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Biological Evaluation of Fungal Deteriorated Archeological Wood (Islamic Period) and the Impact of Using Some Fungicides

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EIGHTY SEVEN fungal isolates were obtained from the surface of biodeteriorated ceiling wood (No.1803, 1539) from the Islamic museum, Cairo, Egypt (Islamic period). Isolates belonging to eight main genera of fungi were identified, in the following frequencies: *Acremonium* 2.3%,, *Alternaria* 11.5%, *Aspergillus* 37.8%, *Botryotrichum* 2.3%,, *Epicoccum* 3.5%,, *Fusarium* 6.9%,, *Penicillium* 29.9% and *Stemphylium* 5.7%. In a series of trials , cellulase production was maximal for all fungal strains when grown on medium containing 4-6 % of wood straw (at pH 4.5-5 after 10-15 days at 30°C, whereas the maximum production of pectinase was attained on medium containing 6% wood straw at pH 4.5-5 after 10-15 days at 30°C -35°C.

Application of the fungicides dichloroxylenol, paracresol and pentachlorophenol are recommended for use at 1000, 500 and 1000 ppm respectively, based on protection of artificially infected wood. Infected wood lost 40.1% of its bending strength, but showed increased density and water absorption compared with non infected wood. The lowest bending strength loss was attained with dichloroxylenol(14.5%) followed by wood treatment with pentachlorophenol or paracresol (34.2%).

Keywords: Wood biodeterioration, Cellulase, Pectinase, Fungicide, Bending strength.

It is well known that wood plays an essential role in man's life and the use of wood has always been considered as a very important part of the history of civilization. The Islamic museum is enriched by having many wooden objects decorated with Islamic ornaments and Arabic writings (Abed-El Razek, 2003). Mourad *et al*. (2009) noted that during the history of civilizations, advanced wood decay is resulted from exposure to various agents over long periods of time. Blanchette (2000) showed that wooden cultural properties are often degraded by microorganisms when moisture, oxygen and other environmental factors are favorable for microbial growth. Archaeological woods recovered from most environments even those that are extreme typically suffer from some form of biodeterioration. Joao *et al*. (2003) screened forty-six fungal strains for pectinase production, all of which were positive for pectinase activity in a cup-

plate assay. *Aspergillus giganteus* produces polygalacturonase (PG) at pH 5.5–6.5 and 55–60°C, and polygalacturonases are pectinolytic enzymes that catalyze the hydrolysis of the plant cell-wall pectin backbone (Danielle & Eleonora, 2010). Harunobu *et al*. (2000) described three kinds of organo- iodine compounds, 3 iodo-2-propynyl butylcarbamate (IPBC), 1-bromo-3-ethoxy carobonyloxy- 1,2-diiodo-1-propene (BECDIP), and 4-chlorophenyl- 3 iodopropargyl formal (CPIP), which are used as anti fungal agents and wood preservatives against *Aspergillus* sp.,while Marc & Evelyne (2007) used a mixture of borax, $Na_2B_4O_7.10H_2O$ and boric acid dissolved in polyethylene glycol (PEG) as a fungicide to treat *the Vasa*- Sweden's famous warship.

Materials and Methods

Samples

Deteriorated valuable ceiling wood (No.1803, 1539) were obtained from the Islamic Museum, Cairo, Egypt. (From Islamic period)

Productive medium

The medium recommended by Ammar *et al*. (1995) was used for production of cellulase and pectinase, whereas the detection for pectinolytic & cellulytic isolates was carried out according to Ammar *et al*. (1998).

Fungicides used

Three commercially available fungicides namely Dichloroxylenol ($C_8H_8Cl_2$), Paracresol ($CH_3C_6H_4OH$) and Pentachlorophenol (C_6Cl_5OH) were applied.

Isolation and microbial cultivation

The biodeteriorated ceiling wood was examined for fungal contents. Their surface was wiped with a sterile wet cotton swab. Then every cotton swab was transferred to an Erlenmeyer conical flask containing 25 ml of sterile tap water and shaken vigorously for 10 min. One ml of this suspension was transferred to the surface of agar solidified Czapek's medium at 28-30°C for 7days.

Identification

Identification of the fungal isolates was accomplished based on colonial characters of the pure culture, microscopic characters and by measuring the dimensions of informative characters using a computerized Carl Zeiss microscope Axioplane 2 and the specific program Axio Vision 4.7. Measurements were compared to those available in identification references (Traute *et al.* 1980 and Alexopoulos *et al*.,1985).

Examination of the biodeteriorated wood ceiling samples using environmental scanning electron microscopy

The collected samples were investigated using an environmental scanning electron microscope (ESEM) Fei quanta 200 in the Center of Research and Conservation of Antiquities, to determine the major forms of microbial decay.

Fermentation process

Fermentation was carried out in 250 ml conical flasks. Each flask contained 100 ml of productive medium (Ammar *et al.*, 1995). The flasks were sterilized at 121° C for 15min then inoculated with 2ml of standard inoculum of each isolate. The inoculated flasks were incubated at 28-30° C for variable time . At the end of an incubation period, the liquid cultures were centrifuged at 3000 rpm for 15 min. At the end of each incubation period, the supernatant was used to determine the levels of cellulase and pectinase activity according to Ammar *et al*. (1998).

Effect of environmental conditions on the cellulase and pectinase production by the selected fungi

Wood concentration

The productive medium (recommended by Ammar *et al*., 1995) was prepared and supplemented with six different concentrations of wood straw (same type as the wood ceiling , *Ficus sycamores* and *Pinus pinea*) ranging between 2 to 12gm /100ml liquid medium at 2 gm intervals.

Incubation period

Incubation was performed for 5, 10, 15, 20 and 25 days for each fungal isolates.

Initial pH

The most potent fungal isolates were inoculated on the productive medium adjusted to pH values at 0.5 intervals ranging from 3 to 6 .

Incubation temperature

The most potent fungal isolates were allowed to grow on the productive medium which incubated at different temperature ranged from 20 to 45°C with 5°C interval.

Determination of minimal inhibitory concentration (MIC) of some fungicides

MIC evaluations were carried out according to Brantner *et al*. (1993).

The impact of using some fungicides on artificially infected wood

Undeteriorated ancient wood was covered with a base layer of animal glue, a carrying layer of calcium carbonate and then with colored layer (elements of coloration layers) to simulate the layers found in biodeteriorated wood samples. It divided into four equal parts then inoculated with 5 ml of mixture spore-suspension of the 18 fungal strains (~ 0.25 X 10^7 spores/ml) for incubation at 28-30˚C under 50-60 % humidity till the fungal growth developed. Optimal MIC of tested fungicide was sprayed on the surface of the infected wood excluding the control region, then incubated as previously mentioned and record any change could be appears.

Wood properties

Density, water absorption and bending strength of the tested wood, were measured according to Wangaard (1981) and Regis (1999).

Results and Discussion

Screening and identification

Eighty seven fungal isolates were obtained from archeological wood, and then subjected to preliminary identification depending on their morphology according to Traute *et al.* (1980) and Alexopoulos *et al.* (1985). The incidence, as a percentage of the fungal strains detected is presented in Fig. 1 . Most commonly found were species in the genus *Aspergillus* (37.8%) followed by *Penicillium* sp. and *Alternaria* sp. at 29.9% and 11.5%, respectively. Five genera, *Fusarium, Stemphylium, Epicoccum, Acremonium,* and *Botryotrichum* accounted for 6.9%, 5.7%, 3.5%, 2.3%, and 2.3%, respectively of the total fungal isolates. These results are in line with those obtained by Abed-El Hameed (1999) who isolated *P. chrysogenun, A. terreus, A. niger, A. flavus, A. versicolor, Mortioella* sp. *Alternaria alternata and Cladosporium herbarum* from wooden coffins in an Egyptian museum .Similarly, Lokma (1999) isolated *Aspergillus niger, Asp. flavus, Asp. sulphorous, Asp. sydowii, Asp. versicolor, Alternaria alternata, Cladosporium herbarum, Acremonium* sp., *Epicoccum* sp. and *Fusarium solani* from some wooden statues in an Egyptian museum.

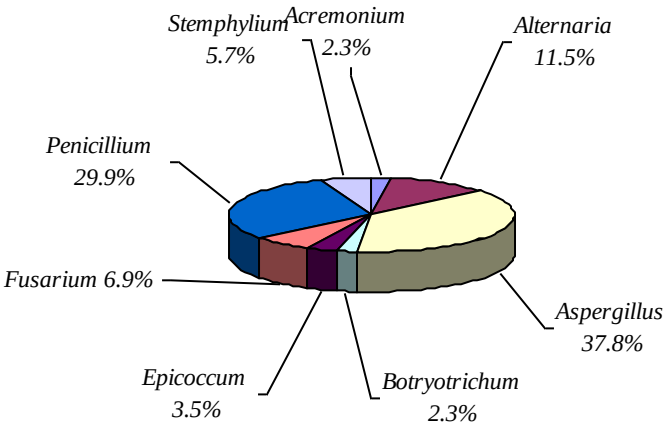
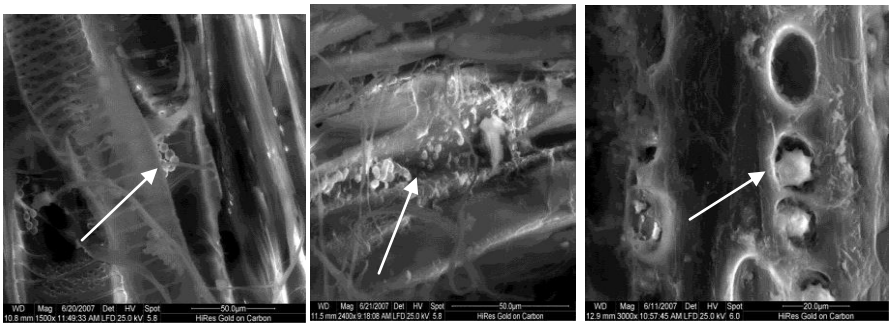


Fig. 1. The incidence percentage of fungal genera isolated from the deteriorated wood No. 1803 and No. 1539 in Islamic museum .

Investigation of biodeteriorated wood ceiling sample using environmental scanning electron microscopy (ESEM)

Growth characteristics of the fungi on biodeteriorated wood samples and the type of degradative system result in different decay patterns as clearly demonstrated via environmental scanning electron microscopy (Fig.2, A & B). The mycelial hypha as well as the conidiophores and conidia spores can be seen attached to the plant cell walls. These figures also show decay of the inner layers of xylem cell walls, separation of longitudinal fibers and dense fungal mycelia. Conidia located inside xylem pits and degradation occurring in the margins are visible in Fig 2-C.



A B C

Fig. 2. Scanning electron micrograph showing fungal filaments, conidiospores and damage in xylem cell walls.

Capability of the isolated fungal strains for cellulase and pectinase production using cup plate technique

Data recorded in Table 1 indicated that strains of eight fungal species, namely *Acremonium strictum, Alternaria geophila, Aspergillus sydowii, Botryotrichum piluliferum, Epicoccum purpurascens, Fusarium mersimoides, Penicillium rubrum* and *Stemphylium botryosum* were the most potent strains for production of cellulose. Strains of *Acremonium strictum, Alternaria geophila, Aspergillus sydowii, Botryotrichum piluliferum, Epicoccum purpurascens, Fusarium mersimoides, Penicillium chrysogenum and Stemphylium botryosum* were most potent for production of pectinase for each genus .Consequently, these strains were selected for further studies.

Effect of some environmental conditions on the cellulase production by the most potent producers fungal strains

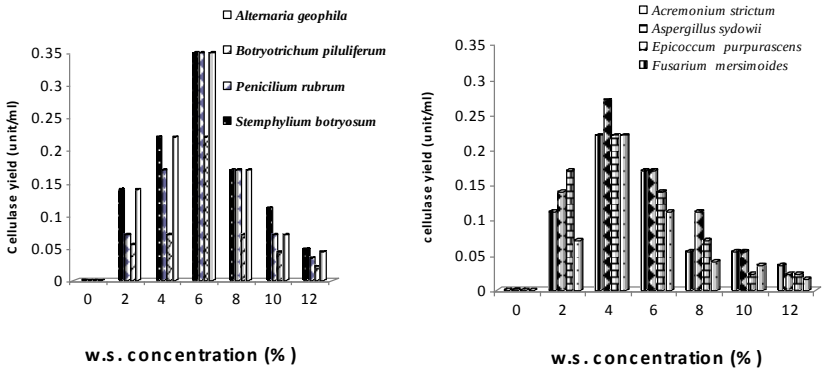
Substrate concentration

The highest yields of cellulase were detected in the presence of 4% and 6% wood straw (w.s) (Fig 3). Optimizing wood straw concentration enhanced the enzyme yield to a range between 0.22-0.35 unit/ml after 7 days at 28-30°C . These results are similar to data obtained by El-Refaie (2005) who found that the maximum cellulase productivity by *Chaetomium* sp. was attained in the presence of 6 % of wood straw.

TABLE 1. Capability of the isolated fungal strains for cellulase and pectinase production using cup plate technique.

Tested organism	Cellulases yield (units/ml)	Tested organism	Pectinase yield (units/ml)
Acremonium strictum	0.111±0.016	*Acremonium strictum*	953±1.73
Alternaria alternata	0.17 ±0.011	*Alternaria alternata*	1901±0.58
Alternaria geophila	0.22 ±0.017	*Alternaria geophila*	2394±4.62
Alternaria tenuissima	0.17 ±0.006	*Alternara tenuissima*	953±1.73
Aspergillus carneus	0.44 ±0.23	*Aspergillus carneus*	601±0.58
Aspergillus niger	0.27±0.017	*Aspergillus niger*	477±13.3
Aspergillus sydowii	0.55±0.029	*Aspergillus sydowii*	953±1.73
Aspergillus terreus	0.111 ±0.011	*Aspergillus terrus*	315±2.88
Aspergillus versicolor	0.111 ±0.0	*Aspergillus versicolor*	315±2.31
Botryotrichum piluliferum	0.35 ±0.029	*Botryotrichum piluliferum*	601±1.73
Epicoccum purpurascens	0.27±0.017	*Epicoccum purpurascens*	301±0.0
Fusarium mersimoides	0.44±0.023	*Fusarium mersimoides*	601±2.31
Penicillium chrysogenum	0.35±0.006	*Penicilium chrysogenum*	1200±5.77
Penicillium islandicum	0.111±0.017	*Penicilium islandicum*	315±5.77
Penicillium lanosum	0.14± 0.023	*Penicilium lanosum*	601±5.77
Penicillium rubrum	0.44± 0.0	*Penicilium rubrum*	315±2.88
Penicillium rugulosum	0.22± 0.017	*Penicilium rugulosum*	953±1.73
Stemphylium botryosum	0.27± 0.017	*Stemphylium botryosum*	315±2.88

Results are expressed as the means ± standard error of three replicates.



(A) **(B)**

Fig. 3. Effect of different wood straw concentrations on cellulase production by the most potent fungal strains (A &B) for 7 days at 28- 30 °C

Incubation period

Strains of *Acremonium strictum, Aspergillus sydowii, Epicoccum purpurascens* and *Fusarium mersimoides* attained the highest yield of cellulase which ranged between 0.17-0.27 unit/ml in the presence of 4% wood straw (w.s.) at 28-30ºC for 10 day *Penicilliun rubrum* gave the lowest activity in the presence of 65 wood strar (w.s.) after 15 day (Fig. 4-8). These results are in agreement with El-Refaie (2005) who (Fig. 4-A). *Alternaria geophila* was precede as a cellulase producer whereas found that *Chaetomium gloosbum* gives the highest yield of cellulase enzyme

after 15 days while, Immanuel & Akila (2007) found the highest yield of the enzyme after 5 days of growth by *Aspergillus niger* & *Aspergillus fumigatus*.

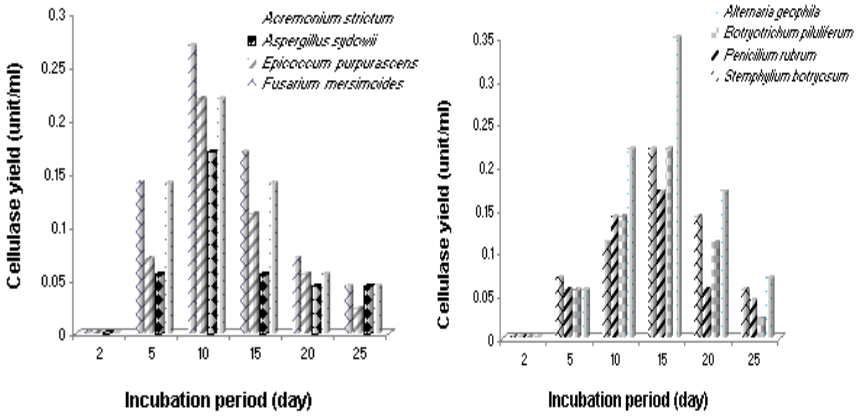


Fig. 4. Effect of different incubation periods on cellulase production by the selected fungal strains on medium containing 4 % w.s. / 28-30°C (A) & 6% w.s. / 28-30°C (B) .

Initial pH value

All the tested fungi gave the highest yield of cellulase at pH 4.5 on medium containing 4% wood straw (w.s.) after 10 day (Fig. 5-A). These results match those of Immanuel & Akila (2007) who also found that *Aspergillus* sp. gave the highest yield of enzyme at pH 4.5. From Fig (5-B) it can be concluded that the optimum pH value capable of promoting cellulase biosynthesis by all the tested fungal strains was 5 on medium containing 6% wood straw (w.s.) after 15 days at 28-30°C. These results are in line with Coral *et al.* (2002) who found an optimum of pH 5 for *Alternaria* sp.

Incubation temperature

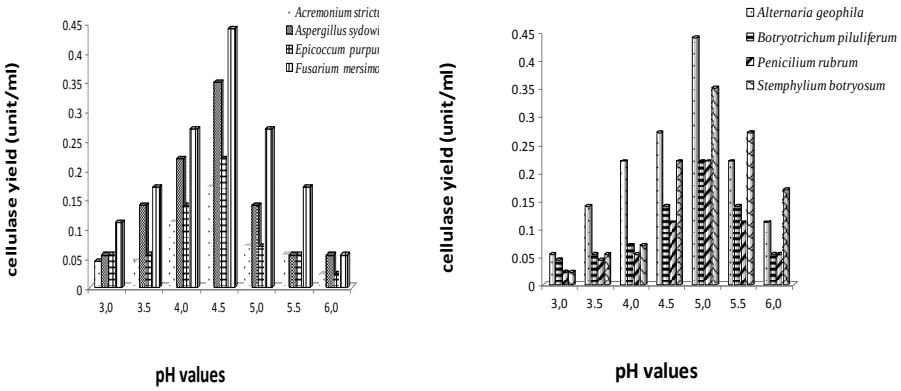
The optimal incubation temperature was 30ºC either in the presence of 4% wood straw (w.s.) at pH 4.5 for 10 days or 6% wood straw (w.s.) at pH 5 for 15 days Fig (6. A&B). These results are in line with El-Hawary *et al*. (2001) who found that the highest enzyme activities were obtained from *Aspergillus niger* at 30°C. On the contrary, Danielle & Eleonora (2010) found that optimal temperature for activity of cellulase was ranged from 50°C to 55°C by *Aspergillus giganteus.*

Effect of some environmental conditions on pectinase production by the most potent fungal strains

Substrate concentration

The fungal strains were able to produce the highest yield of pectinase when grown in media containing 6 % wood straw (w.s.) . The maximum was 5002 unit/ml attained by *Aspergillus sydowii* while the lowest at 1901unit/ml was detected for

Botryotrichum piluliferum and *Penicillium chrysogenum* after 7days (Fig 7-A&B). These results differ from those of El-Refaie (2005) who found that the maximum pectinase productivity by *Chaetomium* sp. was attained in the presence of 4% of wood straw.



A **B**

Fig. 5. Effect of different pH values on cellulase production by the selected fungal strains on medium containing 4 % w.s. for 10 days / 28-30°C (A) & 6 % w.s. for 15 days / 28-30°C (B) .

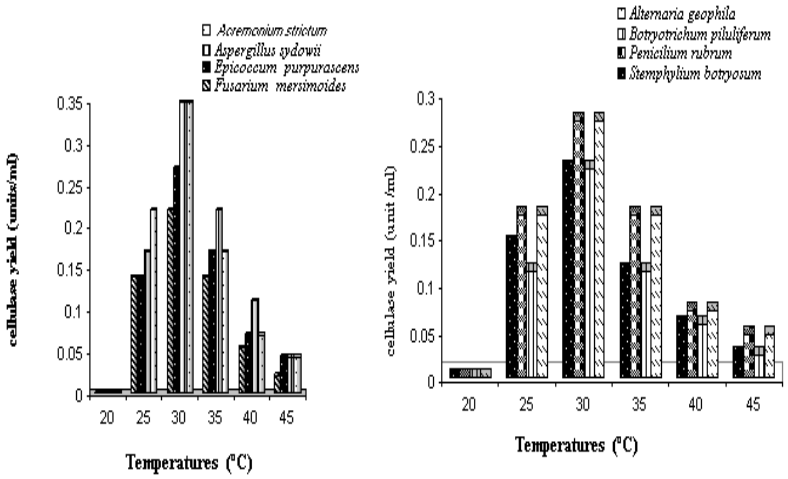
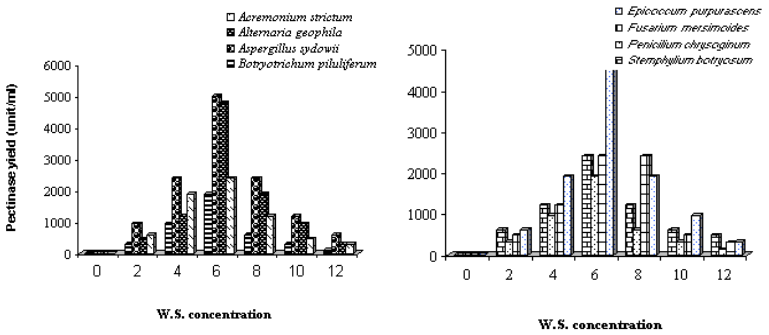


Fig. 6. Effect of different incubation temperatures on cellulase production by the selected fungal strains on medium containing 4 % w.s. at pH 4.5 for 10 days (A) & 6 % w.s. at pH 5 for 15 days (B) .

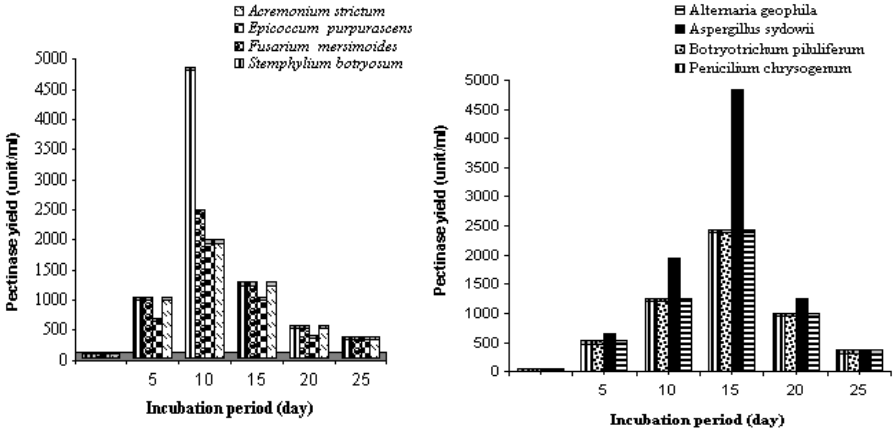


A **B**

Fig. 7. Effect of different wood straw concentrations on pectinase production by the most potent fungal strains (A&B) for 7 days at 28-30 ºC

Incubation period

The maximum pectinase yields of 1901, 1901, 2394, 4777 unit/ml were attained by *Acremonium strictum, Epicoccum, purpurascens, Fusarium mersimoides* and *Stemphylium botryosum* respectively after 10 days. The fungal strains which produced the highest enzyme after 15 days were *Alternaria geophila, Aspergillus sydowii, Botryotrichum piluliferum* and *Penicillium chrysogenum* (Fig. 8- A&B). These results are in agreement with El-Refaie (2005) who found that the *Alternaria geophila* produced the highest yield of pectinase after 15 days.



A **B**

Fig. 8. Effect of different incubation periods on pectinase production by the selected fungal strains (A, B) on medium containing 6 % w.s. at 28-30°C .

Initial pH value

The optimal pH value capable of promoting enzyme biosynthesis by *Acremonium strictum, Epicoccum purpurascens, Fusarium mersimoides* and *Stemphylium botryosum* was found to be pH 4.5 for 10 days. The other strains (*Alternaria geophila, Aspergillus sydowii, Botryotrichum piluliferum* and *Penicillium chrysogenum)* gave the maximum yield of enzyme at pH 5 after 15 days (Fig 9- A & B). These results are in agreement with Yogesh *et al.*(2009) who found that *Aspergillus niger* produced high pectinase at pH 3.8 -5.

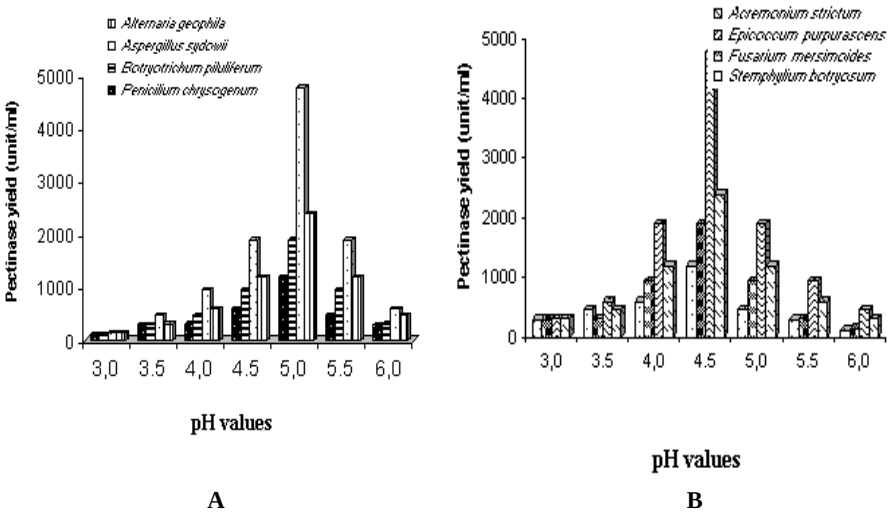


Fig. 9. Effect of different pH values on pectinase production by the selected fungal strains on medium containing 6 % w.s. / 15 days at 28-30°C (A) & 6 % w.s. / 10 days at 28-30°C (B) .

Incubation temperature

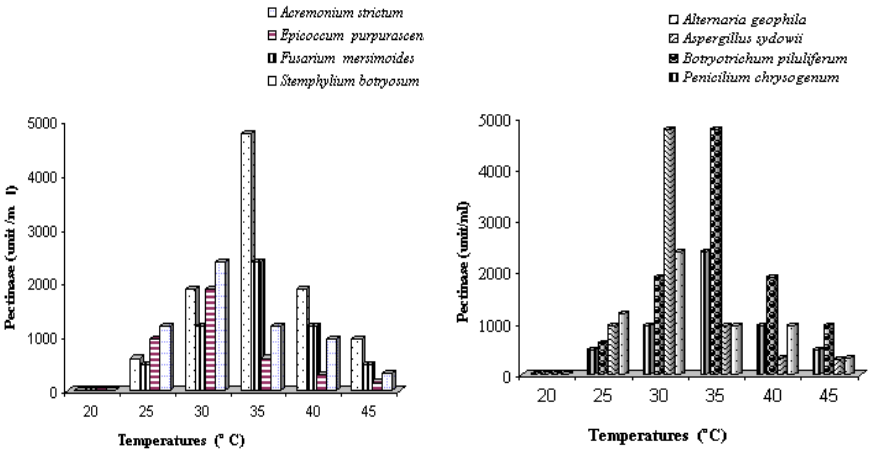
Results revealed that 30ºC at pH 4.5 was the optimal temperature for pectinase produced by *Acremonium strictum* and *Epicoccum purpurascens* whereas it was 35ºC for *Fusarium mersimoides* and *Stemphylium botryosum* at the same pH and 10 days incubation period. Pectinase production by *Alternaria geophila, Aspergillus sydowii, Botryotrichum piluliferum* and *Penicillium chrysogenum* was maximized at 30 ºC & 35ºC (Fig. 10- A&B). Yogesh *et al* (2009) also found that *A. niger* gave high activity of pectinase at 30ºC.

The proper concentration of some fungicides against the fungal strains

Three compounds namely dichloroxylenol (125- 2000 ppm), paracresol (125 - 2000 ppm) and pentachlorophenol (250 - 2000 ppm) were tested for effectiveness as fungicides against 18 fungal strains. The optimal concentration for use of each as a fungicide is recorded in Table 2. Applications of dichloroxylenol, paracresol and pentachlorophenol at 1000, 500 and 1000 ppm, respectively , killed all of the tested fungi. Shash & Arya (1999) and Regis (1999) stated that periodical fungal spraying of pentachlorophenol was useful in preventing fungal attack as it penetrates deeply.

TABLE 2. The proper fungicide concentration (ppm) used for each fungal strain

Fungal strains	Dichloroxylenol		Paracresol		Pentachlorophenol	
	Inh. Zone (mm.	ppm	Inh. Zone (mm.)	ppm	Inh. Zone (mm.)	ppm
Acremonium strictum	26	125	22	500	30	500
Alternaria alternata	23	1000	19	500	35	1000
Alternaria geophila	25	125	25	125	18	250
Alternaria tenuissima	20	125	20	500	20	500
Aspergillus carneus	23	125	18	500	31	1000
Aspergillus niger	33	1000	25	500	25	500
Aspergillus sydowii	23	500	23	500	23	500
Aspergillus terrus	20	1000	18	500	20	1000
Aspergillus versicolor	24	1000	20	500	20	500
Botryotrichum piluliferum	24	125	24	125	40	1000
Epicoccum purpurascens	22	125	23	500	35	1000
Fusarium mersimoides	26	125	26	125	22	1000
Penicillium chrysogenum	28	125	22	500	22	500
Penicillium islandicum	26	1000	22	250	22	250
Penicillium lanosum	23	500	23	500	30	100
Penicillium rubrum	20	500	20	500	20	500
Penicillium rugulosum	22	500	22	500	22	500
Stemphylium botryosum	21	500	21	500	21	500



A **B**

Fig. 10. Effect of different incubation temperatures on pectinase production by selected fungal strains on medium containing 6 % w.s. at pH 4.5 for 10 days (A) & 6 % w.s. at pH 5 for 15 days (B) .

Impact of some fungicides on the artificial infected wood

Figures 11-13 summarize results of the procedural steps which carried out on artificially infected wood. No fungal growth could be detected on the surface of inoculated wood samples till ~ 8 months after fungicides treatment at 28˚-30˚C under 50-60 % humidity when compared to the untreated part (Fig. 13)

Fig. 11. Wood samples divided into four parts and sprayed with a mixed spore suspension (18 fungal strains) .

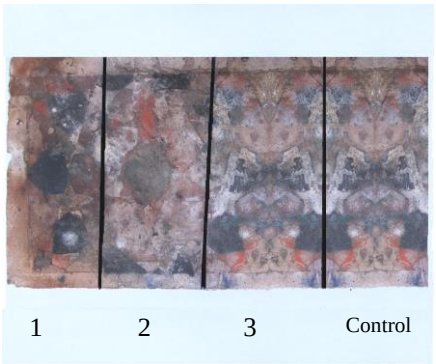


Fig. 12. Fungal growth appears on the surface after 60 days at room temperature (before fungicides treated) .

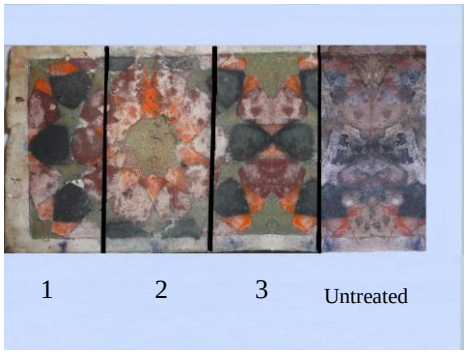


Fig. 13. The infected inoculated wood after 8 months of fungicides treated .

Wood properties

Data presented in Table 3 recorded standard physical properties of wood , including density, water absorption and bending strength measured before and after infection and with or without pretreatment with dichloroxylenol, pentachlorophenol or paracresol. The infected wood resulted in a loss of 40.1% of bending strength. Higher density and water absorpation were detected as compared with non-infected wood. Regarding the effect of wood treatment the data indicated that the lowest bending strength loss was attained with dichloroxylenol (14.5%) followed by wood treatment with pentachlorophenol or paracresol (34.2%). From the previous data, it could be concluded that the wood treatment with di-chloroxylenol was best in improving the physical properties of the infected wood.

TABLE 3. Physical properties of wood sample (before and after infected and treated with fungicides).

Test	Non infected wood	Infected wood	Treated wood		
			With dichlorox-ylenol	With pentachloro-phynol	With Paracresol
Bending strength (MPa) (megapascal)	15.2	9.1	13	10	10
Water absorption (%)	65.1	68.8	65	67	67
Density (gm/cm^3)	0.35	0.40	0.37	0.40	0.40

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التقييم الحيوي لتدهور الأخشاب الأثرية (عصر اسلامي) بالفطريات وتأثير أستخدام بعض المبيدات الفطرية

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قسم الترميم- المتحف الأسلامي **ومركز بحوث وصيانة الآثار- القاهرة- مصر .

تم عزل ٨٧ عزلة فطرية من سطح سقف خشبى مصاب رقم (١٥٣٩ ، ١٨٠٣) بالمتحف الإسلامى القاهرة – مصر – (العصر الاسلامي) وقد تم تصنيف العزلات الفطرية إلى ثمانية أجناس هى: *Acremonium* (2.3%) & *Alternaria* (11.5%) & *Aspergillus* (37.8%) & *Botryotrichum* (2.3%) & *Epicoccum* (3.5%) & *Fusarium* (6.9%) & *Stemphylium* (29.9%) and *Penicillium* (5.7%) . جميع العزلات الفطرية أعطت أعلى انتاجية من انزيم السليوليز على بيئة تحتوى على ٤ – ٦ % من نشارة الخشب عند pH ٤,٥ – ٥ بعد ١٠ – ١٥ يوم / ٣٠° م ، بينما أعلى انتاجية من البكتينيز على بيئة تحتوى على ٦% من نشارة الخشب عند pH ٤,٥ – ٥ بعد ١٠ – ١٥ يوم / ٣٠ – ٣٥° م٠ تم أستخدام دايكلوروزيلينول ، الباراكريزول ، البنتاكلوروفينول كمبيدات فطرية بالتركيزات ١٠٠٠ ، ٥٠٠ ، ١٠٠٠ جزء فى المليون على التوالى لحماية عينة الخشب المصاب . فقد فقدت عينة الخشب المصاب ٤٠,١% من قوة انحنائه ، وكذلك تأثرت كثافته ودرجة إمتصاصه للماء مقارنة بعينة الخشب الغير مصاب . وكان أقل فقد فى قوة الانحناء (١٤,٥%) عند أستخدام المبيد الفطري دايكلوروزيلينول ثم يلية الباراكريزول والبنتاكلوروفينول (٣٤,٢%).