

Studies on protection and preservation of wood archaeological furniture against mould fungi

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SUMMARY This work aimed to isolation and identification of fungi associated with some deteriorated wood artifacts, studying some factors affecting growth of some selected fungi under laboratory conditions and their abilities to colonize the sycamore wood. To investigate the natural decay in term of loss in wood dry weight under conditions of different museum locations using different kinds of woods. Some factors affecting ability of these fungi to produce the cellulolytic enzymes were also investigated in addition to evaluate some consolidatives alone or mixed with some chemical or natural antifungal substances to wood conservations. The most important results could be summarized as follow: 1)- Isolation trials from ten archeological wooden samples showing different types of deterioration and discoloration symptoms found at 10 different museums resulted in isolation of 37 fungal isolates including 16 fungal species belong to 12 genera. 2)- *Aspergillus niger* showed the highest isolation frequency (7 isolates) followed by *A. flavus*, *Fusarium moniliformae* and *Streptomyces* sp. (4 isolates for each); *Chaetomium indicum* (3 isolates); *A. fumigatus*, *Penicillium* sp., *Stachybotrys atra*, *Botryodiplodia theobromae* (2 isolates for each); *Aspergillus sydow*, *Trichoderma harzianum*, *T. album*, *Papulospora* sp., *Mucor racemosus*, *Stemphyllium botryosum* and *Pithomyces chartarum* (1 isolate for each). The three fungi namely *Papulospora* sp., *Stachybotrys atra* and *Chaetomium indicum* were used throughout the present work. Summary 1143) The colored mummy mask at faculty of archaeology museum (sample No.1) was the most contaminated one (yielded 6 fungal species) followed by the wooden object at Tanta museum (sample No.6) which yielded 5 fungal species while the boat in the Egyptian museum at Cairo (sample No.9) was the least contaminated one as it yielded only two fungal species. 4)- Examination with the environmental scanning electron microscope (ESEM) revealed that all tested fungi (*Papulospora* sp., *Stachybotrys atra* and *Chaetomium indicum*) were able to penetrate, colonize and form their fruiting structures on surface of the sycamore wood. 5)- Reduction in wood dry weight was depended greatly on kind of wood and environment at location of museum. Reduction in dry weight of different kinds of woods was ranged between 0.8-4.37% at Menia museum, 0.55-14.81% at Cairo museum, 0.58-6.28% at Tanta museum and 0.79-8.16% at Alexandria museum. Based on percentage of reduction, the different woods could arranged in descending order as follow: The cypress > beech > acacia > oak > pith pine > sycamore > walnut (at el Menia museum), the cypress > beech > acacia > oak > walnut > sycamore > pith pine (at Cairo museum), the sycamore > cypress > pith pine > oak > beech > acacia > walnut woods (at Tanta museum) and the oak > cypress > sycamore > acacia > pith pine > beech > walnut (at Alexandria museum). 6)- Isolation from beech wood kept at Alexandria and Tanta museums yielded *Fusarium* sp, *Botrytis* sp, *Aspergillus niger*, *A. flavus* and *Trichoderma* sp. while, isolation from sycamore wood kept at museum at Cairo yielded *Chaetomium* sp, *Botryodiplodia theobromae*, *Aspergillus niger*, *A. flavus* and *Trichoderma* sp. The same wood kept at El Menia museum produced only *Aspergillus niger* and *A. flavus*. 7)- Czapek's salts medium + powdered cellulose (Czapek's-cellulose) was the best for growing the 3 wood deteriorating fungi tested. Using such medium, the obtained results showed that the urea was the best N-source for *Papulospora* sp., while peptone was the best one for *S. atra* and *Ch. indicum*. All these fungi were completely failed to grow at pH 4, the liner growth, in general, was higher at pH 5.6-6.0 comparing with growth at pH

4.0-5.0 or at pH 6.6-8.6.8)- All tested fungi could not grow at 5°C and 50°C. The optimum temp for growth of *Papulospora* sp., *S. atra* and *Ch. indicum* were 30, 20 and 30°C., respectively. The highest growth was produced at 80.0% R.H. (*Papulospora* sp. and *Ch. indicum*) and at 100.0% R.H. (*S. atra*) while the lowest growth was produced at 14.5% R.H. (*Papulospora* sp. and *S. atra*) and at 100% R.H. (*Ch. indicum*).9)- The growth of the tested wood deteriorating fungi was greatly affected by the surrounded light conditions. The red light, yellow light and darkness conditions resulted in the highest linear growth for *Papulospora* sp., *S. atra* and *Ch. indicum*, respectively.10)- In the reaction mixture, cellobiase and cellulase enzymes showed the highest activity at pH 6.0 (*Papulospora* sp.) whereas theSummary116highest activities for both enzymes, respectively was occurred at pH 6.0 and 5.4 (*S. atra*) and 4.8 and 5.4 (*Ch. indicum*). Activity of both enzymes (*Papulospora* sp. and *Ch. indicum*) and cellulase enzyme only (*S. atra*) was decreased at temp higher or lower than 50°C. whereas activity of cellobiase enzyme (*S. atra*) was increase by elevating temperature up to 60°C.11)— Using Mandels salt medium plus avicel "microcrystalline cellulose", the final pH, in general, was slightly higher in shaken than static cultures of *S. atra* and *Ch. indicum* in particular. Production of cellobiase and cellulase enzymes in both kinds of cultures was higher after 10 days from incubation (*Papulospora* sp.) and after 15 and 20 days for both enzymes, respectively (*Ch. indicum*). However, *S. atra* produces the highest cellobiase after 20, 25 days, and cellulase enzyme after 20 and 10 days in static and shaken cultures, respectively. Production of both enzymes was obviously higher in the shaken culture than the static one (*Papulospora* sp. and *Ch. indicum*) while the opposite was noticed in case of *S. atra*.12)— Applying glucose as carbon source recorded the lowest final pH in growth media, while sawdust of walnut (*S. atra* and *Ch. indicum*) and sawdust of walnut, cypress or sycamore woods (*Papulospora* sp.) recorded the highest pH values. The avicel (*Papulospora* sp.) and sawdust of beech (*S. atra*) were the best C-sources for production of the cellobiase and cellulase enzymes. In case of *Ch. indicum*, glucose and sawdust of walnut wood gave the highest production of both enzymes, respectively.13)- Production of the cellulolytic enzymes was greatly affected by the initial pH values (pH 3-8). Production of cellobiase and cellulase was gradually increased by elevation initial pH up to pH 5 (*Papulospora* sp. and *S. atra*) and up to pH 6 (*Ch. indicum*). Moreover, *S. atra* only failed to produces cellobiase enzyme at 018.14)- The final pH value in the growth medium was successively increased by all tested fungi as their incubation temperature increased from 20 to 30 and 40°C, respectively. Production of both cellobiase and cellulase enzymes was higher at 20°, 30 and 30C for *S. atra*, *Ch. indicum* and *Papulospora* sp., respectively.15 Applying pentachlorophenol (PCP) affected fungal growth and cellulolytic enzymes activities to different extents. The inhibitive effect increased as PCP concentration increased. Growth was completely stopped at 0.02% (*Ch. indicum*) and at 0.05% (*Papulospora* sp. and *S. atra*). Meanwhile, activities of cellulolytic enzymes (cellulase and cellobiase) were completely stopped at 0.01% PCP (*Ch. indicum*) and 0.02% (*S. atra* and *papulospora* sp.).16) All fungicidal treatments tested significantly reduced growth of all tested fungi, the reduction increased as fungicide concentration increased. The fungicides victra, caramba and rizolex-T caused complete inhibition of *Papulospora* sp., *S. atra*Summary118and *Ch. indicum* at 0.5, 0.5 and 2.0ppm, respectively.17)- Growth of *Ch. indicum* and *Papulospora* sp. increased as concentration of propolis (the byproduct of honeybee) increased up to 2% then gradually decreased until complete stop at 5% meanwhile growth of *S. atra* was decreased gradually by elevating its concentration from 1 to 10% and completely stopped at 20%.18)- The tested volatile oils (cumin, clove, lemongrass, and rhubarb) significantly reduced growth of all tested fungi comparing with the control (without oil). The cumin followed by clove oils were the most effective while rhubarb was the least effective in this respect. Ability of all tested fungi to produce cellobiase and cellulase enzymes was completely stopped by using clove oil at 0.063 and 0.125% cone., respectively.19)— All tested plant extracts (Rosslea, cloves, lemongrass, garlic, rhubarb and cumin) whether extracted by water (aqueous) or by acetone and sterilized by filtration or autoclaved significantly reduced growth of tested fungi in comparison with the control (without extract) and reduction increased as their concentration increased. The filtered extracts (whether aqueous or acetonc) were more effective than the autoclaved one against *Papulospora* sp. and *Ch. indicum* while the opposite trend (particularly the aqueous extracts) was noticed against *S. atra*. The aqueous extract of Roselle and

acetic one of clove (whether filtered or autoclaved) at 5% conc., completely suppressed growth of all tested fungi. The filtered aqueousSummary119 extracts of lemongrass, garlic and cumin plants were better than the autoclaved ones for reducing growth of all tested fungi while, the opposite trend was noticed in case of clove and rhubarb extracts.20)- Ability of all tested fungi to produce cellulolytic enzymes (cellobiase and cellulase) was obviously reduced by the autoclaved aqueous Roselle extract used at 1% conc., and reduction was increased as its conc. increased up to 2.5%.21)- Applying paraloid B-72 (3%) or methyl cellulose (1%) to consolidate sycamore wood samples gave the best results for impregnation of the wood cells but not prevent colonization of wood by any of the tested wood decaying fungi (based on the ESEM examination).22)— The reducing, non-reducing and total sugars were obviously higher in the sycamore wood consolidated with paraloid B-72 or methyl cellulose comparing with the non-consolidated wood. Also, sugars content particularly reducing sugars were higher wood consolidated (with paraloid B-72 or methyl cellulose) and inoculated with *Papulospora* sp., *S. atra* or and *Ch. Indicum* in comparison with wood consolidated state alone (without inoculation).23)— Colonization of the sycamore wood by any of the tested fungi, however, was completely prevented or partially decreased by treating wood samples by paraloid B-72 mixed with clove oil (0.125%), crude extract of clove (1g) or PCPSummary120 "pentachlorophenol" (0.15ppm) or methyl cellulose mixed with clove oil (0.125%), Roselle extract (5%) or the fungicide caramba (25ppm) in comparison with the consolidation materials alone. Summary121