five percent of DMSO has also been added to fungal suspension as a cryoprotectant, for storage at very low temperature (-80°C) [9]. However, it has been reported for their antimicrobial effect [10] .

The objective of this study was to investigate the inhibitory effect of DMSO. DMSO was evaluated for mold inhibition on wood by three methods: immersing, indirect compresses and also by spraying treatment. Finally, it was studied the effect of DMSO on non-infected wood by Environmental Scanning Electron Microscope (ESEM) and Fourier transform infrared spectroscopy (FTIR).

Materials and Methods

Test fungi

After an exhaustive search on the occurrence and frequency of different fungi isolated from different archaeological wood objects, it was found that the most occurrent genera were *Aspergillus* (48.21%), followed by *Pencillium* (15.16%) and *Cladosporium* (14.29%) [11]. *Aspergillus parasiticus* isolated from biodeteriorated wood objects found in the excavation of Saqqara and also from Storage area of Cheops's Solar Boat, recorded the maximum polygalacturonase production [11], was selected for this study *Aspergillus parasiticus* was maintained on Potato Dextrose Agar (PDA, Difco). 2-3 $\times 10^4$ spores ml^1 suspension was prepared from 7 day old culture by washing the surface of cultures with sterile 0.85% saline and used as an inoculum in broth dilution method.

Antifungal susceptibility of Dimethyl sulfoxide

Different concentrations of Dimethyl sulfoxide (DMSO), 5, 10, 25, 50, 75 and 100 % (v/v) were prepared to determine the inhibitory effect against tested fungi. The inhibitory effect was measured by the dry weight of fungal mycelia, compared with that of untreated control after an appropriate incubation time at 28°C. The percentage inhibition calculated according to the formula as suggested by Vincent [12]:

$$\text{inhibition Percentage} = \frac{C - T}{C} \times 100 \quad (1)$$

Where, C = Dry weight of the control, T = Dry weight of the treated sample

Application of Antifungal agent on the artificial biodeteriorated wood

Preparation of samples

The wood samples (Sycamore) was cut into 4×2×0.9 cm (length × width× height) and used as a test specimen. The samples were sterilized by autoclaving at 121°C for 15min.

Fungal deterioration

Sterilized wooden samples were inoculated with *Aspergillus parasiticus* and kept in the incubator at 28°C at suitable RH value till the fungal growth was developed.

Mechanical cleaning (Vacuum cleaning)

Biodeteriorated specimens were first dried in a fume hood using silica gel and keep overnight then vacuum cleaning using vacuum cleaner fitted with HEPA filter were applied to reduce the number of mold spores on the decayed object.

Chemical treatment

The samples were treated by three methods, Immersing (in 50% solution of DMSO for 10 minutes), Indirect compresses (acid-free tissue paper covered sample and above it another paper saturated with 50% solution of DMSO) and also by Spraying sample with 50% solution of DMSO. After treatment the sample were dried at 40°C.

Evaluation the effect of DMSO on the standard non infected wood

Colourimetry

The colour coordinates of wood samples before and after DMSO application were determined using Konica minolto colour Spectrophotometer (CM-700d/600d) and the CIEL*a*b* colour system, in Conservation Center- Grand Egyptian Museum. The CIEL*a*b* colour coordinates L (lightness), a (red/green axis), and b (yellow/blue axis) were recorded. The colour changes of the sample after DMSO application were calculated and expressed as ΔL, Δa , Δb . Calculation of the total colour change (ΔE) was done according to Normal 43/93 [13] using the following equation:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (2)$$

Examination of wooden samples using ESEM

The collected samples were investigated using Environmental Scanning Electron Microscope ESEM (FEI Quanta 3D 200i) in House Building National Research Center, to evaluate of the treatment.

Fourier transform infrared spectroscopy (FTIR)

FTIR-6100 (Jasco, Japan), in National Research Center was used to indicate structural changes occurring in the wood. The samples were prepared using the KBr (potassium bromide) pellet technique. Spectra were recorded in the range of 4000–400 cm^{-1} with 4 cm^{-1} resolution and 50 scans per sample.

Statistical analysis

Statistical analysis of data was carried out by using one way analysis of variance (ANOVA) followed by homogenous subsets (Duncuna) using the Statistical Package for the Social Science (SPSS) version 17. Duncan's multiple range tests were used at significance P= 0.05 according to Walter & Duncan [14].

Results

Antifungal susceptibility of Dimethyl sulfoxide

The effect of different concentrations of DMSO on the growth of *A. parasiticus* is given in Table 1. There was complete inhibition of the growth at concentration 50%. Also, 25% of DMSO inhibited growth by 60%. On the other hand low concentrations of DMSO were less effective.

Table 1. Percentage inhibition of *A. parasiticus* at different concentration of DMSO

Percent of inhibition (%)	Dry weight (mg/ml)	Concentration of DMSO (%)
7.3	16.59 ± 1.23[a]	5
34.2	11.78 ± 1.42[b]	10
60.5	7.06 ± 1.70[c]	25
100	0	50
100	0	75
100	0	100
	17.91 ± 1.08[a]	Control

Data are expressed as mean ± SD (n=3), Means within the same column and followed by the same letter are not significantly different from each other according to Duncan's Multiple range test (P = 0.05).

Application of Antifungal agent on the artificial biodeteriorated wood

In this experiment, antifungal agent (DMSO) was used for its ability to inhibit fungal growth in artificial biodeteriorated sycamore wood. After biodeterioration, specimens were dried using silica gel and then vacuum cleaner fitted with HEPA filter was applied. After that the specimen was treated by immersing, indirect compresses and also spraying of 50 % DMSO was applied to evaluate the inhibitory effect of DMSO. Random swaps from biodeteriorated specimen were taken before and after mechanical cleaning, and also after application of DMSO. It was also investigated by SEM (fig.1-4).

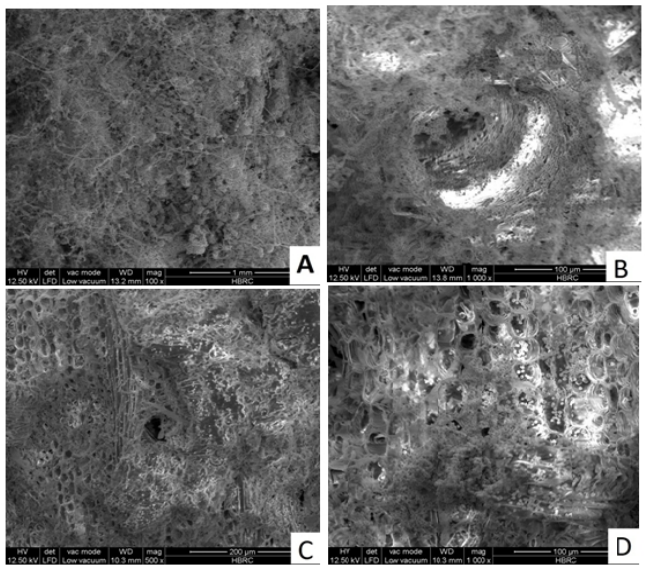

Fig. 1. Transverse section of sycamore wood inoculated with *Aspergillus parasiticus*, **A** and **B** showing extremely dense mycelia on the outer surface hiding the main wood features, (A, Bar 1mm and B, Bar 100 µm). **C** and **D** showing of the hyphae and spores after mechanical cleaning and removal of the dense mycelia, but spores remained on the surface of the wood and within the cells. Wood cells became partially visible. (C, Bar 200 µm and D, Bar 100 µm).

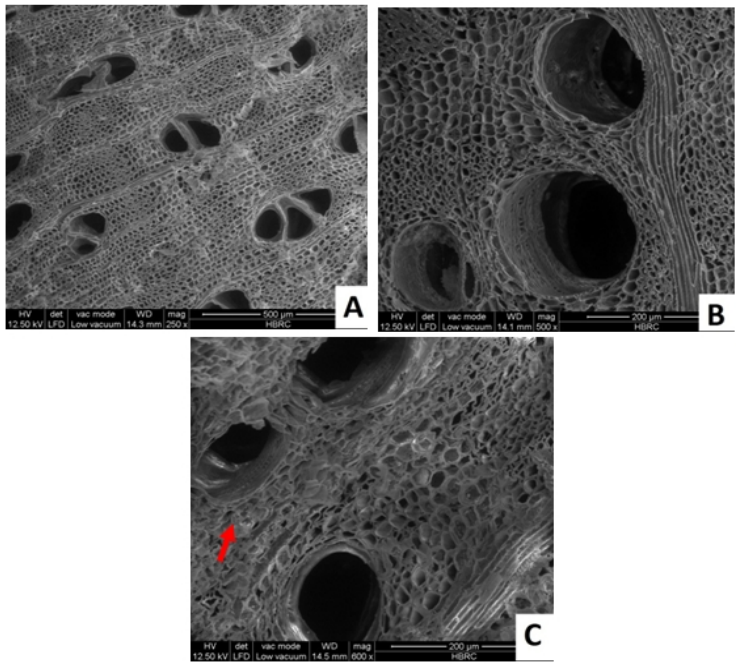

Fig. 2 Transverse section of sycamore wood inoculated with *Aspergillus parasiticus* after immersion in DMSO (antifungal agent), No evidence of hyphae in most parts of the wood surface, (**A**, Bar 500 µm and **B**, Bar 200 µm). Some remains of spores were evident cells surrounding a few vessels (**C**, Bar 200 µm).

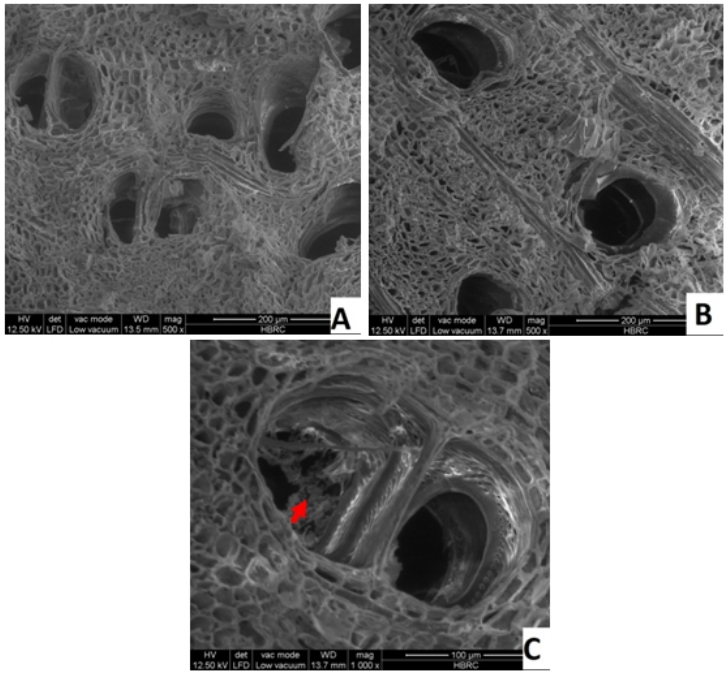

Fig. 3 Transverse section of sycamore wood inoculated with *Aspergillus parasiticus* after applying DMSO (an antifungal agent) by using indirect compresses methods. No evidence of hyphae was shown, **A** and **B** (Bar 200 µm). Slightly chains of spores were evident inside vessel beneath the exposed wood surface, (**C,** Bar 100 µm).

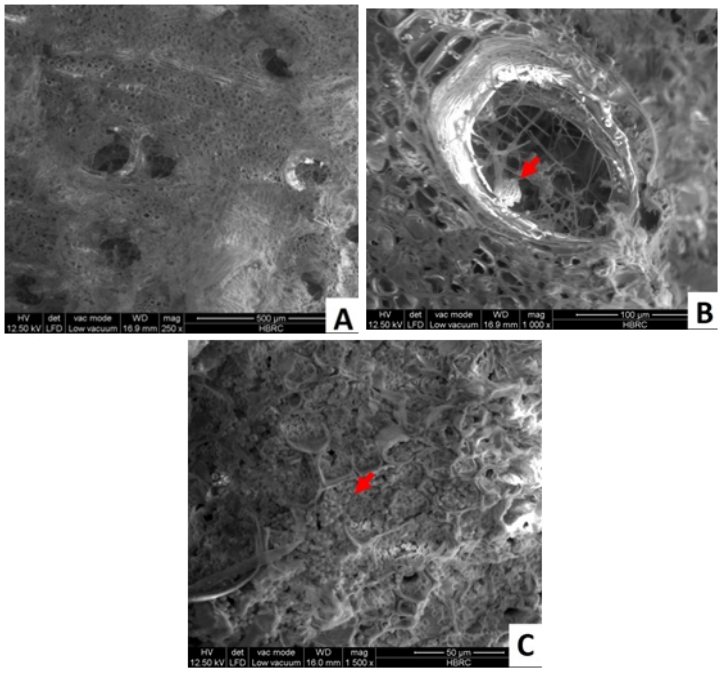

Fig. 4 Transverse section of sycamore wood inoculated with *Aspergillus parasiticus* after spraying DMSO (used as an antifungal agent). (**A**) Showing fungal mycelia on the outer surface (Bar 500 µm). (**B**) Showing conidia of *A. parasiticus* on the wood surface and inside the vessels (Bar 100 µm). (**C**) Showing dense infestation of spores and mycelia on the wood surface (Bar 50 µm).

It was revealed that the immersing and indirect compresses methods are more effective in fungal control in wood decay. It showed no fungal growth on the cultured plates. While spraying method may be needed more than one time to be effective. The spraying technique doesn't distribute the solvent evenly and therefore the removal of fungal mycelia was different in various parts of the sample as shown in figure 4.

Evaluation the effect of DMSO on the standard non infected wood

Colourimetry

After application DMSO, the standard and DMSO applied samples were evaluated by colorimetric measurements with a Konica minolto colour. The co-ordinate a* is the degree of redness and greenness and it takes positive values for reddish colours and negative values for the greenish ones. The co-ordinate b* is the degree of yellowness and blueness and it takes positive values for yellowish colours and negative values for the bluish ones. The co-ordinate L* is the degree of lightness; it is an approximate measurement of luminosity, which is the property according to which each colour can be considered as equivalent to a member of the grey scale, between black and white [15] . Positive value of Δb indicates that the sample is yellower than the standard. Negative value of Δa indicates that the sample is greener than the standard and Negative ΔL values indicate that the analysed area reflects less light than the standard. The colour changes of the sample after DMSO application were calculated and expressed as $\Delta L, \Delta a$, Δb . The evaluation was conducted by calculating the magnitude of the colour difference (ΔE) between the two samples.

Colour values and changes in colour of the standard wood sample before and after applied DMSO were shown in Table 2, the colour change is very low (ΔE= 0.70); this value indicates that no appreciable chromatic variation has been induced in the sample when applied DMSO solvent.

Table 2. Chromatic coordinates and total colour differences after applied DMSO

After applied DMSO	Standard sample	Colour values & changes
55.41 ± 0.93	55.92 ± 0.88	L
7.04 ± 0.58	7.06 ± 1.15	A
20.53 ± 0.79	20.05 ± 1.89	B
-0.51		ΔL
-0.02		Δa
0.48		Δb
0.70		ΔE

Investigation of wood samples using ESEM

The SEM analysis was performed in order to investigate morphological and microstructural characteristics of the wood after applied DMSO. Micrographs of standard wood surface (*Ficus sycomorus* wood without microbial infection) and the wood surface after immersing in DMSO were reported in figure 5 and 6. The SEM micrographs revealed that DMSO had not effect on the characteristic structure of the wood, as well as it remove the extraneous wood components that easily dissolve in DMSO (Fig. 6) compared to standard one (Fig. 5).

Investigation of wood samples using FTIR

The FTIR analysis was done to investigate the changes in the functional groups occurred after applied DMSO on the standard wood. Three samples were analysed by means of FTIR spectroscopy in the transmittance mode. The first sample was taken from the surface of *Ficus sycomorus* wood without microbial infection (standard wood), the second after immersing in DMSO for ten minutes and the third one from DMSO solvent as reference. FTIR spectra (Fig.

7) revealed that there was no difference in the chemical structure of the wood after applied DMSO with compared to the first one.

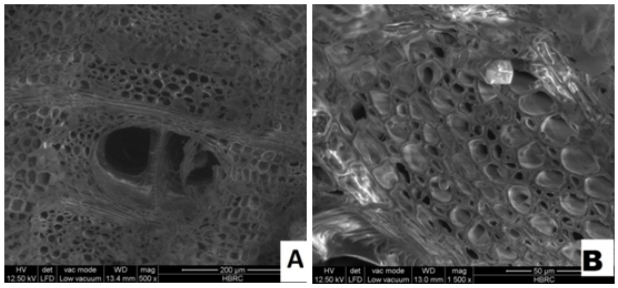

Fig. 5. Transverse section of recently cut sycamore wood without microbial infection (standard) showing; (A) the characteristic structure of wide-banded fibres and axial parenchyma, multiseriate rays and large vessels which could be sometimes in clusters (Bar, 200 µm). (B) A prismatic calcium oxalate crystal which can be occasionally present in chambered cells in axial parenchyma and in ordinary cells in ray parenchyma (Bar, 50 µm).

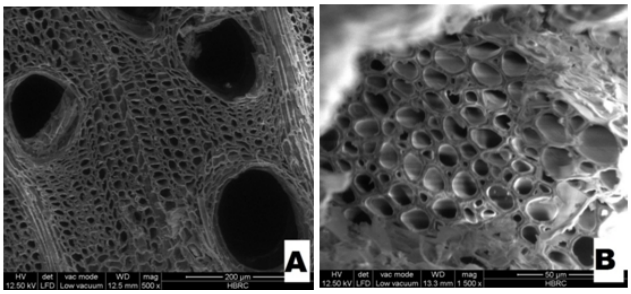

Fig. 6. Transverse section of recently cut sycamore wood without microbial infection after applied DMSO showing in; A and B, the removal of extraneous wood components that easily dissolve in DMSO, without affecting the characteristic structure of the wood (A, Bar 200 µm and B, Bar 50 µm).

It was showed that the entire functional groups of those two samples were similar among each other. In the standard sample, each peak was clearly illustrated same as peak found in DMSO applied wood sample (Fig. 7 A, B). It was indicated that there is no effect of DMSO appeared in the FTIR spectra of second wood sample as compared with first one.

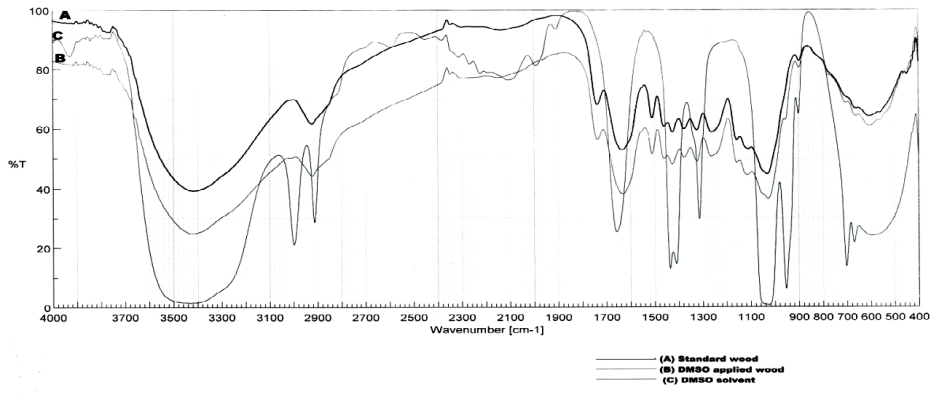

Fig. 7. FTIR spectra of (**A**) the surface of the standard wood
(**B**) The surface of the DMSO applied wood and (**C**) DMSO solvent.

On the other hand, The spectrum of DMSO (Fig. 7 C) exhibits peaks at 1435, 1409, and 1313 cm^{-1} and a broad peak at around 1028 cm^{-1} The peaks at wavenumber 1435 and 1409 cm^{-1} correspond to the antisymmetric bending of CH_3 (δ_{as} CH3), and the peak at 1313 cm^{-1} is identified as a symmetric deformation of CH_3 ($\delta_s CH_3$) group that is attached to the S atom. Abroad peak around 1028 cm^{-1} can be assigned as S=O stretching (*v* SO).

Discussion

A number of chemicals have been used for the treatment of museum artefacts. Biocides are the most effective at eradicating spores and mature organisms. Dimethyl sulfoxide (DMSO) is commonly used as a solvent for antifungal drugs. Five percent of DMSO has also been added to fungal suspension as a cryoprotectant, for storage at very low temperature,-80°C [9] . However, it has been reported for their antimicrobial effect [10, 16, and 17] . So, the present study was carried out to investigate the inhibitory effect of DMSO at different concentration against *Aspergillus parasiticus. In vitro* It was found that 50% of DMSO gave complete inhibition of the growth. Also, 25% of DMSO inhibited growth by 60%. On the other hand low concentrations of DMSO were less effective. Randhawa [18] found that 10% DMSO inhibited the growth of dermatophytes. Hakura *et al*. [19] reported that DMSO is mutagenic and induce membrane damage, and cause formation of abnormal structures, etc in test organism. Sharma & Sharma [20] observed that treatment with Dimethyl Formamide (DMF), DMSO and methanol resulted in increased mycelial width as well as reduction in size, number and germination of fungal conidia and vesicle. Toxicity and effect of DMF, DMSO, methanol and tween 80 on cytology, morphology, reproductive structures, germination of conidia and sporulation of different fungi also reported by various authors [21, 22, 23, 24, 25, 26, and 27] .

In vivo studies, it was shown that treatment with DMSO on biodeteriorated sycamore wood resulted in inhibition of fungal growth. Furthermore, the application of DMSO had no effect on the colour, structure and chemical characteristic of the wood as well as, DMSO removed extraneous wood components that easily dissolve in DMSO. Caroline & Middleton [28] observed that prismatic calcium oxalate crystals occasionally present in chambered cells in axial parenchyma and in ordinary cells in ray parenchyma of *Ficus sycomorus* wood. Hossain [29, and 30] had found that DMSO, in the presence of small amounts of mineral acids, is a powerful delignifying agent for both high-lignin sulphite pulps and wood chips. The delignifying process is conducted at a temperature between 399 and 448 K (126 & 175°C) in an aqueous solution containing 75 w- % DMSO in the presence of catalytic amounts of sulphuric acid or hydrochloric acid.

Conclusions

Fifty percent of DMSO is very efficient for inhibition of fungal growth and hence in the treatment of biodeteriorated wood. The results also showed no significant changes in the wood properties due to the use of this treatment. This indicates that 50% of DMSO can be used for controlling mold growth on wood. Nevertheless, further studies are needed to consider the influence of these treatments on wood after accelerated aging.

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