MICROBIAL STUDIES TO EVALUATE BIODETERIORATION OF OIL PAINTING AND ITS PREVENTION

Abed el Hamid, H .♦♦♦ Darwish, S.S. ♦♦ Bahaa, A.♦

Abstract

Microbiological samples were taken from deteriorated oil paintings exhibited in various locations in addition to private possessions. The results showed that eight fungal and four bacterial species were isolated from these objects. These isolates were studied to measure their ability in degrading cellulose, gelatin, linseed oil and the varnish dammar. They were also tested for their potentiality to produce extracellular cellulases and proteases enzymes. The effect of different concentrations of benlate, coside 101 and some natural oils such as cloves, jojoba and garlic oils on the radial growth of the tested fungi and bacteria was studied. The results showed that all the tested biocides had a negative effect on the growth of the tested isolates and a single biocide is sufficient to kill a number of fungal species effectively.

Key words: oil painting, biodeterioration, fungi, bacteria, cellulases, proteases, natural oils.

1-Introduction

The development of micro-fungi and bacteria on the surface of paintings induces aesthetical, mechanical and biochemical decay. In fact, the growing mycelium spread over the paints, masking design and color, while the growth of hyphae and fruiting bodies inside the

♦♦♦ General Organization for Culture Palaces, Ministry of Culture.
♦♦ Conservation Dept., Faculty of Archaeology, Cairo University.
♦Conservation Dept., Faculty of Archaeology, Cairo University.

support can cause friability and loss of the paint layer. Exoenzyme activities can cause more serious damage by decomposition of some polymers both of the paint layer and of the support, whereas the presence of colored fruiting bodies and the production of colored metabolites provokes the formation of permanent stained patches while organic acids produces irreparably modifications in the structure ([1]). The control of microbial growth over the monuments is not an easy task. Both indirect and direct methods are used to achieve this aim. The indirect methods are those that aim at inhibiting the growth of fungi and bacteria by modifying the microclimatic conditions around the substrate. On the other hand, the direct methods are those which eliminate the existing population of microorganisms by employing suitable biocides ([2]). In order to evolve a suitable conservation programme, it becomes imperative to identify various organisms growing over different oil painting as well as find suitable biocides for their treatment ([3]) and ([4]).

The present research reports the common microorganisms associated with deteriorated valuable oil painting and suitable biocides to prevent or slow down the biodeterioration of these oil paintings.

2-Materials and Methods

2.1- Objects

Objects used in this study, their locations and state of deterioration were shown in Table (1).

[1] **Tiano, P.**, Biodegradation of cultural heritage: Decay mechanisms and control methods" 9th ARIADNE Workshop "Historic Material and their Diagnostic", ARCCHIP, Prague, 22 to 28 April 2002. http://www.arcchip.cz/w09/w09_tiano.pdf.

[2] **Mishra A. K., Garg K., L.**: Microbiological deterioration of stone an over view 3rd Int. symp. On restoration and conservation of monuments, Hyderabad, India ,1995, Pp.217-228

[3] **-Garg, K. L.; Mishra, A. K.; Ajay and Kamal, K., Jain**, Biodeterioration of cultural heritage some case studies3rd Int. symp. On restoration and conservation of monuments, Hyderabad, India, 1995, pp.1-7.

[4] **-Bahadur, A.K**.: Biodeterioration and its control in open-air historical monuments In: Conservation of cultural property in India, Vol. 30, 1997, p. 175-181.

2.2- Collection of samples

Microbiological samples were collected and taken from areas of several objects which showed particularly dense microbial growths or where they appeared to be associated with decay (table 1).

2.3-Chemicals

All the chemicals used are of analytical grade. Glucose / peroxidase kit was from Stan Bio Laboratory, INC.°2930 East Houston Street° San Antonion Texas 78202 USA. Benlate was a product of DU PONT de Nemours and Co. Inc.Switzerland. Natural oils and coside 101 were supplied from National Research Centre, Cairo, Egypt.

2.4- Isolation and purification

Samples were collected from various objects as shown in table (1) and plated out on nutrient agar(NA) for bacteria and potato dextrose agar (PDA) for fungi ([5]). Incubation was carried out at 37^0C for 24 hr (for bacteria) and at room temperature for 72 hr for isolation of fungi. During the incubation period, any emerged microorganism was isolated on to PDA and NA slants. Fungi and bacteria were purified by using the single spore technique of Manandhar ([6]).

2.5- Identification

The identification of fungal isolates were carried out on the basis of their macro and microscopically characteristic sporulation according to the keys of Gilman ([7]); Nelson, et al. ([8]) and Barnett &Hunter ([9]), while that of bacteria were carried out by studying their physiological and biochemical properties according to the keys

[1]**Nwachukwu,S.C.U.and Akpata,T.V.I.**,Isolation of microorganisms by spread plate technique. In: Principles of quantitative microbiology. University of Lagos Press, 2003, pp.3-6.

[6]**Manandhar, J. B.; Hartman, G. L. and T. C. Wang,** Conidial germination and appressorial formation of *Colletotrichum capsici* and C. gloeosporioides isolates from pepper. Pl. Dis. Reptr., 1995, 79: 361-366.

[7]**Gilman , J.C .,** A manual of soil Fungi , 2nd Ed . The Iowa state university press, Ames .Iowa , U.S.A., x+450.(1957) , pp., 14 , 153.

[8] **Nelson, PE ,** Toussoun , T.A.and W.F.O Marasaa :Fusarium spp.Anillustrated manual for identification, the Pennsylvania Univ ,1982, press. Univ., park.

[9] **Brnett , H.L. and Hunter, B.B . ,** I llustrated genera of imperfect fungi . 4th.Ed . Macmillin New York, 1986.

of Cowan and Steal´s ([10]) and Peterh, et al. ([11]). The frequency occurrence of each species was expressed as the percentage of samples containing a given organism.

2.6- Selection of cellulose, gelatin, linseed oil and dammer degrading microorganisms

Fungal and bacterial isolates were studied to measure their ability in degrading cellulose of the support, gelatin of animal glue, linseed oil of the pigment binder and the varnish dammar by using cellulose, gelatin, linseed oil and dammar as carbon sources. Czapek-Dox agar medium ([12]) was used for this purpose. Only sucrose was substituted by the above mentioned carbon sources.

2.7- Growth medium and Substances

Mandels and Weber's medium ([13]) was prepared. It contained (g/L): 1.4 $(NH_4)_2$ SO_4; 2.0 KH_2PO_4; 0.3 urea; 3.0 $CaCl_2$; 0.3 $MgSO_4.7H_2O$; 0.005 $FeSO_4$; 0.0014 $ZnSO_4$; 0.0016 $MnSO_4$; 0.002 $COCl_2$; protease peptone (1%); tween 80 (0.1%) and avicel (1%) with final pH of 5.0. The medium was supplemented individually by cellulose and gelatin as carbon sources instead of avicel.

2.8-Growth in liquid culture

250 ml Erlenmeyer flasks containing 50 ml Mandels and Weber's medium with the adequate carbon source were inoculated by 5% (v/v) of inoculum of the tested fungi and bacteria. The flasks were then incubated at 28^{o}C for 15days. After the incubation period, the content of each flask was filtered. Culture filtrates were subjected to enzyme assay.

2.9-Enzyme assay

2.9.1-Carboxymethyl cellulase (CMC-ase){C_x β(1-4) gluconase} was assayed by following the release of free glucose from

[10] **Cowan and Steal´s ,** Manual for the Identification of Medical Bacteria, Cambridtion – University , press. 3 rd edn , 1974 , p.8

[11] **Peterh, H. A. Sneath ; Nicolas, S . Mair ; M . Elisabeth Sharph and John, G. Holt**, , Bergey´s Manual of systematic Bacteriologe Volume 2 1986 . P.1122-1123

[12] **Riker, A.J. and Riker, R.S.** Introduction to research on plant disease. John, S. Swift, Go. Ino., New York, 1936.

[13] **Mandeles, M. and Weber, J. ,** Adv. Chem. Ser. **,** 1969, 95, 391

carboxymethyl cellulose as a substrate. The activity was defined and measured according to the method of Mandels and Weber ([14]) where the resulting sugars were determined by Somogyi Reagent ([15]) using glucose as standard.

2.9.2-Cellobiase (β- glucosidase) was assayed according to the method of Berghem and Petterson ([16]) using cellobiose as a substrate. The activity of the enzyme was determined by measuring the concentration of the released glucose by using glucose/peroxidase kit according to Teller method ([17]).

2.9.3-Protease activity was measured by some modifications of Keay and Wildi method ([18]). The reaction mixture consisted of 1.0 ml enzyme solution preincubated at 37^{o}C for 5 min. The reaction was started by the addition of 1.0 ml casein 2.0% (w/v), pH 7.0. The reaction mixture was then incubated at 37^{o}C for 10 min and terminated by the addition of 2.0 ml of 0.4 M trichloro acetic acid. This mixture was further incubated at 37^{o}C for 20 min, followed by centrifugation at 13.000Xg for 10 min. To 1.0 ml supernatant, 5.0 ml of 0.4 M Na_2CO_3 and 1.0 ml folin ciocalteau reagent: water (1: 3, v/v) were added to yield a blue colour. The coloured mixture was incubated in an incubator at 37^{o}C for 20 min before the absorbance was read at 666 nm using BAUCH LOMB spectronic 2000 spectrophotometer. A blank was prepared by the same procedure, the trichloro acetic acid being added at zero time and the casein after 10 min incubation. One unit of protease is equivalent to 0.5 µg tyrosine liberated by 1.0 ml enzyme solution under the assay conditions. The amount of tyrosine was determined from tyrosine standard curve.

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[14]**Mandeles, M. and Weber** , 1969, Op.cit.,95-391

[15]**Somogi, N.**, J. Biol. Chem., 1952, 195, 19.

[16]**Berghem, L. E. R. and Petterson, L. G. ,** The mechanism of enzymatic cellulose degradation, Purification of cellulolytic fungi on highly ordered cellulose, Eur. J. Biochem., 1974 ,46: 295-305, Biol. Abst.,

[17]**Teller, J. D. ,** Abstr. 130th . Meeting, Am. Chem. Soc., 1956, P 69 c

[18] **Keay, L. and Wildi, B.C.,** Proteases of genus *Bacillus*. I. Neutral proteases. Biotechnol. Bioeng., XII: 1970,179-212.

2.10- Laboratory studies of biocide efficacy:

The Effect of biocides on the tested fungi and bacteria was studied using agar plates. A disc (5mm) from a pure culture of each of the tested fungi was placed on the center of (PDA) plates containing different concentrations of the biocide, while in the case of bacteria the different concentrations of the biocide were placed in a hole in the center of inoculated (NA) plates. (0.0, 0.5, 2.0, 5.0, 10.0 and 15.0 ppm) for benlate and coside 101, (0.0, 0.125, 0.25, 0.5, 1.0 and 2.5% v/v) for cloves, jojoba and garlic oils were used for this purpose. Petri plates were incubated at 27± 2ºC and the two diameters of every plate were measured. Four replicates were used for each particular treatment.

2.11- Statistical analysis:

Data obtained were subjected to analysis of variance according to procedures obtained by Snedcor and Cochran ([19]).

3-Results and discussion:

3.1- Survey of microorganisms associated with deteriorated oil painting

The results in tables (2&3) showed that without exception all the screened oil paintings were of a highly microbial polluted. These results indicated that there are shortages in precautions and in the maintenance of these paintings. High relative humidity, inadequate air movement, and darkness provide an almost ideal environment for the cultivation of bacterial and fungal spores, microbiological deterioration, and insect attack ([20]).

About 388 representative fungal isolates in addition to 35 bacterial isolates developed on agar media were isolated from different deteriorated oil paintings. The results in table (2) showed that the frequent occurrences of bacterial species ranged from 20% for

[19]**Snedecor, G. W. and W. G. Cochran,** Statistical method 7th. Iowa State Univ. Press. Ames**,**1980, 57 (4): 22254.

[20]**Hino, H., et al**, The Ultrastructure of Bacterial Spores in the Skin of an Egyptian Mummy. Acta Pathologica et Microbiologica Scandinavica, Section B, Microbiology (Denmark), 1982, 90 (1): 2124.

Bacillus stearothermophilus to 28.57% for Bacillus acidocalayius and Bacillus Subtilis. While in table (3), the frequent occurrences of fungal species ranged from 3.87% for Aspergillus terrus to 29.64% for Aspergillus niger. These results agreed with those recorded in the literature on biodeterioration of wall and easel painting (21) (22) ([23]) ([24]) ([25]) ([26]) ([27]) ([28]) ([29]) and ([30]).

3.2 - Selection of cellulose, gelatin, linseed oil and dammer degrading microorganisms

Table (4) represents data on the activities of various representative fungal and bacterial isolates. The degree of decomposition of

[21] **Grant, C., I.C. Wright., W.R.** Springle and M. Greenhalgh: Collaborative investigation of laboratory test methods for evaluation of the growth of pink yeasts on paint films. Int. Biodeterior. Biodegrad., 32, 279-288 (1993).

[22]**Garg, K.L.; Kamal, K.J. and Mishera, A.K.,** Role of fungi in the deterioration of wall paintings. Science of the Total Environment, May 1995, vol. 167, no. 1-3, p. 255-271.

[23]**Cieerri, O.** Microbial degradation of paintings. Applied and Environmental Microbiology, March 1999, vol. 65, no. 3, p. 879-885.

[24]**Arai, H.,** Foxing caused by fungi: twenty-five years of study. International Biodeterioration & Biodegradation, October 2000, vol. 46, no. 3, p. 181-188.

[25]**Gonzalez, J.M. and C. Saiz-Jimenez,** Application of molecular nucleic acid-based techniques for the study of microbial communities in monuments and art works. Int. Microbiol., 8, 189-194 (2005).

[26]**Domenech-Carbo, M. T.; Osete-Cortina, L.; De La Cruz-Canizares, J.; Bolivar-Galino, F.; Romero-Noguera, J.; Fernandez-Vivas, M. A. and Martin-Sanchez, Inés.** Study of the microbiodegradation of terpenoid resin-based varnishes from easel painting using pyrolysis-gas chromatography-mass spectrometry and gas chromatography-mass spectrometry. Analytical and Bioanalytical Chemistry, August 2006, vol. 385, no. 7, p. 1265-1280.

[27] **Imperi,F.,G.Caneva, L.Cancellieri,M.A.Ricci,A.Sodo and P.Visca,**The bacterial aetiology of rosy discolouration of ancient wall paintings.J. Environ. Microbiol., 11, 146-292 (2007).

[28]**Romero-Noguera, J.; Bolivar-GaliIano, Fernando C.; Ramos-Lopez, J.M.; Fernandez-Vivas, María Antonia and Martin-Sanchez, Inés.** Study of biodeterioration of diterpenic varnishes used in art painting: Colophony and Venetian turpentine. International Biodeterioration & Biodegradation, December 2008, vol. 62, no. 4, p. 427-433.

[29]**Obidi,O.F.; Aboaba,O.O. Makanjuola , M.S. and Nwachukwu, S.C.U**., Microbial evaluation and deterioration of paints and paint-products, Journal of Environmental Biology, 2009, 30(5) 835-840

[30]**Romero-Noguera, J.; Martin-Sanchez, I.; Ramos-Lopez, J.M. and Bolivar-Galiano, F.,** Biodeterioration patterns found in dammar resin used as art material. Electronic Journal of Biotechnology, 2010, vol 13, no. 3.

cellulose, gelatin, linseed oil and dammar differed considerably between microorganisms.

3.2.1- Growth on Cellulose-Czapek's agar medium:

Data in Table (4) showed that *Aspergillus niger* gave maximum growth on solid cellulose medium. Similar results were obtained by other researchers ([31]) ([32]) ([33]) ([34]) ([35]) ([36]) and ([37]) who proved that the tested microorganisms are good cellulose decomposers.

3.2.2- Growth on gelatin -Czapek's agar medium:

The results in Table (4) also showed that *Penicillium chrysogenum* had high ability to decompose gelatin while among the tested bacterial species, *Bacillus megaterium* was effective in its ability to biodegrade gelatin. These results completely agreed with that obtained by Abrusci,et al. ([38]).

3.2.3- Growth on linseed oil -Czapek's agar medium:

The maximum growth on linseed oil -Czapek's agar medium was obtained when *Penicillium chrysogenum* and *Aspergillus niger*

[31]**Domsch, K.H.; Gams, W. and Anderson, T.H. Eds (1993).** Compendium of Soil Fungi, Vol. 1,IHW-Verlag, Eching, Germany. Ed. by Basem Samir El-Sharkawy. Supreme Council of Antiquities Press, (Book review), 2009,pp. 53-63.

[32]**Tuomela, M.; Vikman, M.; Hatakka, A. and Itvaara, M.,** Biodegradation of lignin in acompost environment: A review. Bioresour. Technol., 2000,72 (2): 169-183.

[33]**Darwish, S. S.,** Biological studies on some cellulolytic microorganism isolated from old paper manuscripts. Ph D. thesis**, 2001**, Chemistry Dept., Fac. Of science, Cairo Univ., Egypt.

[34] **Anastasi,A.;Varese,G.C.;Voyron, S.; Scannerini, S.and Marchisio, V.F. ,** Systematic and functional characterization of fungal biodiversity in compost and vermicompost. In: Michel F.C., Rynk R.F., Hoitink H.A.J., Eds, Proceedings of the 2002 International Symposium "Composting and Compost Utilization". The JG Press Inc., Emmaus, 2002, pp. 171-182.

[35] **Rocha,M.;Cordeiro,N.,Cunha Queda,A.C.F.and Capela,R.,**Microbiological and chemical characterization during composting of cattle manure and forestry wastes – a study in Madeira Island. In: Michel F.C., Rynk R.F., Hoitink H.A.J., Eds, Proceedingsof the 2002 International Symposium "Composting and Compost Utilization". The JGPress Inc., Emmaus, pp. 156-170.

[36]**Ryckeboer, J. ; Mergaert, J. ; Vaes, K. ; Klammer, S. ; De Clercq,D. ; Coosemans, J. ; Insam, H. and Swings, J.,** A survey of bacteria and fungi occurring during composting and self-heating processes, Ann. Microbiol. , 2003, 53(4), 349- 410.

[37]**Afandy, A. H. ; El-Sonbaty, A.I. and Darwish, S. S. ,** Conservation of old Yemeni books, The horizon; studies in Egyptology in honour of M.A. Nur El-Din (10-12 April 2007); v.3

[38]**Abrusci, C.; Marquina,D. ; Del Amo,A . and Catalina, F.,** Biodegradation of cinematographic gelatin emulsion by bacteria and filamentous fungi using indirect impedance technique,International Biodeterioration & Biodegradation (2007), doi:10.1016/j.ibiod.2007.01.005

were used (table 4). Similar results were achieved by Ramakrishnan and Banerjee ([39]); Kamini, et al. ([40]); Mahadik, et al. ([41]) and Chaturvedi, et al. ([42]).

3.2.4- Growth on dammar -Czapek's agar medium:

The results in Table (4) also showed that all the tested *Aspergillus genus* and *Penicillium chrysogenum* had a good ability to degrade dammar with maximum growth in case of *Aspergillus fumigates*. Many researchers ([43]) ([44]) & ([45]) studied the role of fungi and bacteria in biodeterioration of dammar.

3.3- Enzyme assay:

The results in table (5) showed that the entire tested microorganisms produced various amount of extracellular enzymes (carboxymethyl cellulase, cellobiase and protease) after 15 days. The value of enzyme production depended on the microbial species. The ability of these microorganisms to decompose cellulose and protein was confirmed by other researchers Fagan & Fergus ([46]); Diaz- Ravina, et al ([47]); Botha, et al, ([48]); Domsch, et al. ([49]);

[39]**Ramakrishnan, C.V., Banerjee, B.N.,** Studies on mold lipase – comparative study of lipases obtained from molds grown on Sesamum indicum. Arch Biochem. 1952, 37, 131–135

[40]**Kamini ,N.R.; Mala, J.G.S. and Puvanakrishnan, R., 1998,** Lipase production from *Aspergillus niger* by solid state fermentation using gingelly oil cake. Process Biochem 33(5):505–11

[41]**Mahadik, N.D.;** Puntambekar, U.S.; Bastawde, K.B.; Khire, J.M. and Gokhale, D.V. 2002, Production of acidic lipase by *Aspergillus niger* in solid state fermentation. Process Biochem 38(5):715–21

[42]**Chaturvedi ,M.; Singh ,M.; Rishi, C. M. and Rahul, K.** , Isolation of Lipase Producing Bacteria from Oil Contaminated Soil for the Production of Lipase by Solid State Fermentation using Coconut Oil Cake , International Journal of Biotechnology and Biochemistry, Volume 6 Number 4 , 2010, pp. 585–594

[43] **Domenech-Carbo, et al.,** 2006, op.cit.,

[44]**Romero-Noguera, et al.,** 2008, op.cit.,

[45]**Romero-Noguera, et al.,** 2010, op.cit.,

[46]**Fagan, S.M. and Fergus, C.L.,** Extracellular enzymes of some additional fungi associated with mushroom culture. Mycopathologia, 1984**,** 87: 67-70.

[47] **Diaz-Ravina, M.; Acea, M.J. and Carballas, T.,** Microbiological characterisation of four composted urban refuses. Biol. Wastes, 1989**,** 30: 89-100.

[48] **Botha, W.J.; Eicker, A. and De Meillon, S.,** A comparative physiological study of four weed moulds of *Agaricus bisporus* mushroom compost. Phytophylactica, 1990, 22: 219- 223.

[49]**Domsch, et al.,** 1993, op.cit.

Atkinson, et al. ([50]); Anastasi, et al.([51]); Ryckeboer, et al, ([52]) and Afandy,et al, ([53]).

3.3.1- Carboxymethyl cellulase (CMC-ase):

All the tested microorganisms produced extracellular CMC-ase by different values with maximum amount (0.10 U/ml) for *Aspergillus fumigatus* and minimum (0.02U/ml) for *Bacillus megaterium* as shown in table 5. Many authors {([54]); ([55]); ([56]); ([57]) and ([58])} concluded that all the tested microorganisms had the ability to decompose cellulosic materials and produce cellulases enzymes.

3.3.2- Cellobiase enzyme:

The results in table (5) showed that the highest extracellular cellobiase activity (0.72 U/ml) was obtained from the culture filtrate of *Aspergillus flavus* while minimum amount (0.055 U/ml) was obtained from *Bacillus acidocalayius*. The tested fungi and bacteria were proved to produce cellobiase enzyme by many authors {([59]); ([60]) and ([61])}.

3.3.3- Protease enzyme:

The results showed that *Bacillus acidocalayius* is a good producer of protease enzyme with maximum value (2.9 U/ml). On contrast,

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[50] **Atkinson, C.F.; Jones, D.D. and Gauthier, J. J. (1996b).** Biodegradabilities and microbial activities during composting of oxidation ditch sludge. Compost Sci. Util., 4(1): 84-96.

[51] **Anastasi, et al.,** 2002, Op. Cit.

[52] **Ryckeboer, et al., 2003**, Op.Cit.

[53] **Afandy, et al., 2009,** Op. Cit.

[54] **Domsch,et al, 1993,** Op. Cit.

[55] **Tuomela, et al., 2000,** Op.Cit.

[56] **Rocha,et al, 2002**, Op. Cit.

[57] **Helal, G. A.,** Bioconversion of Straw Into Improved Fodder: Mycoprotein Production and Cellulolytic Acivity of Rice Straw Decomposing Fungi, Mycobiology 33(2) (2005), 90-96 Copyright © 2005 by The Korean Society of Mycology90

[58] **Afandy, et al., 2009,** Op. Cit.

[59] **De Vries, R. P. and Visse,r J.,** *Aspergillus* Enzymes Involved in Degradation of Plant Cell Wall Polysaccharides ,Microbiol Mol Biol Rev. 2001 December; 65(4): 497–522.

[60] **Darwish, S.S. & Sahab, A.F.,** Indoor Airborne Moulds In An Archaeological Museum And Deterioration Of Egyptian Mummy, International Conference on Chemistry, Green and Sustainable Chemistry In Developing Countries, Chem05, 3-5 March, 2008, Faculty of Science, Chemistry Dept., Cairo University.

[61] **Afandy, et al., 2009,** Op. Cit.

Alternaria alternata showed feeble protease activity (0.03 U/ml). Similar results were obtained by Rao, et al. ([62]) and Salleh, et al. ([63]) who proved that microorganisms belonging to the genus *Bacillus* produce most of the commercial proteases enzymes, mainly neutral and alkaline proteases.

3.4- Laboratory biocide efficacy evaluation

This experiment was undertaken to determine the effect of the tested biocides as agar amendment on the inhibition of microbial growth of the isolated fungi and bacteria. Data in Tables (6, 7, 8, 9 &10) indicated that the antimicrobial activity of a biocide against growth of the tested microorganisms was significantly increased as the concentration of the biocide was increased.

3.4.1-Effect of Benlate[64] & Coside 101[65] on the radial growth of tested microorganisms

The results in tables (6&7) showed that 5 ppm of benlate or coside 101 was sufficient to stop growth of all the tested fungi except in case of benlate, *Alternaria alternata* and *Aspergillus terrus* were inhibited at 10ppm and *Aspergillus clavatus* at 15ppm. The recommended dosage of Benlate shouldn't exceed 5 parts per thousand (0.5% concentration). At this concentration, it is toxic to fungi but not toxic to mammals and less phytotoxic ([66]). Whereas, in case of Coside 101 the growth of *Aspergillus terrus* and *Aspergillus niger* was inhibited at 10ppm. The inhibitory effect of these fungicides was proved by other researchers {Darwish ([67]) and

[62]**-Rao, M.B.; Thanksale, A. M.; Ghatge, M.S.and Deshbande, P.P.,** Molecular and biotechnological aspects of microbial proteases, Microbiology and molecular biology, Reviews, 1998(62)3,597-635.

[63]**Salleh, A.B.; Abdul Rahman, N.Z.R. and Basri, M.,** New lipases and proteasea, Nova Science Publishers, 2006,NewYork.

[64] The chemical name of benlate is methyl [1-[(butylamino) carbonyl]-1H-benzimidazol-2-yl]carbamate)

[65]Active Ingredient of Coside 101 is 77.0% (w/w) Copper Hydroxide (Metallic Copper Equivalent 50%) + Inert Ingredients 23.0% (w/w)

[66]**Lever, B.G.,** Crop Protection Chemicals. Ellis Harwood, New York , 1990, p. 192.

[67]**Darwish, 2001,** OP.Cit.

Eziashi, et al. ([68])}. While in case of bacteria the concentration up to 15 ppm couldn't completely inhibit growth of the tested bacteria.

3.4.2-Effect of garlic[69], jojoba[70] and cloves[71] oils on the radial growth of tested microorganisms

The results in table (8) showed that 0.25% of cloves oil was sufficient to inhibit the growth of all tested fungi except *Aspergillus fumigatus , Aspergillus terrus* and *Penicillium chrysogenum* whose growths were stopped at 0.5%. While, the results in table (9) proved that jojoba oil could stop the growth of *Aspergillus niger* and *Penicillium chrysogenum* at 0.5%; *Aspergillus flavus, Aspergillus fumigates* and *Penicillium corylophilum* at 1%; *Alternaria alternata, Aspergillus clavatus* and *Aspergillus terrus* at 2.5%. 0.5% of garlic oil inhibited the growth of all tested fungi except *Alternaria alternate and Aspergillus niger* whose growths were stopped at 0.25% and 1% respectively (table 10). The inhibitory effect of these substances on fungi was studied by other workers {([72]); ([73]); ([74]); ([75]); ([76]) and ([77])}.

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[68]**Eziashi, E. I.; Odigie, E. E. and Airede, C. E.,** Evaluation of crude leaf extracts and benlate solution treatments on oil palm sprouted seeds for the control of *Ceratocystis paradoxa* causing black seed rot, Journal of Medicinal Plants Research, July 2010,Vol. 4(13), pp. 1285-1288, 4.

[69]The main component of clove oil is eugenol (2-Methoxy-4-(2-propenyl) phenol)

[70]Over 97% of jojoba is composed of an array of liquid wax esters, with a combination of mixed tocopherols, free sterols and other unsaponifiables making up the balance

[71]The volatile oil contains allicin (diallyldisulfide-*S*-oxide; diallyl thiosulfinate), allylpropyl disulfide, diallyl disulfide, and diallyl trisulfide as the major components, with lesser amounts of dimethyl sulfide, dimethyl disulfide; dimethyl trisulfide, allylmethyl sulfide, 2,3,4-trithiapentane,bis-2-propenyl tri-, tetra-, and pentasulfides, and other related sulfur compounds.

[72]**Appleton, J. and Tansey, M.,** Inhibition of growth of Zoopathogenic fungi by garlic extract, Mycol, 1975, 67: 882-887.

[73]**Elnima, E. I., Ahmed, S. A., Mekkawi, A. J. and Mossa, J. S.,** The antimicrobial activity of garlic and onion extracts pharmazie,, 1983,38 (11): 747 – 748.

[74]**Bilgrami,K.S.,Sinha,K.K.and Sinha,A.K.**Inhibition of affatoxin production and growth of *Aspergillus flavus* by eugenol, onion and garlic extracts. Indian J. Med. Res.**,**1992,96:171–175.

[75]**Fan, J. J. and Chen, J. H.,** Inhibition of affatoxin – producing fungi by welsh onion extracts, J. food prot. **,** 1999, 62 (4): 414 – 417.

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The results also showed that all the tested oils couldn't completely inhibit the bacterial growth.

4-Conclusion

This study demonstrates the common microorganisms associated with valuable oil paintings, their role in deteriorating various layers of the painting as well as the negative effect of some biocides on the tested fungal and bacterial growth. The results showed that a single treatment of the biocides was sufficient to kill a number of fungal species effectively. A regular cleaning and periodical biocidal spraying will help in saving these paintings from biodeterioration in addition to keep storage and display areas at a constant 50% RH and 20°C, with light levels for display at 50 lux, ultraviolet light at less than 75 μw/lumen and air filtration to 95% of outside levels of pollutants. In practice, however, these levels are rarely consistently achieved and realistic alternatives are often chosen, such as the creation of micro-climate frames or conditioned showcases. In warm humid museums, a glass box, if well built, is efficient in creating a safe microclimate and protecting exhibited paintings from microbial deterioration ([78]).

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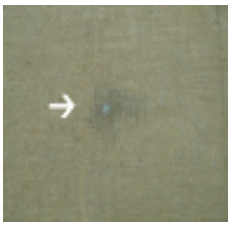
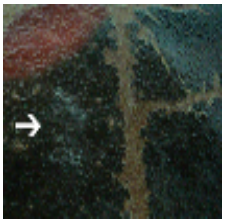
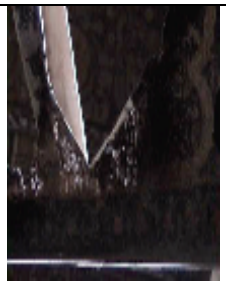
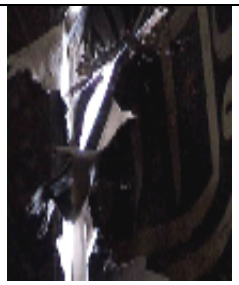
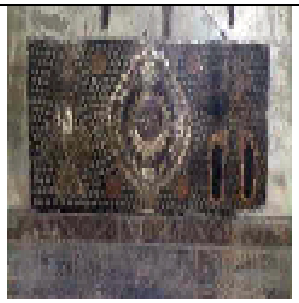
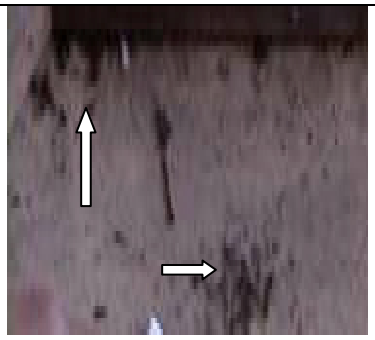
Table (1): Objects used in this study, their locations and state of deterioration

Object no.	Object	location	State of microbial deterioration
1	 **Photo1a:** Oil painting Portrait (no.32/ 4) of Khediwe Ismail made by Liecchi in 1875	El-Gezira museum – Dar El Opera el Masria	  **Photo 1b** **Photo 1c** Microbial growth appeared on the both sides of the portrait
2	 **Photo 2a:** Portrait no. 84 made in 19th century	Hall no. 5 El Manial palace museum - Cairo	 **Photo 2b:** Microbial growth appeared on the paint layer. Most of these growths were removed during periodical cleaning
3	 **Photo 3a:** Portrait of Mohamed Ali Basha made in 19th century	The throne hall- el Manial palace museum - Cairo	  **Photo 3b** **Photo 3c** Microbial growth affected the paint layer and can be seen on the back side of the support

4	 **Photo 4a:** Portrait no. 32 or 34 of the mother of Mohamed Ali Basha made in 19th century	Stores of el Manial palace museum - Cairo	 **Photo 4b:** Dense microbial growth appeared on the paint layer. The original canvas support was highly deteriorated and was treated by adding a new one. Biodeterioration of the lining paste appeared clearly at the back side of the support.
5	 **Photo 5a:** Portrait no. 29 of Khediwe Ismail made in 19th century	Stores of el Manial palace museum - Cairo	  **Photo 5b** **Photo5c** The painting suffered from many aspects of deterioration such as cracking, losing the whole face of the portrait, losing some parts of the paint layer in addition to microbial growth on the wooden support.
6	 **Photo 6a:** Oil painting of a natural view	Stores of el Manial palace museum - Cairo	  **Photo 6b** **Photo 6c** This painting was transferred to el Manial palace after the fire of Jawhara Palace – Salah El Deen Citadel. Yellowish and greenish microbial growth appeared on the back side of the support in addition to cracking and losing some parts of the paint surface.

7	 **Photo 7a:** Canvas wall painting. This painting was applied directly on the ceiling lacking the ground layer	Museum of Jawhara Palace – Alexandria	 **Photo 7b:** Dense greenish microbial growth appeared at the edges of the painting
8	 **Photo 8a:** Paper oil painting. This painting was applied directly on paper support without any preparation layer. The paper support was laid on another canvas one	El Sadat mosque – El Emam-El Shafaey-Cairo	  **Photo8b** **Photo 8c** The painting suffered from cutting and losing a large part of it. Microbial growth appeared on the paint surface.
9	 **Photo 9a:** Paper oil painting. The painting was made on paper which was laid on a canvas. It suffered from losing the lower part of it	El Sadat mosque – El Emam-El Shafaey-Cairo	 **Photo 9b:** Microbial growth appeared on the surface of the paint layer.

10	 **Photo 10a:** Caliphs Panel painting. This painting was applied on wood panel whose thickness 12mm	El Sadat mosque – El Emam-El Shafaey-Cairo	  **Photo 10b:**Dense microbial growths were shown on the both sides of the painting
11	 **Photo 11a:** Canvas oil painting. It was made directly on canvas support without a preparation layer.	Engy Aflaton museum – 15 May-Helwan	 **Photo 11b:** Dense microbial growth appeared clearly on the back side of the canvas.
12	 **Photo 12a:** The Nile and the sand oil painting. made by Engy Aflaton	Engy Aflaton museum – 15 May-Helwan	 **Photo 12b:** There are some microbial growth on the paint surface

13	 **Photo 13a:** Canvas oil painting. This painting was made by the Tahia Haleem	Private possession	  **Photo 13b** **Photo 13c** Biological samples were taken from the paint surface and the canvas
14	 **Photo 14a:** Canvas oil painting. This painting was made by Raghib Aiad	Private possession	 **Photo 14b:**Biological samples were taken from the back side of the support
15	 **Photo15a:** Canvas oil painting. This painting was made by Mahmoud said	Private possession	 **Photo 15b:** Microbial growth affected the paint layer
16	 **Photo 16a:**Canvas oil painting	Private possession	 **Photo 16b:** Biological samples were taken from the back side of the support

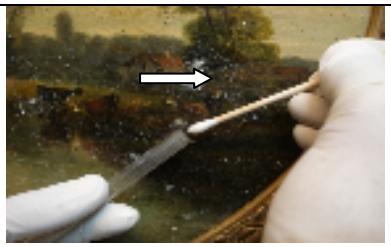
17	 **Photo 17a:** Europe's countryside Canvas oil painting	Private possession	 **Photo 17b:** Microbial growth affected the paint surface

Table (2): occurrence and frequency of bacteria isolated from various oil painting

Object no.	Bacterial species				Total no. of isolates	Frequency %
	Bacillus megaterium	***Bacillus subtilis***	***Bacillus stearothermophilus***	***Bacillus acidocalayius***		
1	--	--	1	1	2	**5.71**
2	--	--	--	--	--	--
3	2	2	--	--	4	11.43
4	**1**	1	**--**	--	2	**5.71**
5	**1**	**1**	**1**	**1**	**4**	11.43
6	**--**	**1**	**1**	**1**	**3**	**8.57**
7	**--**	**--**	**1**	**--**	**1**	**2.86**
8	**--**	**1**	**--**	**--**	**1**	**2.86**
9	**--**	**--**	**--**	**--**	**--**	**--**
10	**--**	**--**	**--**	**--**	**--**	**--**
11	**--**	**--**	**1**	**3**	**4**	**11.43**
12	**--**	**--**	**2**	**2**	**4**	**11.43**
13	**--**	**--**	**--**	**2**	**2**	**5.71**
14	**1**	**1**	**--**	**--**	**2**	**5.71**
15	**2**	**3**	**--**	**--**	**5**	**14.29**
16	**1**	**--**	**--**	**--**	**1**	**2.86**
17	**--**	**--**	**--**	**--**	**--**	**--**
Total no. of isolates	**8**	**10**	**7**	**10**	**35**	
Frequency %	**22.86**	**28.57**	**20**	**28.57**		**100**

Table (3): occurrence and frequency of fungi isolated from various oil painting

Object no.	Fungal species								Total no. of isolates	Frequency %
	Alternaria alternata	*Aspergillus clavatus*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus niger*	*Aspergillus terrus*	*Penicilliium chrysogenum*	*Penicilliium corylophilum*		
1	3	2	5	3	4	--	2	1	20	5.15
2	5	4	6	3	5	1	4	2	30	7.73
3	4	2	4	4	13	--	4	9	40	10.3
4	2	2	32	2	22	14	1	5	80	20.62
5	2	1	4	1	4	--	5	1	18	4.64
6	5	5	4	--	19	--	--	12	45	11.60
7	4	--	--	--	3	--	2	3	12	3.09
8	--	2	5	--	4	--	--	2	13	3.35
9	--	--	--	--	9	--	--	7	16	4.12
10	--	4	4	--	4	--	--	7	19	4.89
11	--	3	--	3	1	--	5	1	13	3.35
12	3	1	3	1	3	--	2	1	14	3.61
13	4	--	2	--	5	--	--	--	11	2.84
14	--	1	4	1	6	--	3	--	15	3.86
15		5		1	6	--	3	3	18	4.64
16	5	--	--	--	2	--	--	3	10	2.58
17	--	--	7	--	5	--	--	2	14	3.61
Total no. of isolates	37	32	80	19	115	15	31	59	388	
Frequency %	9.53	8.25	20.62	4.90	29.64	3.87	7.99	15.21		

Table (4): In-vitro growth of different microbial isolates associated with deteriorated oil painting of various locations using cellulose, gelatin, linseed oil and dammar.

microbial isolates	Linear growth on cellulose		Linear growth on gelatin		Linear growth on linseed oil		Linear growth on dammar	
	(mm)	Densities	(mm)	Densities	(mm)	Densities	(mm)	Densities
Alternaria Alternata	48**	+3**	24	+2	18	+1	19	+1
Aspergillus clavatus	22	+2	24	+3	44	+3	67	+2
Aspergillus flavus	19	+1	25	+3	29	+2	82	+3
Aspergillus fumigatus	26	+1	47	+1	56	+3	90	+3
Aspergillus niger	58	+4	41	+2	73	+3	46	+3
Aspergillus terrus	0.0	0.0	30	+1	50	+3	61	+2
Penicillium chrysogenum	10	+1	52	+2	73	+3	66	+3
Penicillium Corylophilum	32	+2	33	+4	65	+3	15	+1
Bacillus acidocalayius	12	+2	9	+3	8	+2	7	+1
Bacillus megaterium	0.0	0.0	29	+1	7	+1	0.0	0.0
Bacillus stearothermophilus	29	+2	12	+3	25	+2	55	+3
Bacillus Subtilis	10	+1	21	+1	25	+1	0.0	0.0
L.S.D. at 5%	3.130		0.868		5.530		3.205	

*Each figure represents average diameter in (mm) of 4 replicates incubated at 27 ±2 °C for 6 days

**+4 = vigorous growth +3 = heavy growth +2 = moderate growth

+1= weak growth 0= no growth

Table (5): Extracellular CMC-ase, cellobiase and protease enzymes production secreted from tested fungi and bacteria

Organism	Dry weight gm/50 ml	Final pH	CMC-ase U/ml	Cellobiase U/ml	Protease U/ml
Alternaria alternata	1.421	6.1	0.056	0.220	0.03
Aspergillus clavatus	1.307	6.5	0.085	0.100	0.23
Aspergillus flavus	1.323	6.2	0.030	0.720	0.04
Aspergillus fumigatus	1.415	6.8	0.100	0.140	0.16
Aspergillus niger	1.354	6.0	0.075	0.065	0.98
Aspergillus terrus	1.238	6.3	0.055	0.090	0.54
Penicillium chrysogenum	1.549	5.9	0.075	0.080	0.21
Penicillium corylophilum	1.235	5.6	0.085	0.085	0.73
Bacillus acidocalayius	1.212	8.2	0.033	0.055	2.90
Bacillus megaterium	1.123	8.3	0.020	0.064	1.73
Bacillus stearothermophilus	1.233	8.0	0.042	0.062	2.51
Bacillus subtilis	1.207	8.2	0.220	0.073	1.10

Table (6): **Effect of different concentrations of benlate on the radial growth of isolated microbial species associated with deteriorated oil paintings**

benlate conc. ppm / organism	0.0	0.5	2.0	5.0	10.0	15.0
	Radial growth (mm) for fungi / Inhibition Zone (mm) for bacteria					
Alternaria alternate	90	26	23	8	0.0	0.0
Aspergillus clavatus	90	66	51	32	13	0.0
Aspergillus flavus	90	22	12	0.0	0.0	0.0
Aspergillus fumigatus	90	18	11	0.0	0.0	0.0
Aspergillus niger	90	9	7	0.0	0.0	0.0
Aspergillus terrus	90	18	8	6	0.0	0.0
Penicillium corylophilum	90	19	14	0.0	0.0	0.0
Penicillium chrysogenum	90	30	14	0.0	0.0	0.0
L. S. D. at 50 %	Conc. = 2.98 Fungi = 3.22 Interaction = 7.89					
Bacillus acidocalayius	0.0	0.0	12	14	21	32
Bacillus megaterium	0.0	0.0	16	24	37	42
Bacillus stearothermophilus	0.0	0.0	0.0	12	15	20
Bacillus subtilis	0.0	0.0	0.0	19	26	34
L. S. D. at 50 %	Conc. = 1.187 Bacteria = 0.683 Interaction = 1.68					

Table (7): Effect of different concentrations of coside 101 on the radial growth of isolated microbial species associated with deteriorated oil paintings.

Coside 101 conc. ppm / Organism	0.0	0.5	2.0	5.0	10.0	15.0
	Radial growth (mm) for fungi / Inhibition Zone (mm) for bacteria					
Alternaria alternata	90	33	12	0.0	0.0	0.0
Aspergillus clavatus	90	26	9	0.0	0.0	0.0
Aspergillus flavus	90	23	14	0.0	0.0	0.0
Aspergillus fumigatus	90	17	12	0.0	0.0	0.0
Aspergillus niger	90	37	21	7	0.0	0.0
Aspergillus terrus	90	29	11	6	0.0	0.0
Penicillium corylophilum	90	11	0.0	0.0	0.0	0.0
Penicillium chrysogenum	90	17	8	0.0	0.0	0.0
L. S. D. at 50 %	Conc. = 0.574 Fungi = 0.621 Interaction = 1.524					
Bacillus acidocalayius	0.0	9	21	30	32	36
Bacillus megaterium	0.0	12	28	36	41	49
Bacillus stearothermophilus	0.0	12	33	40	46	48
Bacillus subtilis	0.0	8	18	29	37	42
L. S. D. at 50 %	Conc. = 1.17 Bacteria = 2.04 Interaction =2.88					

Table (8): Effect of different concentrations of cloves oil on the radial growth of isolated microbial species associated with deteriorated oil paintings

Cloves conc. % / Organism	0.00	0.125	0.25	0.50	1.00	2.50
	Radial growth (mm) for fungi / Inhibition Zone (mm) for bacteria					
Alternaria alternata	90	12	0.0	0.0	0.0	0.0
Aspergillus clavatus	90	26	0.0	0.0	0.0	0.0
Aspergillus flavus	90	24	0.0	0.0	0.0	0.0
Aspergillus fumigatus	90	11	8	0.0	0.0	0.0
Aspergillus niger	90	0.0	0.0	0.0	0.0	0.0
Aspergillus terrus	90	29	11	0.0	0.0	0.0
Penicillium corylophilum	90	11	0.0	0.0	0.0	0.0
Penicillium chrysogenum	90	17	8	0.0	0.0	0.0
L. S. D. at 50 %	Conc. = 1.588				Fungi = 1.715	
	Interaction = 4.203					
Bacillus acidocalayius	0.0	13	19	24	31	38
Bacillus megaterium	0.0	14	26	35	39	43
Bacillus stearothermophilus	0.0	8	11	16	23	32
Bacillus subtilis	0.0	12	23	29	36	45
L. S. D. at 50 %	Conc. = 1.194				Bacteria = 0.688	
	Interaction =1.688					

Table (9): **Effect of different concentrations of Jojoba oil on the radial growth of isolated microbial species associated with deteriorated oil paintings**

Jojoba conc. % / organism	0.00	0.125	0.25	0.50	1.00	2.50
	Radial growth (mm) for fungi / Inhibition Zone (mm) for bacteria					
Alternaria alternata	90	32	24	20	14	0.0
Aspergillus clavatus	90	30	21	15	7	0.0
Aspergillus flavus	90	42	27	18	0.0	0.0
Aspergillus fumigatus	90	27	20	12	0.0	0.0
Aspergillus niger	90	20	11	0.0	0.0	0.0
Aspergillus terrus	90	35	26	19	11	0.0
Penicillium corylophilum	90	22	15	6	0	0.0
Penicillium chrysogenum	90	15	7	0	0	0.0
L. S. D. at 50 %	Conc. = 0.574 Fungi = 0.622 Interaction = 1.525					
Bacillus acidocalayius	0.0	8	14	19	27	35
Bacillus megaterium	0.0	11	21	28	33	39
Bacillus stearothermophilus	0.0	6	9	12	15	23
Bacillus subtilis	0.0	8	17	27	36	42
L. S. D. at 50 %	Conc. = 1.176 Bacteria = 0.679 Interaction =1.663					

Table (10): **Effect of different concentrations of garlic oil on the radial growth of isolated microbial species associated with deteriorated oil paintings.**

Garlic conc. % / organism	0.00	0.125	0.25	0.50	1.00	2.50
	Radial growth (mm) for fungi / Inhibition Zone (mm) for bacteria					
Alternaria alternata	90	8	0.0	0.0	0.0	0.0
Aspergillus clavatus	90	12	6	0.0	0.0	0.0
Aspergillus flavus	90	22	11	0.0	0.0	0.0
Aspergillus fumigatus	90	11	6	0.0	0.0	0.0
Aspergillus niger	90	14	9	8	0.0	0.0
Aspergillus terrus	90	10	8	0	0.0	0.0
Penicillium corylophilum	90	12	7	0.0	0.0	0.0
Penicillium chrysogenum	90	21	11	0.0	0.0	0.0
L. S. D. at 50 %	Conc. = 3.02					Fungi = 3.25
	Interaction = 7.98					
Bacillus acidocalayius	0.0	6	8	11	18	22
Bacillus megaterium	0.0	10	18	21	32	39
Bacillus stearothermophilus	0.0	12	17	22	29	35
Bacillus subtilis	0.0	8	13	22	28	34
L. S. D. at 50 %	Conc. = 1.417					Bacteria = 0.817
	Interaction = 2.004					