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COMPARATIVE STUDY ON MICROMORPHOLOGICAL CHANGES IN WOOD DUE TO SOFT-ROT FUNGI AND SURFACE MOLD

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ABSTRACT

The current study aims to elucidate and compare ultrastructure alterations that occurred in larch wood (*Larix decidua Mill.*) and beech wood (*Fagus sylvatica*) which were artificially infested with the selected fungi; *Aspergillus niger, Pencillium chrysogenum, Chaetomium globosum,* (soft-rot fungi) *Trichoderma viride* and *Alternaria alternata* (surface mold). Environmental scanning electron microscope were utilized to examine the wood samples which were left three years. Microscopic examination showed differences in the patterns and mechanism of decay in the two wood species and cell types invaded by the selected fungi. *A. nigar* and *P. chrysogenum* can cause wood degradation more aggressively than *C. globosum.* Surface mold fungi, *e.g. T. viride* and *A. alternata* may cause alterations in wood ultrastructurally like soft-rot fungi. The results of this study encourage adding stain fungi and surface mold to wood destroying fungi classification, also recommending examination their enzymatic system.

KEYWORDS: wood, soft rot fungi, surface mold, decay patterns, lignin, SEM.

1. INTRODUCTION

Soft rot decay by fungi can be divided into two categories: Cavity forming (type 1) and erosion (type 2). They both may occur in gymnosperms or angiosperms. While cavities formation (type1) generally takes place within the cell walls of wood following the microfibrillar orientation of cellulose, a complete erosion of the secondary wall with a slight modification of the middle lamella in advanced stages of degradation characterizes the type 2 form of soft rot decay (Daniel and Nilsson, 1997). The type of wood substrate governs the form (type I or type II) of soft rot that occurred (Blanchette, 1995). Angiosperms are at risk more than gymnosperm (Unger et al., 2001) because of the differences in their lignin. Eaton and Hale (1993) reported that there are differences between the erosion of secondary wall layers of angiosperms and gymnosperms. The erosion process in angiosperm often consists of troughs of different sizes, giving the degraded wood a striped appearance. It causes degradation of all secondary cell wall layers. In gymnosperm, the fungus enters the wood cell through the S3 layer, but it appear to cause no degradation. It progresses into the cellulose rich S2 layer. It doesn't affect the middle lamellae, but has a minimal effect on the S1 layer (Jurgens and Blanchette, 2003). Recently, a study has showed differences in hyphae colonization and wood degradation patterns between soft-rot species. It has proved that A. niger produced type I soft-rot decay (cavity formation) and type II (erosion), while P. chrysogenum caused only type II decay in both pine wood and sycamore wood (Hamed, 2013). These differences may be attributed to the nature of lignin and hemicellulose. Previous studies showed that the nature of the lignin has a great influence on wood decomposition by fungi since gymnosperm woods almost consist of guaiacyl lignin, whereas angiosperm woods consist of guaiacyl and syringyl lignin (Whetten & Sederoff 1995, Martínez et al. 2005). Furthermore, the differences of lignin type in wood species, as conifers, e.g. *Larix decidua* (larch) contain guaiacyl lignin that dominates cell walls with the highest concentrations in the latewood tracheids (Baum 2001). While previous investigations confirmed that lignin in angiosperm, e.g. *Fagus sylvatica* (beech) is more variable because the secondary walls of fibers and ray cells have a high content of syringyl units, the compound middle lamella contains a guaiacyl-syringyl lignin. In contrast, vessels contain a guaiacyl lignin in both the secondary walls and the middle lamella (Dence and Lin, 1992). In addition, Hardwoods hemicelluloses contains a high content of xylans and softwoods hemicelluloses are characterized by a high percentage of mannans (Takahashi and Nishimoto, 1973; Rowell et al. 2005).

The present study mainly aims to examine and compare: the ultrastructural alterations in wood cell walls; the resulting decay patterns; and the extent and mechanism of wood decomposition due to the selected fungi. Also, it aims to illustrate the impact of environment on the resulting decay patterns.

2. MATERIALS AND METHODS

2.1. Wood samples

Aged samples of larch wood *(larix decidua Mill.)*, e.g. gymnosperm and beech wood *(fagus sylvatica)*, e.g. angiosperm wood were used to elucidate the type of decay resulting from the inoculation with the selected fungi. Wood samples were prepared with dimensions of 20 × 20 × 20 mm.

2.2. Fungal inoculation

A. niger, p. chrysogenum, C. globosum, T. viride and *A. alternata* were chosen for the experimental part of this study. They were used for the inoculation of wood samples at Conservation Department, Faculty of Archaeology, Cairo University, Giza. In-vitro inoculation followed the methodology described by Darwish et al. (2013). Wood blocks were sprayed until they were covered with spore suspension of each fungi and left for three years.

2.3. Environmental Scanning Electron Microscope

Decay patterns resulting in larch and beech produced by *A. niger, p. chrysogenum, C. globosum, T. viride* and *A. alternata* were evaluated with environmental scanning electron microscope (ESEM; FEI Quanta 250 FEG). Small sticks of the infected wooden samples were sectioned with a scalpel and placed in freshly prepared aqueous 1% w/v $KMnO^4$ and left for an hour at room temperature. The samples were washed with distilled water, then dehydrated by ethanol-acetone series. They were embedded in epoxy resin which polymerization proceeded at 70°C for 24 h. the resin blocks that contain the samples were sectioned with an Ultramicrotome (Reichert FC4) to reveal the wood tissue then these blocks were fixed on aluminum stubs with double-sided cellophane tape and were examined.

3. RESULTS

3.1. Wood samples inoculated with A. niger

Transverse sections of both wood species, i.e. larch *(larix decidua Mill.)* and beech *(fagus sylvatica)*, showed severe decay in ray cells generally (Figs. 1a-

c), and erosion of secondary walls (soft-rot type II) which unusually started at S_1 layer. The lignin –rich regions as the compound middle lamellae and cell corners remained intact in most cells even in the advanced stages of decay, while the secondary wall underwent simultaneous degradation by the removal of all components of the cell wall (Figs. 1b-d). (Fig. 1c) revealed that early wood was highly attacked compared with the late one. The micrographs plotted in (Figs. 1e-f) explain the degradation mechanism of secondary walls as the fungal enzyme diffused from the cell lumen throughout the secondary wall to reach S_1 layer where the erosion began.

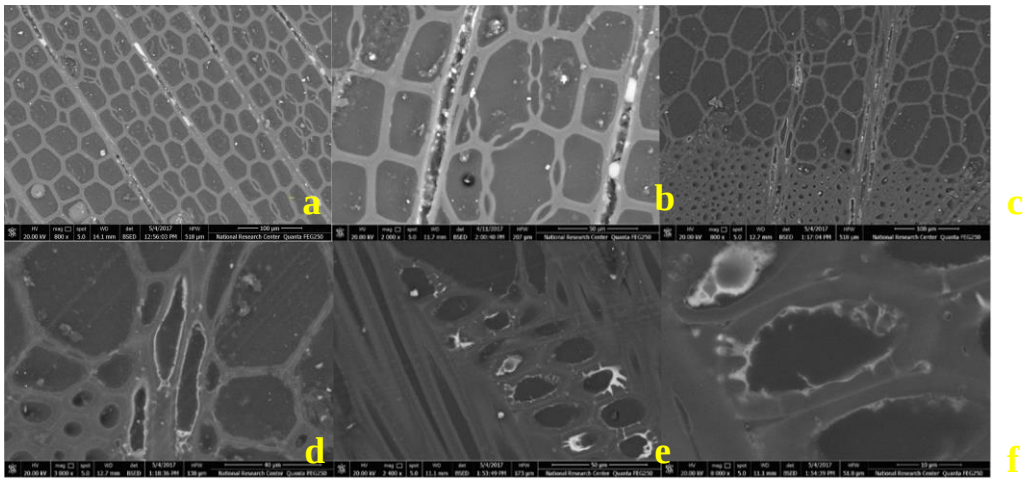

Figure 1: SEM images of Wood samples inoculated with Aspergillus niger; a, b. transverse views of Larch wood showing the decay of ray cells and erosion of cell walls of tracheids that start at S_1 layer. Also, middle lamella and cell corners remain intact. c, d. transverse views of Beech wood showing decay pattern similar to those of Larch wood. e, f. longitudinal views of beech wood showing decay mechanism where the fungal enzyme diffuse through secondary wall to reach S_1 layer.

3.2. *Wood samples inoculated with p. chrysogenum*

The longitudinal sections of larch wood showed erosion of the cell wall (soft-rot type II) in some areas (Figs.2a- b). An advanced stage of decay was noticed in transverse sections since the secondary wall layers were disrupted and dispersed due to the extensive attack on the cell walls. Additionally, the decay pattern of *p. chrysogenum* revealed a typical soft-rot type II (erosion) that started at S_3 layer resulting in a general cell wall thinning from the lumen outwards (Figs. 2c-d). In the micrographs of beech, degradation was evident as ray cells and adjacent cells, especially vessels, gradually became more disrupted, eroded and easily fragmented (Figs. 2e- f).

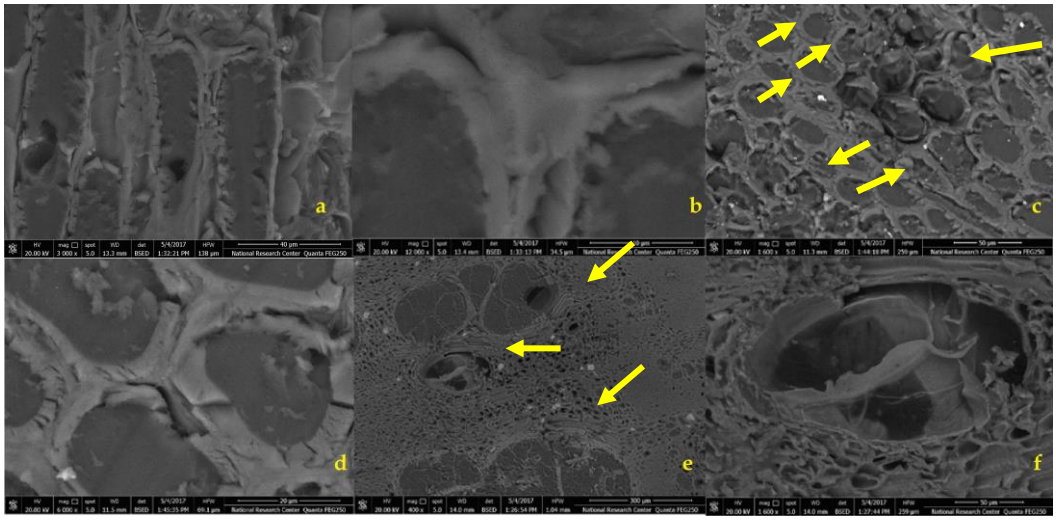

Figure 2: SEM images of Wood samples inoculated with Pencillium chrysogenum; a, b. longitudinal views of larch wood showing the erosion starting at S_3 layer, and c, d. transverse view show the disruption of some regions in wood structure due to extensive erosion. e, f. transverse views of beech wood showing ray cells and adjacent cells, especially vessels, gradually eroded and easily fragmented.

3.3. *Wood samples inoculated with C. globosum*

ESEM micrographs of larch samples illustrated erosion troughs formed in cell walls (soft-rot type II) starting from the lumen outwards (Figs.3 a- b). Beech samples showed the two types of soft rot decay patterns. The formation of cavities within the S2 layer of the secondary wall (Type 1), which is a feature of soft rots, was observed in fiber cells (Figs. 3e- f), whereas erosion troughs (soft-rot type II) were formed in Parenchyma and ray cells (Figs.3d- e). Vessels showed great resistance to decay compared to the other cells (Figs. 3c, d).

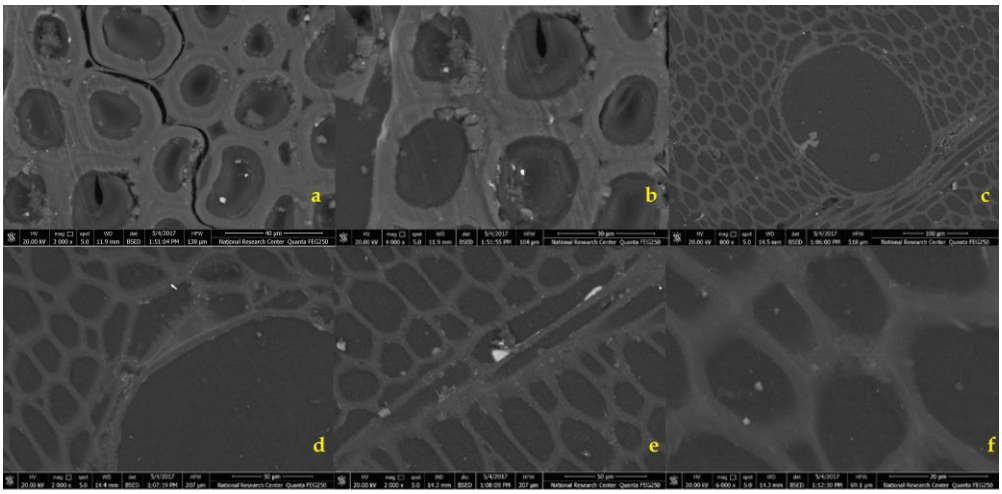

Figure 3: SEM images of Wood samples inoculated with Chaetomium globosum; a, b. transverse views of larch wood showing the erosion troughs starting from the lumen outwards. c-f. transverse views of beech wood; c, d showing the erosion troughs in some cell and the resistance of vessels, whereas e, f. showing cavity formation within the S2 layer in fiber cells.

3.4. *Wood samples inoculated with T. viride*

Erosion troughs could be observed in larch (Fig.4a). Discrete notches of cell-wall erosion were more apparent in parenchyma than tracheids (Fig. 4b). Separations along the fibril orientation of the cell walls due to erosion were clearly observed in tracheids causing gradual breakdown of the cell wall (Fig.4c). Beech samples showed progressive erosion and loss in the middle lamella region within and between fibres (Fig.4d). Loss of cell corners and degradation of middle lamella were also evident in ray cells (Fig. 4e). In some of the fibers and ray cells, the tunnels were observed in the S2 layer but left the S3 and S1 layers intact, indicating cavity formation following the orientation of cellulose microfibrils, similar to the typical soft-rot pattern (type I); (Fig. 4f).

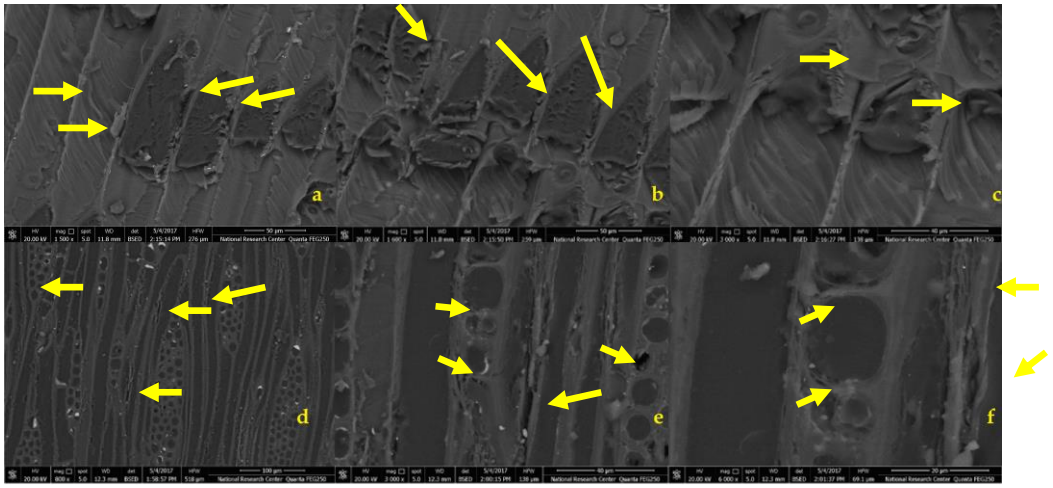

Figure 4: SEM images of Wood samples inoculated with Trichoderma viride; a-c. longitudinal views of larch wood showing the erosion which is more apparent in parenchyma than tracheids. Also, breakdown of cells due to decay d-f. longitudinal views of beech wood; d. showing a progressive loss in the middle lamella region within and between fibres. e, f. showing lose of cell corners and cavity formation in the S2 layer while the S3 and S1 layers left intact.

3.5. *Wood samples inoculated with A. alternata*

In ESEM micrographs of larch and beech, most of the wood surface was intact (Figs. 5a- e). The apparent cracks mostly resulted during samples' preparation (Fig. 5b). An initial degradation started to take place in some cells especially rays, as was observed in longitudinal and transverse sections, which resembled soft-rot type II (erosion) (Figs. 5c, d & f).

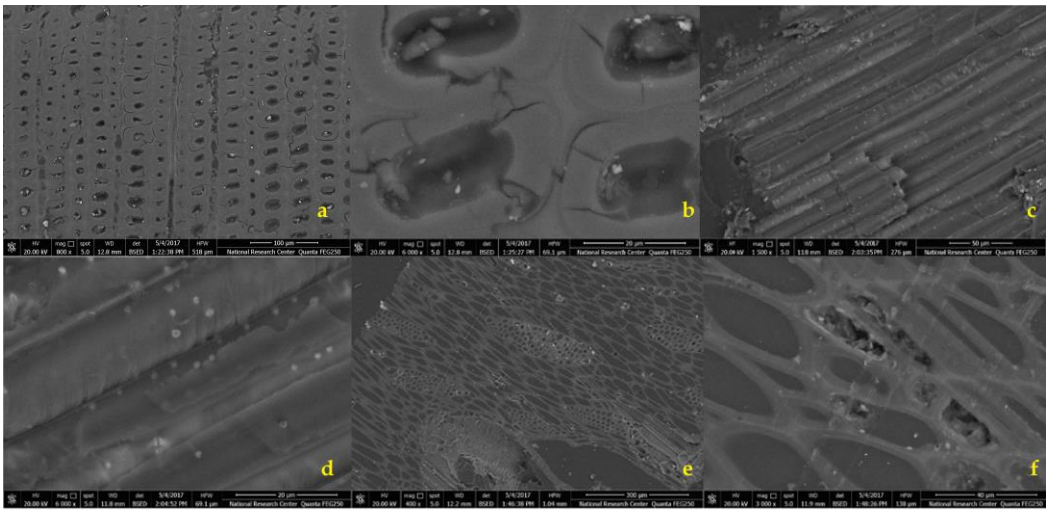

Figure 5: SEM images of Wood samples inoculated with Alternaria alternata; a, b. transverse views of larch wood showing the erosion which is more apparent in parenchyma than tracheids. Added to, breakdown of cells due to decay. c,d. longitudinal views showing the presence of the fungus on wood cells which erode the cell walls following the orientation of cellulose microfibrils. e,f. longitudinal views of beech wood showing initial degradation started to take place in some cells especially rays.

4. DISCUSSION

Visual observations confirmed the significant differences in the patterns, mechanism and degree of degradation between the five selected fungi, namely *A. niger, p. chrysogenum, C. globosum, T. viride* and *A. alternata.* These can be interpreted based on wood cell wall morphology and its chemical composition.

Erosion in secondary cell walls due to *A. niger* began at S_1 layer towards the cell lumen in a sharp difference to the erosion patterns that resulted from soft-rot fungi which beginning at S_3 or S_2 layers outwards. This might be attributed to the large microfibril angle which characterize S_1 layer (Wiedenhoeft and Miller, 2005) or to the fact that hemicellulose concentration increases towards the outer regions of the secondary wall (Levi and Preston, 1965), suggesting the preferential attack of hemicellulose before cellulose by *A. niger.* In addition, lignin- rich regions, middle lamella and cell corners remained unaffected even in advanced stages of decay confirming that *A. niger* acted as a soft-rot fungus, preferentially degrading carbohydrates and leaving lignin untouched in gymnosperm and angiosperm.

Surprisingly, *A. niger* caused only type 2 of soft-rot attack (erosion), although it caused the two types of soft-rot attack (in a previous study). This might be related to the environment, since it produced the two types of attack when the wood was buried in soil, whereas it produced only type 2 under normal conditions.

The findings of the study prove that soft-rot type II (erosion) due to *p. chrysogenum* occurs in both gymnosperm and angiosperm. Similar results, regarding degradation, were reported by Hamed (2013). Also, *p. chrysogenum* shows the ability to degrade cell walls with relatively low lignin content.

Although, it is known that *C. globosum* produces both soft-rot types of attack, the microscopic examination results gave evidence of the occurrence of soft-rot type II (erosion) in larch (gymnosperm), whereas soft-rot attacks, i.e. type I (cavity formation) and type II (erosion), were present in beech (angiosperm). In addition, the decay in fiber and ray cells was greater than vessels in beech wood. These findings seemed to be related to lignin composition of cell walls and the presence of polyphenols. Since softwood and vessels contains guaiacyl lignin, they are particularly resistant to fungal decay (Blanchette et al. 1988; Nilsson et al. 1989; Schwarze et al. 1995). The homogeneous distribution of syringyl lignin throughout the cell walls in fibers and ray cells might explain why they are highly susceptible to decay (Schwarze et al. 2004). Similarly, the nature of hemicelluloses that differ from softwood to hardwood might be a significant factor for the resistance or the susceptibility to decay. A study

showed the ability of *C. globosum* to utilize and remove xylose and xylan, indicating the great susceptibility of beech (hardwoods) to soft rot fungi (Levi and Preston, 1965; Takahashi and Nishimoto, 1973).

Moreover, extensive deterioration of wood structure following infection with *A. nigar* and *p. chrysogenum* compared to deterioration due to *C. globosum* suggested that *A. nigar* and *p. chrysogenum* are more aggressive wood degrading fungi than *C. globosum* for the same period of time.

The study revealed that wood samples inoculated with *T. viride* were severely affected, especially beech samples. Though, *T. viride* was referred to as a surface mold which colonized parenchyma cells consuming their content of nutrients and was not able to decompose wood enzymatically (Unger, 2001). *T. viride* caused cavity formation and erosion that are ultrastructurally like the decay by a soft rot fungus. *A. alternata* also exhibited some features of soft rot. The incipient stages of erosion observed in longitudinal and transverse sections are typically found in the soft-rot attack type II.

The limitation of this study was the great difficulty of getting transverse sections of degraded wood samples inoculated with *T. viride* and *A. alternata.* The damage caused difficulty in their sectioning so the characteristics of decay were mostly observed only in longitudinal sections.

5. CONCLUSION

The present study demonstrates that wood decay depends upon, a. the type of wood and its content of the different cell types that vary in cell morphology and chemical composition, b. enzymatic activity of the fungus, c. the inoculation time. Additionally, *A. nigar* and *P. chrysogenum* are capable of causing wood degradation more aggressively than *C. globosum.* Surface mold fungi, *e. g. T. viride* and *A. alternata,* cause alterations in wood ultrastructurally similar to soft-rot fungi. Therefore, further studies are recommended to develop a new system of decay classification that combines morphological and chemical changes in cell-wall constituents with taxonomy and to study the impact of the environment on the resulting decay patterns.

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