

Natural Durability of *Citharexylum spinosum* and *Morus alba* Woods Against Three Mold Fungi

Maisa M. A. Mansour,^a Mohamed Z. M. Salem,^{b,*} Mohamed H. Khamis,^c and Hayssam M. Ali^{c,d}

The natural durability of wood to mold fungi was tested under laboratory conditions with locally sourced *Citharexylum spinosum* and *Morus alba* woods. The mold fungi were *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger*. Changes in surface elemental composition were evaluated with energy dispersive X-ray spectroscopy (EDX) and the biodeterioration of wood surfaces by scanning electron microscope (SEM). The C peak element of *C. spinosum* wood was affected significantly ($P = 0.0004$) and decreased from 49.91% in the control specimens to 47%, 40.1%, and 40% with *P. selerotigenum*, *A. niger*, and *P. variotii*, respectively. Also, the C peak element of *M. alba* heartwood significantly decreased ($P < 0.0001$) from 51.33% in the control specimens to 41.49%, 45.66%, and 43.66% in wood inoculated with *A. niger*, *P. variotii*, and *P. selerotigenum*, respectively. The elements Al and Cu were observed in high percentages with *M. alba* heartwood inoculated by *P. variotii*. The methanol extract from *M. alba* heartwood showed good inhibition against the growth of *A. niger* at a concentration of 32 $\mu\text{g/mL}$, and the methanol extract from *C. spinosum* wood showed remarkable inhibition against the growth of *P. variotii* at a concentration of 8 $\mu\text{g/mL}$. The results of this study clearly showed the changes that occur in wood samples as a result of fungal infestation.

Keywords: *Morus alba*; *Citharexylum spinosum*; Mold fungi; EDX; SEM; Elemental composition; Methanol extract

Contact information: a: Conservation Department, Faculty of Archaeology, Cairo University, Giza, 12613, Egypt; b: Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt; c: Timber Trees Research Department, Sabahia Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center; Alexandria, Egypt; d: Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; *Corresponding author: zidan_forest@yahoo.com

INTRODUCTION

Wood is a natural organic material that consists of hemicellulose, cellulose, lignin, and minor amounts of extraneous materials, mostly in the form of organic extractives and inorganic minerals such as ash (Pettersen 1984). Generally, wood has an elemental composition of about 50% carbon, 6% hydrogen, 43% oxygen, and trace amounts of nitrogen and several metal ions.

While in storage, wood may deteriorate, discolor, stain, or be left with other devaluing damage as a result of different types of mold that either penetrate or live only on the surface of the wood (Sirmah *et al.* 2009). Mildews or mold fungi are not capable of degrading the structural components of wood, *i.e.*, cellulose, lignin, and hemicellulose; therefore, they do not cause a decrease in wood strength (Viitanen and Ritschkoff 1991; Hukka and Viitanen, 1999; Daniel 2003; Ghosh *et al.* 2008). Biodeteriogenic fungi have

the ability to use substrates such as the simple sugars and starches that are present in ray cells and axial cell lumens to sustain their growth and reproduction, thereby altering wood and wood products (Sequeira *et al.* 2012; Rosado *et al.* 2013). A significantly large growth of mold fungi, however, may act as a medium for other possible means of decay (Scheerer *et al.* 2009; Sterflinger and Piñar 2013).

The natural durability or resistance of wood against decay or mold fungi is primarily dependent on the chemical composition of the wood (Philip *et al.* 1995). Jeloková and Šindler (1997) found that the durability of beech and spruce wood against *Trametes versicolor* and *Serpula lacrymans* was increased by tannins. On the other hand, the sugar contents have a negative effect on the wood durability of beech wood (Jeloková and Šindler 2001). Fungal mycelium utilizes the sapwood in all wood species; some wood species like oak, elm, walnut, and mulberry, which have high natural extractives in heartwoods, are resistant to fungal attack (Kazemi 2007). Several woods, especially the heartwood, contain extractives that are toxic for fungi (Neya *et al.* 2004; Arango *et al.* 2006; Taylor *et al.* 2006; Maranhão *et al.* 2013).

White mulberry (*Morus alba* L.) is native to northern China and has been widely cultivated and naturalized abroad. It is a wild plant available throughout the year, in large numbers in Beni-Suef, Egypt (Hussein *et al.* 2010). The wood has been reported to be durable (Se Golpayegani *et al.* 2010; Se Golpayegani 2011). Different wood, bark, and leaves extracts of *M. alba* have shown antibacterial, antifungal, antioxidant, antiviral, and anti-inflammatory properties (Chung *et al.* 2003; El-Beshbishy *et al.* 2006; Lokegaonkar and Nabar 2011; Salem *et al.* 2013). Many biological compounds have been identified from different extracts of *M. alba*, such as tannins, phytosterols, sitosterols, saponins, triterpenes, flavanoids, benzofuran derivatives, morusimic acid, anthocyanins, anthroquinones, glycosides, stilbenes, 2-arylbenzofurans, and oleanolic acid (Chen *et al.* 2005; Yogisha and Raveesha 2009). Resorcinol, which corresponded to the highest proportion of heartwood extracts (Salem *et al.* 2013; Se Golpayegani *et al.* 2014), has been reported to have preservative effects against basidiomycete fungi (Adikaram *et al.* 2010) and against *Coptotermes formosanus* termites (Yamaguchi *et al.* 2002).

Citharexylum spinosum, also known as *C. quadrangulare* or *C. fruticosum*, belongs to the family Verbenaceae, which includes common plants known as Fiddlewood, is a species of flowering plant native to southern Florida in the United States, the Caribbean, Guyana, Suriname, and Venezuela (Huxley *et al.* 1997; Wagner *et al.* 1999; Mar and Pripdeevech 2014). Iridoid glucoside, the 7- β -O-acetate of lamiide, along with the iridoid glucosides lamiide, lamiidoside, duranterectoside C, 8-epiloganin, and the lignan glucoside (β)-lyonirenisol-3a-O- β -D-glucopyranoside, have been isolated from the aerial parts of *C. spinosum* (Khalifa *et al.* 2002; Balázs *et al.* 2006). Additionally, the extracts and essential oils from the flowers of *C. spinosum* have been shown to exhibit antibacterial and antioxidant qualities (Wei and Shibamoto 2007; Mar and Pripdeevech 2014).

The aim of the present study was to evaluate the natural durability of *Morus alba* and *Citharexylum spinosum* woods against the mold fungi *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger*. These three mold fungi are common molds growing on wood surfaces, where, the wood used in humidified conditions such as in bathrooms or sauna rooms. The evaluation was carried out by elemental analysis using energy dispersive X-ray spectroscopy (EDX) as well as studying the fungal growth on the surface of the wood using a scanning electron microscope (SEM).

EXPERIMENTAL

Chemicals

All of the chemicals used in the present study were of high analytical grade from Fluka and Sigma-Aldrich Co. (USA).

Preparation of Wood Samples

In the present study, locally sourced samples of white mulberry (*Morus alba* L., family: Moraceae) heartwood and Florida fiddlewood (*Citharexylum spinosum* L., family: Verbenaceae) were used. The samples were collected from various locations around Alexandria city, Egypt, during August 2014. Wood samples measuring 20 × 20 × 20 mm were prepared at the Faculty of Agriculture, Alexandria University sawmill workshop and air-dried at room temperature for about three months.

Fungal Inoculation

The mold fungi *Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger* were used to inoculate the wood samples at the Conservation Department, Faculty of Archaeology, Cairo University, Giza. Spore suspensions were prepared separately by adding 10 mL of sterilized distilled water to each plated, 15-day-old PDA culture of each fungus and freeing spores through the use of a camel brush (Darwish *et al.* 2013). Spore suspensions were individually strained through muslin and standardized to contain 1.2×10^6 spores/mL using a haemocytometer slide. Wood blocks (20 × 20 × 20 mm) of each wood type were oven-dried at 105 °C for 24 h, then autoclaved at 121 °C for 20 min. After cooling, three blocks of each wood type were used for each fungus. The wood blocks were sprayed until they were covered with a spore suspension of each fungus and incubated for eight weeks at 25±1 °C and 65% relative humidity (RH). Non-inoculated wood blocks were used as controls. An SEM (FEI Quanta 200 SEM FEG, USA) was used to study the fungal colonization of the wood surfaces by examining the changes in the surface morphology and particle size of the deteriorated samples. The microbial deterioration of both the inoculated and non-inoculated wood samples was studied by measuring the elemental distribution by energy dispersive X-ray spectroscopy (EDX) as well as the microbial growth on wood surface using the SEM (Danilatos and Robinson 1979). EDX analysis was used to measure the changes in the chemical composition of the un-inoculated and inoculated wood surfaces.

Wood Extracts

The methanol extracts of 50-g, air-dried, powdered samples of *M. alba* heartwood and *C. spinosum* wood were used to determine the antifungal activity of extracts by measuring the minimum inhibitory concentration (MIC) against the three tested mold fungi. The MIC values of the extracts were determined according to Eloff (1998), with slight modification. The concentrations of the methanol extracts ranged from 8 µg/mL to 1000 µg/mL.

Statistical Analysis

Analysis of variance (ANOVA) procedure (SAS version 8.2, 2001, USA) in a completely randomized design (CRD) was used to study the statistical differences between the two wood species in terms of changes in surface elemental analysis as affected by the mold fungi *P. selerotigenum*, *P. variotii*, and *A. niger*. Fisher's least

significant difference (LSD) at a 5% level of probability was used to measure the differences among the means of the elemental composition of the wood surfaces.

RESULTS AND DISCUSSION

SEM and EDX Analyses of Wood Inoculated with *A. niger*, *P. variotii*, and *P. selerotigenum*

The surface analysis of *C. spinosum* wood and *M. alba* heartwood samples inoculated with *A. niger*, *P. variotii*, and *P. selerotigenum* was performed by EDX and SEM. Results are shown in Tables 1 and 2 and Figs. 1-6.

Table 1 presents the changes in elemental composition of the surface of *C. spinosum* wood samples as affected by the three mold fungi by comparison with the uninoculated control samples. The C peak element was significantly affected ($P = 0.0004$) and decreased from the 49.91% seen in the control to 47%, 40.1%, and 40% as a result of the wood samples being inoculated with *P. selerotigenum*, *A. niger*, and *P. variotii*, respectively. The results showed that *A. niger* and *P. variotii* consumed a high amount of C in comparison with the control. The change in the O element peak of the wood samples was not significantly affected by inoculation with *P. selerotigenum* (34.55%), *A. niger* (34.67%), and *P. variotii* (35.15%) in comparison with the control (35.76%). The amount of N observed in control samples was 4.85%, and this was completely consumed by the three mold fungi tested. Si element peak in the wood showed significant effects after inoculation with *A. niger* (3.14%) in comparison with the control treatment (1.45%). The P element peak decreased from 3.38% (control) to 1.32% (*A. niger*) and 0.23% (*P. selerotigenum*) but increased by 5.25% in surface wood inoculated by *P. variotii*. Al increased significantly ($P < 0.0001$) from 0.47% in the control to 6.09% as a result of inoculation with *P. selerotigenum*.

Table 1. Elemental Analysis (Atomic %) of *C. spinosum* Wood Inoculated with Three Mold Fungi

Element	Control	Wood + <i>A. niger</i>	Wood + <i>P. variotii</i>	Wood + <i>P. selerotigenum</i>	LSD _{0.05}	P value
C	49.91*	40.10	40.00	47	4.10	0.0004
O	35.76	34.67	35.15	34.55	ns	0.256
N	4.85	0.00	0.00	0.00	1.74	0.0004
P	3.38	1.32	5.25	0.23	2.34	0.005
Al	0.47	0.75	1.84	6.09	1.35	<0.0001
Si	1.45	3.14	0.00	1.78	0.29	<0.0001
S	0.00	1.98	4.13	0.84	0.46	<0.0001
K	0.67	1.59	0.66	0.56	0.63	0.017
Ca	0.89	4.51	0.57	0.56	1.41	0.0005
Zn	0.00	3.44	0.93	2.67	0.82	<0.0001
Mg	1.38	0.00	4.15	0.00	0.10	<0.0001
Cl	0.00	1.40	2.42	0.55	0.16	<0.0001
Mn	0.55	0.00	0.29	0.00	0.39	0.0341
Fe	1.56	0.74	3.76	0.48	0.98	0.0002
Cu	0.00	6.36	0.84	4.67	0.78	<0.0001

*: Values are the mean of three replicates.

ns: Not significant at 0.05 level of probability according to Fisher's least significant difference.

The highest amount of S element observed in the inoculated wood samples was in those samples inoculated by *P. variotii* (4.13%), and the highest amount of Zn element was observed in the wood inoculated with *A. niger* (3.44%). S and Zn elements were not detected in the control samples. Ca element peak was significantly increased ($P = 0.0005$) in the surface of *C. spinosum* wood, from 0.89% in the control to 4.51% in wood inoculated by *A. niger*. The highest amount of Mg element peak was observed in wood inoculated with *P. variotii* (4.15%) and was not observed at all in the wood samples inoculated with *A. niger* and *P. selerotigenum* in comparison with the 1.38% of the control. Furthermore, a significant amount of Cl element was found in the wood samples inoculated by *P. variotii* (2.42%) but was not observed in the control. Cu element was not observed in un-inoculated *C. spinosum* wood samples but was observed in significant ($P < 0.0001$) amounts in wood treated by *A. niger* and *P. selerotigenum* at 6.36% and 4.67%, respectively. The highest amount of Fe element, 3.76%, was observed in wood samples treated with *P. variotii* in comparison with the control amount of 0.69%.

Table 2 shows the changes in elemental composition of the *M. alba* heartwood samples as a result of inoculation by the three mold fungi *A. niger*, *P. variotii*, and *P. selerotigenum* in comparison with the un-inoculated control samples. The presence of C was decreased significantly from 51.33% in the control to 41.49%, 45.66%, and 43.66% in the wood samples inoculated with *A. niger*, *P. variotii*, and *P. selerotigenum*, respectively. The O presence on the surface of the wood samples was not significantly affected in wood inoculated with *A. niger* (36.91%), *P. variotii* (33.73%), and *P. selerotigenum* (34.91%) in comparison with the control (36.24%). N was almost entirely consumed by the three mold fungi, where a small amount was observed in the surfaces of wood samples inoculated by *A. niger* (0.007%) in comparison with the control (1.07%). The presence of P decreased from 2.01% in the control to 1.07% in samples inoculated by *A. niger* and to 0.30% with wood inoculated by *P. variotii* and increased to 3.46% in samples inoculated by *P. selerotigenum*.

Table 2. Elemental Analysis (Atomic %) of Inoculated *M. Alba* Heartwood with Three Mold Fungi

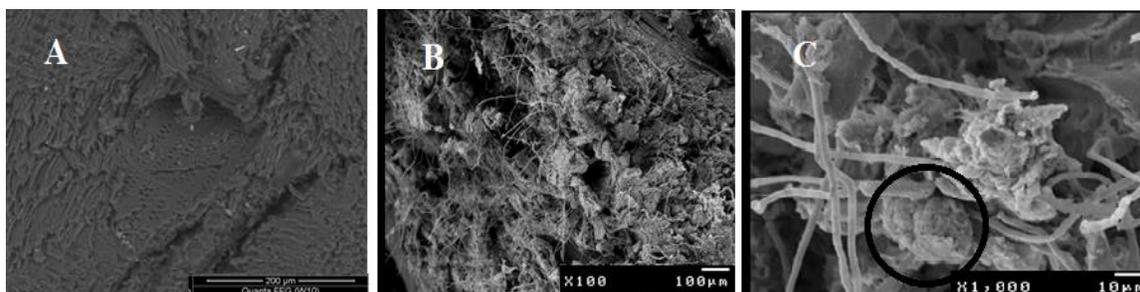
Element	Control	Wood + <i>A. niger</i>	Wood + <i>P. variotii</i>	Wood + <i>P. selerotigenum</i>	LSD _{0.05}	P value
C	51.33*	43.66	45.66	41.49	2.44	0.0006
O	36.24	36.91	33.73	34.91	ns	0.0510
N	1.07	0.007	0.00	0.00	0.015	<0.0001
P	2.01	1.07	0.30	3.46	1.22	0.0462
Al	1.29	0.36	7.81	4.57	2.41	<0.0001
Si	0.00	2.15	2.76	2.26	1.09	<0.0001
S	0.00	1.24	0.92	3.51	0.45	<0.0001
K	2.18	2.84	0.58	0.61	0.47	0.0109
Ca	0.68	5.53	0.65	0.41	4.24	0.0004
Zn	0.00	1.7	0.41	1.29	0.71	<0.0001
Mg	2.1	0.00	0.00	3.12	1.22	<0.0001
Cl	0.00	0.58	0.80	2.13	0.44	<0.0001
Mn	1.53	0.17	0.14	0.00	0.79	0.0242
Fe	0.69	0.34	0.87	0.39	ns	0.6117
Cu	0.00	3.44	5.31	1.84	1.34	<0.0001

*: Values are the mean of three replicates.

ns: Not significant at 0.05 level of probability according to Fisher's least significant difference.

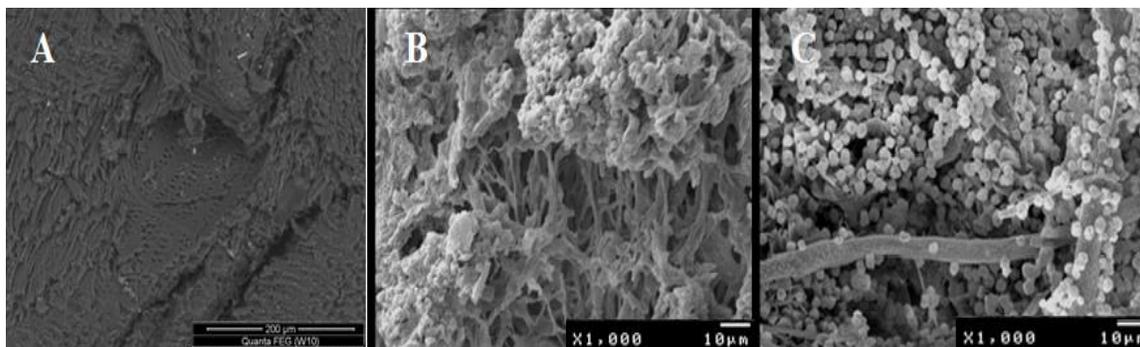
Compared to the 1.29% of the control, the presence of Al was increased significantly (<0.0001) to 7.81% in wood inoculated with *P. variotii* and 4.57% in wood samples inoculated with *P. selerotigenum* but decreased to 0.36% in samples inoculated with *A. niger*. The presence of Ca on the surface of *M. alba* heartwood was increased significantly from 0.68% in the control to 5.53% in samples inoculated by *A. niger*. Mg was observed in wood inoculated with *P. selerotigenum* at 3.12% but was not observed in the wood samples inoculated with *A. niger* and *P. variotii* in comparison with the 2.1% of the control. Si was observed at 2.76%, 2.15% and 2.26% on surface of *M. alba* heartwood inoculated by *P. variotii*, *A. niger* and *P. selerotigenum*, respectively, but it was not observed in the control samples. S, Zn, Cl, and Cu were not observed in the elemental composition of the control wood but were observed with different percentages in the wood samples inoculated with the three mold fungi. For example, Cu was observed at 5.31% and 3.44% in heartwood surface of *M. alba* inoculated with *P. variotii*, and *A. niger*, respectively, but was not observed in the control.

As was reported by Ljaljević-Grbić *et al.* (2013), the mold fungi used in this experiment utilize available sugars, hemicellulose, proteins, and amino acids as the primary means of deteriorating wood materials. The distribution and growth of the fungal hyphae of the three tested fungi over the surface of *C. spinosum* wood, as observed by SEM, are shown in Figs. 1, 2, and 3. The hyphae and spores of *A. niger* (Fig. 1) and *P. variotii* (Fig. 2) inundated the surface of the *C. spinosum* wood, while the anatomical structure of the *P. selerotigenum* wood was partially visible and not completely covered with spores and hyphae (Fig. 3).



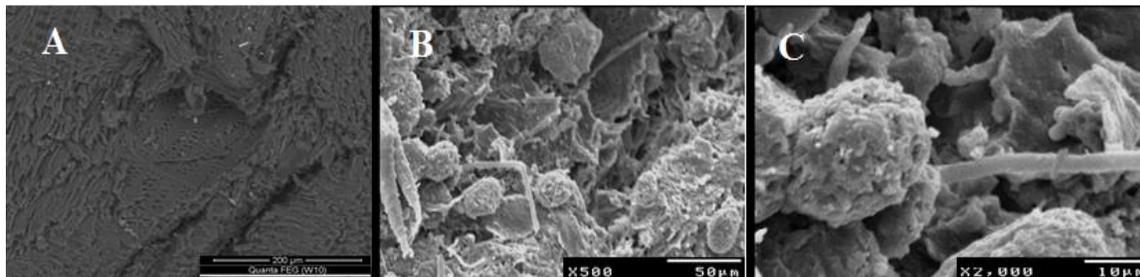
A- Control treatment; B, C- Inoculated samples

Fig. 1. SEM images of the wood surface of *C. spinosum* inoculated with *A. niger*



A- Control treatment; B, C- Inoculated samples

Fig. 2. SEM images of the surface of *C. spinosum* wood inoculated with *P. variotii*

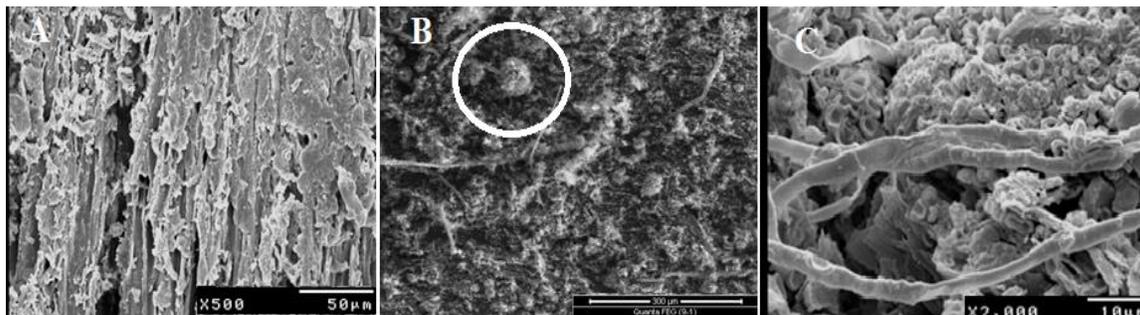


A- Control treatment; B, C- Inoculated samples

Fig. 3. SEM images of the wood surface of *C. spinosum* inoculated with *P. selerotigenum*

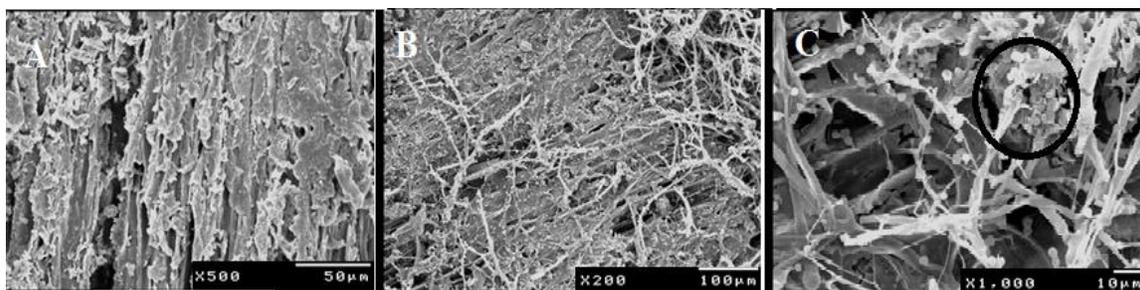
The observed conidiophores of the tested *A. niger* were smooth-walled, hyaline, and turning dark towards the vesicle. The conidial heads were biseriate, and the phialidic conidia were globular and black (Figs. 1 and 4). The fruiting bodies and ascospore formations of *P. variotii* can be clearly seen in Fig. 2C. The mycelium of *P. selerotigenum* typically consists of highly branched, septate, and usually colorless hyphae (Lamsal *et al.* 2013). The sexual reproduction of perfect stage *Penicillium* resulting in the production of cleistothecium ascocarp can be seen in Figs. 3C and 6C.

Figures 4 to 6 show that the surface of *M. alba* heartwood was totally covered by the spores and hyphae of the three tested mold fungi. It has previously been reported that *M. alba* wood from Australia and Asia is nonresistant to decay (Tewari 1978), but two stilbene-type compounds, oxyresveratrol trans-2,3',4,5'-tetrahydro-stilbene 1, a component of heartwood, and its 4'-prenyl derivative, have been isolated from the xylem tissue of the *M. alba* samples inoculated with *Fusarium solani* f. sp. *mori* (Takasugi *et al.* 1978; Takahashi and Shirata 1982). These compounds had previously been extracted from Osage orange wood and shown good antifungal activity (Barnes and Gerber 1955).



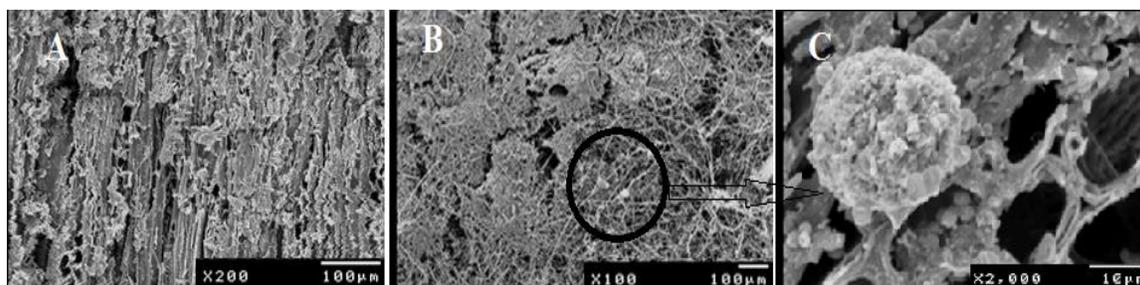
A- Control treatment; B, C- Inoculated samples

Fig. 4. SEM images of the surface of *M. alba* wood inoculated with *A. niger*



A- Control treatment; B, C- Inoculated samples

Fig. 5. SEM images of the surface of *M. alba* wood inoculated with *P. variotii*. The fruiting bodies and ascospore formations are clear in Fig. 5C.



A- Control treatment; B,C- Inoculated samples

Fig. 6. SEM images of the wood surface of *M. alba* inoculated with *P. sclerotigenum*

P. variotii, a soft-rot fungus, is called a green mold because of the color of its spores and mycelium (Yoon *et al.* 2007) and may be recognized by its dense brush-like spore-bearing structures (Pitt 1979; Domsch *et al.* 1980). It produces a yellow discoloration in oak wood during storage and drying (Bauch *et al.* 1991). *P. variotii* produces ample amounts of xylanase (Schmidt *et al.* 1979) and excretes glucuronidase. Additionally, the fungus *P. variotii* may be used to obtain tannins that are suitable for adhesives (Schmidt *et al.* 1984; Schmidt and Weißmann 1986). Also, the sugars found in spent sulfite liquor can be converted by *P. variotii* for use as animal feed (Forss *et al.* 1986).

Species of *Penicillium* are known for their ability to produce extracellular enzymes including cellulase (Krogh *et al.* 2004). Additionally, some *Penicillium* species can degrade pectin, while a greater number of species are able to degrade xylan (Yoon *et al.* 2007). The production of xylanase enzymes by *Aspergillus* spp. has been studied by de Vries and Visser (2001); the hydrolytic enzymes involved include endo-1,4- β -glucanases; exo-1,4- β -glucanase, and 1,4- β -glucosidases for cellulose hydrolysis; and endo-1,4- β -xylanases and β -xylosidases for hemicelluloses hydrolysis (Biely and Tenkanen 1998).

By growing mold fungi over the surfaces of wood, there were large fruiting structures that release vast numbers of spores in nature, as shown in Figs. 1 through 6 (Mansour and Salem 2015; Mansour *et al.* 2015).

In our previously works (Mansour and Salem 2015; Mansour *et al.* 2015), we started to find the changes in the full elemental composition of woods as affected by mold fungi using the EDX technique. For example, the C and O elements compositions of wood (*Acacia saligna*) were decreased compared to the control treatment (unincubated) as affected by the mold fungi; *Trichoderma harzianum*, *Alternaria tenuissima*, and *F. culmorum*.

Most of the research studies were focused in the using of natural extracts or essential oils against the growth of mold fungi, for example, the oils of Garlic, Neem, Clove, Olive and Ajwain caused significant reduction in the growth of *A. niger*, *A. flavus*, *P. variotii*, *Penicillium* sp., *Trichoderma* sp., *Grifola* sp. and *T. viridi* (Hussain *et al.* 2013). Also, the three tested mold fungi showed a variety of growth inhibition responses to fatty acid extracts from the wood, bark, and leaves of *Brachychiton diversifolius* (Salem *et al.* 2014a) and the wood essential oil and methanol and chloroform extracts from the wood and bark of *Delonix regia* (Salem *et al.* 2014b).

Antifungal Activity of Methanol Extracts

According to the MIC values presented in Table 3, the methanol extracts from *M. alba* heartwood showed good inhibition against the growth of *A. niger* at low concentrations (32 µg/mL) and relatively high concentration against *P. variotii* and *P. selerotigenum*, with MIC values of 500 and 250 µg/mL, respectively. Among some extractives used for wood impregnation, extracts of mulberry were most effective relative to wood durability (Kazemi 2007). Phenols, stilbenes, flavonoids, and sterols in the extractives of white mulberry wood have been reported (Venkataraman 1972; Rowe and Conner 1979; Se Golpayegani *et al.* 2010; Sadeghifar *et al.* 2011). The toxicity of extracts from *M. alba* could be related to resorcinol, which corresponded to the highest proportion of the alcoholic extract that has been reported in earlier works (Sadeghifar *et al.* 2011; Salem *et al.* 2013; Se Golpayegani *et al.* 2014). Moracin N, M and P have been isolated before from *M. alba* (Nguyen *et al.* 2009).

The results showed that the heartwood methanol, acetone, and ethyl acetate extracts of *M. alba* had the best inhibitive effect on *Coriolus versicolor*, *Gloeophyllum trabeum* and *Botryodiplodia theobromae* compared to the others studied extracts from other trees. The ethyl acetate extracts (1g/L) showed inhibition rate for three fungi over 85% and at the concentration of 3g/L was 100%, with EC₅₀ value at 0.173g/L, 0.324g/L, and 0.057g/L, respectively (Ying 2009).

Table 3. MIC Values (µg/mL) of Methanol Extracts of *C. spinosum* Wood and *M. alba* Heartwood and Against the Growth of *A. niger*, *P. variotii*, and *P. selerotigenum*

Methanol extract	MIC value (µg/mL)		
	<i>A. niger</i>	<i>P. variotii</i>	<i>P. selerotigenum</i>
<i>M. alba</i> (heartwood)	32	500	250
<i>C. spinosum</i>	250	8	500

The methanol extract from *C. spinosum* wood exhibited remarkable inhibition against the growth of *P. variotii* at low concentrations (8 µg/mL) and at high concentrations. Inhibition against *A. niger* and *P. selerotigenum* growth was found with MIC values of 250 µg/mL and 500 µg/mL, respectively. The antifungal effect of extracts from *C. spinosum* could be related to the active constituents like iridoid glycoside phlomiol, 5-deoxy pulchellose, larniide, durantocide I and lamidoside (Khalifa *et al.* 2002; El-Naggar 2007).

Extracts from wood have been recognized as potential agents against the growth of mold and other wood-destroying fungi, for example, the wood extracts of *Cupressus sempervirens* (Tapondjou *et al.* 2005), and *Maclura pomifera* heartwood wood extracts, morin, oxyresveratrol, and 1,3,6,7-tetrahydroxyxanthoneshown potential fungicidal activity (Barnes and Gerber 1955; Wolfrom and Bhat 1965).

CONCLUSIONS

1. In the present study, the surface elemental changes of *C. spinosum* wood and *M. alba* heartwood were monitored as well as the growth of the three tested mold fungi *P. selerotigenum*, *P. variotii*, and *A. niger* using EDX and SEM techniques.

2. C, mostly in wood carbohydrates and lignin, was significantly decreased and consumed by the three tested mold fungi.
3. C was significantly ($P = 0.0004$) decreased from 49.91% in the control to 47%, 40.1%, and 40% in wood inoculated with *P. selerotigenum*, *A. niger*, and *P. variotii*, respectively.
4. C in *C. spinosum* wood was affected significantly ($P = 0.0004$) and decreased from 49.91% in the control to 47%, 40.1%, and 40% with *P. selerotigenum*, *A. niger*, and *P. variotii*, respectively.
5. C in *M. alba* heartwood was significantly ($P < 0.0001$) decreased, from 51.33% in the control to 41.49%, 40%, and 43.66% in wood inoculated with *A. niger*, *P. variotii*, and *P. selerotigenum*, respectively.
6. The change in O peak element was not significantly affected by inoculation with the three tested mold fungi.
7. Other elements showed different percentages according to wood species and type of mold fungus. For example, Al and Cu were observed in high percentages with *M. alba* heartwood inoculated by *P. variotii*.
8. SEM images showed the distribution and structures of spores and hyphae of the three tested mold fungi.
9. The methanol extract from *M. alba* heartwood showed good inhibition against the growth of *A. niger* at the concentration 32 $\mu\text{g/mL}$, and the methanol extract from *C. spinosum* wood showed remarkable inhibition against the growth of *P. variotii* at the concentration of 8 $\mu\text{g/mL}$.
10. The natural durability between *M. alba* heartwood and *C. spinosum* wood showed variation according to the colonized fungi as shown in the variations of elemental composition.
11. Other work is recommended to study the natural durability of some commercial woods with the same mold fungi.

ACKNOWLEDGMENTS

The authors extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding this research group, No. (RG 1435-011).

REFERENCES CITED

- Adikaram, N., Karunanayake, C., and Abayasekara, C. H. (2010). "The role of pre-formed antifungal substances in the resistance of fruits to postharvest pathogens," in: *Postharvest Pathology*, D. Prusky and M. L. Gullino (eds.), Springer, Berlin, pp. 1-11. DOI: 10.1007/978-1-4020-8930-5_1

- Arango, R. A., Green III, F., Hintz, K., Lebow, P. K., and Miller, R. B. (2006). "Natural durability of tropical and native wood against termite damage by *Reticulitermes flavis* (Kollar)," *Int. Biodeter. Biodegr.* 57, 146-150.
- Balázs, B., Toth, G., Duddeck, H., and Soliman, H. S. (2006). "Iridoid and lignan glycosides from *Citharexylum spinosum* L.," *Nat. Prod. Res.* 20(2), 201-205. DOI: 10.1080/14786410500056694
- Barnes, R. A., and Gerber, N. N. (1955). "The antifungal agent from Osage orange wood," *J. Am. Chem. Soc.* 77(12), 3259-3264. DOI: 10.1021/ja01617a032
- Biely, P., and Tenkanen, M. (1998). "Enzymology of hemicellulose degradation," in: *Trichoderma and Gliocladium*, Vol. 2, G. E. Harman and C. P. Kubicek (eds.), Taylor and Francis, London, UK, pp. 25-47.
- Bauch, J., Hundt von, H., Weißmann, G., Lange, W., and Kubel, H. (1991). "On the causes of yellow discolorations of oak heartwood (*Quercus Sect. Robur*) during drying," *Holzforschung* 45(2), 79-85. DOI: 10.1515/hfsg.1991.45.2.79
- Chen, C. C., Liu, L. K., Hsu, J. D., Huang, H. P., Yang, M. Y., and Wang, C. J. (2005). "Mulberry extract inhibits the development of atherosclerosis in cholesterol fed rabbits," *Food Chem.* 91(4), 601-607. DOI: 10.1016/J.FOODCHEM.2004.06.039
- Chung, K. O., Kim, B. Y., Lee, M. H. Kim, Y. R., Chung, H. Y., Park, J. H. and Moon, J. O. (2003), "In-vitro and in-vivo anti-inflammatory effect of oxyresveratrol from *Morus alba* L.," *J. Pharm. Pharmacol.* 55(12), 1695-700. DOI: 10.1211/0022357022313
- Darwish, S. S., EL Hadidi, N. M. N., and Mansour, M. (2013). "The effect of fungal decay on *Ficus sycomorus* wood," *Int. J. Conserv. Sci.* 4(3), 271-282.
- Danilatos, G. D., and Robinson, V. N. E. (1979). "Principles of scanning electron microscopy at high specimen pressures," *Scanning* 2(2), 72-82. DOI: 10.1002/sca.4950020202
- de Vries, R. P., and Visser, J. (2001). "Aspergillus enzymes involved in degradation of plant cell wall polysaccharides," *Microbiolo. Mol. Biol. Rev.* 65(4), 497-522. DOI: 10.1128/MMBR.65.4.497-522.2001
- Domsch, K. H., Gams, W., and Anderson, T. H. (1980). *Compendium of Soil Fungi*, Vol. 2, Academic Press, London, UK.
- El-Beshbishy, H. A., Singab, A. N., Sinkkonen, J., and Pihlaja, K. (2006). "Hypolipidemic and antioxidant effects of *Morus alba* L. (Egyptian mulberry) root bark fractions supplementation in cholesterol-fed rats," *Life Sci.* 78(23), 2724-2733. DOI: 10.1016/j.lfs.2005.10.010
- Eloff, J. N. (1998). "A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria," *Planta Med.* 64(8), 711-713. DOI: 10.1055/s-2006-957563
- El-Naggar, D. M. (2007). "Antibilharzial study of some extracts from *Citharexylum quadrangular Jacq.*," Ph.D. dissertation, Faculty of Pharmacy for Girls, Al-Azhar University, Nasr City, Cairo, Egypt.
- Forss, K., Jokinen, K., and Lehtomaki, M. (1986). "Aspects of the pekilo protein process," *Paperi ja Puu* 68(11), 839-844.
- Hukka, A., and Viitanen, H. A. (1999). "A mathematical model of mould growth on wooden material," *Wood Sci. Tech.* 33(6), 475-485. DOI: 10.1007/s002260050131
- Hussain, A., Shrivastav, A., and Jain, S. K. (2013). "Antifungal activity of essential oils against local wood degrading cellulolytic filamentous fungi," *Adv. Biores.* 4(2), 161-167.

- Hussein, M. S. H., El-Tawil, O. S., Yassin, N. E. H., and Abdou, A. (2010). "The protective effect of *Morus alba* and *Calendula officinalis* plant extracts on carbon tetrachloride-induced hepatotoxicity in isolated rat hepatocytes," *J. Am. Sci.* 6(10), 762-773.
- Huxley, A., Griffiths, M., and Levy, M. (1997). *Dictionary of Gardening*, McMillan Reference Limited, London, UK.
- Jeloková, E., and Šindler, J. (1997). "Influence of beech and acacia wood extractives on activity of wood-destroying fungi," in: 1st Symposium - Drevoznehodnocujúce huby 97, TU Zvolen, pp. 133-139.
- Jeloková, E., and Šindler, J. (2001). "Testing of some chemical compounds for wood protection," *Drevo* 56, 137-138.
- Kazemi, S. M. (2007). "Impregnation of beech, maple, alder and lime under different treatments of extractives and fungal attack," *J. Biol. Sci.* 7(8), 1463-1467. DOI: 10.3923/jbs.2007.1463.1467
- Khalifa, T. I., El-Gendi, O. D., Ammar, H. A., and El-Naggar, D. M. (2002). "Iridoid glycosides from *Citharexylum quadrangulare*," *Asian J. Chem.* 14(1), 197-202.
- Lamsal, K., Kim, S. W., Naeimi S., Adhikari M., Yadav, D. R., Kim, C., Lee, H. B., and Lee, Y. S. (2013). "Three new records of *Penicillium* species isolated from insect specimens in Korea," *Mycobiology* 41(2), 116-119. DOI: 10.5941/MYCO.2013.41.2.116
- Ljaljević-Grbić, M., Stupar, M., Vukojević, J., Maričić, I., and Bungur, N. (2013). "Molds in museum environments: Biodeterioration of art photographs and wooden sculptures," *Arch. Biol. Sci., Belgrade* 65(3), 955-962. DOI: 10.2298/ABS1303955G
- Lokegaonkar, S. P., and Nabar, B. M. (2011). "In vitro assessment of the antiplaque properties of crude *M. alba* leaf extract," *J. Herb. Med. Toxicol.* 5(1), 71-77.
- Krogh, K. B. R., Morkeberg, A., Friscad, J. C., and Olsson, L. (2004). "Screening genus *Penicillium* for producers of cellulolytic and xylanolytic enzymes," *Appl. Biochem. Biotechnol.* 113(116), 389-401. DOI: 10.1385/ABAB:114:1-3:389
- Mansour, M. M., and Salem, M. Z. M. (2015). "Evaluation of wood treated with some natural extracts and Paraloid B-72 against the fungus *Trichoderma harzianum*: Wood elemental composition, *in-vitro* and application evidence," *Int. Biodeter. Biodegr.* 100(C), 62-69. <http://dx.doi.org/10.1016/j.ibiod.2015.02.009>
- Mansour, M. M. A., Abdel-Megeed, A., Nasser, R. A., and Salem, M. Z. M. (2015). "Comparative evaluation of some woody trees methanolic extracts and Paraloid B-72 against phytopathogenic mold fungi *Alternaria tenuissima* and *Fusarium culmorum*," *BioResources* 10(2), 2570-2584. DOI: 10.15376/biores.10.2.2570-2584
- Mar, A., and Pripdeevech, P. (2014). "Chemical composition and antibacterial activity of essential oil and extracts of *Citharexylum spinosum* flowers from Thailand," *Nat. Prod. Commun.* 9(5), 707-710.
- Maranhão, C. A., Pinheiro, I. O., Santana, L. B. D. A., Oliveira, L. S., Nascimento, M. S., and Bieber, L. W. (2013). "Antitermitic and antioxidant activities of heartwood extracts and main flavonoids of *Hymenaea stigonocarpa* Mart," *Int. Biodeter. Biodegr.* 79, 9-13. DOI: 10.1016/j.ibiod.2013.01.005
- Neya, B., Hakkou, M., Pétrissans, M., and Gérardin, P. (2004). "On the durability of *Burkea africana* heartwood: Evidence of biocidal and hydrophobic properties responsible for durability," *Ann. Forest Sci.* 61(3), 277-282. DOI: 10.1051/forest:2004020

- Nguyen, T. D., Jin, X., Lee, K., Hog, Y.-S., Young, H. K., and Jung, J. L. (2009). "Hypoxia-inducible factor-1 inhibitory benzofurans and chalcone-derived Diels-Alder adducts from *Morus* species," *J. Nat. Prod.* 72, 39–43. doi: 10.1021/np800491u
- Pettersen, R. C. (1984). "The chemical composition of wood," in: *The Chemistry of Wood*, Advances in Chemistry Series 207, R. M. Rowell (ed.), American Chemical Society, Washington, DC, pp. 57-126.
- Philip, R. W., Bruce, A., and Munro, A. G. (1995). "The effect of water soluble Scots pine (*Pinus sylvestris* L.) and Sitka spruce [*Picea sitchensis* (bong) Carr] heartwood and sapwood extracts on the growth of selected Trichoderma species," *Int. Biodeter. Biodegr.* 35(4), 355-367. DOI: 10.1016/0964-8305(95)00053-9
- Pitt, J. I. (1979). *The Genus Penicillium and Its Teleomorphic States Eupenicillium and Talaromyces*, Academic Press, London, UK.
- Rosado, T., Martins, M. R., Pires, M., Mirão, J., Candeias, A., and Caldeira, A. T. (2013). "Enzymatic monitorization of mural paintings: Biodegradation and biodeterioration," *Int. J. Conserv. Sci.* 4(SI), 603-612.
- Rowe, J. W., and Conner, A. (1979). "Extractives in Eastern hardwoods – A review," Forest Products Laboratory, U.S. Department of Agriculture, Madison, WI.
- Salem, M. Z. M., Aly, H. I. M., Gohar, Y. M., and EL-Sayed, A. B. (2013). "Biological activity of extracts from *Morus alba* L., *Albizia lebbek* (L.) Benth. and *Casuarina glauca* Sieber against the growth of some pathogenic bacteria," *Int. J. Agric. Food Res.* 2(1), 9-22.
- Salem, M. Z. M., Abdel-Megeed, A., and Ali, H. M. (2014a). "Stem wood and bark extracts of *Delonix regia* (Boj. Ex. Hook): Chemical analysis and antibacterial, antifungal, and antioxidant properties," *BioResources* 9(2), 2382-2395. DOI: 10.15376/biores.9.2.2382-2395
- Salem, M. Z. M., Ali, H. M., and Mansour, M. M. (2014b). "Fatty acid methyl esters from air-dried wood, bark, and leaves of *Brachychiton diversifolius* R. Br: Antibacterial, antifungal, and antioxidant activities," *BioResources* 9(3), 3835-3845. DOI: 10.15376/biores.9.3.3835-3845
- Sadeghifar, H., Sheikh, A., Khalilzadeh, M. A., and Ebadi, A. G. H. (2011). "Heartwood extractives of Iranian *Morus alba* wood," *J. Chem. Soc. Pak.* 33(1), 104-106.
- SAS (2001). "Users guide: Statistics (release 8.02)," SAS Inst., Cary, NC.
- Scheerer, S., Ortega-Morales, O., and Gaylarde, C., (2009). "Microbial deterioration of stone monuments – An updated overview," *Adv. Appl. Microbiol.* 66, 97-139. DOI: 10.1016/S0065-2164(08)00805-8
- Sirmah, P., Dumarçay, S., and Gérardin, P. (2009). "Unusual amount of (-)-mesquitol from the heartwood of *Prosopis juliflora*," *Nat. Prod. Res.* 23(2), 183-189. DOI: 10.1080/14786410801940968
- Se Golpayegani, A., Thevenon, M. F., Gril, J., and Pourtahmasi, K. (2010). "Natural durability of white mulberry (*Morus alba* L.)," 4th International Research Group Of Wood Preservation (IRG), May 9-13, Biarritz, France.
- Se Golpayegani, A. 2011. "Characterization of mulberry's wood (*Morus alba* L.), considering its usage in making Iranian lute," Ph.D. Thesis, Montpellier II University, France, 288p.
- Se Golpayegani, A., Thévenon, M. F., Gril, J., Masson, E., and Pourtahmasi, K. (2014). "Toxicity potential in the extraneous compounds of white mulberry wood (*Morus*

- alba),” *Maderas-Cienc. Tecnol.* 16(2), 227-238, DOI: 10.4067/S0718-221X2014005000018
- Sequeira, S., Cabrita, E. J., and Macedo, M. F. (2012). “Antifungals on paper conservation: An overview,” *Int. Biodeter. Biodegr.* 74, 67-86. DOI: 10.1016/j.ibiod.2012.07.011
- Schmidt, O., and Weißmann, G. (1986). “Mikrobiologische Behandlung von Larchenrinden-Extrakten zur Herstellung von Leimharzen,” *Holz Roh-Werkst.* 44(9), 351-355. DOI: 10.1007/BF02612742
- Schmidt, O., Ayla, C., and Weißmann, G. (1984). “Mikrobiologische Behandlung von Fichtenrinde-Heiswasserextrakten zur Herstellung von Leimharzen,” *Holz Roh-Werkst.* 42(8), 287-292. DOI: 10.1007/BF02608936
- Schmidt, O., Puls, J., Sinner, M., and Dietrichs, H. H. (1979). “Concurrent yield of mycelium and xylanolytic enzymes from extracts of steamed birchwood, oat husks and wheat straw,” *Holzforschung* 33(6), 192-196. DOI: 10.1515/hfsg.1979.33.6.192
- Sterflinger, K., and Piñar, G. (2013). “Microbial deterioration of cultural heritage and works of art: Tilting at windmills?,” *Appl. Microbiol. Biotechnol.* 97(22), 9637-9646. DOI: 10.1007/s00253-013-5283-1
- Takahashi, K., and Shirata, A. (1982). “Production of antifungal substances in mulberry,” *Jap. Agric. Res. Q.* 16(2), 119-124.
- Takasugi, M., Munoz, L., Masamune, T., Shirata, A., and Takahashi, K. (1978). “Stilbene phytoalexins from diseased mulberry,” *Chem. Lett.* 1241-1242. DOI: 10.1246/cl.1978.1241
- Tapondjou, A. L., Adler, C., Fontem, D. A., Bouda, H., and Reichmuth, C. (2005). “Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val,” *J. Stored Prod. Res.* 41(1), 91-102. DOI: 10.1016/j.jspr.2004.01.004
- Taylor, A. M., Gartner, B. L., and Morrell, J. J. (2006). “Effects of heartwood extractive fractions of *Thuja plicata* and *Chamaecyparis nootkatensis* on wood degradation by termites or fungi,” *J. Wood Sci.* 52(2), 147-153. DOI: 10.1007/s10086-0050743-6
- Tewari, M. C. (1978). “Data on natural durability of timber species (installed in the test yard at New Forest, Dehra Dun) according to 1976 inspection, their treatability and seasoning characteristics,” IRG/WP/3127, International Research Group on Wood Preservation, Stockholm, Sweden.
- Venkataraman, K. (1972). “Wood phenolics in the chemotaxonomy of the moraceae,” *Phytochemistry.* 11(5), 1571-1586. DOI: 10.1016/0031-9422(72)85002-7
- Viitanen, H., and Ritschkoff, A. C. (1991). “Mold growth in pine and spruce sapwood in relation to air humidity and temperature,” Swed. Univ. Agric. Sci. Dept. Forest Prod., Uppsala, Sweden, Report No. 221.
- Wagner, W.L., Herbst, D.R., Sohmer, S.H. (1999). “Manual of the Flowering Plants of Hawaii,” i., Vol. 2, Bishop Museum Special Publication 83, University of Hawai’i and Bishop Museum Press, Honolulu, HI.
- Wei, A., and Shibamoto, T. (2007). “Antioxidant activities and volatile constituents of various essential oils,” *J. Agric. Food Chem.* 55(5), 1737-1742. DOI: 10.1021/jf062959x
- Wolfrom, M. L., and Bhat, H. B. (1965). “Osage orange pigments dVII. 1,3,6,7 Tetrahydroxyxanthone from the heartwood,” *Phytochemistry* 4, 765-768.
- Yamaguchi, H., Yoshino, K., and Kido, A. (2002). “Termite resistance and wood penetrability of chemically modified tannin and tannin-copper complexes as wood preservatives,” *J. Wood Sci.* 48(4), 331-337. DOI: 10.1007/BF00770710

- Ying, L. S. (2009). "Inhibitory effects of heartwood extracts from strong decay-resistant tree species on wood preservation," master thesis, Agricultural University of Hebei.
- Yogisha, S., and Raveesha, K. A. (2009). "In-vitro antibacterial effect of selected medicinal plant extracts," *J. Nat Prod. (India)* 2, 64-69.
- Yoon, J. H., Hong, S. B., Ko, S. J., and Kim, S. H. (2007). "Detection of extracellular enzyme activity in *Penicillium* using chromogenic media," *Mycobiol.* 35(3), 166-169.
DOI: 10.4489/MYCO.2007.35.3.166

Article submitted: March 3, 2015; Peer review completed: June 29, 2015; Revised version received and accepted: July 3, 2015; Published: July 15, 2015.
DOI: 10.15376/biores.10.3.5330-5344