

ORIGINAL RESEARCH ARTICLE

Biological resistance of elm (*Ulmus carpinifolia* var. *Umbelifera*) trees against fungal endophytes and white rot decay fungi

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ABSTRACT

Urban trees are one of the valuable storages in metropolitan areas. Nowadays, a particular attention is paid to the trees and spends million dollars per year to their maintenance. Trees are often subjected to abiotic factors, such as fungi, bacteria, and insects, which lead to decline mechanical strength and wood properties. The objective of this study was to determine the potential degradation of Elm tree wood by *Phellinus pomaceus* fungi, and *Biscogniauxia mediterranea* endophyte. Biological decay tests were done according to EN 113 standard and impact bending test in accordance with ASTM-D256-04 standard. The results indicated that with longer incubation time, weight loss increased for both sapwood and heartwood. Fungal deterioration leads to changes in the impact bending. In order to manage street trees, knowing tree characteristics is very important and should be regularly monitored and evaluated in order to identify defects in the trees.

Keywords: Wood Decay; Impact Bending; Endophytes; Elm Tree; Urban Forestry

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1. Introduction

Urban trees are one of the valuable storages in metropolitan regions. However, trees are exposed to wood decay fungi. Most of the fungi are able to degrade the chemical composition of woody structure of the trees, decreasing their mechanical stability that can cause serious damage to people, particularly during severe weather events^[1,2]. Certain biological agents can naturally deteriorate wood in the presence of a favorable environment. In nature, wood can be rapidly colonized by microorganisms^[3,4]. In the forest, most of the wood decay fungi present were reported in association with sings and fallen, dead wood^[5,6].

Fungi, bacteria, and insects are able to attack wood and consume the cell wall components. In fungi, wood cell wall decomposition is classified as white, brown, or soft rot^[2,3,7,8]. If they consume cellulose and hemicellulose, a brown rot will be created; when lignin is break down, a white rot will be produced. White rot is divided into simultaneous white rot and selective delignification^[9,10]. Decay fungi enter standing trees through wounds or breaks in the bark. Wounds can result from a variety of incidents including storms, pruning, or root cutting. Once the decay fungus breaches the bark, it enters the sapwood and can eventually affect the heartwood. Members of white-rot fungi are able to decompose all structural components in wood cell walls, i.e. the cellulose,

hemicellulose, and lignin^[2,3,11,12]. In the simultaneous rot, carbohydrates and lignin are almost uniformly degraded at the same time and similar rate^[2,13]. In the selective delignification, lignin is preferentially degraded, particularly in the compound middle lamella region (CML) with a separation of individual cells. Cellulose is consumed at the late stage of attack^[14-17].

Fungal attack is responsible for significant decreases in mechanical and physical wood properties, influencing moisture content, electrical conduction, acoustics, convection, elasticity and plasticity in wood^[2,18]. The changes cause significant losses in mechanical properties even before measurable weight loss^[19,20]. Former studies have already shown a close relationship between the degradation of hemicellulose components and losses in mechanical properties^[21,22].

Endophytes are bacterial or fungal microorganisms that colonize healthy plant tissue intercellularly and/or intracellularly without causing any apparent symptoms of disease^[23]. The objective of this study was to examine the role of white rot fungi and endophyte fungi in the wood degradation and their relationship in the wood of *Ulmus carpinifolia* var. *umbelifera* in urban forestry. According to field survey, this fungus was the most frequently in the trees and therefore considered for this study.

2. Materials and methods

2.1 Site study

The study area in this research is located in Shahid-Rajaie Park, Isfahan, Iran, between 30 degrees and 43 minutes to 34 degrees and 27 minutes north latitude, 49 degrees and 36 minutes to 55 degrees, and 31 minutes east of the Caspian Sea from Greenwich (**Figure 1**).

2.2 Tree wood species

This research was conducted on elm (*Ulmus carpinifolia* var. *umbelifera*) species. One infected and living tree selected from green spaces. *Ulmus carpinifolia* var. *umbelifera* was originally cultivated in Iran, where it was widely planted as an ornamental and occasionally grew to a great

size, being known there as “Nalband” Persian.

2.3 Wood samples

Wood samples were achieved from (*Ulmus carpinifolia* var. *umbelifera*) trees at breast height and air-dried to reach $23 \pm 2\%$ moisture content. Samples of $25_l \times 20_r \times 15_t$ mm according to the EN113^[24] were used for determination of mass loss (ML), and $60_l \times 20_r \times 6_t$ mm according to ASTM-D256-04^[25] for testing impact bending strength. The specimens used to assess impact bending strength were cut in cross section. 10 replicate specimens were prepared from different disks for each test. They were kept in a conditioning chamber (25°C , and $40 \pm 3\%$ RH) for 4 weeks before testing.

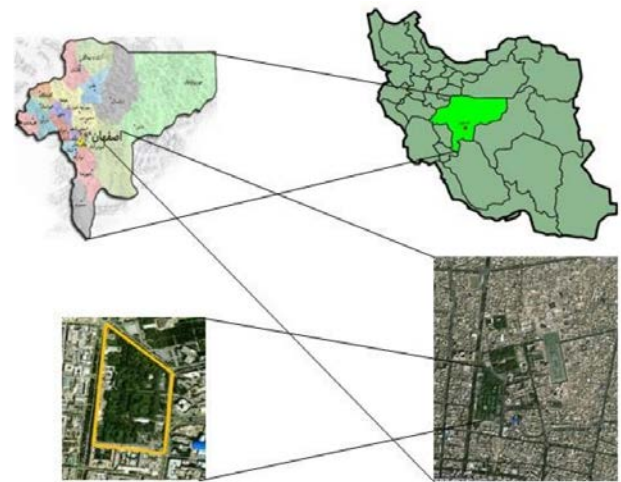


Figure 1. Study area map.

2.4 Decay test

In order to evaluate the degradation capabilities of fungi and endophytes, *Ulmus* wood blocks were cut according to EN113^[24] and then they were initially oven dried at $103 \pm 2^\circ\text{C}$ and weighed prior to fungal exposure. The wood blocks were then sterilized at 121°C for 20 min and exposed to fungi grown in Petrie dish according to EN113. Both heartwood and sapwood were incubated at 25°C and 65% relative humidity until the samples were acclimatized by the *P. pomaceus* fungi, and were then transferred to an incubator for 4 and 8 weeks (under the same conditions). The samples were removed from the incubator and fungal mycelia were removed from the surface of the specimens. The samples were then placed in an oven at $103 \pm 2^\circ\text{C}$ for 24 hours to reach to constant weight and to

determine weight loss for each individual sample to EN113^[24], as follows:

$$\text{Weight loss (Mass loss)} = [(M_2 - M_1) / M_1] \times 100$$

Where, M_1 is the oven-dry weight of sample prior to exposure, and M_2 is the oven-dry weight of sample after exposure to fungus.

2.5 Mechanical tests

The compression strength and unnotched impact bending tests were carried out according ASTM-D256-04^[25] standard and calculated by the following formula:

$$\text{Unnotched impact bending (J} \times \text{m)} = F_{\text{max}} / A$$

Where, F_{max} = force (J), A = cross section area (m^2).

2.6 Statistical analysis

To compare mechanical properties, weight loss and a student t-test was performed (95% confidence level) between decayed and un-decayed samples.

Table 1. Average ML and IB for Elm wood samples exposed to *P.pomaceus* fungi, *B. mediteranae* endophyte *P.pomaceus* + *B. mediteranae* endophyte, *B. nummularia* endophyte, *P.pomaceus* + *B. nummularia* endophyte for 4 and 8 weeks (%) (n = 10).

Test Samples	Mass loss (%)		Impact bending		F*	Sig**
	4 weeks	8 weeks	4 weeks	8 weeks		
Heartwood (control)	0.0		5.87			
Decayed heartwood (<i>P. pomaceus</i>)	2.28	4.02	3.17	2.77	14.70	0.010
Decayed heartwood (<i>P. pomaceus</i> With <i>B. mediteranae</i>)	0.64	4.36	3.08	2.78	17.09	0.000
Decayed heartwood (<i>P. pomaceus</i> With <i>B. nummularia</i>)	0.59	4.91	3.75	3.12	12.51	0.010
Sapwood (control)	0.0		6.17			
Decayed Sapwood (<i>P. pomaceus</i>)	2.56	10.74	3.10	2.55	32.71	0.000
Decayed Sapwood (<i>P. pomaceus</i> With <i>B. mediteranae</i>)	5.16	12.48	3.15	2.87	28.59	0.000
Decayed Sapwood (<i>P. pomaceus</i> With <i>B. nummularia</i>)	1.46	6.37	4.62	2.57	10.30	0.020
Decayed Sapwood (<i>B. mediteranae</i>)	1.12	11.04	3.77	2.60	33.92	0.000
Accession number: MF358883 ^[26]						
Decayed Sapwood (<i>B. nummularia</i>) Ac- cession number: MF358880 ^[26]	2.04	3.59	4.03	2.85	17.04	0.000

*F indicated that the means between two groups are significantly different.

** Sig. indicated that the differences between some of the means are statistically significant.

The results of the T-test **Table 2** indicated that the mentioned white-rot fungus had a significant effect on the weight loss of sapwood and heartwood samples ($p < 0.05$). Because the mean weight loss of decayed. Average mass losses were 4.46%, 3.25% after 8 weeks incubation for *P. pomaceus*

Statistical analysis was performed using the SPSS software program, version 23.

3. Results and discussion

The results of decay and mechanical tests on sapwood and heartwood after 4 and 8 weeks are summarized in **Table 1**. Statistical analysis indicated that there is a significant difference between mass loss and impact bending of sapwood and heartwood after incubation ($P < 0.05$).

Average mass losses were 10.74%, 4.02% after 8 weeks incubation for *P.pomaceus* in sapwood and heartwood, respectively, and 12.48%, 4.36% for *P. pomaceus*+ *B. mediteranae* endophyte for sapwood and heartwood, and 6.37%, 4.91% for *P. pomaceus* + *B. nummularia* endophyte sapwood and heartwood, and 11.04% for *B. mediteranae* endophyte, sapwood, and 3.59% for *B. nummularia*, endophyte sapwood.

(*Pp*), sapwood and heartwood, respectively, and 9.02%, 3.62% for *P.pomaceus*+ *B. mediteranae* endophyte sapwood and heartwood, and 5.01%, 1.87% for *P.pomaceus* + *B. nummularia* endophyte sapwood and heartwood, and 9.89% for *B. mediteranae* endophyte (*Bm*), sapwood, and 2.90% for *B.*

nummularia endophyte (*Bn*), sapwood. Results also indicated that mass loss of sapwood samples exposed to endophyte *B. mediteranae* was higher than

other and then *P. pomaceus* and endophyte *B. mediteranae* had highest reduction, respectively.

Table 2. T-test analysis for average mass loss and impact bending

Tests	Test Samples	Mean	Number	StD	DF	Sig.
Mass Loss (%)	Decayed heartwood (<i>P. pomaceus</i>)	3.25	18	2.77	34	0.383
	Decayed Sapwood (<i>P. pomaceus</i>)	4.46	18	3.43		
	Decayed heartwood (<i>P. pomaceus</i> With <i>B. mediteranae</i>)	3.62	18	1.96	34	0.011
	Decayed Sapwood (<i>P. pomaceus</i> With <i>B. mediteranae</i>)	9.02	18	3.42		
	Decayed heartwood (<i>P. pomaceus</i> With <i>B. nummularia</i>)	1.87	18	0.98	34	0.001
	Decayed Sapwood (<i>P. pomaceus</i> With <i>B. nummularia</i>)	5.01	18	2.73		
	Decayed Sapwood (<i>B. mediteranae</i>)	9.89	18	5.59	34	0.000
	Decayed Sapwood (<i>B. nummularia</i>)	2.90	18	1.69		
Impact bending	Decayed heartwood (<i>P. pomaceus</i>)	4.02	10	1.99	10	0.050
	Decayed Sapwood (<i>P. pomaceus</i>)	10.74	10	5.71		
	Decayed heartwood (<i>P. pomaceus</i> With <i>B. mediteranae</i>)	4.36	10	4.52	10	0.058
	Decayed Sapwood (<i>P. pomaceus</i> With <i>B. mediteranae</i>)	12.48	10	2.21		
	Decayed heartwood (<i>P. pomaceus</i> With <i>B. nummularia</i>)	4.91	10	9.80	10	0.092
	Decayed Sapwood (<i>P. pomaceus</i> With <i>B. nummularia</i>)	6.37	10	2.21		
	Decayed Sapwood (<i>B. mediteranae</i>)	11.04	10	4.25	10	0.464
	Decayed Sapwood (<i>B. nummularia</i>)	3.59	10	2.78		

Figures 2 and 3 display the effects of the cell wall degradation on the impact bending strength after exposure to the white-rot fungi and endophytes. Average decrease of impact bending strength by the fungi was 10.74%, 4.02% (*Pp*), sapwood and heartwood, and 12.48%, 4.36% for (*Pp*) + (*Bm*) endophyte sapwood and heartwood, and 6.37%, 4.91% for (*Pp*) + (*Bn*) endophyte sapwood and heartwood, and 11.04% for (*Bm*) endophyte sapwood, and 3.59% for (*Bn*) endophyte sapwood, respectively, while it was 6.17% sapwood and 5.87% heartwood, for the control sample. Impact strength is the ability of wood to absorb the force of impact bending and characterizes the ability of material to withstand impact loads. Impact strength is expressed as the energy spent while breaking wood with defined dimensions.

This mechanical property is most sensitive to decay and unlike other strength properties that decrease gradually as decay progresses, impact

strength declines rapidly during incipient wood decay^[27]. Trees are vital constituents with a lot of benefits. However, the fracture and falling of the trees due to high loading, especially during storms and severe winds, lead to serious economic and even life-threatening damage, particularly in urban green areas. Trees are always considered as one of the most important indicators in urban planning and management. Therefore, for sustainable management and development, it is very important to learn information regarding the street trees^[28].

In nature, wood undergoes biological decay, primarily by white, brown and soft-rot fungi^[2,3,7,29]. Basidiomycetes are responsible for the majority of wood decay. Soft-rot fungi (ascomycetes and deuteromycetes) degrade wood under wet conditions^[30-32]. *Phellinus* is a genus of fungi in the family Hymenochaetaceae. Many of them cause white rot. It should be noted that any decrease in compression parallel to grain was a result of fungal attack and

changes in the chemical components of the cell walls, especially decrease in lignin content^[2,11] resulting in a reduction in wood density that can also affect strength properties^[32]. Impact bending of the wood samples was also reduced following exposure to *P. pomaceus* fungi, *B. mediteranae* endophyton and *P. pomaceus*+ *B. mediteranae* endophyton (**Table 1**). However, the loss exposure to *P. pomaceus* fungi approximately half of the loss exposure to *B. mediteranae* endophyton and *P.pomaceus*+*B. mediteranae* endophyton, but *B. nummularia* endophyton and *P. pomaceus*+*B. nummularia* endophyton had less weight compared to *P.pomaceus* fungi. According to previous reports^[19,20], decrease in hardness could be due to loss of hemicellulose.

In general, hemicelluloses are responsible for the compression strength perpendicular to grain. The arabinan and galactan are side chain elements of xylan and mannan, the two main hemicellulose

polymers^[33] and may be either more vulnerable to degradation or may have to be removed before the main chain of the polymer can be attacked^[20]. Reduction in hemicellulose affects integrity of the cell wall polymers and decreases the strength against mechanical loads. As shown in **Tables 1** and **2**, *P. pomaceus* fungi, *B. mediteranae* endophyton and *P. pomaceus* + *B. mediteranae* endophyton could reduce the impact load resistance. Cleaved ether bonds in cellulose are responsible for reduction in impact load resistance^[12]. It is also reported that cell wall thinning, bore holes and appearance of micro-cracks in the cell walls due to fungal degradation are other reasons for strength losses; especially impact load resistance^[11,32], indicated that simultaneous white-rot leads to a brittle fracture of the infected wood because of the progressive degradation of the cellulose-rich secondary wall^[11].

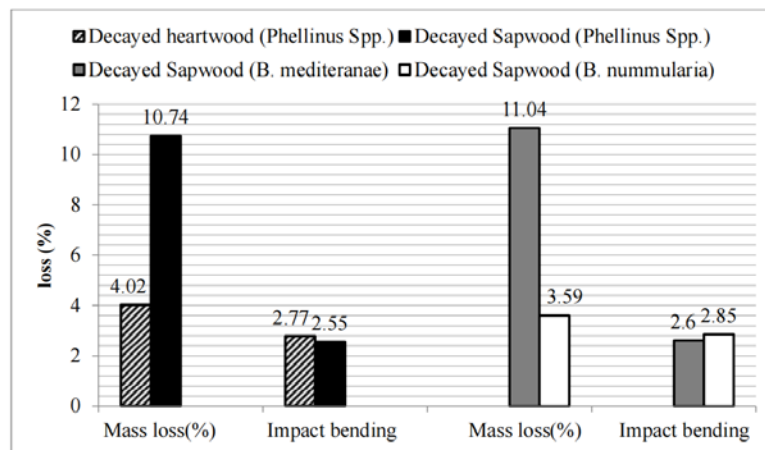


Figure 2. Mean mass loss and mechanical strength.

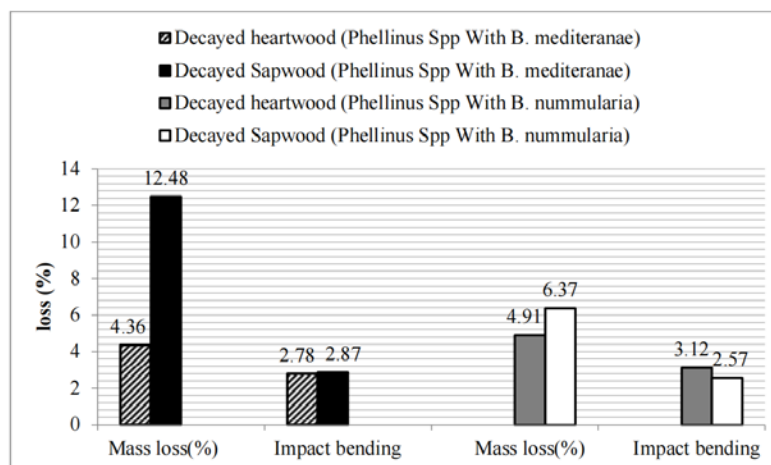


Figure 3. Average mass loss and impact bending.

4. Conclusion

Elm wood subjected to white-rot fungi and endophytes for 4 and 8 weeks of incubation by investigation of mass loss and mechanical properties. Results indicated that *phellinus* and endophytes lead to a significant mass loss which was along with losses in mechanical properties. Overall, under the conditions of the present research, it was concluded that the decay capacity of *phellinus* and was more aggressive than that of endophytes in some test cases.

Conflict of interest

The authors declare that there is no conflict of interest.

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