Comparative Evaluation of Some Woody Tree Methanolic Extracts and Paraloid B-72 against Phytopathogenic Mold Fungi *Alternaria tenuissima* and *Fusarium culmorum*

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Natural compounds from certain timber trees are highly valued and recommended to protect wood and wood products against mold fungi. This study highlighted the use of some natural extracts and Paraloid B-72 against the growth of two mold fungi, namely *Alternaria tenuissima* and *Fusarium culmorum*. From the *in vitro* experiment, the methanol extracts of *Callistemon viminalis* bark were effective against the growth of *F. culmorum*, as were *Magnolia grandiflora* leaves against *A. tenuissima*. Environmental scanning electron microscopy (ESEM) and electron dispersive X-ray spectroscopy (EDX) analysis of treated *Acacia saligna* wood with the two fungi and Paraloid B-72 demonstrated the clear hyphal growth of *F. culmorum* and *A. tenuissima* and changes in elemental chemical composition. After three months, no fungal growth on the wood surface treated with the methanol extract of *M. pomifera* bark was found. After three months of treating wood with Paraloid B-72 at 5% and 10%, the mold growth was visible. Almost all of the wood treated with methanol extracts showed growth of the *A. tenuissima* hypha, as well as some contamination by other microorganisms, except for the wood treated with the methanol extract of *M. pomifera* bark.

Keywords: Alternaria tenuissima; Fusarium culmorum; Wood; Natural extracts; Paraloid B-72; EDX; ESEM

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INTRODUCTION

Biodeterioration of wood is a common problem when wood is attacked by biological pathogens such as fungi, bacteria, and insects. In places where there is poor design and a moist environment (*e.g.*, homes, hotels, schools, and other structural buildings), *Fusarium* and *Alternaria* mold growth has been detected (Fogel and Lloyd 2002; Xu *et al.* 2013). In addition, there are difficulties in killing fungi using natural substances (biocides) or other antifungal treatments because of their thick cell walls (Sterflinger 2010).

Fusarium and *Alternaria* species are the most common hyphomycetes in museums and are components of materials used in paintings (oil, water color, acrylic),

paper (laid-paper, wood pulp paper), and cellulose textiles (cotton, linen) (Meier and Petersen 2006; Błyskal 2009; Mesquita *et al.* 2009; Pangallo *et al.* 2009). According to a survey of the literature by Błyskal (2009) pertaining to microbiological deterioration of keratinous substrates, genera of *Aspergillus*, *Penicillium*, *Chrysosporium*, *Fusarium*, *Microsporum*, *Trichophyton*, and *Acremonium* appeared to be the most common. Additionally, many organisms, *e.g.*, *Penicillium*, *Aspergillus*, and *Alternaria* spp., can cause adverse health effects in archive workers and users (Crous *et al.* 2007; Mesquita *et al.* 2009).

The conidia of *Alternaria* species produce dark brown, green-black, or black colonies (Zhao 2003). *Alternaria* species are the most common airborne allergens and have a wide range of hosts, including wood products (Yang 2005; Vukojević and Grbić 2010; Andersen *et al.* 2011). They were associated with economic losses to wood users in Korea as a mold fungus (Lee *et al.* 2014), and they are the dominant fungal species on the surface of polymeric materials (Lugauskas *et al.* 2003). *Alternaria* species were associated with deteriorated wooden sculptures and photographs that were temporarily stored in the quarantine room of the Cultural Center of Belgrade, in Serbia (Ljaljević-Grbić *et al.* 2013). Sharma and Sharma (1979) described the presence of *A. alternata* in finished leather. *A. tenuissima* has been recorded on a wide range of plant species, usually as a secondary invader rather than a primary parasite, and produces tenuazonic acid, alternariols, tentoxin, altertoxin I, and a number of unknown metabolites (Davies *et al.* 1977; Andersen *et al.* 2002).

Fusarium species can parasitize cultivated plants and can be found in the soil. *Fusarium* species are found in normal mycoflora of common industrial plants, such as rice (O*ryza sativa* L.) and soybeans (*Glycine max* L.). While most species are common in tropical and subtropical areas, some inhabit soil in cold climates (Pitt *et al*. 2000; Yli-Mattila *et al*. 2002). *F. culmorum* is an ubiquitous fungus infecting cereals and grains, and therefore constitutes a major problem for agriculture (Zamir and Farah 2000; Ezekiel *et al.* 2008). Additionally, some strains of *F. culmorum* produce red color on agar and purple on wood (Yang and Gignac 2011).

Different factors should be taken into account when discussing the deterioration of wood materials by fungi, such as surface quality of wood, amount and quality of sapwood and heartwood, nutrient content, permeability of the wood, and surface treatments (Theander *et al.* 1993; Terziev 1996; Terziev and Boutelje 1998; Viitanen and Ahola 1999). Paraloid B-72 (The acrylic resin) is well-known for surface consolidant for many materials such as wood (Yang *et al.* 2007; Vaz *et al.* 2008). In beech and spruce woods, the application of Paraloid B-72 at 2 or 10% did not increase the resistance against brown-rot fungi (Tiralová and Reinprecht 2004). Additionally, it exhibited the weakest resistance against the growth of *Poria vaillantii* and *Gloeophyllum trabeum* (Pohleven *et al.* 2013).

The aim of this work was first to evaluate the *in vitro* antifungal activity of natural extracts against the growth of two mold fungi (*A. tenuissima* and *F. culmorum*). The second objective was to evaluate visual observations of mold growth caused by *A. tenuissima* and *F. culmorum* on wood samples of *Acacia saligna* (Labill.) H. L. Wendl treated with different natural extracts as well as Paraloid B-72 polymer at concentrations of 2, 3, 5, and 10%. The surface elemental analysis of the treated wood samples with *A. tenuissima* and *F. culmorum* was measured by dispersive X-ray spectroscopy (EDX). The microbial growth on the wood surface was evaluated using an environmental scanning electron microscope (ESEM).

EXPERIMENTAL

Materials

Preparation of extracts

In the present study and during 2013, different parts of some hardwood species grown in Alexandria City, Egypt were collected to be used as a natural source of extracts. The species used were *Cupressus sempervirens* L. (wood), *Maclura pomifera* (Raf.) C.K. Schneid (bark), *Morus alba* L. (heartwood), *Callistemon viminalis* (Sol. ex. Gaertn) G. Don. (bark), *Magnolia grandiflora* L. (leaves), and *Dalbergia sissoo* Roxb. (bark). They were kindly identified at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University. After air-drying the materials at room temperature, they were ground into powder using a small laboratory mill. The methanol extracts were prepared by soaking about 50 g of the material in 100 mL of methanol (80%). The extraction was repeated three times. The crude methanol extracts found after filtration were concentrated under reduced pressure at 45 °C using a rotary evaporator, and stored at 4 °C until further use (Salem *et al.* 2013). Eight concentrations (8, 32, 64, 125, 250, 500, 1000, and 2000 μg/mL) were prepared from the methanol extracts dissolved in 10% dimethylsulfoxide (DMSO; Sigma-Aldrich, Germany) and sterilized distilled water (1:1 *v*/*v*).

Antifungal activity of extracts

Two phytopathogenic fungi, namely *Alternaria tenuissima* and *Fusarium culmorum*, were used in the present study. The linear growth of the two fungi was evaluated against the use of prepared concentrations of methanol extracts (Satish *et al.* 2007; Essa and Khallaf 2014). Plates of potato dextrose agar (PDA) medium with a known amount of the concentrated extracts were used as media for growing the tested fungi. For the control treatment, 10% DMSO was used. The prepared plates were inoculated with a 5-mm disc of 7-day-old culture of each of the tested fungi (*A. tenuissima* and *F. culmorum*) and were placed at the center of the petriplates and incubated at 26 ± 1 °C 25±2 ºC for seven days or until the growth in the control treatment reached the maximum. The treatments were repeated three times. The minimum inhibitory concentration (MIC) of the extracts was determined according to Eloff (1998) with slight modification. The methanol extracts from each tree part were diluted to serial concentrations (8, 16, 32, 64, 128, 265, 512, and 1000 μg/mL).

Methods

Comparative study: Visual observation

Acacia saligna (Labill.) H. L. Wendl. is widely grown and distributed in various locations in Egypt. Wood samples of *A. saligna* with dimensions of 0.5 cm × 1 cm × 2 cm were soaked in a solution of Paraloid B-72 polymer (Dow Chemical Co., USA) (diluted in distilled water) and methanol extracts (diluted in 10% DMSO) in concentrations of 2%, 3%, 5%, and 10% for 2 h daily for three sequential days and left to dry at room temperature for 15 days. After that, the treated wood samples were inoculated with a 5-mm disc of each of the tested fungi (*A. tenuissima* and *F. culmorum*) for 2 weeks at 25 °C. After 15 days and 3 months of inoculation, the fungal colonization was visually evaluated. The fungal growth was visually assessed for mildew growth according to GOST 9.048-75 (1975) and Lugauskas *et al.* (2003).

Comparative study: ESEM and EDX analyses

To investigate the effect of the treatments on surface elemental composition (%), wood samples of *A. saligna* were treated with *A. tenuissima*, *F. culmorum*, and by Paraloid B-72 (2, 3, 5, and 10%). The control was untreated. After the incubation period was finished, the hyphal growth on the wood surface was measured by an environmental scanning electron microscope (ESEM; FEI Quanta 200 SEM FEG) operating at an accelerating voltage of 20 KV. Surface elemental composition of wood surfaces treated with *A. tenuissima* and *F. culmorum* was measured by dispersive X-ray spectroscopy (EDX) (Danilatos and Robinson 1979).

Statistical analysis

The effect of different methanol extracts on the linear growth values of *A. tenuissima* and *F. culmorum*, as well as the extract concentrations, were statistically analyzed using the general linear models (GLM) procedure in SAS version 8.2 (2001) in a completely randomized design to test the differences among factors and levels. The comparison among the least square (LS) means with 95% confidence intervals (95% CI) was performed at 0.05 level of probability using the least significant differences ($LSD_{0.05}$) method (Böhm *et al.* 2012).

RESULTS AND DISCUSSION

Antifungal Activities of Different Extracts

Tables 1 and 2 show that the linear growth of fungal mycelia of *Alternaria tenuissima* and *Fusarium culmorum* was significant ($P < 0.0001$) and affected by the type and concentration of extracts as well as the interaction between them. At concentrations of 1000 µg/mL and 2000 µg/mL, the linear growth of the two fungi was completely inhibited.

The linear growth of the two studied fungi reached the maximum at the concentration of 8 µg/mL methanol extract of *C. sempervirens* wood and leaves of *M. grandiflora*. The lowest linear growth (31.55 mm) with *F. culmorum* was observed with the methanol extract of *C. viminalis* bark, and the highest linear growth (59.22 mm) occurred with the methanol extract of *D. sissoo* bark (Table 1).

The statistical results presented in Table 2 show that the lowest linear growth of *A. tenuissima* was observed using the methanol extract of *M. grandiflora* leaves (35.14 mm) followed by the bark of *C. viminalis* (42.96 mm); the highest values were observed from the methanol extracts of *D. sissoo* bark (55.44 mm) and *M. alba* heartwood (53.96 mm), followed by *C. sempervirens* wood (51.59 mm).

According to the values of MICs presented in Table 3, the methanol extracts from *C. viminalis* (bark) and *M. pomifera* (bark) showed inhibition at lower concentrations against the growth of *F. culmorum* and *A. tenuissima* when the MIC value was 8 µg/mL. According to the literature, the MIC values of the extracts against *Alternaria* species have ranged between 1.25 and 25 µg/mL (Díaz-Dellavalle *et al.* 2011); in the present study, these values were between 8 and 32 µg/mL.

Table 1. Linear Growth (means ± SD) of *F. culmorum* as Affected by Natural Extracts at Different Concentrations

Concentration (µg/mL)	Linear growth of fungal mycelia (mm)						Mean linear growth (mm)*
	Extract						
	C.S.	M.P.	M.A.	C.V.	M.G.	D.S.	
Control (10% DMSO)	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00^{A}
8	90.00± 0.00	54.33± 0.57	71.66± 0.57	45.33± 0.57	90.00± 0.00	90.00± 0.00	73.55± 18.69^{B}
32	73.66± 0.57	60.33± 0.57	60.33± 0.57	36.66± 0.57	81.66± 0.57	85.33± 0.57	66.33± 16.82^{C}
64	63.33± 0.57	76.66± 0.57	63.66± 0.57	35.33± 0.57	47.66± 0.57	76.33± 0.57	60.50± 15.31^{D}
125	58.33± 0.57	62.00± 2.00	50.66± 0.57	30.33± 0.57	37.00± 2.00	72.00± 2.00	51.72± 14.81^{E}
250	52.00± 2.00	56.66± 0.57	42.66± 0.57	25.66± 0.57	32.00± 5.19	69.33± 0.57	46.38± 15.35^{F}
500	36.33± 0.57	47.33± 0.57	40.33± 0.57	20.66± 0.57	20.00± 1.00	50.00± 0.00	35.77± 12.14^{G}
1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00^{H}
2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00^{H}
Mean of extract (mm)*	51.51± 32.54^{b}	49.70± 29.68^{c}	46.59± 29.23^{d}	31.55± 25.85^{f}	44.25± 34.51^{e}	59.22± 34.36^{a}	

C.S.: wood of *C. sempervirens*; M.P.: bark of *M. pomifera*; M.A.: heartwood of *M. alba*; C.V.: bark of *C. viminalis*; M.G.: leaves of *M. grandiflora;* D.S.: *D. sissoo*

*****Means with the same letter within the same column (capital letters) or row (small letters) are not significantly different according to LSD at 0.05 level of probability

Table 2. Linear Growth (means ± SD) of *A. tenuissima* as Affected by Natural Extracts at Different Concentrations

Concentration (µg/mL)	Linear growth of fungal mycelia (mm)						Mean linear growth (mm)*
	Extract						
	C.S.	M.P.	M.A.	C.V.	M.G.	D.S.	
Control (10% DMSO)	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00	90.00± 0.00^{A}
8	74.00± 2.00	70.33± 0.57	90.00± 0.00	69.33± 0.57	74.00± 27.71	90.00± 0.00	77.94± 13.07^{B}
32	68.33± 0.57	66.66± 0.57	88.33± 0.57	63.66± 0.57	37.66± 0.57	75.66± 0.57	66.72± 15.74^{C}
64	63.66± 0.57	61.33± 0.57	66.33± 0.57	56.66± 0.57	34.66± 0.57	68.00± 0.00	58.44± 11.57^{D}
125	58.33± 0.57	53.66± 0.57	55.66± 0.57	47.66± 0.57	30.33± 0.57	63.66± 0.57	51.55± 10.96^{E}
250	56.33± 0.57	48.33± 0.57	52.66± 0.57	31.66± 0.57	26.00	61.66± 0.57	46.11± 13.34^{F}
500	53.66± 0.57	39.33± 0.57	42.66± 0.57	27.66± 0.57	23.66± 0.57	50.00± 0.00	39.50± 11.21^{G}
1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00^{H}
2000	0.00	0.00	0.00	0.00	0.00	0.00	0.00^{H}
Mean of extract (mm)*	51.59± 30.00^{b}	47.74± 29.42^{c}	53.96± 33.77^{a}	42.96± 29.61^{d}	35.14± 29.89^{e}	55.44± 32.65^{a}	

C.S.: wood of *C. sempervirens*; M.P.: bark of *M. pomifera*; M.A.: heartwood of *M. alba*; C.V.: bark of *C. viminalis*; M.G.: leaves of *M. grandiflora*; *D.S.: D. sissoo*

*****Means with the same letter within the same column (capital letters) or row (small letters) are not significantly different according to LSD at 0.05 level of probability

Table 3. MIC Values (µg/mL) of Natural Extracts against the Growth of *A. tenuissima* and *F. culmorum*

Extract	MIC (µg/mL)	
	F. culmorum	*A. tenuissima*
C. sempervirens (wood)	32	8
M. pomifera (bark)	8	8
M. alba (heartwood)	8	32
C. viminalis (bark)	8	8
M. grandiflora (leaves)	32	8
D. sissoo (bark)	32	32

MIC: minimum inhibitory concentration

It can be concluded that the methanol extracts of *C. viminalis* bark were highly effective against the growth of *F. culmorum*, and *M. grandiflora* leaves were effective against *A. tenuissima*. Many researchers have shown the biological activity of extracts from *C. viminalis*, and several groups of chemical compounds were identified in the extracts (Wollenweber *et al.* 2000; Parekh *et al.* 2005; Delahaye *et al.* 2009; Islam *et al.* 2010; Salem *et al.* 2013). Different parts of *M. grandiflora* have different biological compounds with potential antifungal activity against *A. alternata*, *Helminthosporium* spp., *Nigrospora* spp., *F. oxysporum*, *F. culmorum*, and *Rhizocotonia solani* (El-Feraly and Chan 1978; El-Feraly 1984; Luo *et al.* 2001; Ahmed and Abdelgaleil 2005). On the other hand, stem bark methanol extract was reported to have very weak or no antifungal activity (Ahmed and Abdelgaleil 2005). Additionally, the antifungal activity of extracts from *M. grandiflora* could be observed with the alkaloid contents (Nakano 1954) or by glycosides (Rao and Davis 1982).

EDX and ESEM Analyses of the Treated Wood Samples with *F. culmorum*, *A. tenuissima*, and Paraloid B-72

Wood material rarely supports active fungal growth unless the surface has been wet for a period of time (Florian 2002), where water at a water vapor pressure (a_w) in the range of 0.8 to 0.98 is available for use by fungi. Materials can be almost saturated, but if the a_w is not in this range, the material will not support fungal activity (Florian 2002).

The primary colonizers of wood materials are biodeteriorate species that utilize available sugars, hemicellulose, proteins, and amino acids (Ljaljević-Grbić *et al.* 2013). *A. tenuissima* rather than *A. alternata* is found predominately in buildings (Nielsen *et al.* 1999; Andersen *et al.* 2002). Furthermore, it was reported that *A. tenuissima* was found on dead branches of *Fagus orientalis* (Selçuk *et al.* 2014).

The surface analysis by EDX and ESEM of wood samples inoculated with *F. culmorum* and *A. tenuissima* is shown in Figs. 1 and 2. It can be seen that hyphal growth of *F. culmorum* and *A. tenuissima* clearly occurred. The changes in elemental chemical composition of the treated *A. saligna* wood with Paraloid B-72 at 2%, 3%, 5%, and 10%, as well as the inoculated wood samples with *F. culmorum* and *A. tenuissima* were compared with the untreated one (control). In the treated wood with 2% Paraloid B-72, there was 59.58% carbon, and it can be seen that little change was found in the wood treated with Paraloid B-72 at 3% (60.44% carbon) and 5% (59.88% carbon) in comparison with the control treatment (59.41%). In contrast, a high amount of carbon was present at 10% Paraloid B-72 (65.48%).

Additionally, there was little change in the element peak of oxygen in the wood treated with Paraloid B-72 at 2% (34.50%), 3% (33.42%), and 5% (33.26%), and the lowest amount was found by Paraloid B-72 at 10% (27.02%) in comparison with the control treatment (38.84%). In the case of the inoculated wood samples, the element peaks of carbon decreased to 56.47% with *A. tenuissima* and 57.96% with *F. culmorum*. On the other hand, the element peaks of oxygen increased to 39.14% with *A. tenuissima* and 37.84% with *F. culmorum*. Other elements such as sodium, magnesium, phosphorus, and calcium did not show valuable changes in the treated wood with *F. culmorum* and *A. tenuissima* in comparison with other treatments.

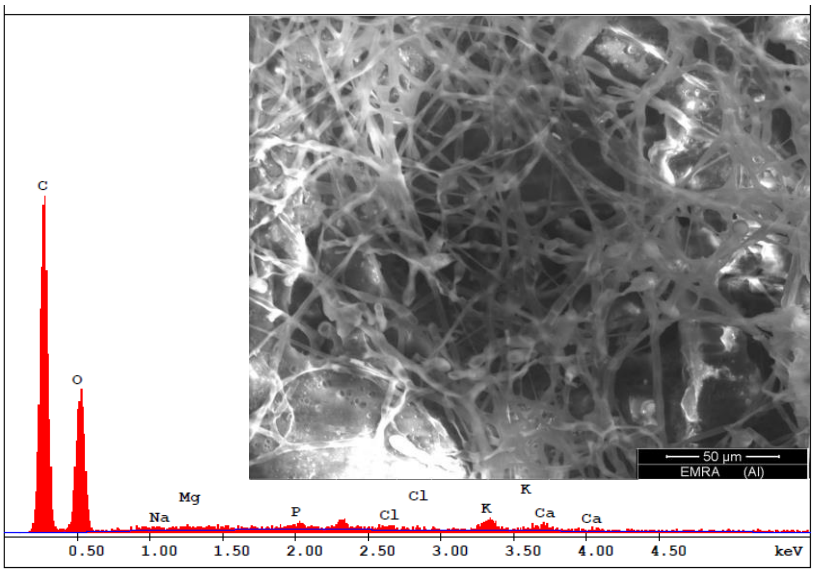

Fig. 1. ESEM and EDX image analysis of the *A. tenuissima* mold on wood

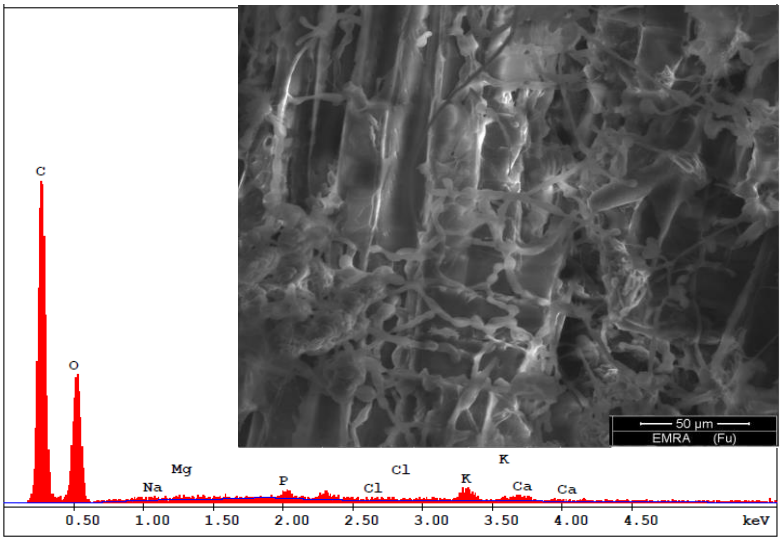

Fig. 2. ESEM and EDX image analysis of the *F. culmorum* mold on wood

Visual Observation of the Wood Samples Treated with Different Extracts and Paraloid B-72 and Inoculation with *F. culmorum* and *A. tenuissima*

According to visual observation of the *A. saligna* wood treated with *F. culmorum* and *A. tenuissima*, as well as Paraloid B-72 at concentrations of 2%, 3%, and 5%, the mycelial growth of *F. culmorum* and *A. tenuissima* on the surface of treated *A. saligna* wood samples was full (coverage around 100%) to more than half (coverage more than 50%), according to the methods described by GOST 9.048-75 (1975) and Lugauskas *et al.* (2003).

Most of the wood specimens treated with Paraloid B-72 and methanol extracts (particularly at the concentration of 10%) showed no observable growth of *F. culmorum* over the wood surfaces. However, the wood samples treated with methanol extract of *C. sempervirens* showed fungal growth of *F. culmorum* over the wood surface. Furthermore, wood treated by the methanol extract of *M. grandiflora* leaves showed some inhibition zones at the concentration of 10% (Fig. 3).

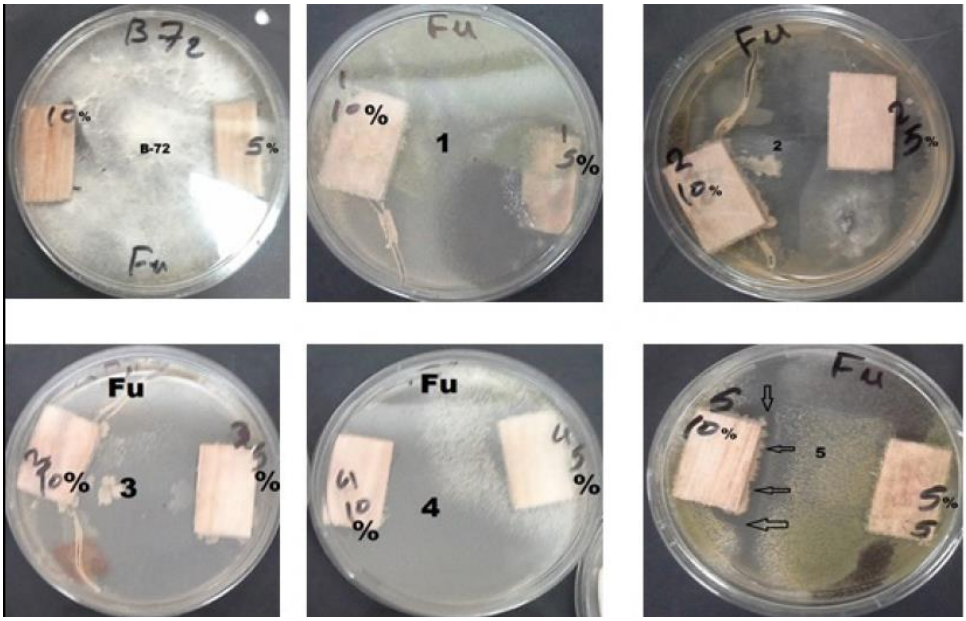

Fig. 3. The visual observations for treated *A. saligna* wood with different methanol extracts and Paraloid B-72 with *F. culmorum* (Fu) at different concentrations (5% and 10%). 1- Methanol extract (ME) of *C. sempervirens* wood; 2- ME of *M. pomifera* bark; 3- ME of *M. alba* heartwood; 4- ME of *C. viminalis* bark; 5- ME of *M. grandiflora* leaves

It can be seen that no fungal growth occurred on the wood surface treated with the methanol extract of *M. pomifera* bark, but the other treatments showed growth of *F. culmorum* over the surface of the treated wood with different extracts and Paraloid B-72 at concentrations of 5% and 10% after three months at room temperature (Fig. 4).

It is noteworthy that among the treated wood samples with the fungus *A. tenuissima*, almost all of the wood treated with methanol extracts showed growth of the fungus hypha with some contamination by other microorganisms (data not shown), except for the wood samples treated with methanol extract of *M. pomifera* bark (Fig. 5). However, the growth of the tested fungi completely covered the treated wood samples with Paraloid B-72 at 5% and 10% after three months of treatment (Fig. 6).

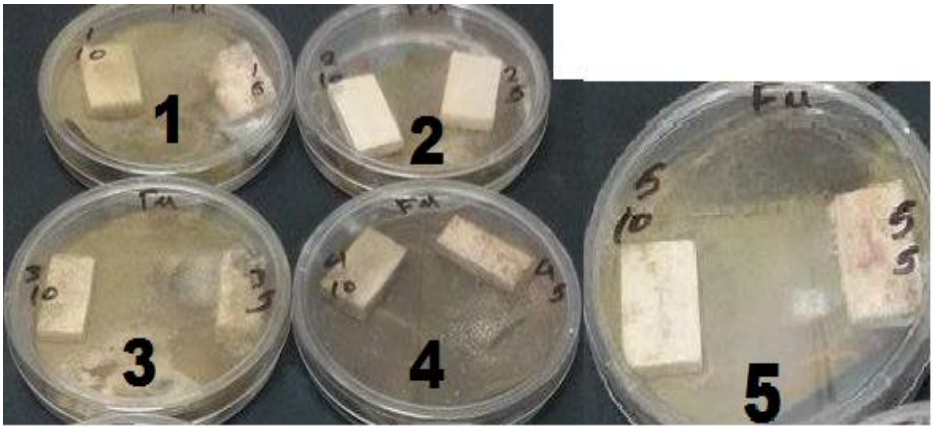

Fig. 4. The visual observation of *F. culmorum* growth on the surface of wood samples treated with different extracts and B-72 at the concentrations of 5% and 10% after three months at room temperature. 1- Methanol extract (ME) of *C. sempervirens* wood; 2- ME of *M. pomifera* bark; 3- ME of *M. alba* heartwood; 4- ME of *C. viminalis* bark; 5- ME of *M. grandiflora* leaves

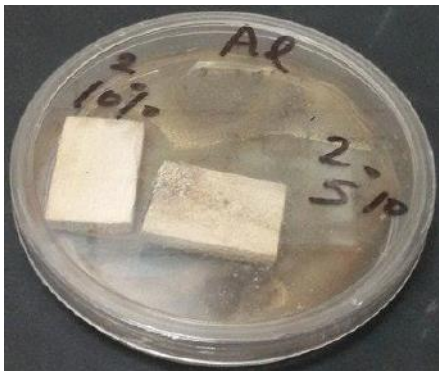

Fig. 5. Visual growth of *A. tenuissima* (Al) on the surface of wood samples treated with methanol extract *of M. pomifera* bark after three months at room temperature

Fig. 6. Visual growth of *A. tenuissima* (Al) and *F. culmorum* (Fu) on the surface of wood samples treated with Paraloid B-72 after three months of inoculation at room temperature

Strong antifungal activity of ethanol extracts and the isolated compounds morin, oxyresveratrol, and 1,3,6,7-tetrahydroxyxanthone from *M. pomifera* have been previously shown (Barnes and Gerber 1955; Wolfrom and Bhat 1965; Mahmoud 1981; Delle Monache *et al.* 1984). More recently, different solvent extracts of wood, bark, and leaves

from *M. pomifera* were shown to have strong antibacterial activity (Mohamed *et al.* 2014).

A. tenuissima was the dominant species isolated from various wood species (Sivanesan 1991). It was reported that among the genus *Alternaria*, two species, *A. alternata* sensu lato (s.l.) and *A. tenuissima*, are involved in the discoloration of wood and wood products (Yang 2005; Vukojević and Grbić 2010; Andersen *et al.* 2011; Lee *et al.* 2014). Furthermore, fungal strains including *Alternaria* and *Fusarium* genera were isolated and identified after wood preservative treatments (Bridžiuvienė and Raudonienė 2013).

According to the biodeterioration caused by *Alternaria* species, Sohail *et al.* (2011) reported that *Alternaria* species can produce a variety of enzymes capable of hydrolyzing cellulose to glucose. Also it has developed lignocellulolytic enzyme systems and has been found to be very destructive molds in museums, especially for wooden frames (Garg *et al.* 1995).

CONCLUSIONS

1. This study reported on natural extracts from some woody trees and their use for protecting wood against attacks by two mold fungi, namely, *Alternaria tenuissima* and *Fusarium culmorum*. Additionally, their efficacy was compared with Paraloid B-72, which can be used as a consolidation polymer as well as an antifungal agent.
2. According to the *in vitro* experiment, the methanol extract of *C. viminalis* bark was highly effective against the growth of *F. culmorum*, and so was the extract of *M. grandiflora* leaves against *A. tenuissima*.
3. X-ray spectroscopy and ESEM analyses showed changes in surface element components as well as the hyphal growth of the tested fungi.
4. Visual observations of *A. saligna* wood treated with *F. culmorum* and *A. tenuissima* as well as Paraloid B-72 at concentrations of 2%, 3%, and 5% showed that the mycelial growth of *F. culmorum* and *A. tenuissima* on the surface of treated wood samples could be considered full (coverage around 100%) to more than half (coverage more than 50%).
5. Most of the wood samples treated with Paraloid B-72 and methanol extracts, especially at a concentration of 10%, showed no fungal growth of *F. culmorum* over the wood surfaces, except for *C. sempervirens* wood treated with methanol extract. Almost all of the wood treated with methanol extracts showed growth of the fungus *A. tenuissima* hypha with some contamination by other microorganisms, except for the wood treated with the methanol extract of *M. pomifera* bark.
6. On the wood treated by the methanol extract of *M. grandiflora* leaves, some inhibition zones were observed around a concentration of 10%.
7. After three months, no fungal growth on the wood surface treated with methanol extract of *M. pomifera* bark was observed, while the other treatments showed obvious growth of *F. culmorum* on the surface of the treated wood.

8. The tested fungi completely covered the wood samples treated with Paraloid B-72 at 5% and 10% after three months of treatment.
9. Other work was recommended on the same extracts with the same wood to show the effect of the combined use of the extracts and the Paraloid B-72 as antifungal agent (Mansour and Salem 2015) as well as possible application in the protection of the wood.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for the funding of their work through the research group project No. RGP-010.

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Article submitted: November 26, 2014; Peer review completed: March 1, 2015; Revised version received and accepted: March 1, 2015; Published: March 10, 2015.
DOI: 10.15376/biores.10.2.2570-2584