ΕΘΝΙΚΟ ΜΕΤΣΟΒΙΟ ΠΟΛΥΤΕΧΝΕΙΟ
ΣΧΟΛΗ ΧΗΜΙΚΩΝ ΜΗΧΑΝΙΚΩΝ
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Thesis for the degree of Doctor of Philosophy

Evaluation of the effectiveness of materials used for the microbial decontamination of wooden works of art

Rehab G. El Gamal

National Technical University of Athens, Greece
Athens, July 2018.

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Ph.D. Thesis

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ΕΘΝΙΚΟ ΜΕΤΣΟΒΙΟ ΡΟΛΥΤΕΧΝΕΙΟ
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ΔΙΔΑΚΤΟΡΙΚΗ ΔΙΑΤΡΙΒΗ

Της

ΡΗΧΑΜΠ ΓΚΑΛΑΛ. ΕΛ ΓΚΑΜΑΛ

Αξιολόγηση της αποτελεσματικότητας υλικών που χρησιμοποιούνται για τη μικροβιακή απολύμανση ξύλινων έργων τέχνης.

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Rehab El Gamal

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The State Scholarship's Foundation (I.K.Y.) in Greece is gratefully acknowledged for financially supporting this work.

Table of Contents

Summary

This study includes an introduction, aims of the study and seven chapter. The details of these chapters are as follow:

Chapter one: Chemistry and types of wood

This chapter includes wood chemistry, which contains cellulose, hemicellulose, and lignin. Wood also contains elements such as calcium, potassium, magnesium, phosphorus, sulfur, sodium, silicon, and cadmium contribute to the chemical composition of wood. This chapter also contains types of wood, which include hardwood (oak, maple, Mahogany, Walnut, Rosewood, Teak, and Cherry) and softwood (Pine, ash, Hickory, beech, birch, cedar, redwood, Hemlock, fir and Spruce).

Chapter Two: Fungal deterioration of archaeological wood

This chapter explains the fungal deterioration of archaeological wood. It explained Physiological requirements of wood-destroying and wood-inhabiting fungi. In addition to the chemical composition of wood, several other factors greatly influence decay rates. These factors include nutrients (wood components), temperature, moisture, oxygen, carbon dioxide and hydrogen ion concentration. The most common aspects of deterioration resulted from these factors staining of wood with different colors. The mechanism of wood decay is explained. White-rot fungi, Brown-rot fungi, and Soft-rot fungi were also explained. Some negative changes such as weight loss, strength loss and discoloration were observed.

Chapter Three: Materials and methods

Four archaeological sites in Islamic Cairo were used for the isolation of fungi used in this study (The Mosque of Sabiile and Koutab Suleiman Agha Selehdar dated back to 1837–1839 AD, The Mosque of Abu Haribh dated back to 1480 and 1481 AD, The Mosque of El Musafir Khana which was built in 1779 AD and the second in 1783 AD, and The Mosque of El Mouayed Sheikh Al-Mahmoudi dated back to 1415 to 1421 AD. New pitch pine samples were prepared, to be used in all the experimental studies. Isolation of and identification of fungi were done. Three fungi were identified (*Aspergillus niger*, *Aspergillus flavus*, and *Penicillium*

chrysogenum). Chitosan, propiconazole and tebuconazole fungicides are used with different concentrations. Artificial fungal deterioration before and after fungicides treatment was applied. Investigation techniques used for the evaluation of fungicides used are Measurement of color change with UV spectrophotometer, Fourier transform infrared spectroscopy (FTIR) and X-Ray diffraction (XRD) for the determination of wood crystallinity.

Chapter Four: Results and discussion of chitosan fungicide

The best results obtained from the color measurement proved that the total color difference in the infected samples increased with increasing incubation time. The total color difference in the treated samples decreased with increasing concentration of chitosan. FTIR analysis confirmed that for all three fungi studied, infected samples treated with chitosan were more amorphous during the incubation times than the infected and untreated samples. X-ray diffraction analysis showed that with all three fungi studied, the peak positions of cellulose I ((101), (10Í) and (002)) in infected samples decreased compared to the control samples at all incubation times. The peak positions of the infected, treated samples increased with increasing concentration of chitosan. The crystallinity index, measured with either the first or the second method, decreased, but it increased after the third and fourth months of incubation with any of the fungi studied.

Chapter Five: Results and discussion of the fungicide propiconazole

For measurement of color, the results revealed that the whiteness in the infected samples with fungi decreased up to the second month and re-increased to the fourth month. The yellowness of the untreated infected samples increased. The treated samples with propiconazole fungicide gave good resistance against fungal deterioration. For FTIR analysis, Treatment with propiconazole at the concentrations used and infected with *Aspergillus flavus* negatively affected hemicellulose content and enhanced

cellulose depolymerization and oxidation. However, it protected lignin from fungal decay. For the fungicide-treated, infected samples, the crystallinity index increased compared to the control sample. The increase in the crystallinity index decreased with increasing incubation time.

Chapter Six: Results and discussion of the fungicide tubeconazole

For color measurement, the second (0.50%) of tubeconazole fungicide gave better results than the first concentration (0.25%) for both whiteness and yellowness indices. The results obtained by FTIR showed that increased tebuconazole concentrations were needed to achieve acceptable protection against *Aspergillus niger* due to oxidation of this fungicide, especially at low concentrations of fungicide. The crystallinity index, measured with all concentrations used decreased with increasing the incubation time.

Chapter Seven: Conclusions

Fungi play an important role in the deterioration of archeological wood in Egypt, especially in certain environments. *Asperigllus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* were the fungi identified from different sites in historical Cairo, Egypt. The investigation techniques used in this study gave good indication on the effectiveness of fungicides used for the protection of wood against fungal deterioration. Many fungicides are used for the preservation of wood against fungal deterioration, but chitosan, propiconazole, and tebuconazole fungicides were used in this study at limited concentrations. Chitosan was used at 0.25%, 0.50%, and 0.75%. Propiconazole and tebuconazole were used at 0.25% and 0.50%. These limited concentrations gave good resistance to fungal deterioration. The best results were obtained with chitosan, followed by tebuconazole and propiconazole.

Περίληψη

Αξιολόγηση της αποτελεσματικότητας των υλικών που χρησιμοποιούνται για τη μικροβιακή απολύμανση των ξύλινων έργων τέχνης".

Η παρούσα μελέτη περιλαμβάνει μια εισαγωγή, τους στόχους της μελέτης και επτά κεφάλαια. Αναλυτικά τα κεφάλαια έχουν ως εξής:

Κεφάλαιο Ένα: Χημεία και είδη ξύλου

Το κεφάλαιο αυτό ασχολείται αρχικά με τη χημική σύσταση του ξύλου, η οποία χαρακτηρίζεται από κυτταρίνη, ημικυτταρίνη και λιγνίνη. Το ξύλο περιέχει επίσης στοιχεία όπως το ασβέστιο, το κάλιο, το μαγνήσιο, ο φώσφορος, το θείο, το νάτριο, το πυρίτιο και το κάδμιο. Στο κεφάλαιο αυτό αναλύονται επίσης οι δύο τύποι ξύλου: σκληρό (δρυς, σφενδάμι, μαόνι, καρύδι, τριαντάφυλλο, τικ και κερασιά) και μαλακό (Πεύκο, φλαμουριά, καρυδιά, οξιά, σημύδα, κέδρος, ερυθρόδεντρο, δέντρο κώνειο, έλατο και ερυθρελάτη)

Κεφάλαιο Δύο: Μυκητολογική φθορά αρχαιολογικού ξύλου

Αυτό το κεφάλαιο εξηγεί τη φθορά του αρχαιολογικού ξύλου από μύκητες. Εξηγούνται αναλυτικά οι φυσιολογικές απαιτήσεις των μυκήτων που καταστρέφουν το ξύλο και αναπτύσσονται μέσα σε αυτό. Εκτός από τη χημική σύνθεση του ξύλου, τα ποσοστά αποσύνθεσής του επηρεάζονται και από πολλούς άλλους παράγοντες, οι οποίοι περιλαμβάνουν τις τα θρεπτικά συστατικά (συστατικά ξύλου), τη θερμοκρασία, την υγρασία, το οξυγόνο, το διοξείδιο του άνθρακα και τη συγκέντρωση ιόντων υδρογόνου. Οι πιο κοινές μορφές της φθοράς ήταν αποτέλεσμα αυτών των παραγόντων οι οποίοι προκάλεσαν χρώση του ξύλου με διαφορετικά χρώματα Εξηγείται επίσης ο τρόπος της αποσύνθεσης του ξύλου. Εξηγήθηκαν επίσης οι μύκητες λευκής σάρωσης, οι μύκητες Brown-rot και οι μύκητες Soft-rot. Παρατηρήθηκαν, τέλος, μερικές αρνητικές αλλαγές όπως η απώλεια βάρους, η απώλεια αντοχής και ο αποχρωματισμός.

Κεφάλαιο 3: Υλικά και μέθοδοι

Τέσσερις αρχαιολογικοί χώροι στο Ισλαμικό Κάιρο χρησιμοποιήθηκαν για την απομόνωση των μυκήτων που χρησιμοποιήθηκαν σε αυτή τη μελέτη. Πρόκειται για το Τέμενος Sabiile και Koutab Suleiman Agha Selehdar (περί το 1837-1839 μ.Χ.), το Τέμενος του Abu Haribh (1480-1481 μ.Χ), το El Musafir Khana (μεταξύ 1779 μ.Χ. και 1783 μ.Χ.), και το Τέμενος El Mouayed Sheikh Al-Mahmoudi (1415 έως το 1421 μ.Χ). Δείγματα νέας πεύκου ετοιμάστηκαν ώστε να χρησιμοποιηθούν σε όλες τις πειραματικές μελέτες. Ολοκληρώθηκε επίσης η απομόνωση και ταυτοποίηση των μυκήτων. Ταυτοποιήθηκαν τρεις μύκητες (*Aspergillus niger, Aspergillus flavus* και *Penicillium chrysogenum*) Χρησιμοποιήθηκαν μυκητοκτόνα χιτοζάνης, προπικοναζόλης και τεβουκοναζόλης με διαφορετικές συγκεντρώσεις. Εφαρμόστηκε τεχνική επιδείνωση των μυκήτων πριν και μετά τη θεραπεία με μυκητοκτόνα. Οι τεχνικές έρευνας που χρησιμοποιήθηκαν για την αξιολόγηση των χρησιμοποιούμενων μυκητοκτόνων ήταν η μέτρηση της αλλαγής χρώματος με φασματοφωτόμετρο UV, φασματοσκοπία υπερύθρου με μετασχηματισμό Fourier (FTIR) και διάθλαση ακτίνων X (XRD) για τον προσδιορισμό της κρυσταλλικότητας του ξύλου.

Κεφάλαιο Τέσσερα: Αποτελέσματα και σχολιασμός του μυκητοκτόνου χιτοζάνης

Τα καλύτερα αποτελέσματα που προέκυψαν από τη μέτρηση χρώματος απέδειξαν ότι η ολική διαφορά χρώματος στα μολυσμένα δείγματα αυξήθηκε με την αύξηση του χρόνου επώασης. Η συνολική διαφορά χρώματος στα επεξεργασμένα δείγματα μειώθηκε με την αύξηση της συγκέντρωσης της χιτοζάνης. Η ανάλυση FTIR επιβεβαίωσε ότι για τους τρεις μύκητες που μελετήθηκαν, τα μολυσμένα δείγματα που υποβλήθηκαν σε αγωγή με χιτοζάνη ήταν πιο άμορφα κατά τους χρόνους επώασης από τα μολυσμένα και μη επεξεργασμένα δείγματα. Στους τρεις μύκητες που μελετήθηκαν, οι θέσεις αιχμής της κυτταρίνης I ((101), (10α) και (002)) σε μολυσμένα δείγματα μειώθηκαν σε σύγκριση με τα δείγματα ελέγχου σε όλους τους χρόνους επώασης

Οι θέσεις αιχμής των μολυνθέντων, επεξεργασμένων δειγμάτων αυξήθηκαν με αυξημένη συγκέντρωση χιτοζάνης. Ο δείκτης κρυσταλλικότητας, μετρούμενος είτε με την πρώτη είτε με τη δεύτερη μέθοδο, μειώθηκε, αλλά αυξήθηκε μετά τον τρίτο και τέταρτο μήνα επώασης με οποιονδήποτε από τους μύκητες που μελετήθηκαν.

Κεφάλαιο πέμπτο: Αποτελέσματα και σχολιασμός του μυκητοκτόνου προπικοναζόλης

Για τη μέτρηση του χρώματος, τα αποτελέσματα αποκάλυψαν ότι η λευκότητα στα μολυσμένα δείγματα με μύκητες μειώθηκε μέχρι τον δεύτερο μήνα και αυξήθηκε εκ νέου στον τέταρτο μήνα. Η ωχρότητα των μη επεξεργασμένων μολυσμένων δειγμάτων αυξήθηκε. Τα υποβληθέντα σε αγωγή δείγματα με το μυκητοκτόνο προπικοναζόλη έδειξαν καλή αντίσταση ενάντια στη φθορά. Για την ανάλυση FTIR, η θεραπεία με προπικοναζόλη στις συγκεντρώσεις που χρησιμοποιήθηκαν και μολύνθηκε με *Aspergillus flavus* επηρέασε αρνητικά την περιεκτικότητα σε ημικυτταρίνη και τον ενισχυμένο αποπολυμερισμό και οξείδωση της κυτταρίνης. Ωστόσο, προστάτευσε την λιγνίνη από τη μυκητιακή φθορά. Για τα μολυσμένα με μυκητοκτόνα δείγματα, ο δείκτης κρυσταλλικότητας αυξήθηκε σε σύγκριση με το δείγμα ελέγχου. Η αύξηση του δείκτη κρυσταλλικότητας μειώθηκε με την αύξηση του χρόνου επώασης.

Κεφάλαιο έξι: Αποτελέσματα και σχολιασμός του μυκητοκτόνου σωληνοζόλη

Για τη μέτρηση των χρωμάτων, το δεύτερο (0,50%) μυκητοκτόνο σωληνικόλης έδωσε καλύτερα αποτελέσματα από την πρώτη συγκέντρωση (0,25%) για τους δείκτες λευκότητας και ωχρότητας. Τα αποτελέσματα που ελήφθησαν από το FTIR έδειξαν ότι χρειάστηκαν αυξημένες συγκεντρώσεις τεβουκοναζόλης για να επιτευχθεί αποδεκτή προστασία έναντι του *Aspergillus niger* λόγω της οξείδωσης αυτού του μυκητοκτόνου, ειδικά σε χαμηλές συγκεντρώσεις μυκητοκτόνου. Ο δείκτης κρυσταλλικότητας, μετρούμενος με όλες τις χρησιμοποιούμενες συγκεντρώσεις μειώθηκε με την αύξηση του χρόνου επώασης.

Κεφάλαιο 7: Συμπεράσματα

Οι μύκητες διαδραματίζουν σημαντικό ρόλο στη φθορά του αρχαιολογικού ξύλου στην Αίγυπτο, ειδικά σε ορισμένα περιβάλλοντα. Οι *Asperigllus niger, Aspergillus flavus και Penicillium chrysogenum* ήταν οι μύκητες που εντοπίστηκαν σε διαφορετικές τοποθεσίες στο ιστορικό Κάιρο της Αιγύπτου. Οι τεχνικές έρευνας που χρησιμοποιήθηκαν σε αυτή τη μελέτη έδωσανισχυρέσ ενδείξεις σχετικά με την αποτελεσματικότητα των μυκητοκτόνων που χρησιμοποιούνται για την προστασία του ξύλου

από τη φθορά των μυκήτων. Πολλά μυκητοκτόνα χρησιμοποιούνται για τη συντήρηση του ξύλου κατά της φθοράς των μυκήτων, αλλά στη συγκεκριμένη μελέτη χρησιμοποιήθηκαν χιτοζάνη, προπικοναζόλη και τεβουκοναζόλη σε περιορισμένες συγκεντρώσεις. Η χιτοζάνη χρησιμοποιήθηκε σε 0,25%, 0,50% και 0,75%. Η προπικοναζόλη και η τεβουκοναζόλη χρησιμοποιήθηκαν σε 0,25% και 0,50%. Αυτές οι περιορισμένες συγκεντρώσεις έδωσαν καλή αντίσταση στην επιδείνωση των μυκήτων. Τα καλύτερα αποτελέσματα ελήφθησαν με χιτοζάνη, ακολουθούμενη από τεβουκοναζόλη και προπικοναζόλη.

Introduction

The main chemical components that constitute wood cell walls are cellulose, lignin, and hemicelluloses. Cellulose is a carbohydrate macromolecule that represents 40-45 wt.% of the wood, being slightly higher in hardwoods than in softwoods. It is built up from β-D-glucose units linked together by 1-4 bonds, and the average degree of polymerization ranges from 8,000 to 10,000. The cellulose macromolecule is linear as a result of the bond arrangement. In their native state, cellulose molecules are arranged in a crystalline lattice through intermolecular H-bonds and van der Waals forces, forming microfibrils. A cellulose microfibril has both crystalline and non-crystalline (amorphous) parts. Microfibrils are further arranged in larger structural elements called fibrils (Fengel and Wegener 1989). Due to the strong covalent bonds and H-bonds within and among the cellulose molecules, cellulose fibrils are able to resist high tensile stresses. Crystallinity is an important attribute of cellulose, with a significant effect on its physical, mechanical, and chemical properties (Andersson et al. 2003; Schenzel et al. 2005; Agarwal et al. 2010). According to the literature, 60-70% of the cellulose in wood is crystalline (Stamm 1964; Fengel and Wegener 1989; Hedges 1989).

Lignin is a polyphenolic, branched polymer amounting to about 20% and 30% in hardwoods and softwoods, respectively. It has a heterogeneous, amorphous structure made from a combination of three phenyl propane units: p-coumaryl alcohol (or p-hydroxyphenyl), sinapyl alcohol (syringyl) and coniferyl alcohol (guaiacyl). The composition of lignin varies between wood species and also shows a difference between softwoods and hardwoods (Fengel and Wegener 1989).

Hemicellulose is a collective term for carbohydrate components of wood other than cellulose. It includes a wide variety of compounds composed of various sugar units. The most common compounds are xylose and mannose. The types of hemicelluloses present vary greatly between softwoods and hardwoods, and also among species (Fengel and Wegener 1989).

Besides the main components described above, wood also contains low-molecular-weight components, of which the organic part is referred to as extractives and the inorganic part is called ash (Zobel and van Buijtenen 1989). Extractives include a high number of compounds of varying chemical composition. This group can be divided further into phenolic compounds, terpenes, fatty acids, alcohols, monosaccharides, and disaccharides (Zobel and van Buijtenen 1989).

The remaining components consist of various extracellular compounds. When exposed to even moderate environmental conditions, wood deteriorates rapidly through a variety of biotic processes. It is important to consider environmental factors such as moisture content, wood temperature, accessible nutrients, and the types of wood-decaying fungi present. Fungi are widespread in nature and cause deterioration of wooden artifacts in a range of terrestrial and aquatic environments. The structure of wood acts as a substrate/template for fungal growth and development. During the damage and decay process, fungal hyphae may grow on the wood surfaces and into the wood via the xylem rays, and then spread further. Depending on the type of decay, the fungal hyphae may be located in the cell lumen or even within all layers of the cell wall (Blanchette 1994; Blanchette et al. 2004; Jingran et al. 2014, El-Gamal et al. 2016).

The wooden artifacts in most places in Egypt suffer from biological deterioration (through microorganisms and insects), and this is due to

moisture, high temperature, bad ventilation, air pollution, and so on. Due to these factors, some forms of deterioration can be found on wooden artifacts—such as color changes, stains, deformations, and so on.

Most librarians, archivists, and the personnel of museums and archeological sites share the conviction that fungi should be eliminated. At the same time, worldwide trade in wet wood has increased considerably and the wooden artifacts that are generally traded in the dry state have required the use of fungicides, which would provide protection during storage and transport lasting up to six months.

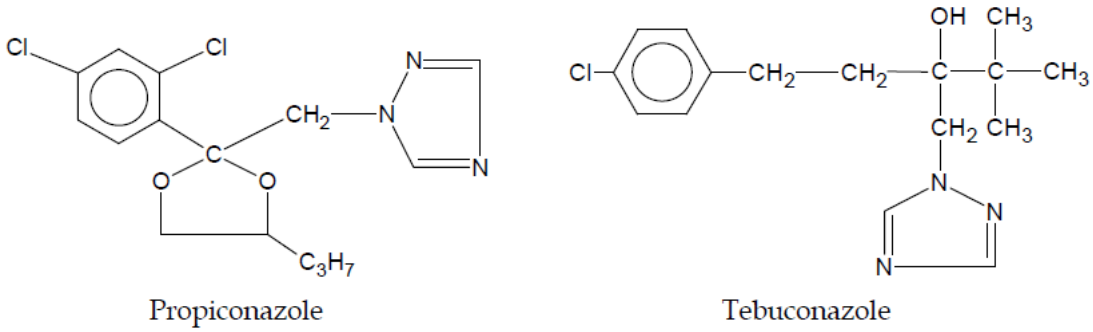
These conditions have led to a search for suitable fungicides that would give long-term protection of wooden artifacts. The results in this study concern evaluation of three fungicides: chitosan, propiconazole, and tebuconazole. Application of fungicides is considered one from the most important protection procedures for wooden materials prior to fungal attacks (Reinprecht, 2010). Chitosan has become a vital biocide and is used in different fields because of its high antimicrobial activity against various microorganisms (Dutta et al. 2009; El-Gamal et al. 2016). Special interest has been shown in the use of chitosan for the control of wood-degrading fungi. This may be due to the fact that chitosan toxicity has an effect on membrane permeability and on the architecture of the mycelium (Singh et al. 2006; El-Gamal et al. 2016). It was found that increasing concentrations of chitosan cause an increase in chitin deposition, which reflect changes occurring at the morphological and ultrastructural levels within the cell wall. Increase in the chitin content of the mycelium in the presence of increasing concentrations of chitosan suggested that chitosan treatment enhances deposition in the cell wall (Vesentini et al. 2006; El-Gamal et al. 2016).

Chitosan has been widely used because of its biodegradability, biocompatibility, antimicrobial effects, lack of toxicity, and antitumor

properties (Ravi Kumar, 2000; El-Gamal et al., 2016). Ahonkhai et al. (2007) reported that chitosan is freely available, is cheap, and has antifungal and bacteriostatic properties. Chitin is found naturally in the exoskeleton of crustaceans and insects (Mcknight et al., 1988; El-Gamal et al., 2016). Chitosan occurs naturally in fungi of the order Mucorales. Chitosan (as a polymer) is characterized according to the degree of deacetylation (DD), which is determined from the proportion of D-glucosamine and N-acetyl-D-glucosamine. Structurally, chitosan is a straight-chain copolymer composed of D-glucosamine and N-acetyl-D-glucosamine. It is the most abundant basic polymer and is structurally similar to cellulose, which is composed of only one monomer (glucose). The solubility of chitosan depends on the amount of protonated amino groups in the polymeric chain, and therefore on the proportion of acetylated and non-acetylated D-glucosamine units. It is soluble after stirring in acids such as acetic, nitric, hydrochloric, perchloric, and phosphoric (Anthonsen and Smidsrød, 1995; Klug et al., 1998; Kubota et al., 2000; Guibal, 2004; Kurita, 2006; Rinaudo, 2006; Sankararamakrishnan and Sanghi, 2006; Yang, 2013; El-Gamal et al., 2016).

In recent years, chitosan has been investigated as a chemical for protection of wood against fungal decay. Schmidt et al. (1995) found that treatment with 2% chitosan considerably reduced the amount of wood decay by two brown-rot fungi. It has been used in aqueous solution, employing an impregnation technique (Eikenes et al., 2005; El-Gamal et al., 2016). Triazoles such as propiconazole and tebuconazole have been used as organic fungicides (see below Chemical formula, IUPAC name and chemical structure) for protection of wood against all types of wood-degrading fungi in the 20th century and already currently used for their characteristic properties especially their stability in environment and their low toxicity (Reinprecht, 2010).

Fungicide	Chemical formula	IUPAC name
Tebuconazole	$C_{16}H_{22}ClN_3O$	(Alpha-[2-(4-chlorophenyl)-ethyl]-alpha-(1,1- dimethylethyl)-1H-1,2,4-triazole-1-ethanol)
Propiconazole	$C_{15}H_{17}Cl_2N_3O_2$	±cis/trans(1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]- methyl]-1H-1,2,4-triazole),



Chemical structure (Reinprecht, 2010)

Propiconazole and tebuconazole are becoming more widely used for the protection of wood against microorganisms. These are organic triazole biocides that are effective against wood decay fungi. They are soluble in some organic solvents (Lebow 2010; Kalawate and Pandey 2012). They have low solubility in water. They are stable and leach-resistant in wood (Kalawate and Pandey 2012). They are also stable, are non-volatile, are better at resisting leaching by water, and are therefore suitable for long-term protection of wood (Terebesyov and Ryparov, 2016).

Tebuconazole and Propiconazole as used in wood preservation contain racemic mixtures of isomers (Evans et al., 2007). Propiconazole and tebuconazole play an important role in disruption process of cell membranes for fungi (Reinprecht, 2010, Fettig et al., 2014). Curative

fungicides, such as propiconazole and tebuconazole can stop existing fungal infections by limiting spore germination (Battaglin et al., 2011).

Propiconazole and tebuconazole are widely used for agriculture and medical purposes (Battaglin et al., 2011, Hagiwara et al., 2016). They are not experiment before this study for the protection of archaeological wood against microbiological attack. This study aims to use the two fungicides (propiconazole and tebuconazole) for the protection of wooden artifacts against fungal deterioration. Before the application of new materials for the conservation treatment of archaeological object, experimental study should be used to verify the effectiveness of the conservation materials before their application on archaeological objects. Experimental studies on propiconazole and tebuconazole have occurred to verify their effectiveness in the protection of wood against fungal deterioration. Different concentrations have been used from propiconazole and tebuconazole fungicides. Some authors used high concentration (5%) from tebuconazole (Schubert et al., 2012). Obanda (2008) has used tebuconazole with low concentration (0.02%). This low concentration gave inhibition for some species of fungi.

In this study, low concentrations of propiconazole and tebuconazole fungicides were used (0.25%, 0.50%), Since from the conservation point of view, the fungicide should give inhibition to microorganisms with low concentration (Abdel-Maksoud, 2006).

Analyses and investigations are considered to be the most important tools for evaluation of the environment, and of materials that are used in conservation processes.

The X-ray diffraction method has been used more and more extensively in the past twenty years for the characterization of different crystalline and non-crystalline materials of archeological, historical, and artistic interest (Franceschi, 2014). Abdel-Maksoud and Al-Saad(2009), Abdel-Maksoud

(2011), and Marutoiu et al. (2013) have used X-ray diffraction for the determination of wood crystallinity.

Fourier transform infrared (FTIR) analysis can be used for different purposes. It has been used successfully for detection and identification of microorganisms. Some of these studies have shown that discrimination is possible not only at the genus level, but also at the species level and even at the strain level (Salman et al., 2010).

In this work, attenuated total reflectance (ATR) Fourier transform infrared radiation is used in order to follow the changes in wood treated with fungicides. Jelle and Hovde (2012) found that the ATR-FTIR technique makes it possible to study materials that are not transparent to IR radiation in a pristine condition. That is, the extensive, time-consuming, and often cumbersome sample preparation by pressing thin KBr pellets—as in traditional FTIR transmittance spectroscopy, which might even change the sample material in question, is avoided. The ATR technique is based on a special reflectance set-up where the sample material is pressed directly onto various crystals with high refractive indices. Also, the use of FTIR spectroscopic techniques is showing promise as a valuable tool because of its sensitivity, rapidity, low cost, and simplicity (Salman, et al., 2010). Many authors (Bugheanu, et al., 2010; Picollo, et al., 2011, Gelbrich, et al., 2012, and El-Gamal et al, 2016) have been used FTIR for studying the bio-deterioration of wood or for the evaluation of pesticides.

Color measurement of wood treated with fungicides and infected with different fungi is vital in the field of conservation. The color measurement is used to detect the ability of fungicides to be used against fungi with long incubation times. Esteves and Pereira (2009), Sahin and Mantanis (2011), Monacoa et al. (2011), and Ozgenc et al. (2012) used UV spectrophotometry to measure color parameters including the whiteness

and yellowness indices calculated from the CIE Lab color system (El-Nagar et al. 2012). The accurate measurement of color using spectrophotometry requires calibrating to achieve the required traceability to SI measurement units (Hunt 1999). Uncertainty is the degree of doubt that may affect the final results, and it arises from systematic and random errors during execution of the measurements (El-Nagar et al. 2012; ISO Guide 1993).

Spectrophotometry is a very important tool for laboratory testing in many fields, especially archeology, chemistry, and physics (Bruke and Mavrodineanu,1983), The basic operation of UV/visible spectrophotometers depends on the light emitted from aD65 illuminant source, which is mainly a tungsten lamp for the visible spectrum and a deuterium lamp for the ultraviolet spectrum (Owen, 2000). Measurement of absorbance generally follows the Beer-Lambert law (Decusatis, 1997). For accurate and precise measurements, the instrument should be calibrated to achieve the required traceability to SI measurement units, and all results should be accompanied by uncertainty values that result from all possible sources of error during the measurements. These errors might be due to the sample itself, or the instrument, in addition to the environmental testing conditions (El-Nagar et al. 2012).

Aims of the study

In this study we wanted to:

1. Isolate and identify mold fungi from monuments at different locations and in different environments in historical Cairo, Egypt;
2. Study the effects of mold fungi (that have been isolated and identified) on selected properties of pitch pine wood;
3. Evaluate the efficiency of different concentrations of the fungicides chitosan, propiconazole, and tebuconazole for the protection of wood from mold fungi.

Chapter one

Chemistry and types of wood

Chapter one: Chemistry and types of wood

1.1 Wood chemistry

The chemical constituents of dry wood species are so-called structural substances and non-structural substances. The structural substances are cellulose, hemicelluloses, and lignin. Non-structural substances are mostly low-molecular-mass compounds, e.g. extractives, some water-soluble organics, and inorganics, as shown in Fig. 1.

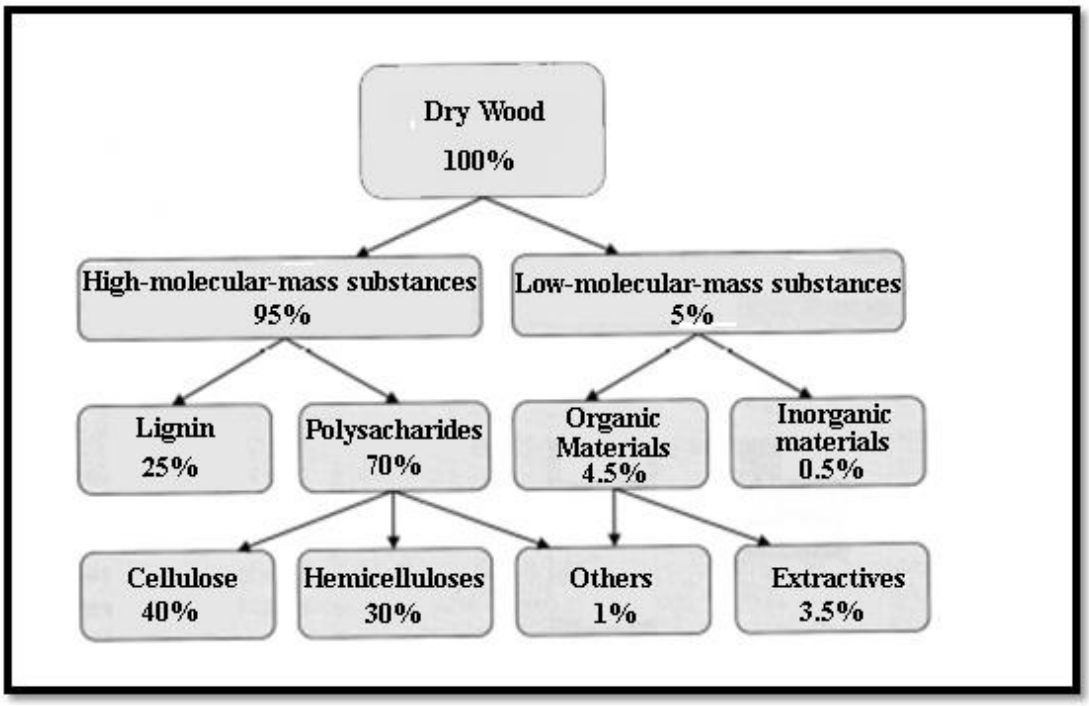


Fig. 1. General classification and content of the chemical components of wood (After Yang and Jaakkola, 2011).

The gross chemical composition of softwood and hardwood is presented in Fig. 2. Softwood usually differs from hardwood with regard to chemical composition, for example, in pine and birch. The cellulose content in pine and birch is more or less the same, but pine usually contains less hemicelluloses and more lignin. Furthermore, the chemistry of hemicelluloses and lignin also differs in pine and birch. On the other hand, the cellulose, hemicelluloses, and lignin are not uniformly distributed in wood cells, and their relative mass proportions can vary widely, depending on the morphological region and the age of the wood. In the following text, the chemistry of cellulose, hemicelluloses, and lignin is introduced.

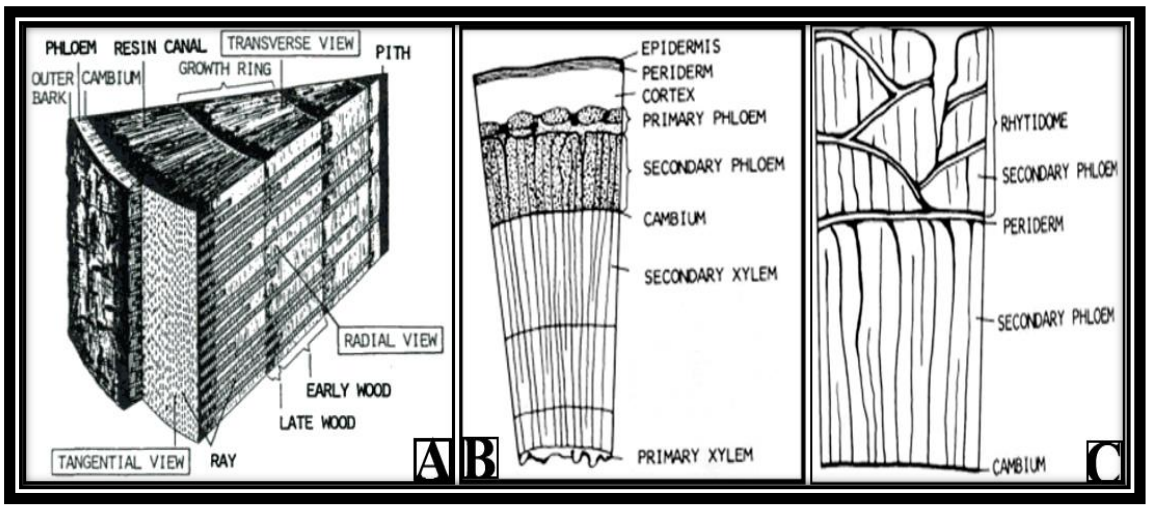


Fig. 2. The structure of wood. A. Stem.B. Yang stem.C. Wood bark. (After Yang and Jaakkola, 2011).

1.1.1 Cellulose

Cellulose is the world's most abundant and important biopolymer. It is a polydispersed linear homopolysaccharide consisting of -D-glucopyranose moieties linked together by (14)-glycosidic bonds. The degree of polymerization (DP) of native wood cellulose is of the order of 10,000. Because of the strong tendency for intra- and intermolecular hydrogen bonding, bundles of cellulose molecules aggregate to microfibrils, which form either highly ordered (crystalline) or less ordered (amorphous) regions. Microfibrils are further aggregated to fibrils and finally to cellulose fibers. The structure of a cellulose molecule is shown in the Fig. 3.

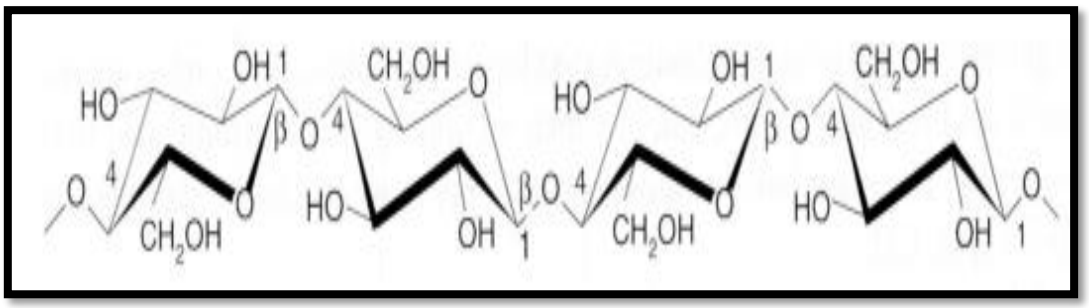


Fig. 3. A segment of a cellulose chain (after Yang and Jaakkola, 2011).

The tight fiber structure created by hydrogen bonds results in the typical material properties of cellulose, such as high tensile strength and insolubility in most solvents. X-ray and other diffraction methods have played a decisive role in the analysis of the crystalline structure of cellulose. It is commonly accepted that native cellulose has parallel structure. For isolation of cellulose from wood, direct nitration of wood yields undegraded cellulose trinitrate, which is soluble in organic solvents. On the other hand, the glycosidic linkages are easily cleaved by strong mineral acids, so cellulose can be hydrolyzed to simple sugars. However, for a complete hydrolysis of cellulose, concentrated acid solutions must be used in order to bring about the necessary swelling and at least a partial destruction of the ordered regions (Yang and Jaakkola, 2011).

1.1.2 Hemicelluloses

Apart from cellulose, hemicelluloses are other major naturally occurring carbohydrate-based polymers, which are heteropolysaccharides and are clearly less well-defined than cellulose. The building units of hemicelluloses are hexoses, pentoses, or deoxyhexoses. These units exist mainly as six-membered (pyranose) structures either in the α- or ß- forms.

The type and content of hyemicelluloses varies among hardwoods and softwoods. Softwood hemicelluloses mainly include galactoglucomannans and arabinoglucuronoxylan. Galactoglucomannans are built up of a mainly linearbackbone chain of (14)-linked and partially acetylated -D-glucopyranose and –mannopyranose units, which are substituted at C-6 with a variable number of single -D-galactopyranose units. Arabinoglucuronoxylan consists of a linear framework of (1-4)-linked -D-xylopyranose units with branches of both 4-*O*-methyl--D-glucuronic acid and -L-arabinofuranose. Unlike xylan in hardwood, there are no acetyl groups. Hardwood hemicelluloses mainly include glucuronoxylan and glucomannan. Glucuronoxylan is composed of the same framework as the arabinoglucuronoxylan, but it contains much fewer uronic acid substituents. No arabinose units are present, and the xylose residues are partially acetylated.

Glucomannan has the same linear framework as galactoglucomannans, except that itis unsubstituted and has a higher glucose-to-mannose ratio. For a special type of

wood, e.g. larch, arabinogalactan content might reach 10-20% by mass. The structures of the different hemicelluloses are shown in Fig. 4.

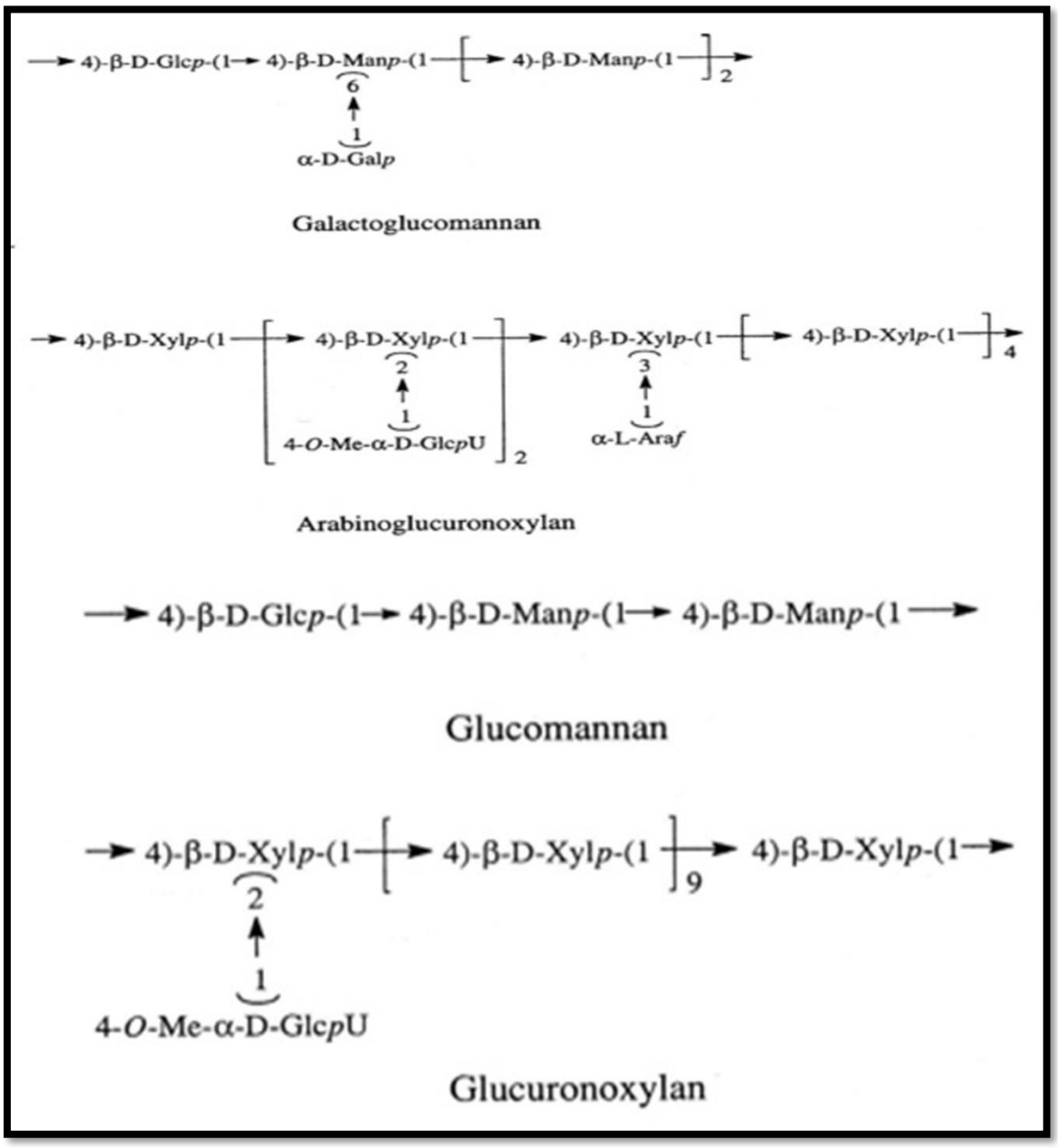


Fig. 4. Partial chemical structure of hemicelluloses from wood (Stenius, 2000).

Hemicelluloses are usually isolated by successive extractions with dimethyl sulfoxide and aqueous alkali. However, degradation caused by the alkali cannot be completely avoided. Galactoglucomannans are easily depolymerized by acids - and especially the bond between galactose and the main chain. The acetyl groups are much more easily cleaved by alkali than by acid. For arabino glucuronoxylan, the arabinose side chains can be easily hydrolyzed by acids due to their furanosidic structure, which

is less resistant to hydrolysis. Unlike all other wood hemicelluloses, larch arabinogalactan is extracellular and can be extracted quantitatively from the heartwood with water. Reliable analysis of hemicelluloses is based on the separate determination of the polysaccharide constituents using chromatography. The extractive-free wood sample is subjected to an acid hydrolysis, after which the liberated monosaccharides are separated and quantified.

1.1.3 Lignin

Lignin is an amorphous polymer, and the chemical structure of lignin is irregular in the sense that different structural elements are not linked to each other in any systematic order. In general, lignins are roughly classified into softwood lignin, hardwood lignin, and grass lignin. Besides the native lignin, there are several methods that can be used to separate lignin, so various forms of lignin are available, e.g. milled wood lignin, dioxane lignin, enzymically liberated lignin, Kraft lignin, and alkali lignin. Although native lignins behave as an insoluble and three-dimensional network, the isolated lignins show maximum solubility insolvents such as dioxane, acetone, methyl cellosolve, tetrahydrofuran, dimethyl formamide, and dimethyl sulfoxide.

Lignin can be defined as a polyphenolic material arising mainly from enzymicdehydrogenative polymerization of three phenylpropanoid units (p-hydroxycinnamylalcohols), as shown in Fig. 5. The proportions of the precursors in lignins vary with their botanical origin. Normal structural elements of softwood lignins are derived principally from trans-coniferyl alcohol (90%) with the remainder consisting mainly of trans-p-coumaryl alcohol. In contrast, hardwood lignins are mainly composed of trans-coniferyl alcohol and trans-sinapyl alcohol in varying ratios (about 50% for each alcohol). A simplified representation of a segment of softwood lignin is shown in Fig. 6.

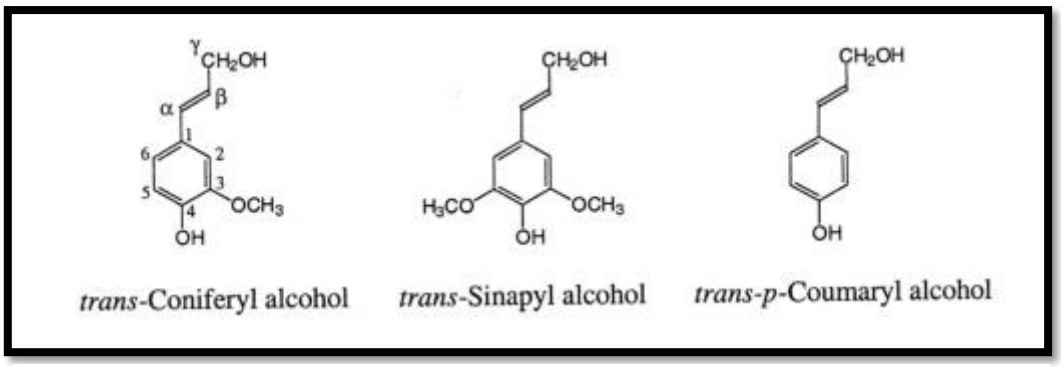


Fig. 5. The building units of lignin (Kollmannand Cote,1986).

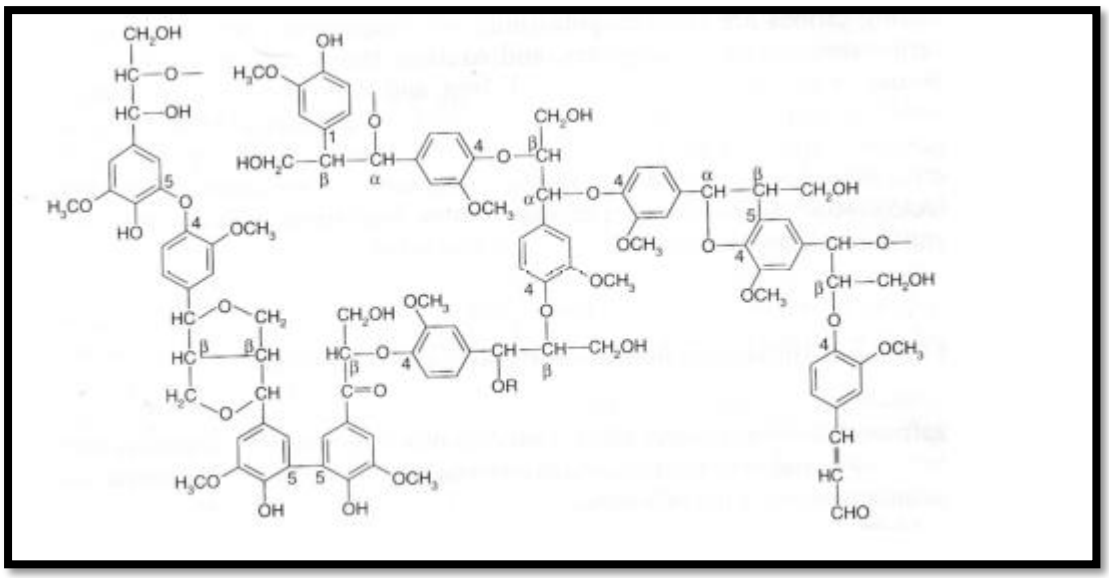


Fig. 6. Simplified representation of a segment of soft wood lignin (Yang and Jaakkola, 2011).

The structural building blocks of lignin are joined together by ether linkages (C-O-C) and carbon-carbon bonds (C-C), and the former ones predominate both in softwood and in hardwood. The predominant bond types and frequencies are shown in Fig. 6, and the frequency of these groups can vary according to the morphological location of lignin. As with its precursors, the lignin polymer contains several functional groups in the side chain, e.g. methoxyl groups, phenolic hydroxyl groups, and aldehyde groups. Only relatively few of the phenolic hydroxyl groups are free, because most of them form linkages to the neighboring phenylpropane units.

1.1.4 Inorganic elements in wood

Elements such as calcium, potassium, magnesium, phosphorus, sulfur, sodium, silicon, and cadmium contribute to the chemical composition of wood (NYSERDA Report 13, 2013).

1.2 Types of wood

Historically, the classification of wood has always been in terms of either hard wood (from any leaf-bearing tree) and soft wood (any cone-bearing tree). These terms can be confusing, since some leaf-bearing trees can have very soft wood and some coniferous trees can have very hard wood. To make this easier, below you will find a list of different types of trees, their classification, and the individual wood characteristics (http://www.hoovedesigns.com/woods.html).

Despite what one might think based on the names, not all softwoods have soft, lightweight wood: nor do all hardwoods have hard, heavy wood. To define them botanically, softwoods are those woods that come from gymnosperms (mostly conifers), and hardwoods are woods that come from angiosperms (flowering plants). In the temperate part of the northern hemisphere, softwoods are generally needle-leaved evergreen trees such as pine (Pinus) and spruce (Picea), whereas hardwoods are typically broadleaf, deciduous trees such as maple (Acer), birch (Betula), and oak (Quercus). Softwoods and hardwoods not only differ in terms of the types of trees from which they are derived, but they also differ in terms of their component cells. Softwoods have a simpler basic structure than do hardwoods, because they only have two cell types and relatively little variation in structure between these cell types. Hardwoods have greater structural complexity because they have both a greater number of basic cell types and a far greater degree of variability between the cell types. The single most important distinction between the two general kinds of wood are that hardwoods have a characteristic type of cell called a vessel element (or pore) whereas softwoods lack these. An important cellular similarity between softwoods and hardwoods is that in both kinds of wood, most of the cells are dead at maturity, even in the sapwood. The cells that are alive at maturity are known as parenchyma cells and can be found in both softwoods and hardwoods. (Botanist, 2010).

1.2.1 Hardwoods

1.2.1.1 Oak

Oak (Fig. 7A) is the most widely used hardwood. There are more than 60 species of oak grown, which can be separated into two basic varieties; white and red. The red variety is also known as black oak (which is a reference to its bark) (http://www.hoovedesigns.com/woods.html).

Properties

Oak is a heavy, strong, light-colored hardwood. It is ring porous, due to the fact that more and larger conductive vessels are laid down early in the summer, rather than later. Prominent rings and large pores give oak a coarse texture and prominent grain. Oak also has conspicuous medullary rays, which can be seen as "flakes" in quarter- sawed oak lumber.

Uses

Oak is the most popular wood used to craft country designs. It is also used for many transitional and contemporary pieces (http://www.hoovedesigns.com/woods.html).

1.2.1.2 Maple

There are 115 species of maple.

Properties

Maple (Fig. 7B) is so hard and resistant to shocks that it is often used for bowling-alley floors. Its diffuse, evenly sized pores give the wood a fine texture and even grain. Maple that has a curly grain is often used for violin backs (the pattern formed is known as the fiddleback figure). Burls, leaf figures, and bird's-eye figures found in maple are used extensively for veneers. The bird's-eye figure in maple is said to be the result of stunted growth and is quite rare.

Uses

Maple is used extensively for American colonial furniture, especially in medium- and lower-priced categories. It can also be stained to simulate cherry wood, which it resembles (http://www.hoovedesigns.com/woods.html).

1.2.1.3 Mahogany

Also known as Honduras, mahogany is a tropical hardwood indigenous to South America, Central America, and Africa. There are many different grades and species sold under this name, which vary widely in quality and price. Mahogany that comes from the Caribbean is thought to be the hardest, the strongest, and of the best quality. Logs from Africa, though highly figured, are of slightly lesser quality. Philippine mahogany has a similar color, but it is not really mahogany at all. It is a much less valuable wood, being less strong and not as durable or as beautiful when finished.

Properties

Mahogany (Fig.7C) is strong, with a uniform pore structure and poorly defined annual rings. It has a reddish-brown color and may display stripe, ribbon, broken stripe, rope, ripple, mottle, fiddleback, or blister figures. Crotch mahogany figures are widely used and greatly valued. Mahogany is an excellent carving wood and finishes well.

Fig.7: Hardwoods. A. Oak. B. Maple. C. Mahogany. D. Walnut. E. Rosewood. F. Teak. G. Cherry.

Uses

Mahogany is used extensively in the crafting of Georgian, Empire, and Federal reproduction furniture. Mahogany is also used in styles ranging from Victorian furniture reproductions to Contemporary.

1.2.1.4 Walnut

Walnut (Fig. 7D) is one of the most versatile and popular cabinet-making woods. It grows in Europe, America, and Asia. There are many different varieties.

Properties

Walnut is strong, hard, and durable, without being excessively heavy. It has excellent woodworking qualities and takes finishes well. The wood is light to dark chocolate brown in color, with a straight grain in the trunk. Wavy grain is present toward the roots, and walnut stumps are often dug out and used as a source of highly figured veneer. Large burls are common. Walnut solids and veneers show a wide range of figures, including strips, burls, mottles, crotches, curls, and butts. European walnut is lighter in color and slightly finer in texture than American black walnut, but they are otherwise comparable.

Uses

Walnut is used in all types of fine cabinet work, especially eighteenth-century reproductions.

1.2.1.5 Rosewood (Fig. 7E)

It is very hard and has a dark reddish-brown color. It is fragrant and close-grained. It is hard to work and takes high polish. It is used in musical instruments, piano cases, tool handles, art projects, veneers, and furniture.

1.2.1.6 Teak

True teak (Fig. 7F) is indigenous to Southeast Asia, but similar wood species also grow in Africa.

Properties and uses

Teak is a yellow to dark brown hardwood that is extremely heavy, strong, and durable. Often strongly figured, teak may show straight grain, or mottled or fiddleback figures. It carves well, but because of its high value, it is often used as a veneer. Scandinavian modern and oriental furniture styles are often crafted from teak.

1.2.1.7 Cherry

Cherry (Fig. 7G) is grown in the eastern half of the USA. It is sometimes called fruitwood. The term “fruitwood” is also used to describe a light-brown finish on other woods.

Properties

A moderately hard, strong, closed grained, light to red-brown wood, cherry resists warping and checking. It is easy to carve and polish.

Uses

Cherry veneers and solids are used in a variety of styles. Cherry has been called New England mahogany and is often used to craft eighteenth century, Colonial, and French Provincial designs.

1.2.2 Softwoods

1.2.2.1 Pine

Pine (Fig. 8A) is a softwood that grows in most areas of the Northern Hemisphere. There are more than 100 species worldwide.

Properties

Pine is a soft, white or pale-yellow wood that is lightweight, is straight grained, and lacks figure. It resists shrinking and swelling. Knotty pine is often used for decorative effect.

Uses

Pine is often used for country or Provincial furniture. Pickled, whitened, painted, and oil finishes are often used on this wood.

1.2.2.2 Ash

There are 16 species of ash (Fig. 8B), which grow in the eastern United States. Of these, white ash is the largest and most commercially important species.

Properties

Ash is a hard, heavy, ring porous hardwood. It has a prominent grain that resembles oak, and a white to light-brown color. Ash can be differentiated from hickory (pecan), which it also resembles, by white dots in the darker summerwood. These can be seen with the naked eye. Ash burls have a twisted, interwoven figure.

Uses

Ash is widely used for structural frames and steam-bent furniture pieces. It is often less expensive than comparable hardwoods.

1.2.2.3 Hickory

There are 15 species of hickory in the eastern United States, eight of which are commercially important.

Properties

Hickory is one of the heaviest and hardest woods available. Pecan is a species of hickory that is sometimes used in furniture. It has a close grain without much figure.

Uses

Wood from the hickory is used for structural parts, especially where strength and thinness are required. Decorative hickory veneers are also commonly used.

1.2.2.4 Beech

The American beech is a single species that grows in the eastern half of the United States.

Properties and uses

Beech is a hard, strong, heavy wood with tiny pores and large conspicuous medullary rays, similar in appearance to maple. This relatively inexpensive wood has reddish-brown heartwood and light sapwood. Beech is often used for frames, and a variety of bent and turned parts. Quarter-sliced and half-round cut beech veneers are commonly used.

1.2.2.5 Birch

There are many species of birch (Fig. 8C). The yellow birch is the most important commercially. European birch is fine-grained, rare, and expensive.

Properties and uses

Birch is a hard, heavy, close-grained hardwood with a light-brown or reddish-colored heartwood and cream or light sapwood. Birch is often rotary- or flat-sliced, yielding straight, curly, or wavy grain patterns. It can be stained to resemble mahogany or walnut.

1.2.2.6 Cedar

Several species of cedar (Fig. 8D) grow in the southern United States, Central America, and South America.

Properties and uses

Cedar is a knotty softwood that has a red-brown color with light streaks. Its aromatic and moth-repellent qualities have made it a popular wood for lining drawers, chests, and boxes. Simple cases and storage closets are also constructed from this light, brittle wood.

1.2.2.7 Redwood

Indigenous to the Pacific United States, redwood trees grow to more than 300 feet tall and to be 2,500 years old.

Properties and uses

The best-quality redwood comes from the heartwood, which is resistant to deterioration due to sunlight, moisture, and insects. It is used to craft outdoor furniture and decorative carvings. Redwood burls have a “cluster of eyes” figure. They are rare and valuable.

1.2.2.8 Hemlock

Hemlock is light in weight and is uniformly textured. It machines well and has low resistance to decay and is non-resinous. It is used for

construction lumber, planks, doors, boards, paneling, sub-flooring, and crates.

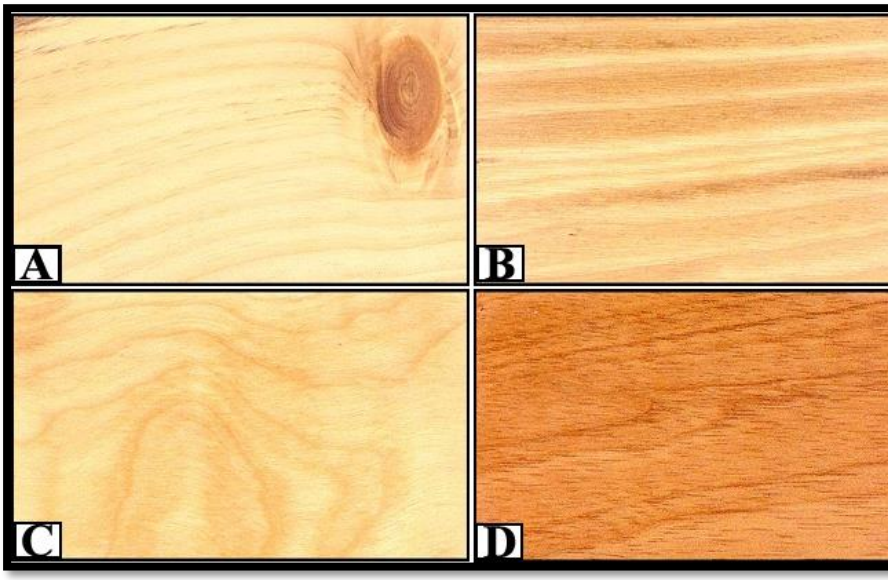


Fig.8.Softwoods. A. Pine. B. Ash. C. Birch. D. Cedar.

1.2.2.9 Fir

Fir works easily and finishes well. It is uniform in texture and non-resinous. It has low resistance to decay. It is used in furniture, doors, frames, windows, plywood, veneer, general millwork, and interior trim.

1.2.2.10 Spruce

Spruce is strong and hard. It finishes well and has low resistance to decay. It has moderate shrinkage and is light in weight. It is used for masts and spars for ships, aircraft, crates, boxes, general millwork, and ladders.

Chapter two

Fungal deterioration of archaeological wood

Chapter Two: Fungal deterioration of archaeological wood

Woods are susceptible to rapid deterioration by a variety of organisms. The wood in living trees and also in used products, or as archaeological material, can start to decay and decompose, with attack by organisms, which is termed biodegradation. This biodegradation is one of the major challenges, because it can mean heavy economic loss. Fungi and insects are the major causes of biodegradation. Wood materials are subject to various biological attacks if preventative measures are not taken. Proper preservative treatment can reduce the damage and increase the longevity of wood (Shupe et al, 2008).

Archaeological wood that survives for long periods is found in environments that protect it from decomposition. Although the decay processes are restricted, these woods are rarely free from attack. Over long periods, microbes that are able to grow under adverse conditions degrade the wood, and various compounds, such as salts, cause a slow chemical deterioration. Most, if not all, historic and archaeological woods have been affected by degradation to some degree (Blanchette, 1991).

It is essential to improve our understanding of the microbes and processes that affect archaeological woods, and to improve our knowledge of the structural and chemical changes that occur in wood from degradation. This review provides information about biodeterioration mechanisms affecting wood, and gives a wide variety of examples of deterioration found in archaeological wood from different environments (Blanchette, 2000).

2.1. Physiological requirements of wood-destroying and wood-inhabiting fungi

In addition to the chemical composition of wood, several other factors greatly influence decay rates.

2.1.1 Nutrients

The basic nutritional requirements of wood-rotting fungi are satisfied by the structural carbohydrates and certain extraneous materials in wood. Degradation of lignin does not occur in the absence of the metabolism of wood carbohydrates, and lignin is probably of limited imporatance as a carbon source (Kirk et al, 1976).Among the non-structural

nutrients in wood, nitrogen plays the most important role. The requirements are evidently not great; wood usually contains about 0.03-0.1% nitrogen (Merrill and Cowling, 1966). Wood-decay fungi have an efficient mechanism for its metabolism and reuse (Levi et al. 1968). Decay rates were found to be highly correlated with the nitrogen content of the individual annual increments. In addition to the growth factors such as thiamine, certain micronutrients of inorganic nature are needed. Iron, zinc, copper, manganese, and molybdenum are needed. It has also been shown that phosphorus, potassium, sulfur, and magnesium are required in larger amounts (Côté, 1968).

2.1.2 Temperature

Temperature is one of the many ecological parameters affecting the activities of wood-decaying fungi. Most of these fungi are mesophilic, are generally unable to grow above 401°C, and have temperature optima of 24-30°C (Cartwright and Findlay, 1950; Jennison, 1952; Findlay, 1967). Even the majority of tropical wood decay fungi have temperature optima of 30°C or less. Some wood-decaying fungi have special tolerance of higher temperatures, however, being unable to grow below 20°C and with optima above 40°C.

Jensen (1969) studied the effect of fluctuating temperatures on the growth of four heart-rot fungi, and concluded that if temperature fluctuations were small, growth of the organisms was stimulated, whereas if the fluctuations were large, the growth of the organisms was suppressed. Stain fungi can withstand high temperatures for brief periods, but prolonged exposure to elevated temperatures is fatal. On the other hand, some are very resistant to low temperatures and survive exposure to frost.

It has been found that the soft-rot fungi as a group appear to grow well at temperatures that are higher than most wood-destroying Basidiomycetes prefer. Almost half of the test group that was investigated showed optimum growth at temperatures of 34°Cor higher (Côté, 1968).

2.1.3 Moisture

The determination of moisture requirements of fungi under laboratory conditions is difficult, because water is produced during respiration. The moisture content of the wood can therefore be changed by the fungus. Generally speaking, moisture content from 35% to 50% appears to be the most favorable for fungal growth. This condition means that the cell walls are saturated with water, with a layer offer water lining the

cell lumen. The remainder of the cell cavity is then available for gas exchange, and the liquid layer facilitates the diffusion of enzymes from the hyphae into the cell wall.

There have been reports of attack by fungi at moisture levels below the fiber saturation point, when there is no free water in the cell lumen. In the absence of moisture, no fungi will grow, but at moisture contents as low as 15% some fungi have been reported to become established. Generally, however, it is safe to assume that wood will not be subject to attack by the common fungi at moisture contents below the fiber saturation point. It should be borne in mind that moisture content figures are average levels for an entire board or sample, and moisture gradients are present. In cases where the circulation of air is unevenor restricted, it is possible that there will be pockets where moisture levels are much higher than the average value. This condition exists when wood is exposed to rain oris in contact with the soil, and fungi can get a start under such conditions. In addition, it should be noted that the fiber saturation point for different woods has a rather wide range, 20-35% moisture content, with a few instances below or above these levels. Another consideration is that the spread of decay and the continued growth of fungi probably require different conditions. Spores probably require a level of moisture content to germinate that is considerably different from that required for mycelial growth. It is recommended that wood in service should be maintained at a moisture content level that is 2-3% below the known fiber saturation point, to provide a desirable safety factor (Côté, 1968).

It is commonly believed that wood shrinks as it loses moisture and swells as it gains moisture. This is partially true. Actually, wood will change dimensions only between two precise moisture conditions. One condition is when the wood is devoid of moisture. This is termed the oven dry condition. The second condition is when the wood fibers are saturated with moisture. This point usually occurs at about 30% moisture content for most Louisiana species. As wood is dried from an original green condition, sometimes with more than 100% moisture content, moisture is first lost from the cell cavities. No shrinkage will occur until the wood is reduced to a moisture content of about 30% (the fiber saturation point). If drying continues below 30% moisture content, water is removed from the cell walls and shrinkage occurs. The amount of shrinkage or swelling depends on the species, density, and board direction. Pressure treatment with waterborne preservatives raises the moisture content above the fiber saturation point, and shrinkage will occur as the wood dries down to its in-service moisture content. In many applications, such as deck boards, this shrinkage is not a majorconcern.When

dimensional stability is critical, it is imperative that the lumber be kiln-dried after treatment (KDAT). Any KDA Tlumber that you buy should be kept under a roof, or at least under cover and off the ground. The dimensional stability of different wood species is affected by width and density differences between earlywood and latewood in the growth rings. For example, in species with wide, dense latewood bands and low-density earlywood bands, the differential shrinking and swelling of the bands with changes in moisture content can cause large stresses in the wood that can result in raised grain and a defect known as shelling. Raised grain will tend to be more pronounced on flat-grainedlumber.Shelling is an extreme case of raised grain, in which the latewood bands separate from the earlywood bands to form a knife-like or spear-like edge. This is one reason why it is often recommended that deck lumber be placed pith-side down (or bark-side up). If the two sides of particular board are of equal quality, it is better to place the board bark-side up. If, however, the pith-side is clearly the better side, place this side up. Moisture greatly affects lumber in use and can quickly lead to deterioration. Moisture can also allow wood to be attacked by insects, hinder the performance of finishes and paints, and induce surface stains (Shupe et al., 2008).

2.1.4 Oxygen

Since respiration is involved in the metabolism of fungal organisms, oxygen is essential for growth. The end-products of respiration are carbon dioxide and water. When there is insufficient oxygen, organic products such as alcohol and oxalic acid are formed. Experiments have shown that in ordinately high concentrations of carbon dioxide will retard growth, as most fungi require free access to atmospheric oxygen for optimum growth. In the absence of oxygen, no fungus will grow. Wood attacked by soft-rot fungi is often attacked under very wet conditions, such as in cooling towers. In this type of situation, the oxygen requirements of the organism can be satisfied by the oxygen dissolved in the water with which it is in contact (Côté, 1968).

2.1.5 Carbon dioxide (CO_2)

A heart rot fungus attacking a tree must often grow in a near-anaerobicatmosphere, with high CO_2 concentrations (Zeikus and Ward, 1974). Wood-degrading fungi evidently vary in their capacity to grow in atmospheres high in CO_2. It has been stated that several wood-inhabiting fungi continue to grow in 70% CO_2, whereas many litter-decomposing fungi are totally inhibited by 30% CO_2.Inhibition of growth by CO_2only occurs at low O_2 tensions. CO_2 also affects spore germination. Morton and French (1974) found that

germination of basidiospores of *Inonotusrheades* (*Polyporusdryophilus*) was stimulated by CO_2. Removal of CO_2 prevented germination.

2.1.6 Hydrogen ion concentration

Another condition that can be considered to be a physiological requirement is the hydrogen ion concentration of the wood medium. Wood-rotting fungi show a decided preference for a pH on the acid side, between 4.5 and 5.5. Optimum growth of both the white-rot and the brown-rot fungi takes place at this level. At the same time, it is interesting to note that both of these groups will make the wood in which they grow more acidic, simply as a result of their metabolism. Brown-rot fungi increase the acidity more than white-rot fungi do (Côté, 1968).

2.2 Characteristics of wood-staining fungi

The deterioration of wood by staining fungi is one of the most important sources of financial loss in lumber processing. It is particularly critical for products that are to be given clear or natural finishes. Although many stains of wood are of biological origin, some are due to oxidation of wood constituents or caused by contact with chemically reactive materials such as iron. Cartwright and Findlay (1950) devoted an entire chapter of their book on decay of timber to the various aspects of stains in wood. They considered the non-biological causes mentioned above, but concentrated on the discolorations brought about by incipient decay, surface molds, and sap-staining fungi. The non-biological causes of discoloration can be of considerable economic significance in certain cases, but the fungal causes are of greater general concern to the wood processor. Since the wood-staining fungi derive their nourishment only from the contents of parenchyma cells rather than from the polysaccharides and lignin, and since they bore only minute holes through the cell wall, their effects on the mechanical strength of wood are not as detrimental as is attack by wood-rotting fungi (Fig. 4.11). Consequently, the determination of the type of causal organisms responsible for staining can be very critical. In structural applications, a blue-stained member may be used without great concern unless shock loading is likely to occur. Incipient decay, however, may have already reduced the overall strength of the wood by a substantial amount. Yet both may appear, superficially, similar. Stained wood should always be suspected of containing decay organisms because the conditions that are favorable for the growth of sap-stain fungi are also favorable for the development of decay fungi. A few comparative characteristics are useful in distinguishing between discolorations

typical of decay-causing organisms and those due to the more innocuous staining fungi. Sap-stains, as implied by the name, are confined to sapwood. Stains associated with incipient wood decay are not always restricted to sapwood but are likely to be more highly concentrated there. Decay is generally found in patches or streaks, whereas overall discoloration is characteristic of staining fungi. Sap-stain can also appear as a wedge-shaped area on a log cross-section. This is due to the concentration of hyphae in the ray parenchyma cells.

Color differences, as well as pattern, provide further evidence for identification. Bluish-gray stain in sapwood is a good indication that sap-stain fungi are present. Colors ranging through green, pink, yellow, orange, or blackish are also found. Discolorations caused by decay fungi in softwood are more typically reddish-brown, while in hardwoods they may be white or dark-brown spots or streaks. Zone lines may be present with incipient decay, but these well-defined, dark outlines are not present with stain fungi.

A test for weakened fibers has been suggested as another method of determining whether a stain or a decay fungus has caused discoloration. A knife point inserted into the stained region will break fibers weakened by incipient decay but prying of this sort reveals little weakening by staining fungi (Côté, 1968).

2.3 Mechanism of wood decay

Wood-destroying fungi are grouped into three categories: brown rot, white rot and soft rot. These different fungi will attack the three different, main chemical components of wood: cellulose, hemicellulose, and lignin. When wood-degrading fungi metabolize wood, a decrease in strength occurs. The extent of the loss in strength will vary depending on the type of fungus involved, the wood species, and lumber dimensions. Louisiana and most of the Deep South are classified as a severe-risk area for wood decay (Fig. 9). Not all fungi that attack wood cause degradation. In fact, many are classified as wood-staining or mildew (mold) fungi because they discolor or stain the wood rather than cause it to decay. These fungi typically develop because of poor lumber-drying practices or excessively wet conditions. Stain fungi do not cause loss of strength but result in a lower grade of some grading lumber, and are considered to unfavorable consumers because of their appearance. Stain is not as important for structural-grade lumber. Structural integrity is more important than aesthetic appeal in certain situations, such as rural fencing or construction. Consumers should be wary if

they notice stain fungi, even though no loss of strength may have occurred, because conditions that favor stain fungi are often ideal for wood-degrading organisms. “Dry rot” is a frequently misused term. Wood with dry rot appears to be dry, but it must have been wet for decay to occur. Some mycelia can “wick” moisture from a distance, however.

2.3.1 White-rot fungi

White-rot fungi are common wood degraders in forest ecosystems and have the capacity to produce extracellular enzymes that degrade all cell wall components. The types of enzymes produced and the sequence of their production govern the amount and extent of cellulose, lignin, and hemicellulose degradation from wood. Some species are very efficient degraders of cellulose, while others degrade extensive amounts of lignin without there being significant cellulose degradation (Blanchette, 1991). Different forms of white rot result when cell wall components are degraded from the wood in varying amounts. Although white-rot fungi are some of the most prevalent wood-decaying organisms in nature, they are not often found attacking archaeological wood (Blanchette, 1991).

Fig. 9: Map of deterioration hazard zones for the USA as developed by the USDA Rural Electrification Administration (REA) and adopted by the American Wood Protection Association (AWPA). (Source: REA. 1973. Pole Performance study staff report. U.S. Department of Agriculture (after Shupe et al., 2008).

2.3.2 Brown-rot fungi

These fungi will preferentially attack softwoods but will also attack hardwood lumber and logs (Fig. 10). It is imperative to use treated wood or brush-apply a preservative and water repellant to prevent brown rot on wood used outside. The appearance of any mushroom-like bodies, which is an indication of advanced wood decay and substantial loss of strength, is important for the consumer.

Brown-rotted wood will develop a reddish-brown color and have a charred appearance. It also displays more than average shrinkage upon drying and is friable (soft).

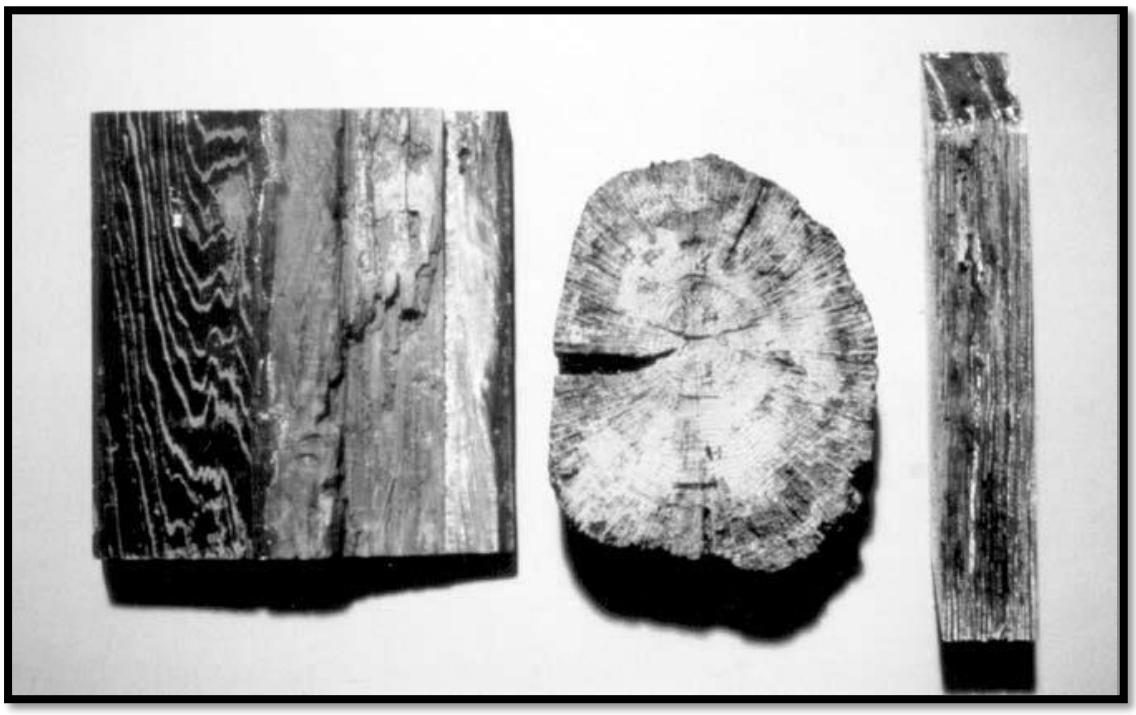


Fig. 10: Fungal decay of wood by brown-rot decay fungi. (Source: Forest Products Society; Shupe et al., 2008).

2.3.3 Soft-rot fungi

These fungi are classified as Ascomycota and Deuteromycota, and can be distinguished from other decay fungi by the decay patterns they produce in wood (Blanchette, 1994; Nilsson et al., 1989). Typically, soft-rot fungi produce cavities that spiral within the secondary wall of wood cells, following the microfibrillar orientation of cellulose (type-I attack) (Daniel and Nilsson, 1998). In transverse sections of wood, holes of varying

sizes can be observed in the secondary walls, whereas in radial or tangential sections they are elongated cavities, often with pointed ends. In some woods, a different form of attack occurs (type-II attack) and the entire secondary wall is gradually eroded, leaving a relatively intact middle lamella. Soft-rot fungi are often associated with waterlogged woods, and the term soft rot is used because the affected wood surface appears soft in wet environments. Soft rot can also occur under non-waterlogged conditions, and the advanced stages of decay appear brown and crumbly and may look similar to brown-rotted wood. Soft-rot fungi are often found in environments that are not conducive to the growth of brown-rot and white-rot fungi. Since soft-rot fungi can completely degrade the entire secondary cell wall, they have the capacity to degrade some lignin as cellulose and hemicellulose are metabolized. The highly lignified middle lamella, however, is not degraded and persists even in the most advanced stages of decay (Blanchette, 1991).

2.4 Damage in wood due to decay

Toughness and weight loss have been considered to be the most sensitive indicators of the degree of wood deterioration caused by decay. Other negative effects are observed and experienced due to unexpected changes in the wood properties after infestation. These changes are:

- Weight loss
- Strength loss
- Increased permeability
- Increased electrical conductivity
- Reduction in volume
- Changes in pulping quality
- Discoloration
- Reduction in caloric value (http://www.cals.ncsu.edu/course/pp318/profiles/decay/decay.htm)

Chapter Three

Materials and Methods

Chapter Three: Materials and methods

3.1 Samples

3.1.1 Archeological samples

The fungi studied were isolated from the following sites:

A. The Mosque of Sabiile and Koutab Suleiman Agha Selehdar (Fig. 11), which is located in El Muizz Street, Islamic Cairo. It was established in 1837–1839 AD (1253–1255 AH).

B. The Mosque of Abu Haribh (Fig. 12), which was built by Prince Sayf al-Din Akjmas Ishaqi El Zahery between 1480 and 1481 AD (885–886 AH).

C. The Mosque of El Musafir Khana (Fig. 13), situated at Darb Almsmt, El Gamaliya, and created by Mahmud Muharram. The first section was built in 1779 AD (880 AH), and the second in 1783 AD (884 AH).

D. The Mosque of El Mouayed Sheikh Al-Mahmoudi, inside Bab Zuwayla, close to the El Soukary neighborhood. The construction of themosque took about six years, from 1415 to 1421 AD (818–824 AH).

3.1.2 Preparation of new sample

New pitch pine samples were prepared, to be used in the experimental studies to evaluate the efficiency of the fungicides used. Pitch pine has been used in this study because it is a common wood used in the historical Cairo in Egypt. El-Hadidi (2015) informed that throughout the Coptic (since fourth century AD) and Islamic periods (since seventh century AD) domes and ceilings, which varied from very simple to highly sophisticated decorated types composed of several layers, were made of wood, but not much was published on wood identification, and it is usually presumed to have been built using ‘pitch pine.

The pitch pine has been used in the historical Islamic buildings in historical Cairo for its good characteristics. Miller (1999) said that the wood of pitch pine is moderately heavy to heavy, moderately strong, stiff, and hard, and moderately high in shock resistance. Shrinkage ranges from moderately low to moderately high.

According to these good characteristics, the pitch pine wood was used in outdoor woodwork in historical buildings such as doors, windows, carpentry, etc., because it has a good resistant against surrounding environmental conditions.’

3.2 Isolation and identification of fungi

Isolation of fungi was done according to Abdel-Maksoud (2011). Pieces of deteriorated wood were placed in sterile plastic bags and were transferred to the laboratory, where their inner parts were exposed and sterile swabs were used to wipe along surfaces contaminated with fungi (Fig. 14). The swabs were then gently wiped onto the surface of a potato-dextrose agar (PDA) medium in Petri dishes, and cultures were incubated at 28°C for 1–2 weeks. Individual fungal colonies were then recovered and transferred to Petri dishes with malt extract agar (MEA). Colonies from the established subcultures were used for the assessment of the morphological characteristics of the fungi isolated (Benjamin et al., 1966, Barnett et al., 1972) and for their grouping into distinct species/morphotypes.

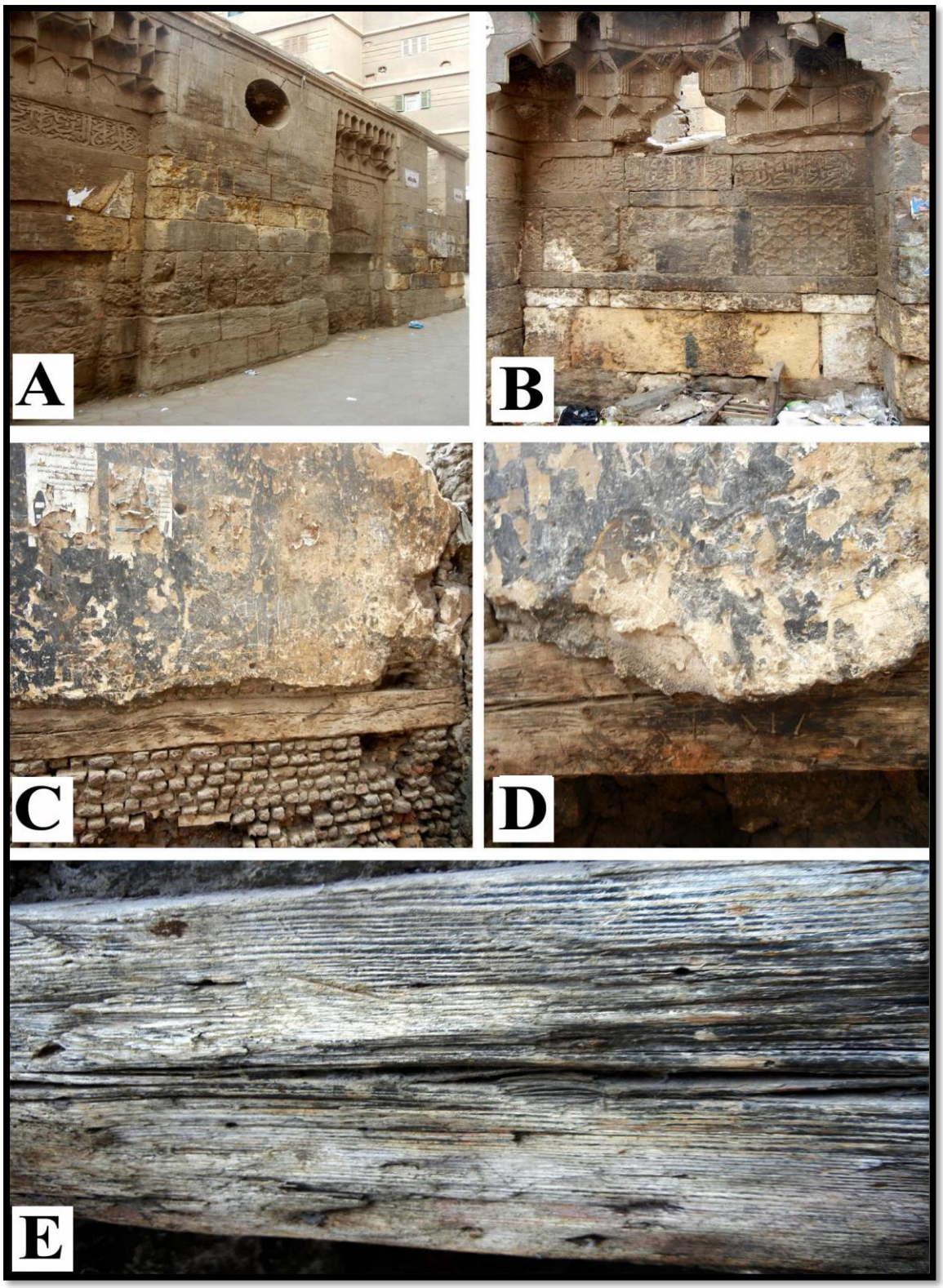


Fig. 11. Mosque of Sabiile and Koutab Suleiman Agha Selehdar: A-E. Aspects of deterioration caused by biological, chemical, and physical factors.

Fig. 12: Abu Haribh mosque: A-D. Different aspects of the deterioration processes of wooden artifacts.

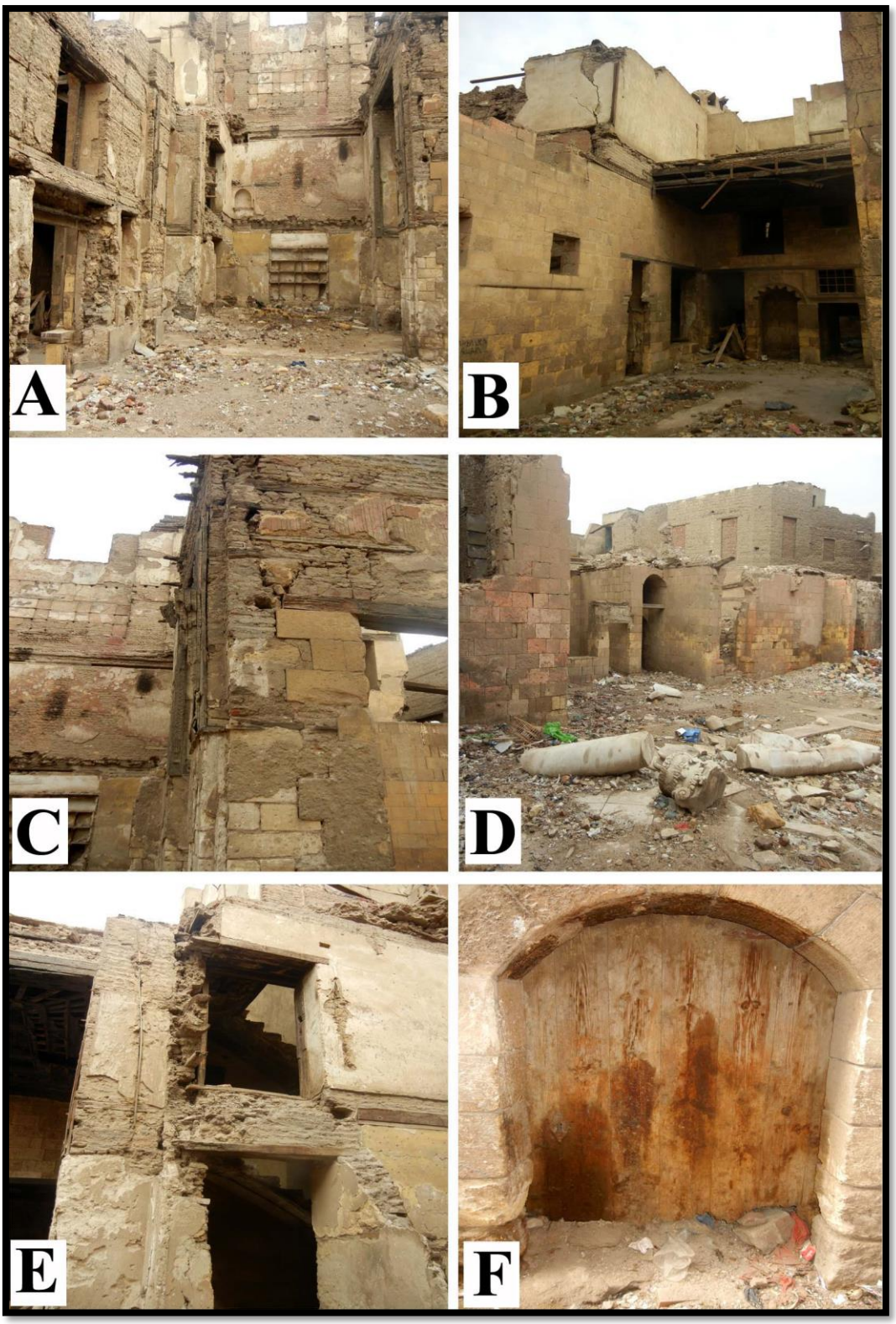


Fig. 13. El Musafir Khana: A-F. Aspects of deterioration of wooden artifacts such as biological attack and darkness from pollutants.

Fig. 14. Historical samples used for the isolation and identification of the fungi studied.

In addition, and for verification of initial taxonomic determinations based on morphological features, total genomic DNA of representative strains from each species/morphotype was extracted from mycelium. For the isolation of fungal DNA, 50 mL of potato-dextrose medium was inoculated with stock cultures, which were grown on MEA slants for 7 days at27°C. Biomass was obtained after 2 days of incubation at 27°C by centrifugation at 4,000× g for 10 min and washed twice with sterile double-distilled water. Disruption of the cells was performed in a ceramic mortar using liquid nitrogen and a pestle, until a white powder was obtained. Genomic DNA was extracted from 100 mg of biomass powder according to the instructions of the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St Louis, MO, USA). Elution was performed in two steps (100 μL each) and the presence of genomic DNA was detected by 1% agarose-gel electrophoresis.

PCR primers used by White et al. (1990) defined the amplification reactions for the ITS1-5.8S-ITS2 rDNA region: ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The primers were constructed by Eurofins Genomics (Ebersberg, Germany). The following PCR conditions were used for ITS amplification. The reaction was initiated at 95°C for 2 min followed by 35 cycles of 95°C for 20 s,48°C for 30 s, and70°C for 15 s, with a final extension step at 70°C for 2 min. PCR products were checked by agarose-gel electrophoresis using 1 kb DNA Ladder RTU (NIPPON Genetics EUROPE, Düren, Germany). The PCR products were

directly sequenced by Eurofins Genomics. All fragments were read in both directions and the nucleotide sequences were submitted to the GenBank database. Their accession numbers are as follows: KU243044, KU243045,KU243046, and KU243047.

Alignment of sequences was carried out using Clustal Omega software (http://www.clustal.org/omega/). Phylogenetic relationships were inferred by using the maximum likelihood method based on the Kimura 2-parameter model (Kimura, 1980). Initial tree(s) for the heuristic search were obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated by the maximum composite likelihood (MCL) approach. The branch swap filter was set to 'strong', and all sites were used for the analysis, including gaps. Bootstrap values were derived from a total of 10,000 replicates. Pertinent analyses were conducted through the use of MEGA 6 software (Tamura et al., 2013). Eleven additional sequences from related fungal taxa (i.e. related to this study's material) were retrieved from GenBank, and were included in the phylogenetic analysis. Among them, *Mucor fragilis* was used as out group.

3.3 Fungal strains

In this study, the three active strains *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* were isolated from wood samples taken from the different locations mentioned above.

For fungal growth prior to infestation of wood samples, Czapek-Doxagar (CZA) was used: sucrose 30 g/L, sodium nitrate 2 g/L, dipotassium phosphate 1X/L, magnesiumsulfate0.5 g/L, potassium chloride 0.5 g/L, ferrous sulfate 0.01 g/L, and agar 20 g/L (final pH: 7.3 at 25°C). CZA was poured into Petri dishes, and was further inoculated with fungal spores, which were uniformly distributed onto the surface of the medium with a wrapped glass spreader. Wood samples (treated and untreated, with and without fungicide) were put at the center of each Petri dish.ThePetri dishes were sealed by using Parafilm to prevent any possible contamination and drying of agar over an incubation period of four months at 28°C.

3.3.1 Sample incubation

Fungal cultures were incubated at 28°C for four months. After this incubation period, the wood samples were picked out and cleaned mechanically with a brush to remove mycelia. The samples were conditioned at 21 ± 2°C and 65% relative humidity (RH) for 72 h before measurements.

3.4 Fungicides used

3.4.1 Chitosan

Chitosan (Fig. 14) is a linear polysaccharide composed of randomly distributed β-(1→4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is made by treating the chitin shells of shrimps and other crustaceans with an alkaline substance, such as sodium hydroxide.

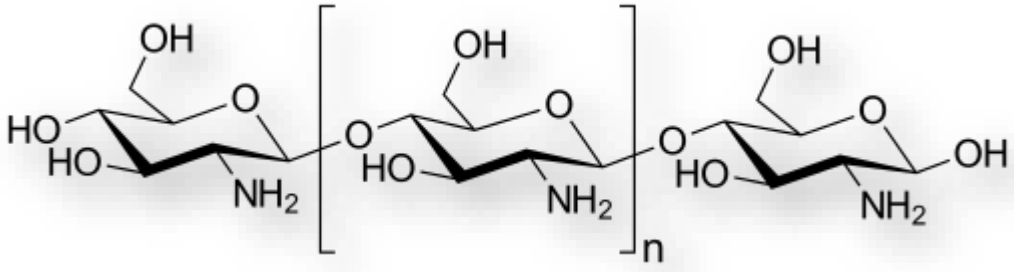


Fig. 14. The chemical composition of chitosan (https://en.wikipedia.org/wiki/Chitosan).

3.4.2 Propeconazole

Propiconazole (Fig. 15) is a triazole fungicide and is also known as a DMI, or demethylation inhibiting fungicide, due to its binding with and inhibition of the 14-alpha demethylase enzyme from demethylating a precursor to ergosterol. Without this demethylation step, the ergosterols are not incorporated into the growing fungal cell membranes, and cellular growth is halted.

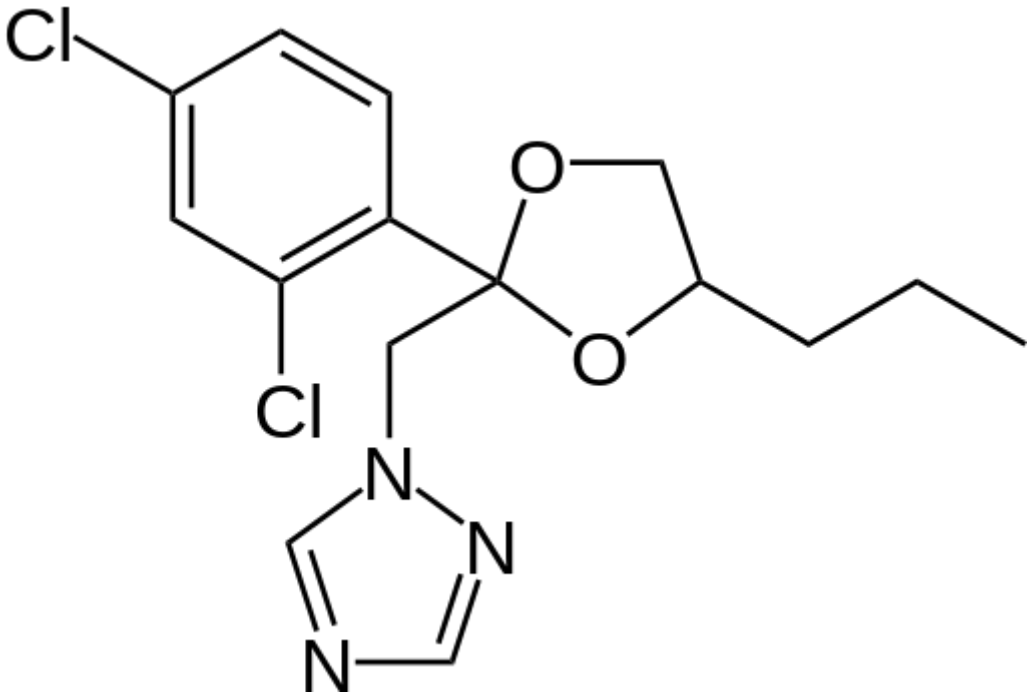


Fig. 15. The chemical composition of propiconazole (https://en.wikipedia.org/wiki/Propiconazole#/media/File:Propiconazol.svg)

3.4.3 Tebuconazole

Tebuconazole (Fig. 16) is a triazolefungicide.

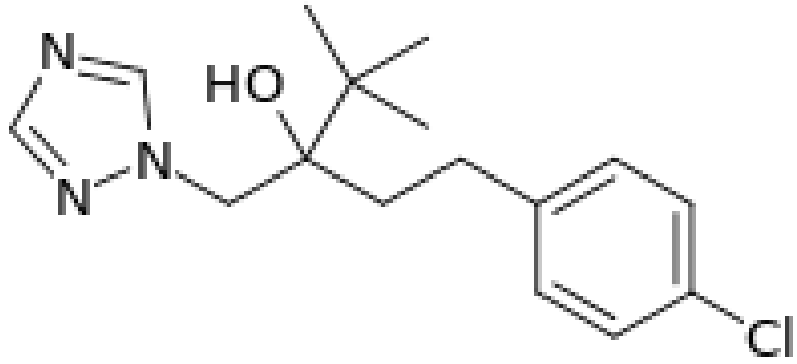


Fig. 16. The chemical composition of tebuconazole (https://en.wikipedia.org/wiki/Tebuconazole).

Chen (2009), Schubert et al. (2012) mentioned that propiconazole and tebuconazole have been used as fungicides for wood protection.

Propiconazole and Tebuconazloe are triazole-based fungicides that are commonly used to control fungi and are also incorporated into wood preservative formulations (Lebow et al., 2013, Alonso et al., 2014).

3.5 The application of fungicides to the wood samples

The method of application of fungicides used was the impregnation technique. The samples were soaked with fungicides until saturation. Upon removal of excess fungicide, the samples were allowed to dry at room temperature, and were then transferred to Petri dishes (Abdel-Maksoud, 2006).

Chitosan [deacylated chitin, poly(D-glucosamine)] dissolved in 2% acetic acid was used in this study. The concentrations used were 0.25%, 0.50%, and 0.75%. Chitosan (low molecular weight) was purchased from Aldrich. The control samples were treated with acetic acid alone (2%).

Propiconazole($C_{15}H_{17}C_{l2}N_3O_2$) and **tebuconazole** ($C_{16}H_{22}ClN_3O$) fungicides dissolved in toluene were used in this study. The concentrations used were 0.25% and 0.50%. These fungicides were also purchased from Aldrich.

3.6 Investigation techniques

3.6.1 Measurement of color change with UV spectrophotometry

Color changes caused by the effect of accelerated incubation cycles were measured by using the CIE L*a*b* system (Abdel-Maksoud andAl-Saad, 2009; Abdel-Maksoud and Marcinkowska, 1999; Abdel-Maksoud and Marcinkowska, 2000). The L* scale measures lightness, and varies from 0 (black) to 100 (perfect white). The a* scale measures red-green, with '+a' meaning more red and '-a'meaning more green. The b* scale measures yellow-blue, with '+b'meaning more yellow and '-b' meaning more blue. The total color difference (ΔE) is calculated according to the following equation (Billmeyer and Saltzman, 1981):

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

Measurements were made using a Macbeth Color Eye 7000 UVspectrophotometer (GretagMacbeth, USA). This method was used with chitosan.

3.6.1.1 Whiteness (WI) and yellowness (YI) indices

Whiteness index(WI) and yellowness index (YI) resulting from accelerated incubation cycles were measured with a double-beam spectrophotometer(ColourEye^{TM} 3100, SDL, UK). The two indices were calculated using the following equation:

$$WI = L - 3b + 3a$$

$$YI = 142.86 * b/L$$

where L is the brightness, the red-green CIE component, and the yellow-blueCIE component (Caivano and Buera 2012; Ibrahim et al. 2013). This method was used with propiconazole and tebuconazole.

3.6.1.2 Calibration of spectrophotometer with neutral density filters

The spectrophotometer was calibrated for photometric and wavelength scales by multiple readings with standard neutral density glass filters of known nominal values. Reflectance calibration was done using standard ceramic white tile of known reflectance value (anideal white surface reflects 100 of all incident light while an ideal black one absorbs 100% of incident light) at temperature of23.5ºC and 42% relative

humidity (ASTM E275-01; Abd-El mageed et al. 2016). For reliable results, the doubts from the associated errors were taken into consideration in uncertainty estimation procedures (El-nagar 2012).

3.6.2 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared attenuated total reflection spectroscopy (FTIR-ATR) has been used extensively to investigate adsorption and reactions on surfaces. This method of analysis was used in accordance with Jadoul et al.(1996); Pouliot et al. (1999); Xie et al. (2002); Velkova and Lafleur (2002); Kazarian and Chan (2006); Liao et al. (2006); Dias et al. (2008); and Russeau et al. (2009). A significant advantage of the ATR technique is that the wood sample does not require any preparation, thereby minimizing possible damage to the sample (Terinte et al., 2011). The infrared spectra were obtained using a JASCO-ATR-FT/IR-6100 Fourier transform infrared spectroscope.

3.6.3 X-Ray diffraction (XRD) for the determination of wood crystallinity

The crystallinity of selected samples that were either untreated or treated with chitosan at different concentrations, and of samples infected by the fungi under study, was determined using a Lab XRD6000 X-ray diffractometer (Shimadzu, Japan). The results for four peaks were selected in the fitting of each diffractogram, three peaks for the cellulose crystalline peaks (101), (10$\bar{1}$), and (002). In this study, the position (2θ degrees), the full width at half maximum (FWHM) (2θ degrees), and intensity were obtained for each fitted peak.

3.6.4 Peak position (2θ degrees)

The positions of the peaks in an X-ray diffractogram reflect the dimensions of the crystallite of the cellulose unit cell.

3.6.5 Peak width (2θ degrees)

Measurement of the degree of crystallinity by using peak width (the width at half height parameters in 2θ degrees) was done in accordance with Hermans and Weidinger (1961). The peak width was calculated by taking the difference between the peak position and the half height intersection and multiplying by two. These parameters should represent the width of the peak at half its height if no other peaks have interfered.

3.6.6 Peak intensity (2θ degrees)

There are two methods for evaluation of the intensity of untreated, incubated and untreated, and incubated and treated wood samples. These methods were applied as follows:

First method. This method was used in accordance with Hermansand Weidinger (1961). In this method, the intensities of the ($10\bar{I}$) and (002) peaks were normalized against the (101) peak intensity.

Second method. This method was used in accordance with Segalet al. (1959)and Terinte et al. (2011). By this method, the crystallinity index can be calculated according to the following equation:

$$\mathbf{CrI} = [(\mathbf{I}_{002} - \mathbf{I}_{am})/(\mathbf{I}_{002})] \times 100$$

Where **CrI** = crystallinity index, $\mathbf{I}_{002}$ =intensity at approximately 22.6^{o} 2θ, and $\mathbf{I}_{am}$ = intensity at approximately 19^{o} 2θ.

This crystallinity index, which is based on the two-phase model for cellulose, has no absolute theoretical significance, but it is as good as any other approach for the relative ranking of cellulose I crystallinities. This method was therefore used in ranking the crystallinities of the cellulose examined.

Chapter Four

Results and Discussion of Chitosan Fungicide

Chapter Four: Results and discussion of chitosan fungicide

4.1 Identity of the fungal strains isolated from wooden artifacts

The main morphological features of the strains isolated were as follows:

A. Strain HRa — MEA 25°C, 7 d: colony diameters 8.5–9.0 cm, plane to floccose; conidial heads radiate to columnar, yellowish to green to olive green; mycelium white; reverse uncolored. Stipes 160–730 × 6.4–21.5 µm, rough, pale brown; vesicles globose to subglobose to ellipsoidal, 20.5–55.0 µm wide. *Aspergillus biseriate*, less of tenuniseriate, or both in the same vesicle; metulae 8.0–15.4 × 3.7–7.2 µm, phialides 7.6–13.0 × 2.2–4.4 µm; conidia globose, smooth to finely rough, 3.8–5.9 × 2.7–3.3 µm.

B. Strains A2 and HRb — MEA 25°C, 7 d: colony diameters 8.5–9.0 cm, granular, conidial heads radiate or splitting into columns, brownisholive to green brown, later dark brown to black; mycelium white, submerged; reverse color cream. Stipes 330–2,300 × 4.8–21.5 µm, uncolored to light brown, smooth; vesicles globose, 25.0–70.0 µm wide. *Aspergillus biseriate*; metulae rarely septate, 12.8–36.0 × 4.2–10.4 µm; phialides 5.7–11.6 × 2.9–4.4 µm. Conidia subglobose to globose, rough, 3.3–5.5 × 2.6–5.1 µm.

C. Strain 503 — MEA 25°C, 7 d: colony diameters 30–35 mm, plane, velutinous, margin entire, mycelium white, margin thin; conidiogenesis abundant, grayish-green to dull green; no exudates or soluble pigments; reverse yellow to dull green. Conidiophores borne from aerial and superficial mycelium; stipesseptate, apices vesiculate, 45–130 × 3.4–3.7 µm, smooth, thin walled; penicillium mostly irregularly terverticillate or quaterverticillate; metulae in verticils of 2–6, smooth, 7.4–13.0 × 2.4–5.2 µm; phialides in verticils of 5–9, ampulliform, smooth, 6.8–7.2 × 2.5–2.8 µm; conidia subglobose to broadly ellipsoidal, 2.5–3.1 × 2.4–2.8 µm, smooth, thick-walled, borne on long irregular columns.

The above morphological descriptions led to the following initial taxonomic assessments: *A. flavus* for strain HRa, *A. niger* for strains A2 and HRb, and *P. chrysogenum* for strain SO3.

Since identification to the species level of fungi of these two genera is often difficult and ambiguous, the four strains were also subjected to sequence analysis of their ITS-5.8S region. The sequences obtained (GenBank accession numbers: KU243044,

KU243045, KU243046, andKU243047) were compared to published sequences selected on the basis of their highest homology (99–100%, through the use of the BLASTN algorithm; NCBI). The phylogenetic tree constructed byincluding all these sequences confirmed the identity of three out of the four strains examined, while the fourth strain (HRb) was grouped together with *A. tubingensis* (Fig. 15). It is noteworthy that *A.niger* and A. tubingensis are among the several morphologically indistinguishable species of the section Nigri(Someren et al., 1991; Samson et al., 2007); hence, the use of molecular markers was necessary for their discrimination (Peterson, 2008).

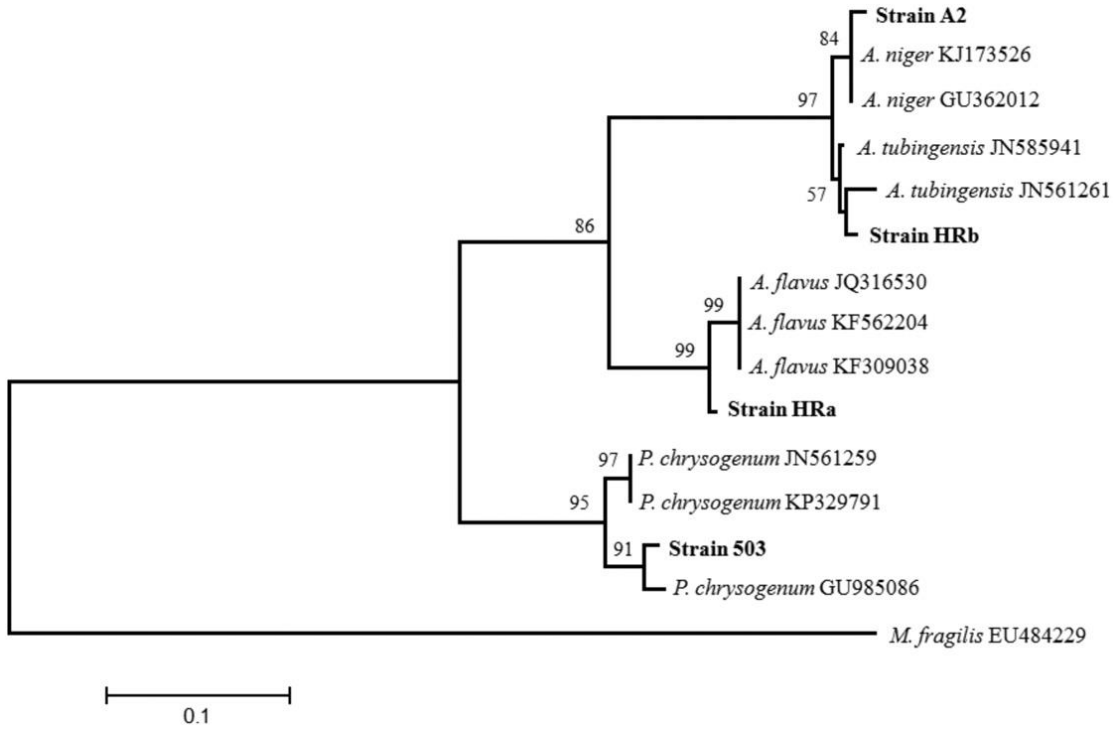


Fig. 15. Phylogenetic relationships among fungal strains of the genera *Aspergillus* and *Penicillium* isolated from the inner part of wooden artifacts as inferred by using the maximum likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (−3274.0695) is shown. Sequences derived in the context of the present study are marked in bold, whereas other highly similar sequences were obtained from GenBank. The percentages of replicate trees in which fungal material clustered together in the bootstrap test (10,000 replicates) are shown next to the branches when values ≥50% (Felsenstein, 1985). *Mucorfragilis* was used as outgroup.

4.2 Change of color

4.2.1 Lightness (L* value)

It was clear from the data obtained (Appendix) that the lightness of the samples infected by the fungi isolated decreased in relation to those of the control samples, and also decreased with increasing incubation times. The highest reduction in lightness was obtained with *A. niger*, followed by *P. chrysogenum* and *A. flavus*. The data obtained from the incubated samples treated with chitosan showed resistance of the samples to fungal infection. The concentration of chitosan used was important in combatting the fungi studied and in improving lightness compared to infected samples that were not treated. The best results for lightness were obtained with the third concentration, followed by the second concentration and then the first. It should be mentioned that the capacity of chitosan for disinfection decreased with increase in incubation time.

It was clear from the data obtained (Appendix) that the degree of red color in the infected samples caused by the fungi being studied increased to a great extent compared to the control sample. The degree of red color (a* value) in the infected samples decreased with increasing incubation time until the 4th month of incubation. The highest increase in red color value was obtained with *P. chrysogenum*, followed by *A. niger* and then *A. flavus*. We also noticed that the red color of the treated samples decreased compared to samples that were only infected with fungi but not treated. The reduction in the red color of treated samples decreased with increasing concentration of chitosan. It should also be mentioned that the red color values of the samples that were both treated and infected with fungi increased with increasing incubation time at all concentrations used.

4.2.3 Hue: more yellow (b* value)

It was clear from the data obtained that the b* value of the infected samples corresponded to yellow, which increased with increasing incubation time. The highest increase in yellow color value was obtained with *P. chrysogenum*, followed by *A. niger* and then *A. flavus*. The yellow color of the samples treated with chitosan increased with all chitosan concentrations and with all incubation times, relative to the control samples. The yellow color values of samples treated with chitosan decreased compared to the samples that were only infected with fungi but not treated. The yellow color values of the treated samples decreased with increasing concentration of chitosan. The results

confirm that the yellow color values of the samples that were both treated and infected with fungi increased with increasing incubation time at all concentrations used.

4.2.4 Total color difference (ΔE)

It was clear from the data obtained that the total color difference in the infected samples increased with increasing incubation time. The total color difference in the treated samples decreased with increasing concentration of chitosan. Mold fungi cause discoloration of wood. Blanchette et al. (2004) mentioned that these fungi do not degrade lignified cell walls but use the contents of ray parenchyma cells to grow on the surfaces of wood and slightly into the wood. He also stated that staining fungi have pigmented mycelia that penetrate into the wood through the rays, bordered pits, and cell lumen.

Morrell and Smith (1988) observed that the graying of wood surfaces is almost exclusively the result of growth of dark-colored fungi. The fungi that colonize weathered wood surfaces can grow on most carbon-containing materials. Growth of molds occurs after spores germinate on the surface of wood; then hyphae ramify through the outer few millimeters of wood by penetrating the cell Lumina, bordered pits, and rays. The hyphae of fungi that colonize softwoods are most prominent in rays and resin ducts, where they grow by metabolizing sugars, starch, and resin acids. The walls of the ray parenchyma and epithelial cells surrounding resin ducts are often destroyed, leaving elongated, open channels that increase the permeability of the affected wood. This effect may contribute to pronounced fluctuations in the surface moisture content of wood.

4.3 FTIR analysis

It was clear from the data obtained (Figs. 16-18) that the band at approximately 3,572 cm^{-1} (control sample) could be assigned to O=H stretching vibration, which gives considerable information about the hydrogen bonds. Relative to the control samples and for all incubation times (1 month to 4 months), the peak characteristics of hydrogen bonds from the spectra of amorphous cellulose became sharper and of lower intensity, and the peak shifted to higher wavenumber values for all samples treated at different chitosan concentrations and it applied to samples incubated with any of the three fungi studied.

Compared to the control samples, the wave number of the infected samples shifted to lower values with *A. flavus*, and to higher values with *A. niger* and *P.chrysogenum*. Ciolacuet al. (2011) stated that the broad band in the 3,600–3,100 cm^{-1} region, is due to OH-stretching vibration, gives considerable information about the hydrogen bonds. The peak characteristics of hydrogen bonds from the spectra of amorphous cellulose became sharper and of lower intensity compared to the initial cellulose samples. Shang et al. (2013) stated that the FTIR spectra of wood samples with fungal decay show large changes in peak intensity and peak position compared to corresponding samples without fungal decay.

For all the samples studied and for all incubation times, the band at approximately 2,941 cm^{-1} was assigned to the C=H stretching vibration. This peak also showed the presence of amorphous cellulosic samples. For all the fungi studied and for all incubation times, the peak intensity from the incubated samples treated with chitosan decreased relative to those from the control samples and the infected samples. Bugheanu et al. (2010) stated that the cellulose components are susceptible to microorganisms. Free cellulosic components are a favorite substrate for microorganisms, and a decrease in the corresponding peak intensity is always assigned to this phenomenon.

Lojewska et al. (2005) found that the intensity of the band at around2,900 cm^{-1}comes from the C=H stretching vibrations, which could change depending on the conditions used. In this study, the amount of amorphous cellulose increased with increasing concentration of chitosan.

The bands at 1,659 cm^{-1} for the control samples and 1,685 cm^{-1} for the infected samples were assigned to C-C bonds. The intensity of this peak from the infected samples increased compared to the peak from the control samples. It also increased with increasing incubation time. The intensity of the band increased because of the appearance of C-C bonds, which indicated that there was a decomposition process. For incubated, treated samples, the intensities of the peak decreased relative to the control samples, and this also indicated the potency of chitosan to protect against infection by the fungi studied. Bugheanu et al. (2010) reported that changes in the band at approximately 1,650 cm^{-1} are due to the different moisture levels in the samples. The source of moisture in the samples studied may have been from the wood samples and chitosan, which was dissolved in 2% acetic acid.

The FTIR absorption band at approximately 1,434 cm^{-1} for the control samples, 1,428 cm^{-1} for infected samples, and 1,424 cm^{-1} for most of the incubated, treated samples was assigned to an asymmetricCH2bending vibration. This band is known as the "crystallinity band", meaning that a decrease in its intensity reflects a reduction in the degree of crystallinity of the samples. We noticed that the intensity of the band at 1,428 cm^{-1} for most of the incubated samples treated with chitosan increased more than for the control samples and infected samples. This band reflects the increase in amorphous cellulose.

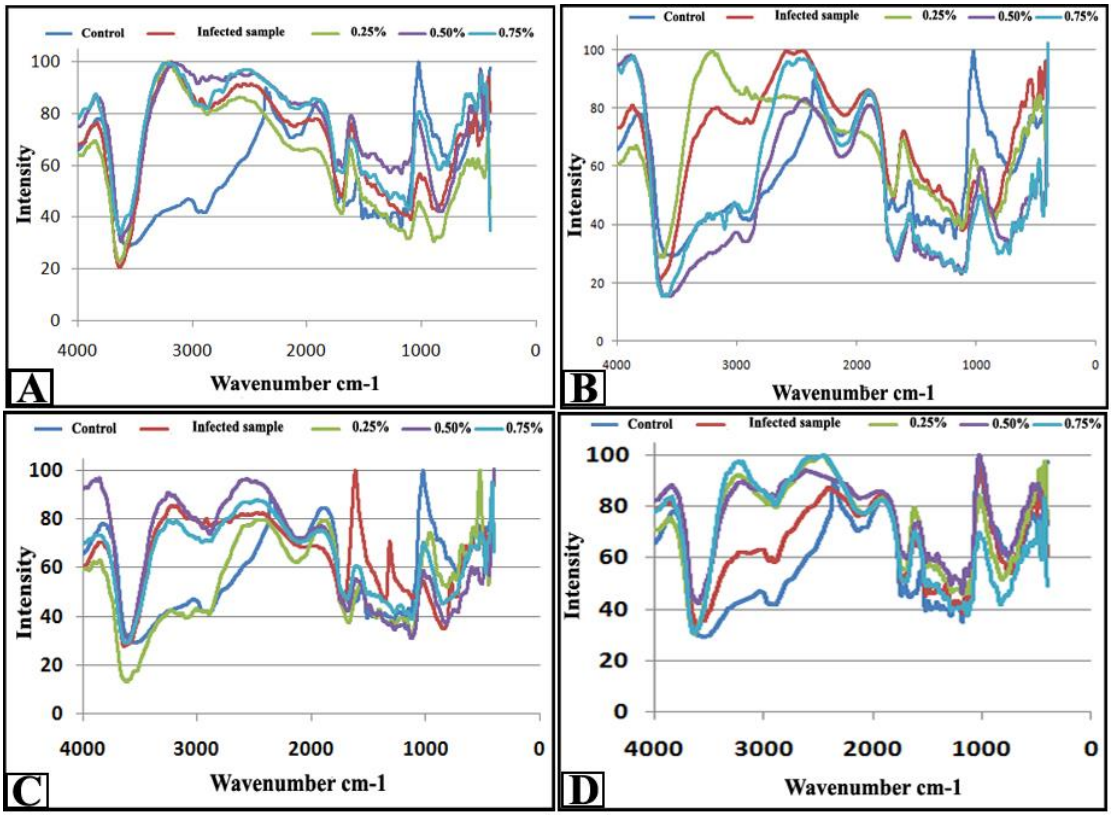


Fig. 16: FTIR of wood samples treated with chitosan at different concentrations and infected with *Aspergillus niger* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

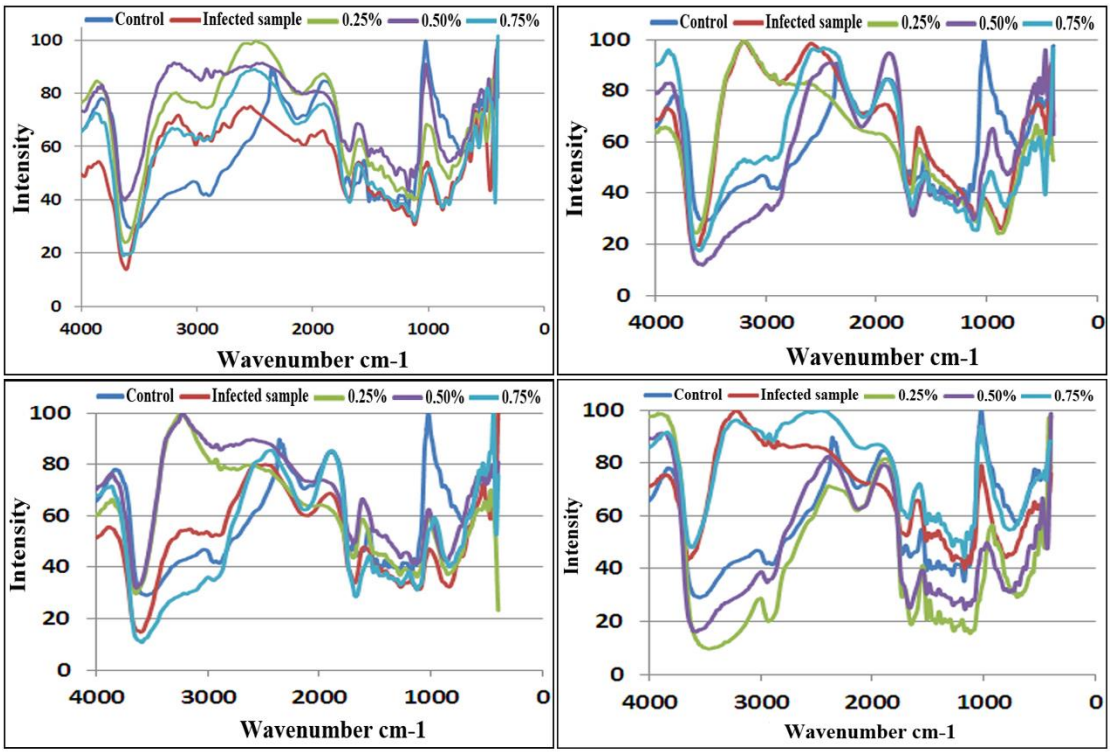


Fig. 17: FTIR of wood samples treated with chitosan at different concentrations and infected with *Aspergillus flavus* at for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

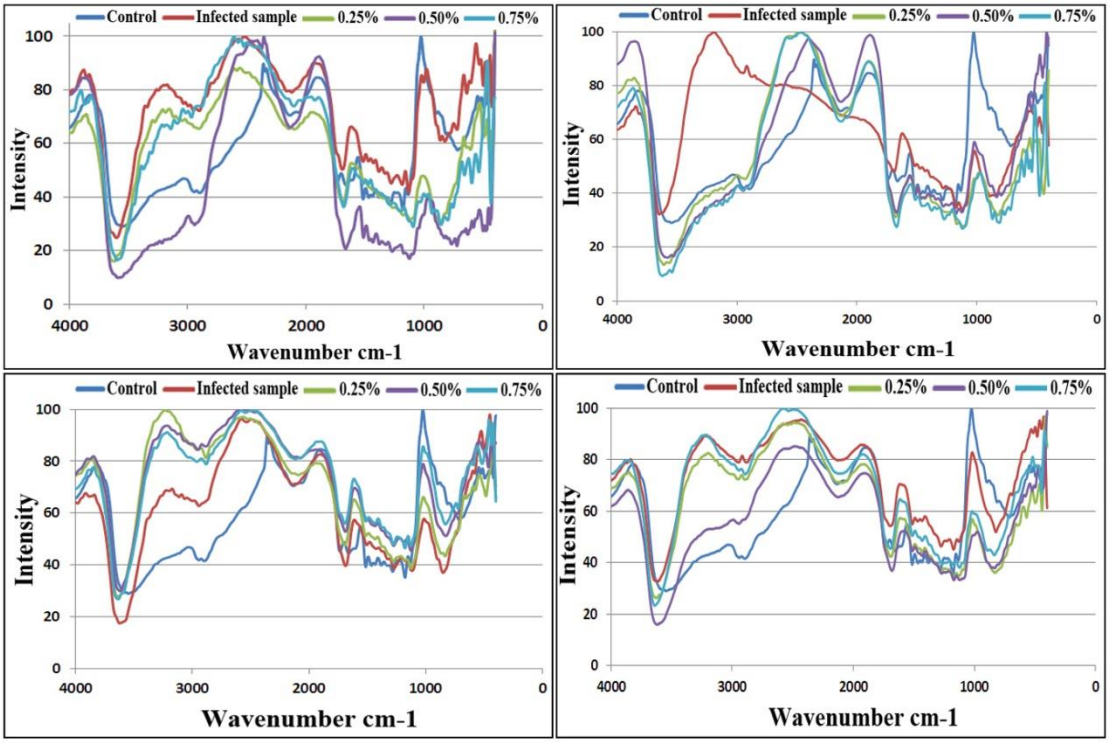


Fig. 18: FTIR of wood samples treated with chitosan at different concentrations and infected with *Penicillium chrysogenum* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

4.4 XRD analysis of wood treated with chitosan and infected with different fungi

Poletto (2012) explained that there are several ways for the cellulose chains to crystallize. The degree of crystallinity of native cellulose is source-dependent; the crystallinity of wood cellulose is typically around 60%. There are six known crystal polymorphs of cellulose: I, II, III, IV, V, and VI. In nature, polymerization and crystallization of cellulose occurs at the same time and its native crystalline form with parallel chains is called cellulose I. This study focuses on cellulose I.

4.4.1 Peak position (2θ degrees)

It was clear from the data obtained (Figs. 19-21) that the positions of the(101), (10Í), and (002) peaks of the control sample were at 14.4, 16.4,and 22.58 (2θ), respectively. The positions of these peaks after infection with any of the fungi studied shifted to a lower value for all incubation times (from 1 to 4 months).The values for the positions of the (101),(10 Í), and (002) peaks increased after treatment with chitosan (at all concentrations) and infection with any of the fungi studied, relative to the control samples. We also noticed that the increase in the peak positions (101), (10Í), and (002) of the treated and infected samples increased with increasing concentration of chitosan.

4.4.2 Peak width

The peak width at half maximum is a measurement of the degree of crystallinity of a material. It was clear from the data obtained (Figs. 19-21) that the peak widths for cellulose I ((101), (10Ī), and (002)) of the control sample were 0.09, 0.08, and 0.4 mm, respectively. The peak widths for samples infected with the fungi studied increased relative to the control sample. The increase in the peak width of infected samples increased with increasing incubation times (from 2 to4 months). The peak widths of the samples that were treated and infected with fungi increased relative to the control and infected samples that had not been treated. The increasing in the peak widths of the treated and infected samples increased with increasing concentration of chitosan. It can be said that the samples that were treated and infected with any of the fungi led to an increase in the peak width, which may have been due to the water used as a solvent for chitosan. The increase in peak width became reduced after three and four months of incubation, perhaps due to reduction in moisture content of the wood samples.

4.4.3 Peak intensity

From the data obtained (Figs. 19-21), the crystallinity of the control samples was 3.6 by the first method and 51.4 by the second method. This value decreased after infection with the fungi under study. The decrease in intensity increased with increasing incubation times, and this may have been due to the fact that mold fungi cause microstructural changes in cellulose and reduce the crystallinity values (in those cases where lignin has already been degraded by other fungi).

The crystallinity index of the samples that were treated at the first chitosan concentration (0.25%)and infected with fungi was higher than that of the control and infected samples.

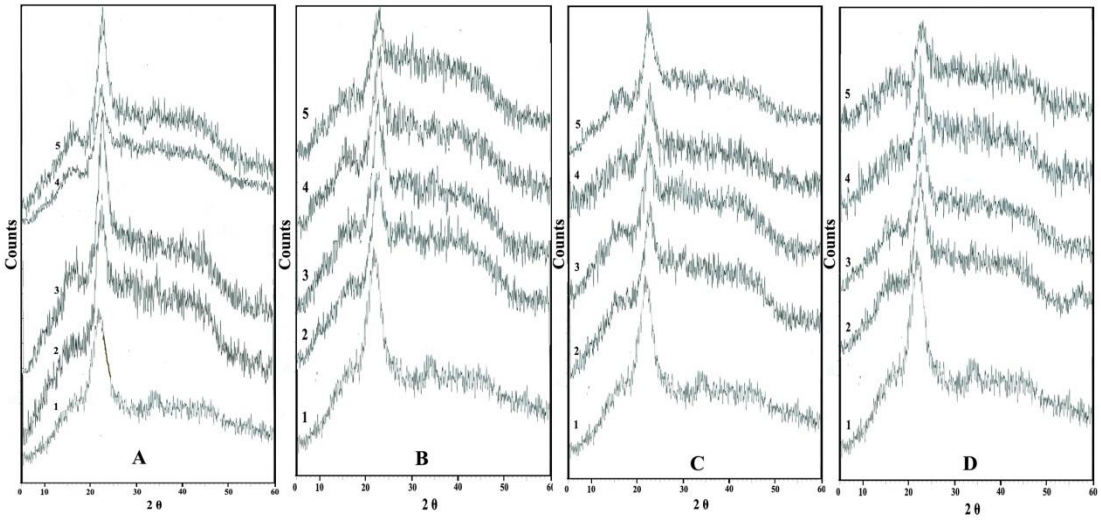


Fig. 19: X-ray diffraction patterns of wood samples treated with chitosan at different concentrations and infected with *Aspergillus niger* (1: control sample; 2: infected sample; 3: treated with 0.25% chitosan and infected; 4: treated with0.50% chitosan and infected; 5: treated with 0.75% chitosan and infected) for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

The crystallinity index by the first and second methods decreased after 2 months and started to increase again after three and four months. We noticed (from measurement of peak intensity) that it decreased with increasing concentration of chitosan. Goh et al. (2012) showed that the crystalline phase of cellulose with no microbial infection has a higher intensity than cellulose that has been infected.

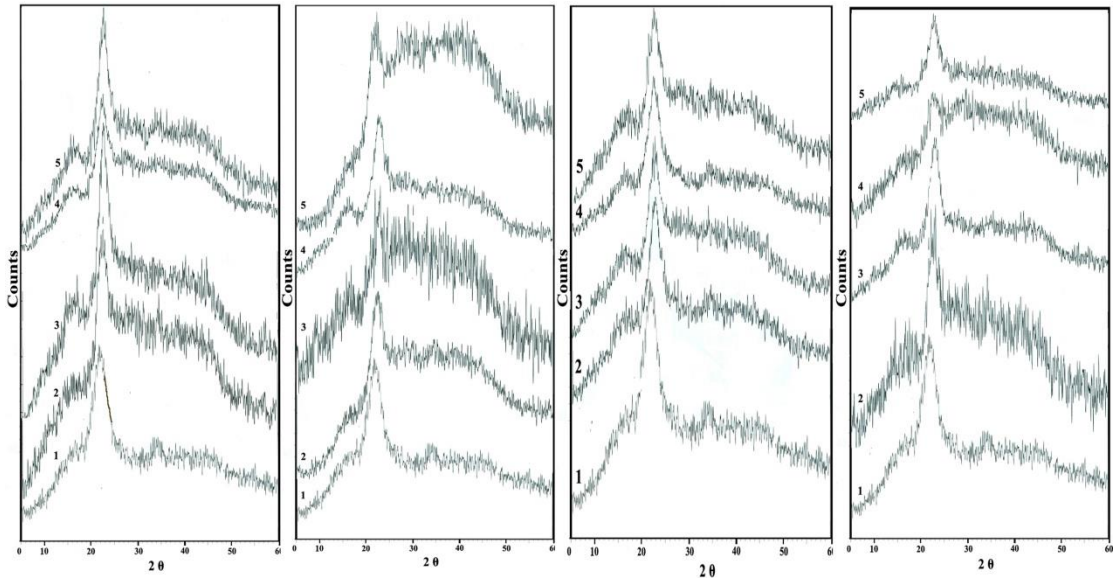


Fig. 20: X-ray diffraction patterns of wood samples treated with chitosan at different concentrations and infected with *Aspergillus flavus* (1: control sample; 2: infected sample; 3: treated with 0.25% chitosan and infected; 4: treated with 0.50% chitosan and infected; 5: treated with 0.75% chitosan and infected) for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

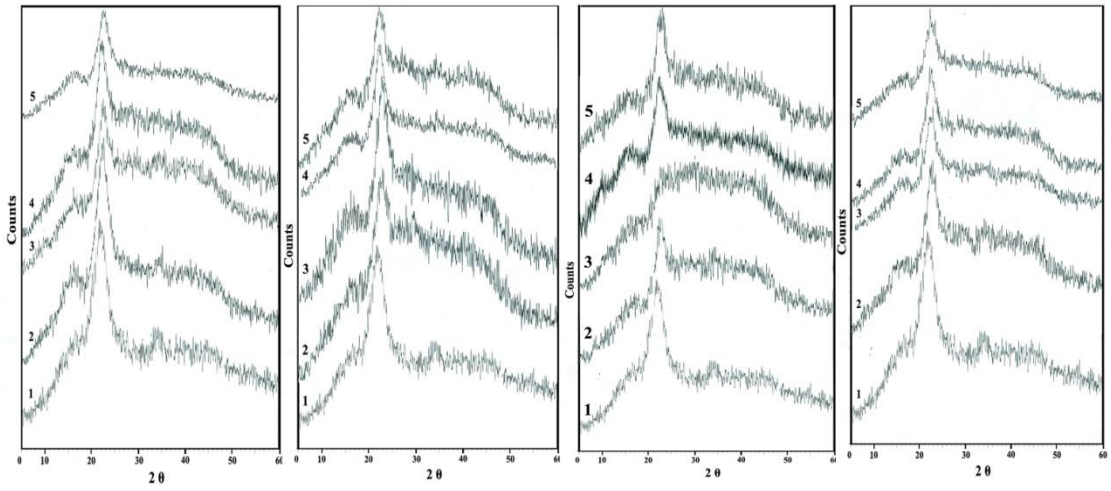


Fig. 21: X-ray diffraction patterns of wood samples treated with chitosan at different concentrations and infected with *Penicillium chrysogenum* (1: control sample, 2: infected sample, 3: treated with 0.25% chitosan and infected; 4: treated with 0.50% chitosan and infected; 5: treated with 0.75% chitosan and infected) for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

Chapter Five

Results and discussion of the fungicide propiconazole

Chapter Five: Results and discussion of the fungicide propiconazole

5.1 Measurement of color changes by spectrophotometry

5.1.1 Calibration of spectrophotometer

The method of calibration is based on ASTM 275-01 and ASTM 925-02. The UV-VIS spectrophotometer was calibrated for wave length and photometric accuracy over the wavelength range 200–800 nm.

5.1.1.1 Wavelength calibration

The peaks from the standard Holmium filter glass and deuterium filter glass were compared with the readings given by the instrument over the UV and visible range(200 nm to 1,100 nm). The results are given in Table 1.

Table 1: Wavelength calibration of double-beam spectrophotometer

Filter type	Certified peak position	Measured peak position	Wavelength error (measured− certified)
RM-HG 9115	333.80	334.0	0.20
	360.78	361.78	1.00
	445.81	445.70	−0.11
	453.63	453.47	−0.16
	460.16	460.03	−0.13
	536.62	536.44	−0.18
RM-DG 9114	513.42	513.19	−0.23
	573.19	572.72	−0.47
	684.54	684.38	−0.16
	740.82	739.15	−1.67
	806.97	807.06	0.09
	879.15	879.45	0.30

5.1.1.2 Photometric calibration

Photometric calibration (Table 2) for the UV-VIS spectrophotometer, where the absorbance measurements were performed by multiple readings of a standard neutral density glass filter of nominal absorbance, temperature was 23.5 ^{o}C ± 1 ^{o}C and the humidity was42% ± 5%.

Table 2: Photometric calibration of double-beam spectrophotometer

Wavelength (nm)	Filter no.	Nominal filter absorbance (AU)	Certified filter absorbance	Average measure of absorbance	Error (measured−certified)
440	14438	0.04	0.0406	0.055	0.0144
465			0.0402	0.054	0.0138
546			0.039	0.049	0.0100
590			0.0387	0.048	0.0093
635			0.0384	0.046	0.0076
440	13427	0.1	0.1497	0.15	0.0003
465			0.1356	0.136	0.0004
546			0.1357	0.135	−0.0007
590			0.1435	0.142	−0.0015
635			0.1450	0.393	0.0248
440	13898	0.3	0.3403	0.338	−0.0023
465			0.3028	0.301	−0.0018
546			0.3050	0.303	−0.0020
590			0.3263	0.324	−0.0023
635			0.3305	0.328	−0.0025
440	13724	0.5	0.5525	0.553	0.0005
465			0.5021	0.503	0.0009
546			0.5162	0.517	0.0008
590			0.5452	0.545	−0.0002
635			0.5297	0. 530	0.0003
440		1.0	1.0612	1.056	−0.0052
465			0.9785	0.974	−0.0045

546	13693		0.9926	0.990	−0.0026
590			1.0359	1.033	−0.0029
635			0.9887	0.986	−0.0027
440	13852	2.0	2.1371	2.137	0.0000
465			1.9885	1.987	−0.002
546			2.0092	2.001	−0.008
590			2.0487	2.039	−0.010
635			1.9381	1.929	−0.010

Tables 1 and 2 show the systematic error when the measured value was compared with the nominal values from a standard neutral glass filter. To obtain accurate results, the error found in these tables was used for the reading of wood samples in order to cancel the systematic error.

To calibrate the spectrophotometer for measurement of reflectance, the regularly used standard white ceramic tile that was supplied with the spectrophotometer, which was measured against a calibrated spectrophotometer traceable to NIST –USA for reflectance measurements (Fig. 22). Reflectance measurements are very important for color determination according to the CIE Lab color system, and, in turn, for determination of the whiteness and yellowness indices.

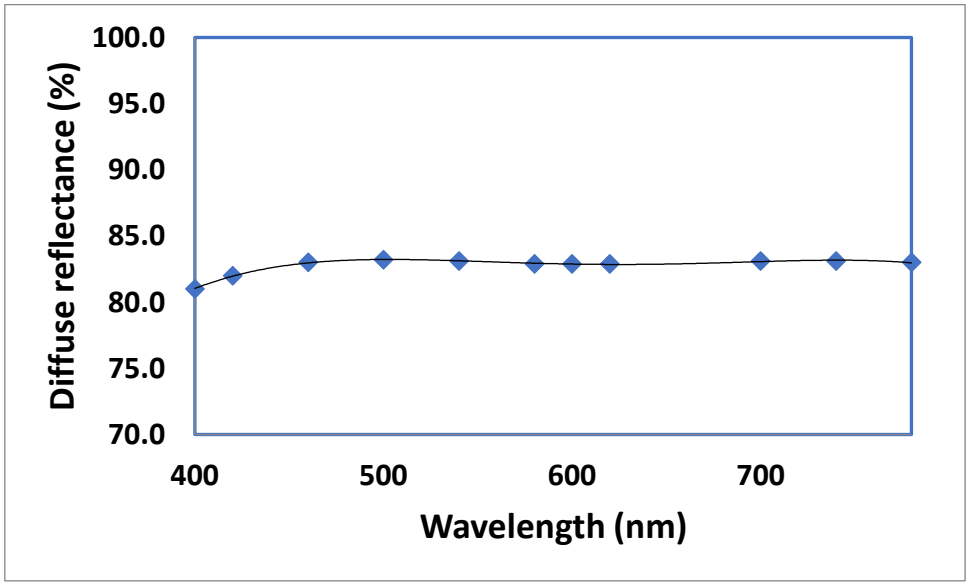


Fig. 22: Calibration of the standard white tile that was supplied with the spectrophotometer, which is used for reflectance measurements.

The results (Fig. 22) showed that there was deviation from 100% reflection at all the visible wavelengths studied (400–780 nm). These errors were excluded from the actual measured values before calculating the CIE Lab parameters in addition to the whiteness and yellowness of wood samples.

5.1.1.3 Uncertainty of color measurements using the standard white tile

To estimate the uncertainty of the whiteness and yellowness of our measurements in this research, we focused on the standard white tile and we classified our uncertainty parameters in two main categories, namely:

1. Uncertainty of repeatability (uncertainty type A), which represents the statistical analysis of measurements (precision).

$$UA=\text{standard deviation} \div \sqrt{\text{number of measurements}} \quad \text{(Equation 1)}$$

2. The budget of all possible sources of error (uncertainty type B), systematic and random errors (Table 4), that were produced from the instrument's calibration, resolution, drift, and uncertainty type A.

$$UB=\sqrt{U_A^2 + U_{cal1}^2 + U_{drift1}^2 + U_{drift2}^2 + U_{cal2}^2 + U_{resolution}^2 + U_{cal}^2} \quad \text{(Equation 2)}$$

where:

U_A = uncertainty of repeatability,

U_{cal1} = uncertainty of calibration of spectrophotometer,

U_{cal2} = uncertainty of calibration for white tile,

U_{drift} = the drift raised from the maximum error of the spectrophotometer in two successive years (2016 and 2017) (subscript1 means spectrophotometer and subscript2 means white tile),

$U_{resolution}$ (U_{res})= half of the lowest reading of the spectrophotometer.

In this research, we focused on the diffuse reflection of the white tile. The measurements were repeated five times and the readings were as follows (Table 3):

Table 3: Uncertainty type A for the whiteness of the standard white tile

S	Diffuse reflection	Average	Variance	Standard deviation	U_A	% U_A(of average)
1	82.9	83.0	0.003	0.106	0.0475	0.057
2	82.9					
3	82.9					

4	83.0					
5	83.0					

Table 4: Uncertainty budget U_B of diffuse reflection measurement of the white tile

Symbol	Value	%	Divisor	Correlation coefficient	Result
U_A	0.0475	0.0570	1	1	0.057
U_{cal1}	0.006	0.2808	2	1	0.14
U_{cal2}	1.000	1.2063	2	1	0.603
U_{res1}	5E-05	0.0023	1.7321	1	0.001
U_{drift1}	0.1	0.0010	1.7321	1	6E-04
U_{drift2}	1	0.0100	1.7321	1	0.006

By applying equation 2, we obtained the result U_B= 0.6528 and to expand the uncertainty value to obtain a confidence level of 95%, we used the coverage factor (2 as the standard Gaussian distribution standard curve).

U_{Exp} = 1.3056 (K=2 for 95% confidence level)

Contribution values as shown in Figure (23). We found that the most predominant source of uncertainty was the uncertainty of calibration of both the spectrophotometer and the standard white tile. On the other hand, the resolution of the spectrophotometer had values that could be neglected as a source of error when compared to the other contributions.

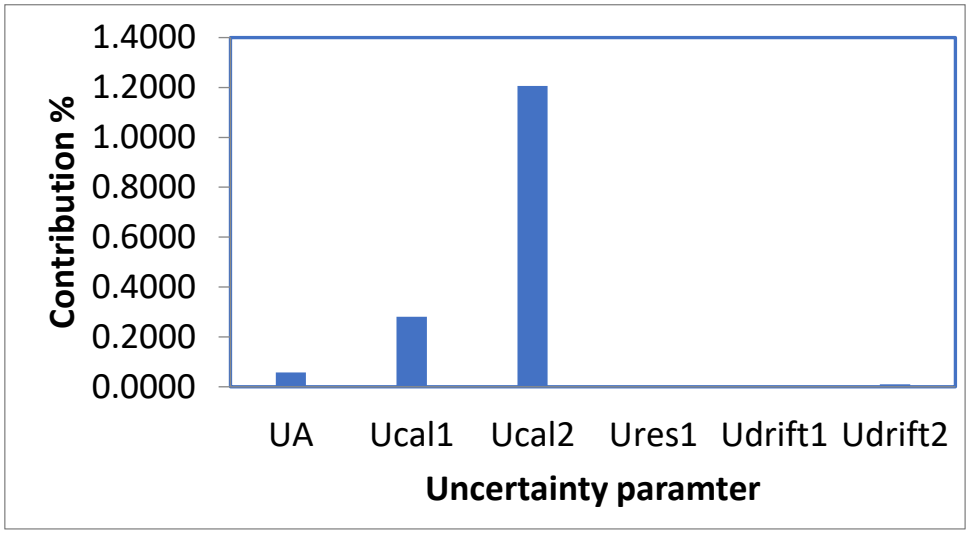


Fig. 23: Uncertainty parameters

From these uncertainty studies, we can reduce the uncertainty of the whiteness measurements by paying more attention to the following points:

- calibrate the instrument used and apply the correction of reading whenever needed;
- make all measurements traceable to the CIE color measurement system;
- use the instrument with the lowest uncertainty values (Vik, 2017).

5.1.2 Effect of fungicide treatment on whiteness and yellowness indices

Most fungi feeding on the dead or decayed parts of wood do not ingest their food, but they absorb it. The hyphae secrete enzymes to break down the wood material and make it suitable for absorption. Fungi grow on the surface and within the substrate itself. This activity of fungi changes all the chemical, physical, and mechanical properties of the wooden substrate.

Fungi are organisms that live on organic materials such as wood. The mycelium grows on the surface or within the substrate. The hyphae obtain nutrients by osmosis through the hyphal walls, causing the disintegration of the organic matter they utilize. Fungi secrete enzymes to break down carbohydrates to simple sugars. The organic substrate is broken down into the necessary nutrients, which are absorbed through the hyphae walls. Any amount of fungal growth for any period of time will decompose the substrate on which it feeds, however, but to cellulose is generally observed only after an extended period of growth. The shorter the period of exposure, the less the damage (http://cool.conservation-us.org/coolaic/sg/bpg/pcc/12_mold-fungi.pdf, signed June 27, 2017).

Fungi can cause stains on the surface of wood at an advanced state of degradation. These stains may be caused by metabolic processes, such as acids being produced during the hydrolysis of the cellulose or other nutrient matter; chemicals being produced during the digestive process, and excreted by-products; or simply from pigments being present in the fungal structure itself. Certain molds are known to produce pigments—and may cause extensive color changes in the substrate, even though their growth is limited. It has been noted that some *Penicillium sp.* produce yellow stains in some cases and pink stains in others (http://cool.conservation-us.org/coolaic/sg/bpg/pcc/12_mold-fungi.pdf, signed June 27, 2017).

Figure 24 shows interaction between ageing time, the action of the fungicide propiconazole at two concentrations (0.25 and 0.50% w/v), and infection by three different fungi, namely *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* (with an ageing time of up to 4 months). The data obtained for *Aspergillus niger* (Fig. 24A) revealed that most of the samples had a marked whiteness increment up to the second month, with no remarkable changes for further periods of ageing. For yellowness (Fig. 24B),the infected wood sample that was treated with a low concentration of propiconazole (0.25%) had a higher degree of yellowness than the other samples, but a slight decrease in yellowness was noted beyond 3 months of ageing.

The data for *Aspergillus flavus* (Fig. 24C) showed that the whiteness index of the samples did not change significantly due to treatment with the two different concentrations of propiconazole. The only marked change was for the sample treated with 0.25% propiconazole from the first to the second month. Regarding yellowness (Fig. 24D), the data showed the same trend as in Fig.24B.

It was clear from the data obtained with *Penicillium chrysogenum* (Fig. 24E and F) that there was a remarkable change in whiteness in the control sample and sample treated with 0.25% propiconazole, from first to the second month. It can be added that the whiteness of the infected samples changed up to 4 months. For yellowness index (Fig. 24F), the data showed the same trend as seen in Fig. 24B and D.

Sandoval et al. (2010) attempted to explain the color changes seen in wood when infected by microorganisms. They reported that microorganisms can cause discoloration of wood. Fungal attacks reduce the quality of wood, since they modify the color. Blue staining is caused by microscopic fungi that commonly infect only sapwood, using sapwood compounds such as simple sugars and starch. As the fungi grow, the fungal hyphae suppress water transportation in the host, causing discoloration of the wood. They cannot grow in heartwood, and in most wet woods that do not contain the necessary food substances. Blue-stain fungi are prone to cause bluish or grayish discoloration of wood, but they do not lead to decay.

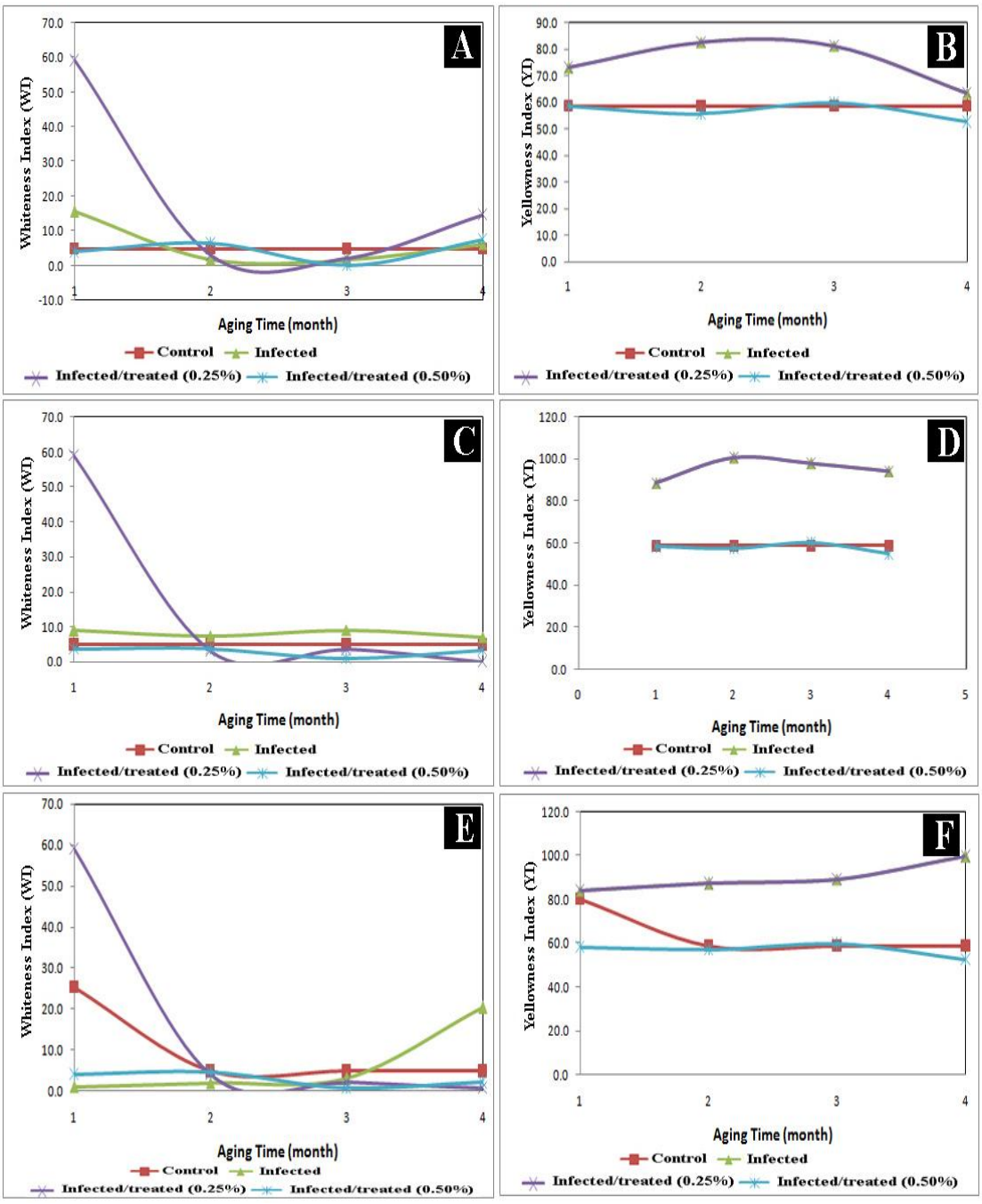


Fig. 24: Effect of treatment of wood samples (that have been infected with different fungi) with propiconazole on the whiteness and yellowness indices. A and B: *Aspergillus niger*; C and D: *Aspergillus flavus*; E and F: *Penicillium chrysogenum*.

5.2 FTIR analysis

5.2.1 Propiconazole with *Aspergillus flavus*

The results obtained from propiconazole at the concentrations used on *Aspergillus flavus* were as follows (Fig. 25):

The band at 3,571.5 cm^{-1} to 3,647.7 was assigned to O-H stretching in cellulose, hemicellulose, and lignin. It was clear that all the frequencies of these bands were shifted to higher wavenumbers with the increase in the intensities compared to the control sample, indicating breakdown of some hydrogen bonds and formation of more hydroxyl groups. This probably resulted from a hydrolysis effect. We also noticed broadening of these bands with a decrease in frequency after treatment relative to infected samples, especially after 3 and 4 months. **At 0.50%** propiconazole, the frequencies of this band (3,571.5 $cm^{-}1$) gradually shifted with increasing incubation period to higher values compared to the control sample. This can be explained by *Aspergillus flavus* secreting cellulase enzymes that broke down hydrogen bonds at early stages of infection. However, with fungicide-treated wood we found that the frequencies decreased compared to the infected samples. It can be said that propiconazole could reduce the secretion of enzymes or inhibit their activities. Edwards (2006) said that Propiconazole is a fungicide. It inhibits an enzyme involved in ergosterol biosynthesis, which is critical to the formation of the cell walls in fungi, thereby slowing or stopping fungal growth.

The band at 1,739.5 cm^{-1} was assigned to unconjugated C=O stretching in ester groups of hemicellulose. We noticed the disappearance of the band in infected and treated samples at the propiconazole concentrations used, except for the sample treated at 0.50% after 4 months of infection. This indicated that both infection by *Aspergillus flavus* and treatment with propiconazole strongly affected hemicelluloses in wood and hydrolyzed its ester groups to acidic ones.

The band at 1,645.9 cm^{-1} to 1,706.7 $cm^{-1}$was assigned to a**dsorbed O-H and C=O conjugated stretching of cellulose.** A new broad band centered around 1,700 cm^{-1} for 0.25% propiconazole and 1,706 $cm^{-1}$for 0.50% propiconazole appeared after fungal infection. This band is assigned to acidic carbonyl groups resulting from hydrolysis of the ester groups of hemicellulose. Broadening of this band may result

from O-H bending of absorbed water together with C=O conjugated stretching of some cellulose oxidation.

The band at 1,511.9 cm^{-1} for both 0.25% and 0.50% propiconazole was assigned to **aromatic skeletal vibration in lignin**. We noticed that this band disappeared or decreased in intensity after fungal infection, indicating decomposition of the aromatic skeleton of lignin. On the other hand, the increase in intensity after treatment with fungicide reflected the good effect of propiconazole on lignin content.

The bands from 1,465.6 cm^{-1} to 1,380.7 cm^{-1}were/are assigned to **plane deformation of C-H in carbohydrates and lignin**. These bands showed a decrease in or absence of absorption at these wave numbers, showing that advanced decay had occurred in cellulose as well as lignin and indicating that a depolymerization process had occurred. Treatment protected cellulose and lignin from decomposition.

The bands from 1,284.4 cm^{-1} to 1,164.8 cm^{-1} were assigned to a**symmetric C-O-C vibration in carbohydrates.** The band around 1,275 $cm^{-1}$is assigned to syringyl ring and C-O stretching in xylan and hemicellulose. The decrease in intensity of this band in the infected wood after 3 and 4 months suggested a decrease in lignin and adjacent hemicelluloses in the ultrastructure of the wood. Treatment with 0.25% and 0.50%propiconazoleprotected wood from such decay. Bands in the1,164–1,183 cm^{-1}range are related to C-O-C stretching in cellulose and hemicelluloses. Disappearance of bands around 1,175 cm^{-1} after treatment indicated advanced breaking of cellulose chains and showed that depolymerization had occurred. Appearance of the bands in infected and treated wood after 4 months may have been due to crosslinking reactions.

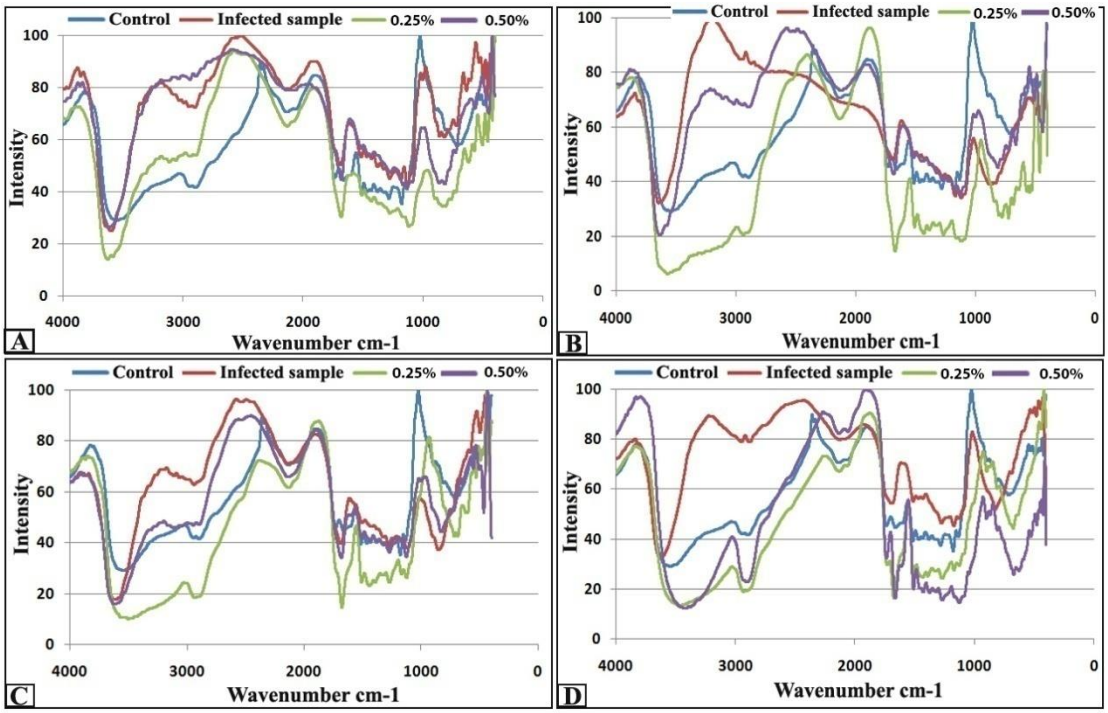


Fig.25: FTIR of wood samples treated with propiconazole at different concentrations and infected with *Aspergillus flavus* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and (D) after 4 months.

5.2.2 Propiconazole with *Aspergillus niger*

The results obtained with propiconazole at the concentrations used on *Aspergillus niger* was (Fig. 26) as follows:

The band at 3,571.5 cm^{-1} to 3,647.7was assigned to **O-H stretching in cellulose, hemicellulose, and lignin**. With0.25% and 0.50% propiconazole, all the frequencies of this band for the infected wood sample were shifted to higher values compared with that of the control sample. This was due to cleavage of some hydrogen bonds. After 3 and 4 months, wood samples treated with 0.25% propiconazole and all samples treated with 0.50% propiconazole showed a decrease in wave number, which was accompanied by increased intensities, indicating the presence of carboxylic acid and hydroxyl groups. This can be explained by *Aspergillus niger* performing some oxidation reactions to form the alcohol monolog of propiconazole, which was further oxidized to form the carboxylic acid analog of propiconazole. These compounds are the primary oxidation products of the methyl groups on the tertiary butyl moiety.

The band at 1,739.5 cm^{-1} was assigned to **unconjugated C=O stretching in ester groups of hemicellulose**. The results with 0.50% propiconazole showed that in the infected samples, the intensity of this band decreased compared with that of control

sample. However, in the treated samples after 1 and 2 months, the intensities slightly increased compared to those of the corresponding infected samples, indicating the low effect of the fungicide. On the other hand, a sharp increase in intensity with a shift to lower wavenumber was found in the samples that were treated for 3 and 4 months. At 0.50% propiconazole, infection with *Apergillus niger* had a clear role in hemicellulose hydrolysis. This was confirmed by a decrease in the band from ester groups at 1,739.5 $cm^{-1}$and the appearance of a new one around 1,700 cm^{-1}, which was assignable to acidic carbonyl groups. The results also showed the good effect of treatment with 0.5% propiconazole on hemicellulose content.

The bands at 1,664.3.9 cm^{-1}, 1,647.5 cm^{-1},and 1,706.7 cm^{-1} were assigned to **adsorbed O-H and C=O conjugated stretching in cellulose.** A new broad band centered around 1,707 cm^{-1} appeared after fungal infection and treatment with propiconazole for 1 and 2 months. This band was assigned to acidic carbonyl groups resulting from hydrolysis of some ester groups of hemicellulose.

The band at 1,511.9 cm^{-1} for both 0.25% and 0.50% propiconazole was/is assigned to **aromatic skeletal vibration in lignin**. The results at 0.25% showed that except for the samples treated for 3 and 4 months, all the intensities of these bands decreased compared to that of the control sample, indicating decomposition of the aromatic skeleton of lignin. At 0.50% propiconazole, a decrease in the intensities of these bands after fungal infection indicated decomposition of the aromatic skeleton of lignin. The results also showed that treatment had an inhibitory effect on the decomposition of lignin.

The bands at 1,468.5 cm^{-1}, 1,561.7 cm^{-1},and 1,381.7 cm^{-1}were/are assigned to **plane deformation of C-H in carbohydrates and lignin**. **At 0.25%** propiconazole, except for the samples treated for 3 and 4 months, all the intensities of these bands decreased compared to that of the control sample. **At 0.50%** propiconazole, the decrease or absence of absorptions at these wave numbers showed that advanced decay had occurred in both cellulose and lignin, which indicated that a depolymerization process had occurred.

The bands at 1,284.4 cm^{-1}, 1,176.4 cm^{-1},and 1,164.8 cm^{-1} were assigned to **asymmetric C-O-C vibration in carbohydrates.** At 0.25%propiconazole, except for the samples treated for 3 and 4 months, all the intensities of these bands for both infected and treated samples decreased compared to that of the control sample. The increase in the treated samples after 3 and 4 months may have resulted from the C-O

bonds of hydroxyl and carboxylic groups formed from fungal oxidation of propiconazole. At 0.50%propiconazole, the decrease in intensity of the band around 1,270 cm^{-1} in the infected wood suggested a decrease in lignin and adjacent hemicelluloses in the ultrastructure of the wood. Disappearance of the bands between 1,160 and 1,176 cm^{-1} or a decrease in their intensities indicated advanced breakage of cellulose chains and showed that depolymerization had occurred. Increase in the intensities of these bands after treatment reflected the good effect of 0.5% propiconazole.

5.2.3 Propiconazole with *Penicillium chrysogenum*

The results obtained with propiconazole at the concentrations used on *Penicillium chrysogenum* was (Fig. 27) as follows:

Theband at 3571.5 cm^{-1} to 3647.7 was assigned to **O-H stretchingin cellulose, hemicellulose, and lignin**. At 0.25% propiconazole, except for the samples treated for 3 and 4 months, all the frequencies of these bands were shifted to higher wavenumbers, with an increase in intensity compared to that of the control sample. This indicated breakdown of some hydrogen bonds and formation of more hydroxyl groups. This probably resulted from a fungal hydrolysis effect in infected samples and/or oxidation of propiconazole in treated samples. This reflected the weak effect of propiconazole. Broadening of this band in the samples treated for 3 and 4 months, with an increase in intensity and a decrease in frequency compared to corresponding infected samples, reflected the formation of more hydroxyl groups from formation of more hydrogen bonds as a result of propiconazole oxidation. At 0.50% propiconazole, except for the O-H stretching band of the sample treated for 4 months, little change was found in treated samples compared to corresponding infected samples. However, in the sample treated for 4 months, the band broadened and the frequency sharply decreased with increased intensity, as a result of propiconazole oxidation.

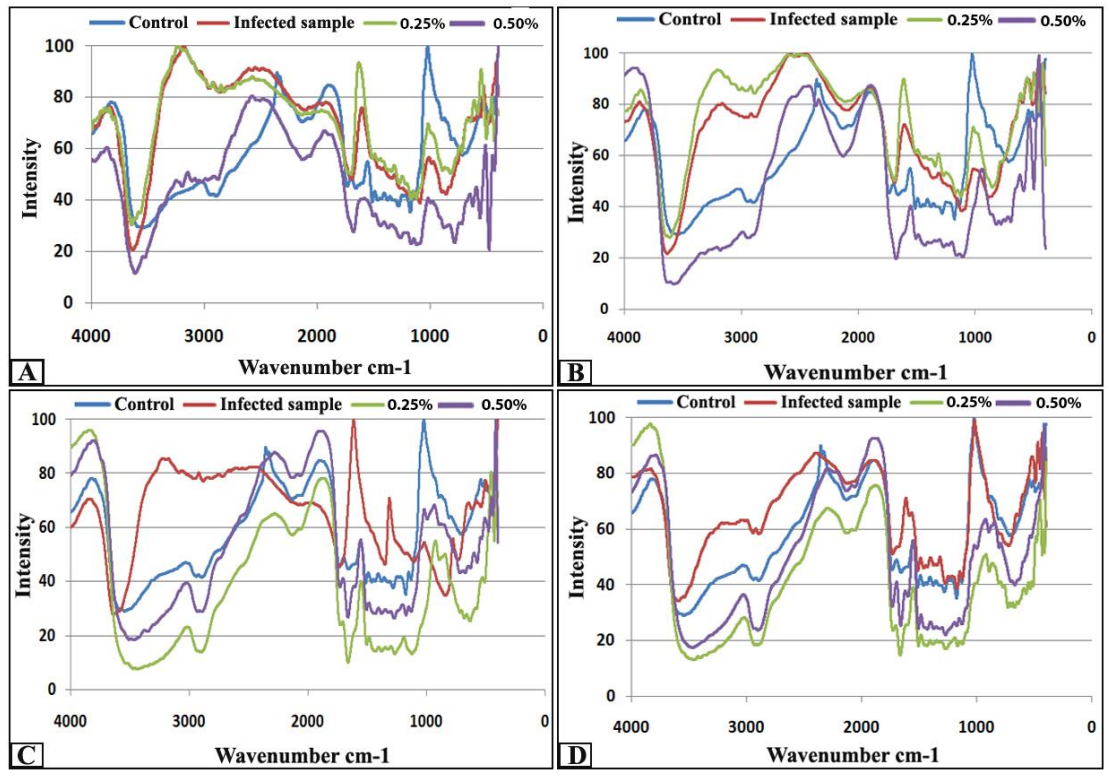


Fig.26: FTIR of wood samples treated with propiconazole at different concentrations and infected with *Aspergillus niger* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

The band at 1,739.5 cm^{-1} was assigned to u**nconjugated C=O stretching in ester groups of hemicellulose**. At 0.25%propiconazole, the intensity of this band increased after treatment compared to that after infection, indicating the good effect of 0.25% propiconazole on hemicellulose. At 0.50%propiconazole, except for the sample treated for 4 months, the intensities of this band in infected and treated samples were almost the same, and they were lower than that of the control, indicating the lack of effect of 0.5% propiconazole on hemicellulose.

The bands at 1,664.3 cm^{-1} and 1,647.5 cm^{-1} were assigned to a**dsorbed O-H and C=O conjugated stretching in cellulose.** At the concentrations used, we noticed that in all the infected and treated samples, new bands appeared around 1,680 cm^{-1}, which were assignable to carbonyl stretching in oxidized cellulose in addition to carbonyl groups resulting from propiconazole oxidation in treated samples.

The band at 1,511.9 cm^{-1} for both 0.25% and 0.50% propiconazole was assigned to a**romatic skeletal vibration in lignin**. At 0.25%propiconazole, we noticed

a decrease in the intensity of this band after fungal infection, indicating decomposition of the aromatic skeleton of lignin. On the other hand, the increase in intensity after treatment with fungicide reflected the good effect of 0.25% propiconazole on lignin content. At 0.50%, the intensity of this band decreased after fungal infection and after treatment. These results reflected the decomposition of the aromatic skeleton of lignin. It was also noticed that the increase in propiconazole concentration is not recommended for *Penicillium* treatment.

The bands at1,468.5 $cm^{-1}$and 1,381.7 cm^{-1} were/are assigned to **plane deformation of C-H in carbohydrates and lignin**. At 0.24% propiconazole, the decrease in absorption at these wave numbers in infected samples showed that advanced decay had occurred in both cellulose and lignin, indicating that a depolymerization process had occurred. Treatment with propiconazole protected cellulose and lignin from decomposition. At 0.50% propiconazole, except for the sample treated for 4 months, the intensities of this band in infected and treated samples were lower than that of the control, indicating the lack of effect of 0.5% propiconazole on wood components and that a depolymerization process had occurred.

The bands from 1,284.4 cm^{-1} to 1,176.4 cm^{-1} were assigned to a **symmetric C-O-C vibration in carbohydrates.** At 0.25% propiconazole, a decrease in band intensity around 1,275 cm^{-1} in the infected wood samples suggested a decrease in lignin and adjacent hemicelluloses in the ultrastructure of wood. A decrease in band intensity at1,165–1,186 cm^{-1} in infected samples indicated breakage of cellulose chains, i.e. That depolymerization had occurred. Treatment with 0.25% Propiconazole kept wood from such these decays. **At 0.50%** propiconazole, except for the sample treated for 4 months, the intensities of these bands for infected and treated samples were lower than that of the control, indicating the lack of effect of 0.5% propiconazole on wood components and that a depolymerization process had occurred.

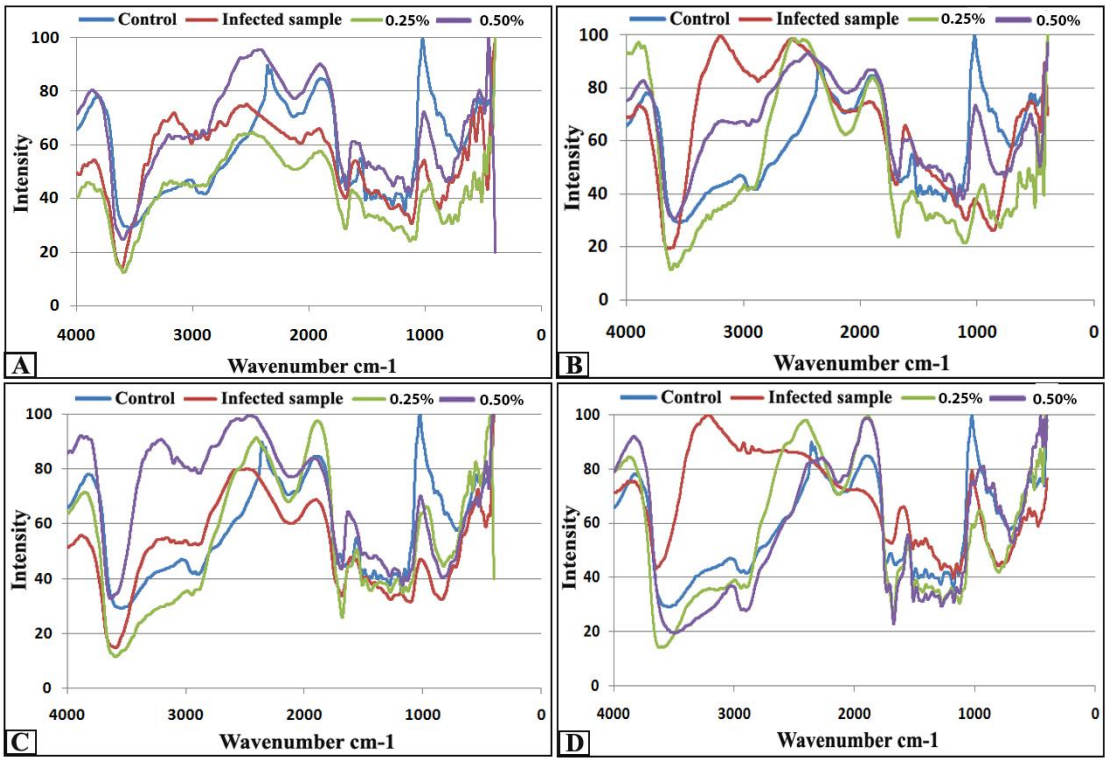


Fig.27: FTIR of wood samples treated with propiconazole treated at different concentrations and infected with *Penicillium chrysogenum* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and (D) after 4 months.

These results were confirmed by Karlsson et al. (2011) who used propiconazole as a fungicide with concentration 0.6%. They have used this material because of it good durability and has an effectiveness for the protection of wood against fungi.

5.3 X-ray diffraction for determination of the crystallinity of wood

5.3.1 Peak position (2 θ degrees)

It was clear from the data obtained (Figs. 28–30) that the positions of the peaks (10), (10Í), and (002) of the control sample were at 14.4 (2 θ), 16.4 (2 θ), and 22.58 (2 θ), respectively. The positions of these peaks shifted to lower values after infection with any of the fungi studied. The highest shift was obtained with *Aspergillus flavus*, followed by *Penicillium chrysogenum* and *Aspergillus niger* for peaks (101) and (10 Í), but for peak (002) the highest shift was obtained with *Aspergillus niger*, followed by *Aspergillus flavus* and *Penicillium chrysogenum*. We also noticed that the shift of the peaks to lower values decreased with increase in incubation times.

The data obtained (Figs. 28-30) showed that the peak positions of the infected samples treated with propiconazole at different concentrations increased with increasing concentration of fungicide used. The increase in peak positions decreased with increasing incubation times. The results also showed that the highest increase in peak position was obtained with *Aspergillus niger*, followed by *Aspergillus flavus* and *Penicillium chrysogenum* using the first and second concentrations of propiconazole.

5.3.2 Peak width

The peak width at half maximum is a measurement of the degree of crystallinity of a material. It was clear from the data obtained (Figs. 28-30) that the peak width of cellulose I (101, 10Ī, and 002) was 0.09, 0.08, and 0.4 mm respectively. The peak width of samples infected with *Aspergillus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* increased compared to the control sample. The peak width of cellulose I (101, 10Ī, and 002) in samples infected with any of the fungi studied increased compared to the control sample. The peak width increased with increasing incubation time. The increase in peak position was explained by Howell (2006), who described initial degradation of the amorphous hemicelluloses and widening of the crystallites by non-enzymatic mechanisms, such as through hydroxyl radicals. These non-enzymatic processes increase the amount of amorphous material present in the wood. The peak width of the samples that were treated with propiconazole (Figs.1-3) at the first and second concentrations and infected with any of the fungi studied increased after the first and second weeks and decreased after the third and fourth weeks of incubation. It was clear from the data obtained that the peak width increased in the samples infected with any of the fungi studied. It can be said that the fungicide-treated samples infected with any of the fungi led to an increase in the peak width, which may have been due to the solvent used with propiconazole.

5.3.3 Peak intensity

It was clear from the data obtained (Figs. 28-30) that the crystallinity of the control sample was 3.6 by the first method and 51.4 by the second method. This value decreased after infection with any of the fungi studied. The decrease in crystallinity increased with increasing incubation time. The crystallinity index of the sample infected with *Aspergillus niger* was 3.5, 3.44, 3.4, and 3.38 by the first method and 50.8, 50.2, 49.8, and 49.5 by the second method, respectively, after 1, 2, 3, and 4 months, respectively. The crystallinity index of the sample infected with *Aspergillus*

flavus was 3.53, 3.50, 3.47, and 3.52by the first method and 50.4, 50.0, 49.4, and 49.5by the second method, respectively, after 1, 2, 3, and 4 months. The crystallinity index of the sample infected with *Penicillium chrysogenum* was 3.50, 3.48, 3.51, and 3.54by the first method and 50.4, 50.6, 50.7, and 50.80by the second method, respectively, after 1, 2, 3, and 4 months. For the fungicide-treated, infected samples (Figs. 1–3), the crystallinity index increased compared to the control sample. The increase in the crystallinity index decreased with increasing incubation time.

Niemenmaa (2008) stated that fungi degrade amorphous cellulose more readily than crystalline cellulose regions. The remaining cellulose after decay showed an increase in crystallinity. Fungi were found to preferentially degrade polysaccharides in wood and to produce large amounts of ·OH. The study suggested that fungi produce extracellular ·OH as part of their wood-degrading system. It should be noted that wood decay only altered the degree of crystallinity, but not the lattice structure of cellulose.

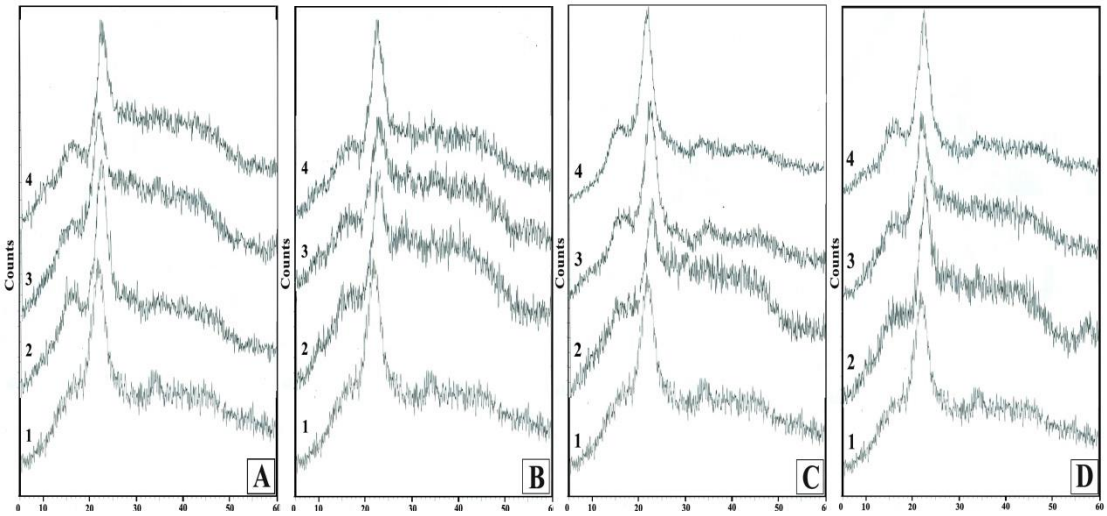


Fig.28: X-ray diffraction patterns of wood samples treated with propiconazole at different concentrations and infected with *Aspergillus niger* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and (D) after 4 months (1: control sample; 2: infected sample; 3: sample treated with 0.25% propiconazole and infected; 4: sample treated with 0.50% propiconazole and infected).

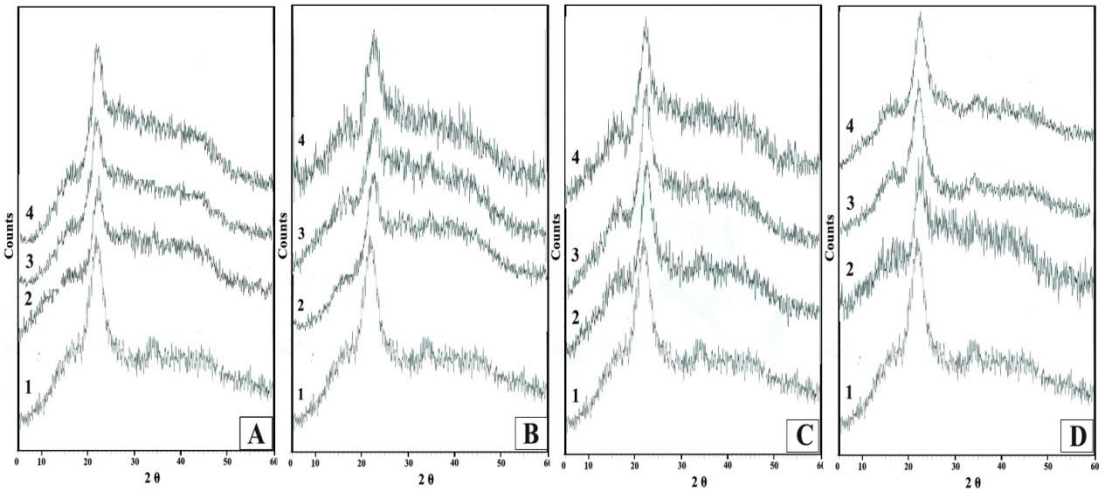


Fig.29: X-ray diffraction patterns of wood samples treated with propiconazole at different concentrations and infected with *Aspergillus flavus* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months (1: control sample; 2: infected sample; 3: sample treated with 0.25% propiconazole and infected; 4: sample treated with 0.25% propiconazole and infected).

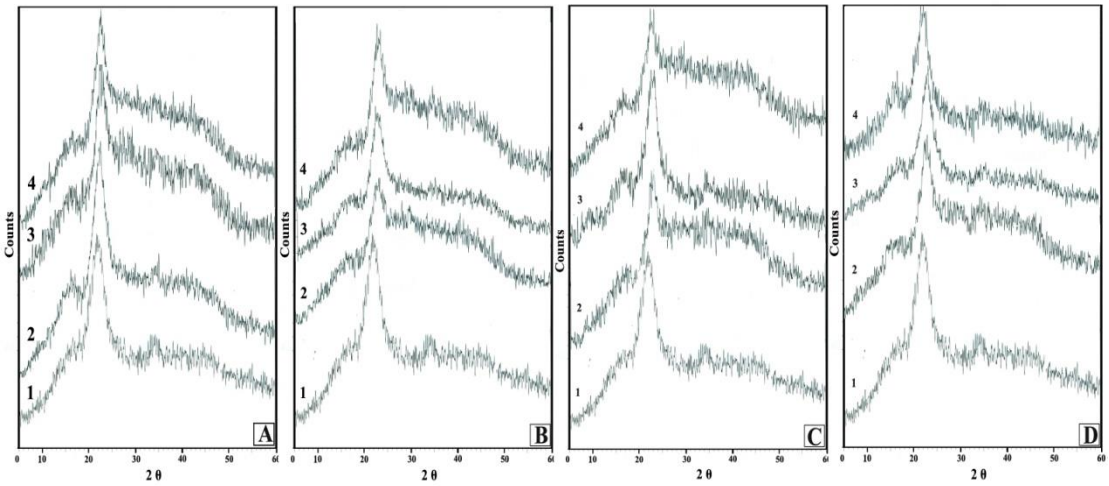


Fig. 30: X-ray diffraction patterns of wood samples treated with propiconazole at different concentrations and infected with *Penicillium chrysogenum* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months (1: control sample; 2: infected sample; 3: treated with 0.25% propiconazole and infected, 4: treated with 0.50% propiconazole and infected).

Chapter Six

Results and discussion of the fungicide tubeconazole

Chapter Six: Results and discussion of the fungicide tubeconazole

6.1 Effect of fungicide treatment on whiteness and yellowness indices

The results obtained concerning the effect of treatment conditions and the action of ageing on *Asperigllus niger* and treatment with the fungicide tebuconazole fungicide are shown in Fig. 31A and Fig. 31B. The whiteness decreased up to the second month and then increased to the fourth month (Fig. 31A). The untreated, infected sample showed a marked reduction in whiteness up to the second month; the nit increased for the rest of the ageing period. Because the amount of aqueous material in the sample was higher than in the other samples, fungal growth was higher. The results also showed the yellowness of the samples studied (Fig. 31B), with no remarkable change, but the yellowness of the untreated sample and the treated, infected sample (0.25% tubeconazole) was higher than for the other samples, due to the action of fungicide. Regarding the effect on the yellowness index, the sample treated with the lower concentration of tebuconazole (0.25%) and the infected, untreated sample showed a higher degree of yellowness than that treated with 0.50% tebuconazole. This can be attributed to the washing effect with the low concentration of fungicide and the low effect of that concentration on the fungus together with the high solids content of the fungicide materials. The results also revealed (Fig. 31B) that the untreated ,infected sample and the sample with the low concentration of tebuconazole (0.25%), which had a low content of solids and no shading film on the surface of the sample, had a higher degree of yellowness than the control sample and the sample treated with the higher concentration of tebuconazole (0.50%). None of the samples showed any marked change during the four months of ageing.

The data obtained show the effect of ageing time up to 4 months for the wood samples infected with *Aspergillus flavus* (Fig. 31C and 31D). These samples were treated with the same two concentrations of tebuconazole fungicide (0.25% and 0.50% w/v aqueous solution) or untreated. It is clear from Fig. 31Cthat the whiteness decreased with ageing until the second month, then increased. This could be attributed to many mechanisms and effects, including treatment with the fungicide material, the solids content of its residue on the surface, drying effects, oxidation of lignin, growth of the fungus itself. All of the above-mentioned factors may contribute to changing the topography and color of the wooden surface, and in turn change the interaction with light on the surface. The data obtained also show the effect of treatment, ageing time,

and the action of the fungus *Aspergillus flavus* on the yellowness index of the wood samples (Fig. 31D). The yellowness of the sample that was not treated and the sample that was treated with 0.25% tebuconazole increased up to the second month then decreased. The yellowness of most cellulosic materials may be due to oxidation. Raw wood samples mostly have colors ranging from pale yellow to dark brown, and the trend in the figure showed that there was no marked change in yellowness in the control sample and that treated with 0.50% tebuconazole. This finding indicates that the higher concentration of tebuconazole preserved the wood samples from the surrounding environmental and fungal effects.

The results obtained also showed the interaction between *Penicillium chrysogenum* and tebuconazole for four months (Fig. 31E and 31F). There was no remarkable change in the whiteness index in the control sample and the sample treated with 0.50% tebuconazole (Fig. 31E), but for the untreated sample and the infected samples there was a remarkable increase in whiteness due to the growth of *hyphae* on the surface of the wood due to the high moisture content, especially after 3 months. The yellowness index of all samples showed no remarkable change (Fig. 31F), but the control sample and that treated with 0.25% tebuconazole had lower values due to the depression of fungal growth on the surface.

6.2 FTIR analysis

6.2.1 Tebuconazole with *Aspergillus flavus*

The results obtained with *Aspergillus flavus* and tebuconazole at the concentrations used were as follows (Fig. 32):

The band at 3,571.5 cm^{-1} to 3,647.7 was assigned to **O-H bond stretching in cellulose, hemicellulose, lignin**. At 0.25%tebuconazole, except the for the samples that were treated for 3 or 4 months, all the frequencies had shifted to higher wavenumbers with an increase in intensity compared to the control sample. This probably resulted from fungal hydrolytic enzymes that cleaved some of the hydrogen bonds and formed more hydroxyl groups. The broadening of these bands with a decrease in frequency after treatment for 3 and 4 months reflects the ability of 0.25% tebuconazole to inhibit the activity of fungal enzymes. At 0.50%tebuconazole, all frequencies measured at all ageing times reflected the ability of this concentration of the fungicide to inhibit the hydrolytic activity of fungal enzymes.

The band at 1,739.5 cm^{-1} was assigned to u**nconjugated C=O stretching in ester groups of hemicellulose**. At 0.25% tebuconazole, except for the samples that were treated for 3 or 4 months, the ester groups of hemicellulose had disappeared, indicating that there was complete hydrolysis of ester groups to acidic ones. This result showed the good effect of 0.25% tebuconazole on hemicellulose content. At 0.50% tebuconazole, infection with *Aspergillus flavus* had a strong role in hemicellulose hydrolysis. This was confirmed by disappearance of the band for ester groups at 1,739.5 cm^{-1} and appearance of a new one at around 1,706 cm^{-1}, which could be assigned to acidic carbonyl groups. The results also showed the good effect of treatment with 0.5% tebuconazole on hemicellulose content.

The band at 1,645.9 cm^{-1} to 1,706.7 $cm^{-1}$was/is assigned to **adsorbed O-H and C=O conjugated stretching in cellulose.** At 0.25%tebuconazole, in infected samples a new broad band around 1,706 cm^{-1} arose, resulting from hydrolysis of the ester groups of hemicellulose. This band was also associated with a absorbed O-H bending. With 0.50% tebuconazole, it was clear that all the hydrolyzed hemicellulose samples gave a band at around 1,706 cm^{-1}, assignable to acidic carbonyl groups.

The band at 1,511.9 cm^{-1} for both 0.25% and 0.50% tebuconazole was/is assigned to **aromatic skeletal vibration in lignin**. Disappearance of these bands or a decrease in their intensity after fungal infection indicated decomposition of the aromatic skeleton of lignin. The results also showed that treatment with the fungicide had a positive effect on lignin content.

The bands from 1,465.6 cm^{-1} to 1,380.7 cm^{-1} were assigned to plane deformation of **C-H bondsin carbohydrate and lignin**. The results with the concentrations of tebuconazole used showed disappearance or decrease in band intensity in fungally infected samples due to depolymerization of carbohydrate and lignin. On the other hand, the intensities of these bands increased after treatment, reflecting the role of the fungicide in protecting wood from decay.

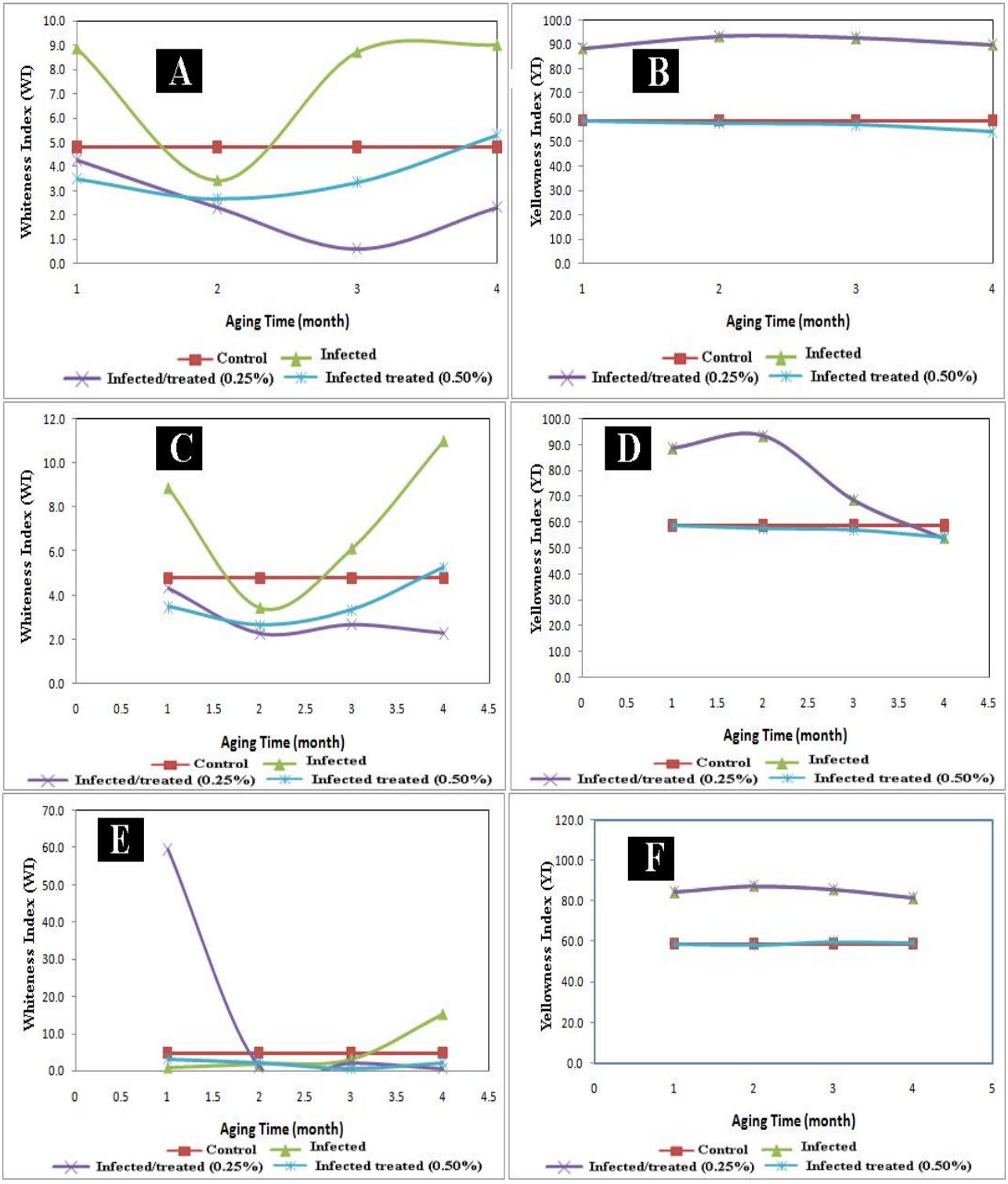


Fig. 31: Effect of treatment of wood samples (that have been infected with different fungi) with tebuconazole on the whiteness and yellowness indices. A and B: *Aspergillus niger*; C and D: *Aspergillus flavus*; E and F: *Penicillium chrysogenum*.

The bands from 1,284.4 cm^{-1} to 1,164.8 cm^{-1} were assigned to **asymmetric C-O-C vibration in carbohydrates.** At 0.25% tebuconazole, the decrease in band intensity around 1,275 cm^{-1} when the samples were infected for 3 or 4 months suggested a decrease in lignin and adjacent hemicelluloses. Treatment with 0.25% tebuconazole inhibited the effects of the fungus. At 0.50%, the intensity of the band at around 1,275 cm^{-1} increased in the treated samples, indicating that treatment with 0.5%tebuconazoleinhibited the effects of the fungus. Disappearance of some bands (at 025% and 50%) in the 1,164 -1183 cm^{-1} range in both infected and treated samples indicated that advanced breakage of cellulose chains and depolymerization had occurred. The appearance of bands in infected and treated wood after 4 months may have been due to crosslinking reactions.

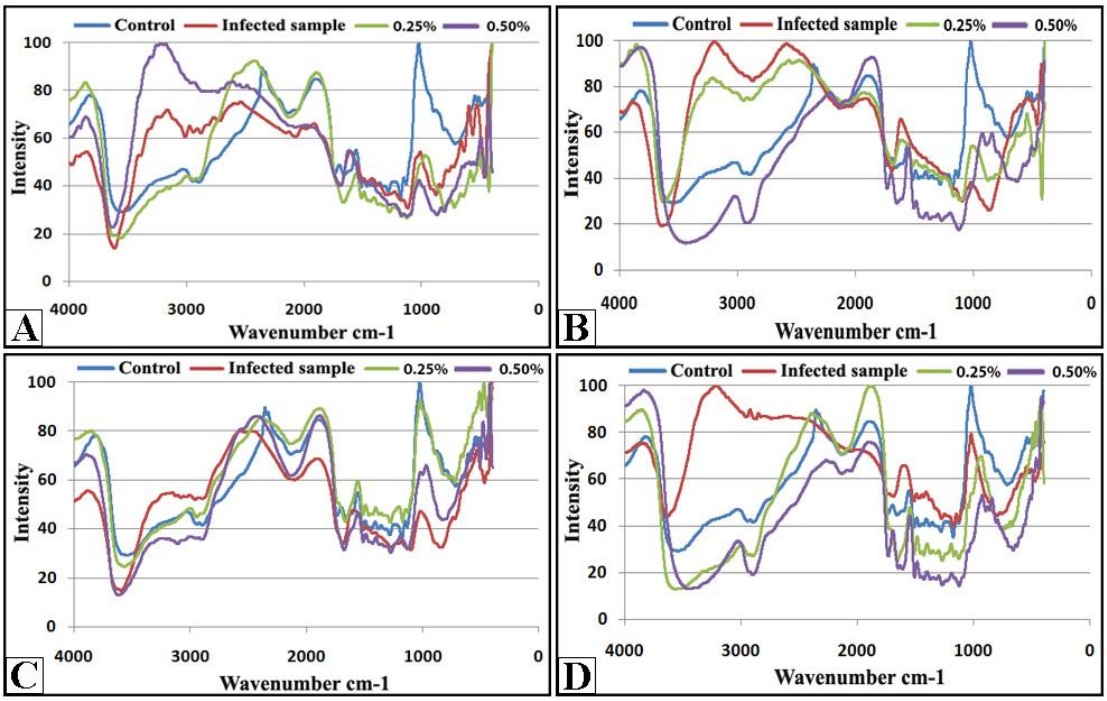


Fig. 32: FTIR of wood samples treated with tebuconazole at different concentrations and infected with *Aspergillus flavus* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

6.2.2 Tebuconazole with *Aspergillus niger*

The results obtained with *Aspergillus niger* and tebuconazole at the concentrations used were as follows (Fig. 33):

Theband at3,571.5 cm^{-1} to 3,647.7 $cm^{-1}$was/is assigned to stretching of **O-H bonds in cellulose, hemicellulose, and lignin**. For 0.25% tebuconazole, treated

samples showed little change in the frequency and intensity of these bands after one or two months compared to the infected samples, indicating a weak effect of the fungicide on *Aspergillus niger*. With the higher concentration (0.50%), except for the band of the sample treated for four months, all the bands of the samples shifted to higher frequencies compared to that of the control sample. The intensity of the bands of samples treated for one or two months decreased compared to those of the corresponding infected samples, indicating a weak effect of the fungicide on *Aspergillus niger*. On the other hand, the bands in samples treated for 3 and 4 months (25% AND 50%) broadened and shifted to lower wave number with an increase in intensity, indicating the presence of carboxylic acid and hydroxyl groups. This can be explained by *Aspergillus niger* oxidizing the methyl groups of tebuconazole to form the alcohol, which was further oxidized to form carboxylic acid.

The band at 1,739.5 cm^{-1} was assigned to u**nconjugated C=O stretching in ester groups of hemicellulose**. In samples treated with 0.25% tebuconazole, after 1 and 2 months the intensity of this band had decreased compared to those of the corresponding infected samples, showing no effect of the fungicide on the fungus and that the fungicide had a negative effect on hemicellulose content. At 0.50% tebuconazole, the intensity of this band in all treated samples was higher than that of the corresponding infected ones. However, in samples treated (with the concentrations used) for three and four months, the intensity increased compared to that of the control sample and those of the corresponding infected samples. This was probably due to ester formation resulting from the reaction between the hydroxyl groups of cellulose or those of oxidized tebuconazole and the carboxylic groups resulting from the final oxidation step of cellulose or tebuconazole and ester groups of remaining hemicellulose.

The bands at 1,664.3 cm^{-1}, 1,647.5cm^{-1},and 1,706.7 cm^{-1}were/are assigned to **adsorbed O-H and C=O conjugated stretching in cellulose.** At the concentrations used, a new broad band in the1,696–1,717 cm^{-1} range appeared after fungal infection and fungicide treatment. This band was/is assigned to acidic carbonyl groups resulting from hydrolysis of some ester groups of hemicellulose in infected samples or resulting from oxidation of tebuconazole in treated samples. We noticed that oxidation of tebuconazole increased with time, as seen from increasing band intensity with increasing incubation period.

The band at 1,511.9 cm-1 for both 0.25% and 0.50% tebuconazole was assigned to **aromatic skeletal vibration in lignin**. At 0.25% tebuconazole, except for

the samples treated for 3 or 4 months, the intensity of these bands decreased compared to that of the control sample—indicating decomposition of the aromatic skeleton of lignin. At 0.50% tebuconazole, in the treated samples the intensity of these bands increased compared to that of the infected sample, indicating a good effect of the fungicide on the aromatic skeleton of lignin.

The bands at 1,468.5 cm^{-1} and 1,381.7 cm^{-1} were assigned to plane deformation of **C-H bonds in carbohydrate and lignin**. At 0.25% tebuconazole, except for the samples treated for 3 or 4 months, the intensities of these bands decreased compared to that of the control sample. At 0.50%tebuconazole, the intensities of these bands in the treated samples increased compared to those in the infected samples, indicating that the fungicide had a good effect.

The bands at 1,284.4 cm^{-1}, 1,176.4 cm^{-1},and 1,164.8 cm^{-1} were assigned to **asymmetric C-O-C vibration in carbohydrate.** At 0.25% tebuconazole, except for the samples treated for 3 or 4 months, the intensities of the bands in both infected and treated samples decreased compared to that of the control sample. The increase in the samples treated for 3 or 4 months may have resulted from C-O bonds of hydroxyl and/or carboxylic groups formed from fungal oxidation of tebuconazole. At 0.50% tebuconazole, the intensities of these bands in the treated samples increased compared to that of the infected sample, indicating that the fungicide had a good effect.

In regard to dry wood: tebuconazole is an effective fungicide in solvent-borne wood preservatives and is also effective against molds such as *Aspergillus niger*. The substance is almost unbleachable, stable in exposure to light and heat, not volatile, and warrants long-term effectiveness (Unger et al., 2001).

6.2.1. Tebuconazole with *Penicillium chrysogenum*

The results obtained with *Penicillium chrysogenum* and tebuconazole at the concentrations used were as follows (Fig. 34):

Theband at3,571.5 cm^{-1} to 3,647.7cm^{-1} was assigned to stretching of **O-H bonds in cellulose, hemicellulose, and lignin**.

All the samples treated with 0.25% or 0.50% tebuconazole showed gradual shifts with time to lower wave numbers, which was accompanied by an increase in intensity compared to the corresponding infected samples and the control sample. This resulted

from formation of more hydroxyl groups and more hydrogen bonds due to oxidation of tebuconazole.

The band at 1,739.5 cm^{-1} was assigned to u**nconjugated C=O stretching in ester groups of hemicellulose**. With all concentrations of tebuconazole used, and except for the sample that was treated for one month (which showed a poor effect of tebuconazole), all the other treated samples showed an increase in intensity compared to that of the control sample or to those of corresponding infected samples. This was probably due to ester formation resulting from reaction between the hydroxyl groups of cellulose or of oxidized tebuconazole and the carboxylic groups resulting from the final oxidation step of cellulose or tebuconazole and ester groups of remaining hemicellulose.

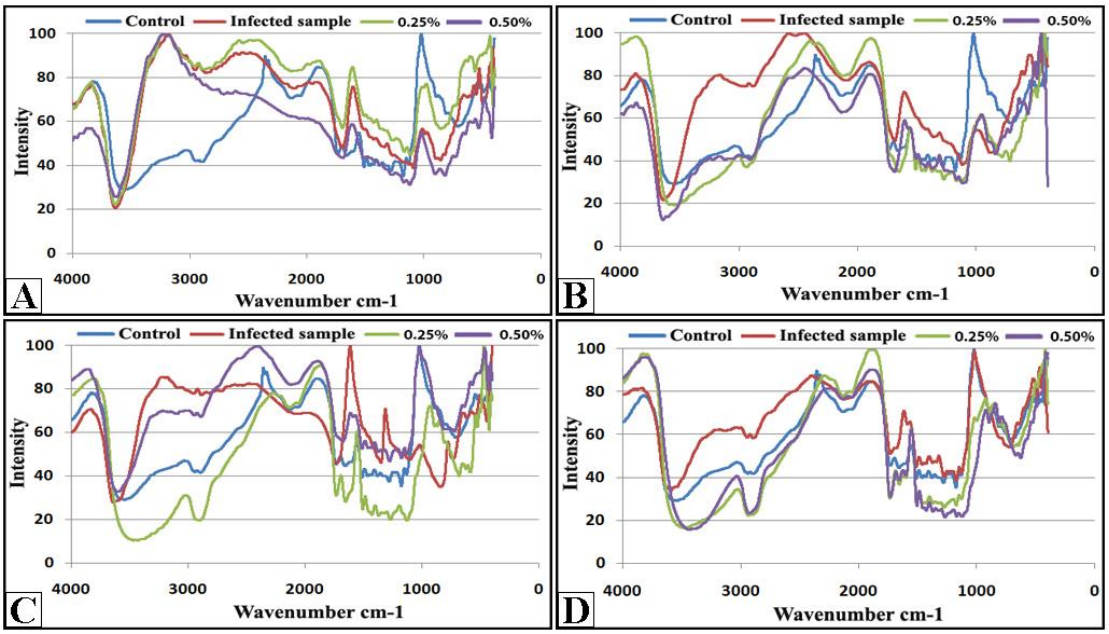


Fig. 33: FTIR of wood samples treated with tebuconazole at different concentrations and infected with *Aspergillus niger* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, (D) and after 4 months.

The bands at 1664.3 cm^{-1} and 1647.5 cm^{-1} were assigned to a**dsorbed O-H and C=O conjugated stretching in cellulose.** In the samples that were treated with 0.25% tebuconazole, new bands appeared at 1,680–1,617cm^{-1}. With 0.50% tebuconazole, except for the sample that was treated for four months, all the treated samples showed new bands in the 1,680–1,650 cm^{-1}region, assignable to carbonyl stretching conjugated band of oxidized cellulose and also to carbonyl groups resulting from tebuconazole

oxidation. The sample treated for four months showed a strong band at 1,725.9 cm^{-1}, assignable to carboxylic groups of oxidized tebuconazole. Some researchers have found that tebuconazole is oxidized by some fungi first to hydroxyl groups, then to carbonyl groups, and finally to carboxylic groups (Woo, et al., 2010).

The band at 1,511.9 cm-1 for both 0.25% and 0.50%tebuconazolewas/is assigned to **aromatic skeletal vibration in lignin**. The intensity of this band increased after treatment with the fungicide. This reflected the good effect of 0.25 % tebuconazole on lignin content.

With 0.50%tebuconazole, except for the sample that was treated for one month, the intensity of this band increased after treatment, reflecting the good effect of this concentration of tebuconazole on lignin content. The substantial increase in the intensity of this band indicated that as decay progressed, extensive loss of carbohydrate occurred and lignin concentrations increased in the remaining wood (Blanchette, 2000; Pandey and Pitman, 2003).

The bands from 1,468.5 $cm^{-1}$and 1,381.7 cm^{-1} were assigned to **plane deformation of C-H bonds in carbohydrate and lignin**. The intensities of these bands in the samples treated with 0.25% tebuconazole, except for the treated sample given 1 month of incubation, increased relative to that of the infected sample, indicating a good effect of the fungicide on these bands.

The bands from 1284.4 cm^{-1} to 1176.4 cm^{-1} were assigned to **asymmetric C-O-C vibrationin carbohydrate.** Compared to the control sample and corresponding infected samples, the intensities of these bands increased after treatment with 0.25%tebuconazole, except for the sample treated with 0.50% tebuconazole and incubated for one month. This may have resulted from C-O bonds of hydroxyl and carboxylic groups being formed from fungal oxidation of tebuconazole and the quiet good effect of the fungicide on the stability of these bonds.

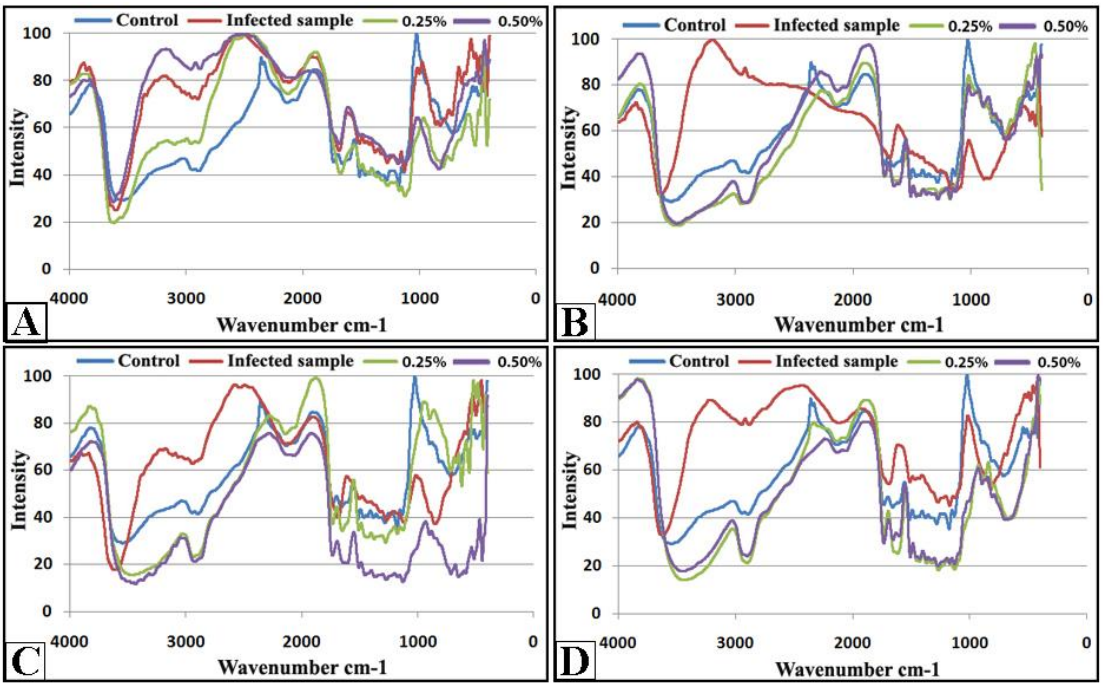


Fig. 34: FTIR of wood samples treated with tebuconazole at different concentrations and infected with *Penicillium chrysogenum* for different lengths of time: (A) after 1 month, (B) after 2 months, (C) after 3 months, and(D) after 4 months.

Stirling (2013) used tebuconazole for the preservation of wood. It had good resistance against fungi.

6.3 X-ray diffraction analysis (XRD) of samples treated with tebuconazole and infected with different fungi

6.3.1 Peak position (2 θ degrees)

The results revealed that the position of the (101) peak increased aftertreatment with tebuconazole at either of the concentrations and infection with any of the fungi (Figs. 35 - 40). The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with *Aspergillus niger* was 14.76, 14.72, 14.67 and 14.61 (2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration of tebuconazole (0.50%), it was 14.78, 14.72, 14.68, and 14.63 (2 θ).

The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with *Aspergillus flavus* was 14.70, 14.66, 14.62, and 14.58 (2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration of tebuconazole (0.50%) it was 14.76, 14.71, 14.66, and 14.62 (2 θ).

The peak position value of the wood sample treated with tebuconazole at the first concentration (0.25%) and infected with *Penicillium chrysogenum* was 14.72, 14.68, 14.62, and 14.59 (2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration of tebuconazole (0.50%), it was 14.73, 14.69, 14.65, and 14.60 (2 θ).

The peak position (10Í) increased after the treatment with tebuconazole at either of the concentrations and infection with any of the fungi. The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with *Aspergillus niger* was 16.74, 16.70, 16.67, and 16.62 (2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration (0.50%) it was 16.76, 16.71, 16.66, and 16.61 (2 θ).

The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with *Aspergillus flavus* was 16.73, 16.69, 16.64, and 16.60(2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration (0.50%), it was 16.73, 16.70, 16.65 and 16.60 (2 θ).

The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with *Penicillium chrysogenum* was 16.70, 16.66, 16.62, and 16.58(2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration (0.50%), it was 16.70, 16.65, 16.60, and 16.56 (2 θ).

The peak position (002) increased aftertreatment with tebuconazole at either concentration and infection with any of the fungi. The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with

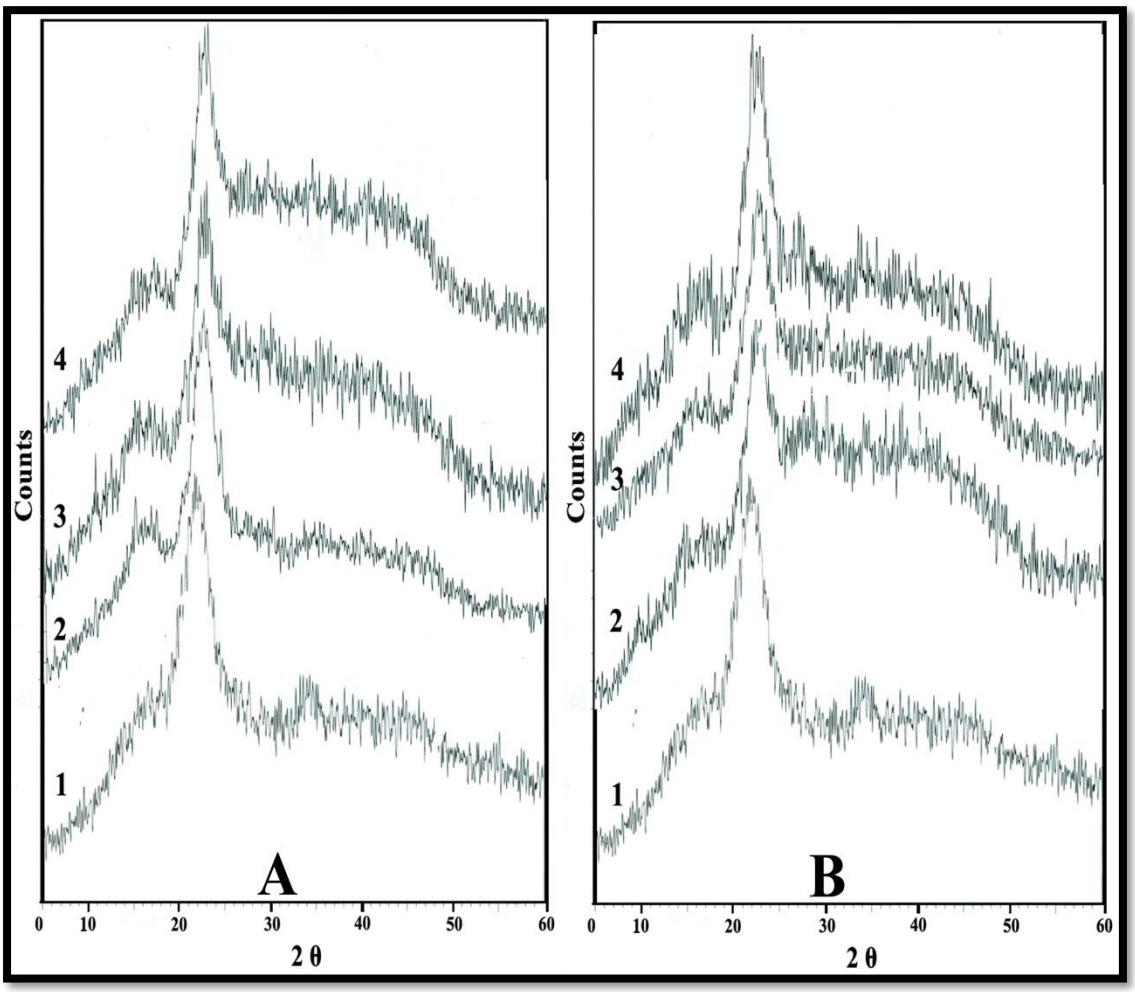


Fig. 35: X-ray diffraction patterns of wood samples treated with tebuconazole at different concentrations and infected with *Aspergillus niger* for different lengths of time: (A) after 1 month, (B) after 2 months (1: control sample; 2: infected sample; 3: sample treated with 0.25% tebuconazole and infected; 4: sample treated with 0.50% tebuconazole and infected).

Aspergillus niger was 22.88, 22.83, 22.76, and 22.74 (2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration (0.50%), it was 22.89, 22.82, 22.78, and 22.73 (2 θ).

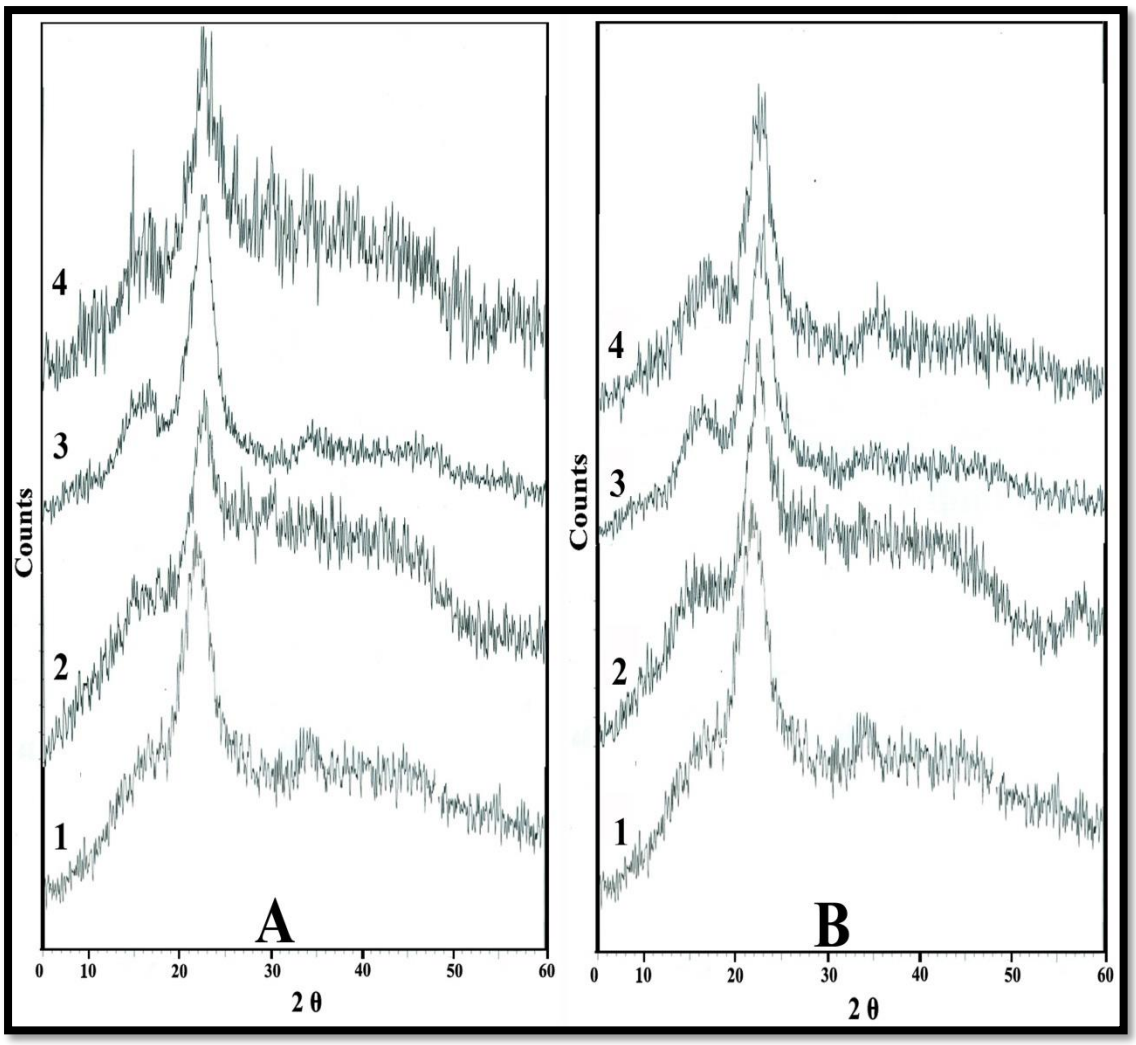


Fig. 36: X-ray diffraction patterns of wood samples treated with tebuconazole at different concentrations and infected with *Aspergillus niger* for different lengths of time: (A) after 3 months, (B) after 4 months (1: control sample; 2: infected sample; 3:sample treated with 0.25% tebuconazole and infected; 4: sample treated with 0.50% tebuconazole and infected).

The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with *Aspergillus flavus* was 22.86, 22.80, 22.76, and 22.71 (2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration (0.50%), it was 22.87, 22.81, 22.76, and 22.70 (2 θ).

The peak position value of the wood samples treated with tebuconazole at the first concentration (0.25%) and infected with *Penicillium chrysogenum* was 22.85,

22.79, 22.74, and 22.70 (2 θ) after 1, 2, 3, and 4 months, respectively. At the second concentration (0.50%), it was 22.86, 22.80, 22.70, and 22.68 (2 θ).

6.3.2 Peak width

The peak width (Figs. 35-40) of samples infected with *Aspergillus niger* and treated with tebuconazole at the first concentration (0.25%) was 0.13, 0.15, and 0.58 mm; 0.14, 0.16, and 0.61 mm; 0.12, 0.14, and 0.56 mm; and 0.11, 0.13, and 0.54 mm for 101, 10Ī, and 002after 1, 2, 3, and 4 months, respectively. With tebuconazole at the second concentration (0.50%), the peak width was 0.13, 0.16, and 0.64 mm; 0.14, 0.17, and 66 mm; 0.12, 0.14, and 0.62 mm; and0.11, 0.13, and 0.56 mm for 101, 10Ī, and 002after 1, 2, 3, and 4 months, respectively.

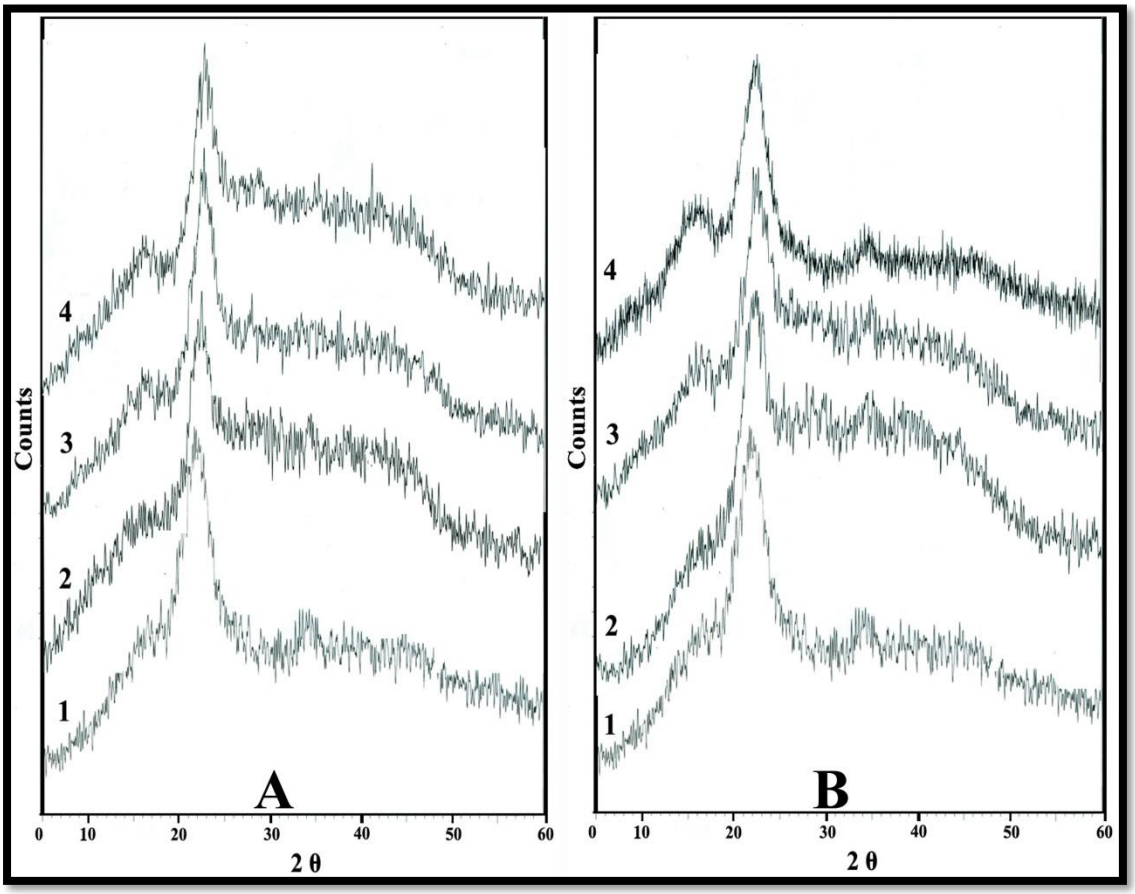


Fig. 37: X-ray diffraction patterns of wood samples treated with tebuconazole at different concentrations and infected with *Aspergillus flavus* for different lengths of time: (A) after 1 month, (B) after 2 months (1: control sample; 2: infected sample; 3: sample treated with 0.25% tebuconazole and infected; 4: sample treated with 0.50% tebuconazole and infected).

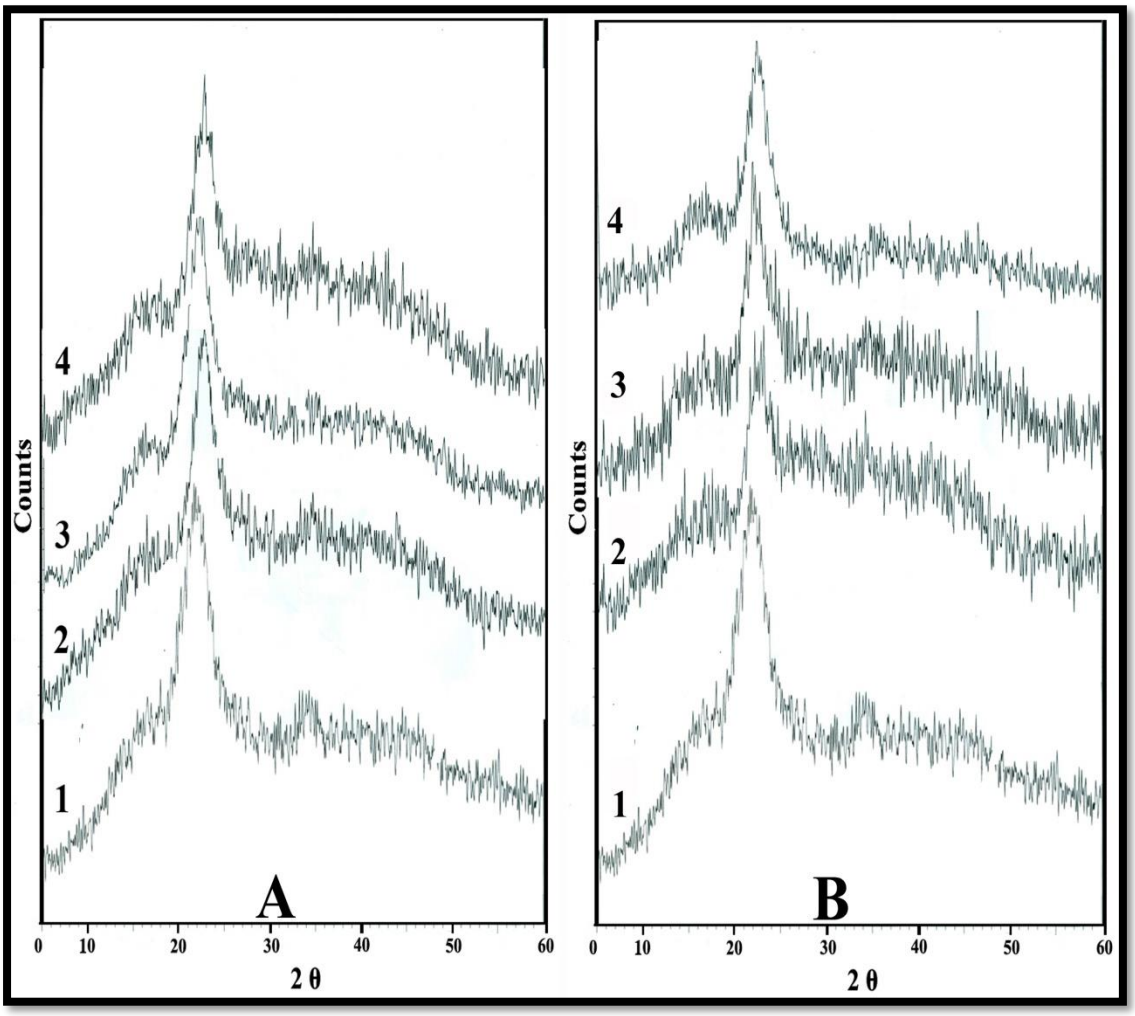


Fig. 38: X-ray diffraction patterns of wood samples treated with tebuconazole at different concentrations and infected with *Aspergillus flavus* for different lengths of time: (A) after 3 months, (B) after 4 months (1: control sample; 2: infected sample; 3: sample treated with 0.25% tebuconazole and infected; 4: sample treated with 0.50% tebuconazole and infected).

The peak width of samples infected with *Aspergillus flavus* and treated with tebuconazole at the first concentration (0.25%) was 0.13, 0.12, and 0.61 mm; 0.12, 0.13, and 63 mm; 0.11, 0.11, and 0.57 mm; and 0.10, 0.10, and 0.51 mm for 101, 10Ī, and 002 after 1, 2, 3, and 4 months, respectively. At the second tebuconazole concentration (0.50%), the peak width was 0.14, 0.13, and 0.64 mm; 0.15, 0.11, and 62 mm; 0.13, 0.10, and 0.57 mm; and 0.11, 0.10, and 0.51 mm for 101, 10Ī, and 002 after 1, 2, 3, and 4 months, respectively.

The peak width of samples infected with *Penicillium chrysogenum* and treated with tebuconazole at the first concentration (0.25%) was 0.14, 0.15, and 0.57 mm; 0.13, 0.14, and 0.53; 0.11, 0.12, and 0.50 mm; and 0.10, 0.11, and 0.48 mm for 101, 10Ī, and 002 after 1, 2, 3, and 4 months, respectively. At the second concentration (0.50%), the peak width was 0.15, 0.14, and 0.65 mm; 0.14, 0.13, and 0.60 mm; 0.11, 0.11, and 0.55 mm; and 0.10, 0.10, and 0.50 mm for 101, 10Ī, and 002after 1, 2, 3, and 4 months, respectively.

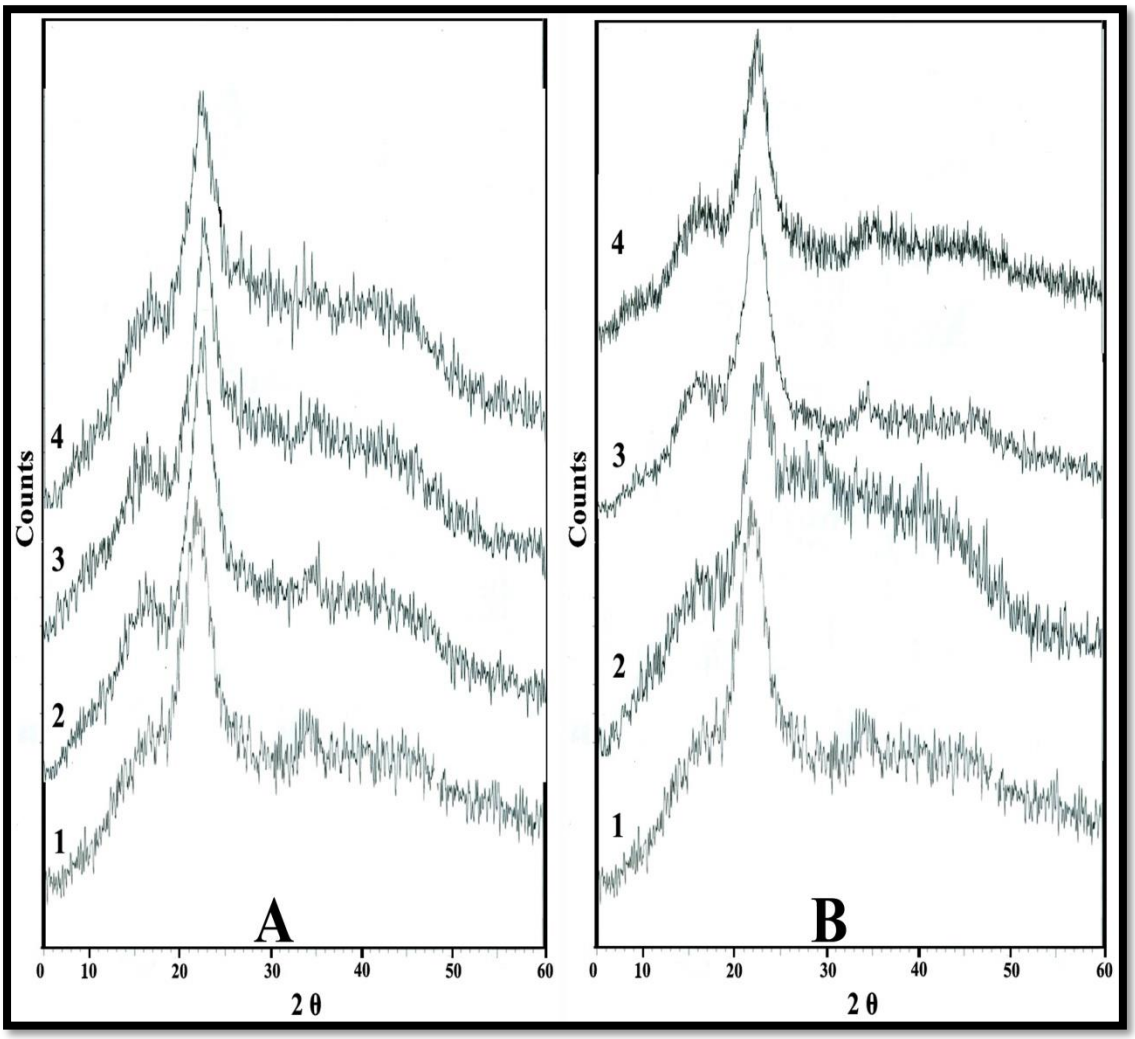


Fig. 39: X-ray diffraction patterns of wood samples treated with tebuconazole at different concentrations and infected with *Penicillium chrysogenum* for different lengths of time: (A) after 1 month, (B) after 2 months (1: control sample; 2: infected sample; 3: sample treated with 0.25% tebuconazole and infected; 4: sample treated with 0.50% tebuconazole and infected).

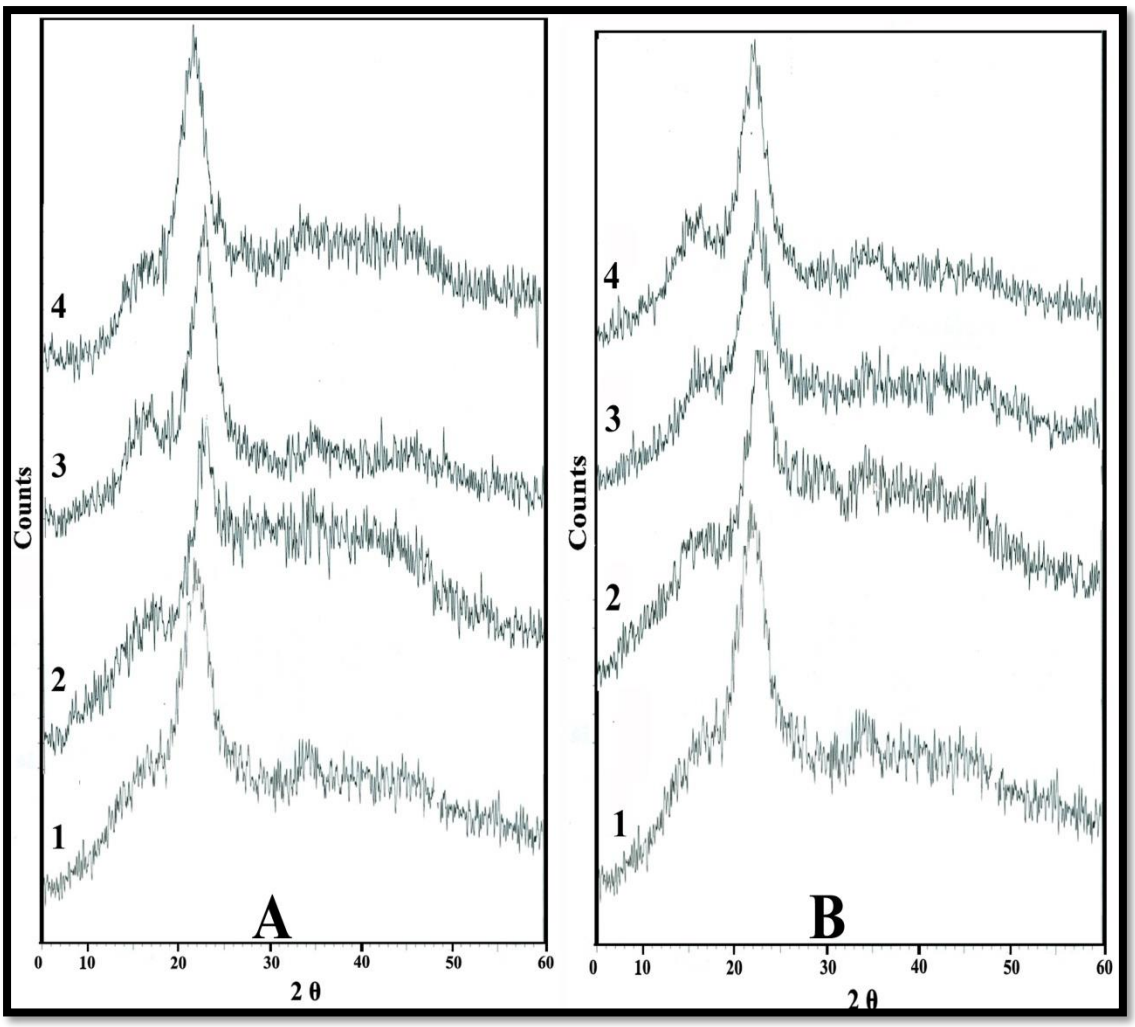


Fig. 40: X-ray diffraction patterns of wood samples treated with tebuconazole at different concentrations and infected with *Penicillium chrysogenum* for different lengths of time: (A) after 3 months, (B) after 4 months (1: control sample; 2: infected sample; 3: sample treated with 0.25% tebuconazole and infected; 4: sample treated with 0.50% tebuconazole and infected).

6.3.3 Peak intensity

The Peak intensity (Figs. 35-40) for the samples infected with *Aspergillus niger* and treated with tebuconazole at the first concentration (0.25%), the crystallinity index by the first method was 4.1, 4.0, 3.8, and 3.6 after 1, 2, 3, and 4 months, respectively. By the second method, it was 51.70, 51.30, 51.10, and 50.67. For the samples infected with *Aspergillus niger* and treated at the second concentration (0.50%), the

crystallinity index by the first methodwas4.1, 4.0, 3.7, and 3.6 after 1, 2, 3, and 4 months, respectively. By the second method, it was 52.90, 51.60, 51.20, and 50.76.

For the samples infected with *Aspergillus flavus* and treated with tebuconazole at the first concentration (0.25%), the crystallinity index by the first method was 4.0, 3.8, 3.7, and 3.6after 1, 2, 3, and 4 months, respectively. By the second method, it was 51.80, 51.30, 51.08, and 50.70. For the samples infected with *Aspergillus flavus* and treated at the second concentration (0.50%), the crystallinity index by the first method was4.1, 4.0, 3.8, and 3.7, and by the second method it was 51.70, 51.20, 50.80, and 50.40.

For the samples infected with *Penicillium chrysogenum* and treated with tebuconazole at the first concentration (0.25%), the crystallinity index by the first method was 4.0, 3.9, 3.7, and 3.6, and by the second method it was 51.60, 51.10, 50.80, and 50.50.For the samples infected with *Penicillium chrysogenum* and treated with tebuconazole at the second concentration (0.50%), the crystallinity index by the first method was 4.0, 3.8, 3.7, and 3.6, and by the second method it was 51.70, 51.30, 50.80, and 50.30 after 1, 2, 3, and 4 months, respectively.

Chapter Seven

Conclusions

Chapter Seven: Conclusions

- Archaeological sites in Egypt contain wooden artifacts that suffer from deterioration caused by different factors including physical factors (moisture, temperature, and light), chemical factors (air pollutants, dust, and particulate matter), and biological factors (insects and microorganisms). Because of the factors mentioned above, we noticed many forms of deterioration, such as stains from different sources, discoloration, deformation, etc.
- Fungi play an important role in the deterioration of archeological wood in Egypt, especially in certain environments. *Asperigllus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum* were the fungi identified from different sites in historical Cairo, Egypt.
- The lightness of the samples infected with any of the fungi studied decreased with increasing incubation time. The resistance to fungi of the samples treated with chitosan increased with increasing concentration of fungicide. The percentage loss of lightness with *A. niger* infection and treatment of samples with chitosan at different concentrations was higher than the percentage loss of whiteness in similarly treated samples infected with *A. flavus* or *P. chrysogenum*. *A. flavus* gave the highest increase in a* value for the samples treated with chitosan at any of the concentrations used, followed by. *chrysogenum* and then *A. niger*. The a* value of the samples treated with chitosan decreased with increasing concentrations of chitosan, but the samples treated at all concentrations had higher a* values than the control samples. The yellowness (b* value) of samples infected with fungi increased with increasing incubation time. The yellowness of the samples treated with chitosan was higher than that of the control samples, but this value decreased with increasing concentrations of chitosan after one month for all the fungi studied, after two months for *A. flavus*, and after three months for *A. niger* and *A. flavus*.
- It is very important to take into consideration all possible sources of uncertainty when measuring the color components and related parameters, including whiteness and yellowness. Special care should be taken regarding the uncertainty of calibration because it was found to be the predominant contributor to the final whiteness and yellowness values. By achieving the most accurate results with the lowest uncertainty, we obtained accurate and precise results concerning the effect of the

three fungi studied—namely *Asperigllus niger*, *Aspergillus flavus*, and *Penicillium chrysogenum*—and the efficiency of fungicides in conditioning wooden artifacts.

- The results revealed that the whiteness of the samples infected with fungi decreased up to the second month and then increased to the fourth month. The yellowness of the untreated, infected samples increased. The samples treated with tebuconazole and propiconazole fungicides gave good resistance against fungal deterioration. The higher concentration(0.50%) gave better results than the lower concentration (0.25%), for both whiteness and yellowness indices. Tebuconazole gave better results than propiconazole at both concentrations regarding whiteness and yellowness.
- FTIR analysis showed that treatment of wood with the fungicide chitosan conferred resistance to fungal growth, since the intensity of the band at 1,685 cm^{-1} for the infected samples, assigned to C-C bonds, increased more than for the infected, treated samples, irrespective of incubation time. FTIR analysis confirmed that for all three fungi studied, infected samples treated with chitosan were more amorphous during the incubation times than the infected, untreated samples.
- Treatment with propiconazole at the concentrations used negatively affected hemicellulose content and enhanced cellulose depolymerization and oxidation. However, it protected lignin from fungal decay. So, it is unfavorable to use propiconazole for treatment of wood infested by *Aspergillus flavus*. On the other hand, tebuconazole had a slight effect on all the main components of wood and can be used safely to treat wood infested by *Aspergillus flavus*.
- The results also showed that increased propiconazole and tebuconazole concentrations were needed to achieve acceptable protection against *Aspergillus niger* due to oxidation of these fungicides by the fungus, especially at low concentrations of fungicide. Good results were obtained in treatment of wood infected by *Aspergillus niger*, and the treatment was safer for all the main components of wood (cellulose, lignin, and hemicellulose) at 0.50%than at 0.25%. High propiconazole and tebuconazole concentrations are not recommended for *Penicillium chrysogenum* treatment, and the low concentration (0.25%) is sufficient to have an inhibitory effect.
- X-ray diffraction analysis showed that with all three fungi studied, the peak positions of cellulose I ((101), (10Í) and (002)) in infected samples decreased compared to the

control samples at all incubation times. The peak positions of the infected, treated samples increased with increasing concentration of chitosan. The X-ray diffraction analysis showed that after the first and second months, the peak position and peak width increased after chitosan treatment and infection with any of the fungi. This increase was less after the third and fourth months, but it was still higher than for the control sample. The crystallinity index, measured with either the first or the second method, decreased, but it increased after the third and fourth months of incubation with any of the fungi studied. The highest concentration of chitosan tested (0.75%)conferred high resistance to fungal growth, and this concentration can be recommended for use with historical wooden artifacts.

- The crystallinity of cellulose increased after the treatment with propiconazole compared to the control and infected samples. The band at 2,941 cm^{-1} of the infected samples treated with propiconazole decreased in intensity compared to the control samples at most concentrations used, with most of the fungi studied, and with most incubation times. This indicated the presence of anamorphous cellulosic area, and showed evidence of dehydration with decrease in the intensity of absorption at 2,941 cm^{-1}approximately. It can be also said that the treatment of wood with propiconazole increased the crystallinity of cellulosic are as in some cases in infected, treated samples. The intensity of the band at approximately 1,660 cm^{-1} in infected samples treated with propiconazole decreased compared to the control and infected samples, indicating that this fungicide conferred resistance to the fungi studied. The use of fungicide (propiconazole) increased the crystallinity of cellulose; this was clear from the band at 1,430 cm^{-1}approximately, since the intensity of this band increased in the infected, treated samples relative to the control and infected samples.
- Many fungicides are used for the preservation of wood against fungal deterioration, but chitosan, propiconazole, and tebuconazole fungicides were used in this study at limited concentrations. Chitosan was used at 0.25%, 0.50%, and 0.75%. Propiconazole and tebuconazole were used at 0.25% and 0.50%. These limited concentrations gave good resistance to fungal deterioration.
- The best results were obtained with chitosan, followed by tebuconazole and propiconazole.
- Propiconazole and tebuconazole fungicides are widely used to resist the biological damage of non-archaeological wood. They are rarely used on archaeological wood.

This experimental study is the first study carried out on these fungicides for their use in the protection of archaeological wood. Low concentrations from these fungicides were used to accept the requirements of using new materials for the conservation treatment. From the conservation point of view, it is often recommended to use low concentrations, especially if they produced positive results. The fungicides used in this study gave good results at low concentrations.

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Appendix: Change of color of wood samples treated with chitosan at different concentrations against infection by selected strains of *Aspergillus flavus*, *A. niger* and *P. chrysogenum*, and their subsequent incubation for 1, 2, 3 and 4 months

Appendix: Change of color of wood samples treated with chitosan at different concentrations against infection by selected strains of *Aspergillus flavus*, *A. niger* and *P. chrysogenum*, and their subsequent incubation for 1, 2, 3 and 4 months

Samples	Incubation: 1 month											
	Aspergillusniger				*Aspergillusflavus*				*Penicilliumchrysogenum*			
	Color values			Total color difference	Color values			Total color difference	Color values			Total color difference
	L	a	b	ΔE	L	a	B	ΔE	L	a	B	ΔE
Control	67.20	6.84	27.63	0.0	67.16	6.84	27.63	0.0	67.16	6.84	27.63	0.0
Infected sample before treatment	49.30	13.96	25.20	57.10	54.34	18.54	33.70	66.57	51.40	13.50	30.30	61.17
Sample treated with (0.25% chitosan)	63.70	6.20	27.10	69.50	65.17	6.30	27.20	70.90	64.50	6.10	27.01	70.19
Treated sample (0.50% chitosan)	65.40	6.40	27.31	71.17	66.85	6.40	27.93	72.73	65.90	6.35	27.30	71.61
Treated sample (0.75% chitosan)	66.80	6.55	27.47	72.50	67.03	6.70	27.50	72.76	66.70	6.42	27.44	72.40
Samples	Incubation: 2 months											
	Aspergillusniger				*Aspergillusflavus*				*Penicilliumchrysogenum*			
	Color values			Total color difference	Color values			Total color difference	Color values			Total color difference
	L	a	b	ΔE	L	a	B	ΔE	L	a	b	ΔE

Control	67.16	6.84	27.63	0.0	67.16	6.84	27.63	0.0	67.16	6.84	27.63	0.0
Infected sample before treatment	40.20	10.52	23.30	27.73	45.20	16.20	35.41	25.11	46.20	12.20	28.23	21.64
Treated sample (0.25%)	62.98	5.87	26.20	4.52	64.81	5.41	27.94	2.77	63.85	5.79	26.45	3.51
Treated sample (0.50%)	64.59	6.02	25.94	3.18	66.21	5.99	27.88	1.14	64.76	5.98	26.45	2.81
Treated sample (0.75%)	65.71	6.12	25.41	2.75	66.20	6.10	27.70	1.21	66.19	5.55	26.65	1.89
Samples	**Incubation: 3 months**											
	Aspergillus niger				***Aspergillus flavus***				***Penicillium chrysogenum***			
	Color values			**Total color difference**	**Color values**			**Total color difference**	**Color values**			**Total color difference**
	L	**a**	**b**	**ΔE**	**L**	**a**	**b**	**ΔE**	**L**	**a**	**b**	**ΔE**
Control	67.16	6.84	27.63	0.0	67.16	6.84	27.63	0.0	67.16	6.84	27.63	0.0
Infected sample before treatment	34.20	7.57	19.50	33.96	40.15	-10.20	42.02	30.97	39.98	7.30	31.52	27.46
Treated sample (0.25%)	60.51	5.02	25.42	7.13	62.97	4.78	28.70	4.79	61.89	4.89	26.20	5.80
Treated sample (0.50%)	63.80	5.57	25.01	3.81	64.04	5.12	28.20	3.61	63.08	5.11	25.80	4.79
Treated sample (0.75%)	64.59	5.88	24.80	2.91	65.31	5.45	27.80	2.32	64.46	5.23	25.48	3.81

Samples	Incubation: 4 months											
	Aspergillusniger				*Aspergillusflavus*				*Penicilliumchrysogenum*			
	Color values			Total color difference	Color values			Total color difference	Color values			Total color difference
	L	a	b	ΔE	L	a	b	ΔE	L	a	b	ΔE
Control	67.16	6.84	27.63	0.0	67.16	6.84	27.63	0.0	67.16	6.84	27.63	0.0
Infected sample before treatment	29.80	5.40	13.24	40.06	34.51	-14.54	46.20	38.34	32.98	5.26	34.56	34.91
Treated sample (0.25%)	58.11	4.95	24.15	9.88	58.30	4.20	26.70	9.29	57.70	4.26	28.10	9.82
Treated sample (0.50%)	60.41	5.20	23.70	7.98	61.40	4.92	26.20	6.24	59.10	4.89	27.70	8.29
Treated sample (0.75%)	61.50	5.61	23.10	7.35	61.95	5.10	25.90	5.76	60.56	5.02	27.13	6.86