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The mycobiota associated with paper archives and their potential control

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Abstract. *El-Nagerabi SAF, Elshafie AE, Al-Hinai UA. 2014. The mycobiota associated with paper archives and their potential control. Nusantara Bioscience 6: 19-25.* Historical collections kept in archives and libraries represent a cultural and artistic heritage of innumerable value. Recently in Oman, more than seventy thousand documents were collected from different countries and displayed as archives showed evident sign of mold contamination. The objectives of the present study were to screen these archives for mold invasion and a test for the effective control measure. For this, 102 samples were collected from documents of different sources and incubated on potato dextrose agar (PDA) at ambient temperature (25°C±2). The isolated fungi were identified microscopically and confirmed with DNA extraction, PCR and DNA sequencing. Twenty-two fungal species belonging to 11 genera were recovered. The genus *Penicillium* (46.8%) was the most prevalent, followed by *Aspergillus* (30.7%), *Cladosporium* (7%), *Rhizopus* (4%), and *Chaetomium* (3.5%) whereas the remaining 6 genera represent only 8%. Eleven species were previously reported from similar substrates, whereas 11 species and one genus are new records for the mycoflora of archives. Sodium hypochlorite at 0.3-5.2% completely inhibited the fungal growth of the 10 tested fungal isolates with minimum inhibition concentration at 0.7%. Fumigation of books with 0.7-5.2% sodium hypochlorite completely inhibited all fungi without evident damage to the documents or ink discoloration. Therefore, sodium hypochlorite can be recommended as effective and eco-friendly disinfectant for archives comparable to other hazardous chemicals.

Key words: Archives, biological degradation, books, ethylene oxide, sodium hypochlorite

INTRODUCTION

Archives and libraries are documentation of human thought and cultural heritage on paper, parchment and other photographic and electronic supports (Maggi et al. 2000). A modern library has in its collections of rare books and archives, periodicals, newspapers, maps, video tapes, digital discs, hard discs, CD's and DV's (Byers 1983; Adams 2011). Paper is a wood-based material extracted from the tree and made of different organic, inorganic constituents with chemical additives to improve the paper quality (Guggenheim and Martin 1995; Mabee and Roy 2003; Doncea et al. 2010; Adams 2011; Chen et al. 2011; Area and Cheradame 2011; Henniges et al. 2012). Liquid ink contains different chemicals, dyes and pigments are used to produce text, design, map or an image (Agha-Aligol et al. 2007). All of these materials (paper and ink) are excellent substrates for the fungal growth under favorable conditions (Byers 1983; Nittérus 2000). They are prone to physical, chemical and biological degradation and damage depending on their chemical nature and storage conditions (Area and Cheradame 2011). It is subject to degradation by different enzymes of fungi and bacteria (Benoit et al. 2012). Therefore, biodeterioration of library materials and art work is a worldwide problem. The literature on the fungal contamination of the library materials during 1919-1977 was reviewed by Zyska (1997). Since then no review was carried out in this field and few studies were conducted. Biological degradation of paper by molds and insects are the most frequent cause of biological problems (Eduardo 2001; Borrego et al. 2010). The fungi produce different types of enzymes such as cellulose-degrading enzymes of *Trichoderma reesei* (Martinez et al. 2008; Area and Cheradame 2011; Kubicek et al. 2011; Van den Brink and de Vries 2011), and pectin degradation enzymes by *Aspergillus* and *Rhizopus* species (Benoit et al. 2012). The apparent growth of these fungi depends on the environmental conditions such as humidity, pH, temperature and nutrients, which are prevailed in archives and libraries (Fassatiová et al. 1987; Fassatiová 1995; Borrego et al. 2010). Fungal contamination occurs in books, manuscript, archives, libraries, and museums around the world. Zyska (1997) reported 84 fungal genera and 234 species from library materials worldwide. In Prague, 88 fungal species were isolated from the atmosphere, archive documents, walls and shelves (Fassatiová et al. 1987). More than fifty fungal species were isolated and identified from air and dust samples of 10 French archives associated with occupational symptoms (Roussel et al. 2012). Many species of fungi were isolated from libraries in Warsaw, Poland (Gutarowska et al. 2012), about 77 species and 30 fungal genera on the documents from eight stores in Russia (Mokeeva and Budarina 1991), bio-aerosol fungi from the Doctorate Library of the University of Perugia, Italy (Ruga et al. 2008), and 21 genera and 33 species of fungi from 163 damaged archives in Assiut City, Egypt (Abdel-Mallek 1994). From the dust sample collected from mortgage registers in the court of the Polish, the genus *Penicillium*,

as well as *Cladosporium herbarum*, *Geotrichum candidum*, *Cephalosporium glutineum*, *Mucor racemosus*, *Trichoderma viride*, and *A. niger* were recovered (Krysinska-Traczyk 1994). *A. ustus*, *A. nidulans*, *A. versicolor*, seven *Penicillium chrysogenum* strains, *A. alternata*, *C. cladosporioides*, *Mucor racemosus*, *Phoma glomerata*, and *Trichoderma longibrachiatum* were recovered from cinematographic films of Spanish archives in Madrid, Barcelona, (Abrusci et al. 2005). The most frequent species are *Cladosporium indicum*, *Alternaria alternata*, *Fusarium* sp., *Penicillium* sp., *Chaetomium* sp., *Aspergillus* sp., (Abdel-Mallek 1994; Rakotonirainy et al. 1999; Nittérus 2000; Rakotonirainy and Lavedrine 2005). Some of these fungi were associated with many health risks and human diseases such as Aspergillosis of *Aspergillus fumigatus* (Abdel-Mallek 1994; Krysinska-Traczyk 1994; Srikanth et al. 2008; Gutarowska et al. 2012).

Different methods were continuously assessed for controlling the fungal growth on books and archives such as UV, gamma radiations and some chemical disinfectants (Hanus 1985; Nittérus 2000). The UV radiation has been restricted because it does not penetrate the paper surface and may cause paper aging (Hanus 1985). Gamma rays penetrate efficiently but may cause paper aging and cancer (Pavon 1975; Justa 1992). Although large number of toxic chemicals has been utilized to sanitize papers, some of these chemicals result in pigment discoloration and damage the books and artworks. Carbo gasoline, formaldehyde, thiabendazole, ethylene oxide, and essential oils were commonly used against mold growth on books and archives (Beebe 1911; Rakotonirainy et al. 1999; Rakotonirainy and Lavédrine 2005). However, most of these chemicals have adverse effects on paper and mild fungal control. Nonetheless, thiabendazole at 10% was very effective on fungi and does not damage the paper and artwork (Rakotonirainy et al. 1999). Formaldehyde (formalin, methyl aldehyde) at 1.5% has been used in the treatment of 8.1 million books in Russia, but it was restricted due to its toxicity and irritation effect (Nittérus 2000). Ethylene oxide was a powerful sterilant in museum fumigation (Brokerhof 1989). For almost 60 years, thymol as a fungicidal has been used in paper conservative practice (Nittérus 2000). On the other hand, since 1787, sodium hypochlorite solution (NaOCl, household bleach), as an alternative to SO_2 in winemaking, it is used for controlling the fungal and bacterial contamination (Yoo et al. 2011). At high concentrations, sodium hypochlorite cause skin burns and eye damage, nonetheless, at less than 4.0% it was classified as a moderate oxidizing hazard by the National Fire Protection Association (NFPA). 0.4% is the minimum inhibitory concentration of sodium hypochlorite against *Penicillium expansum* (Cerioni et al. 2013). Therefore, sodium hypochlorite has been suggested as an effective alternative chemical agent against the fungal growth with negligible damage on paper and artwork (Ebling 2007; Yoo et al. 2011).

In Oman, recently around 70,000 documents were collected from India, UK, Tanzania, and Pakistan by the National Records and Archives Authority. They represent a big challenge of evident fungal contamination and their subsequent control measure. Therefore, the present study was designed to identify the fungal flora invading these books and archives and searching for simple, cheap, and eco-friendly control measures. This will participate effectively in the development of national and international strategy for the collection and storage of rare books and archives which are useful to mankind.

MATERIALS AND METHODS

Sample collection and isolation of fungi

For this study, sterile cotton swabs were used to collect 102 samples from the books and archives of the National Records and Archives Authority, Muscat, Oman. The swabs were streaked onto Potato Dextrose Agar (PDA) and incubated at room temperature (25±2°C) for 10 days. The developed fungal isolates were isolated in pure cultures for further identification to the species level.

Morphological identification of the isolated fungi

The fungal isolates developed in the growth media were identified macroscopically and microscopically based on their characteristics on the growth media and the morphology of sexual and asexual structures. The identification of the isolated fungi was confirmed using many taxonomic books, monographs and taxonomic papers (eg. Raper and Fennell 1965; Ellis 1971, 1976; Pitt 1979; Sutton 1980; Samson et al. 1995; Barnett and Hunter 1998, 2003). For non-sporulating fungi, mycelial fragments were inoculated on Malt Extract Agar (MEA) and incubated at 28ºC±2ºC to stimulate sporulation and were then identified to species level following the same identification method.

Molecular identification of the isolated fungi

The morphological identification of the isolated fungi was confirmed with the help of molecular techniques through DNA extraction, purification, polymerase chain reaction (PCR), and DNA sequencing. The internal transcribed spacer region (ITS) was used for the identification of the isolated fungi as described by many authors (Eberhardt 2010). Two primers namely ITS1 and ITS4 were consumed.

Isolation and purification of the genomic DNA of fungi

The DNA of the fungal isolates was extracted using Soil DNA Extraction Kits prepared by MO BIO Laboratory, Inc., (Carlsbad, California, USA) as per the manufacturer protocol with some modifications. The mycelial mat of 6 day-old fungal culture was harvested from the surface of the growth media with a sterile disposable loop, aseptically transferred into 2 ml Bead Solution tube and gently vortex. Solution S1 was heated to 60ºC and 60 µL were added, vortex, 200 µL of Solution IRS (inhibitor Removal Solution) were added, vortex at maximum speed for 15 min. The supernatant was transferred to a clean microcentrifuge tube, 250 µL of Solution S2 were added, vortex for 5 min., centrifuged for 1 min. at 10000 xg and the entire supernatant was transferred to a clean microcentrifuge tube. To the tube

contents, 1 ml of Solution S3 was added, vortex for 5 sec., 700 µL were loaded onto a spin filter and centrifuged at 10000 xg for 1 min., and this step was repeated until all the supernatant has passed through the spin filter. From solution S4, 300 µL were added, centrifuged for 30 sec. at 10000 xg, the flow through was discarded and centrifuged again for 1 min. Spin filter was placed in a new clean tube, 50 µL of Solution S4 were added to the center of the white filter membrane, centrifuged for 30 sec., the spin filter was discarded and the isolated DNA in the tube is then ready and frozen at -20ºC for further uses.

Polymerase chain reaction

Polymerase chain reaction (PCR) was adopted to amplify the extracted DNA samples. For the identification of the ITS region of the isolated fungi, a set of forward primer (ITS1) and reverse primer (ITS4) were consumed (Martin and Rygiewicz 2005). Each PCR reaction mixture contains 10 µL of Promega PCR master mix, 0.4 µL ITS1 primer, 0.4 µL ITS4 primer, 2 µL of DNA sample and nuclease-free water to make the final volume of 25 µL. The amplification was carried out by automated thermal cycle with thermocycling of 95°C for 10 minutes (heating and denaturing); 35 cycles at 95°C for 30 seconds; 55°C, 30 seconds; and 72°C, 60 seconds, 72°C, 10 minutes (extension).

Detection and purification of PCR product

For testing the quality of PCR product, a volume of 20 µL of PCR was checked by gel electrophoresis in 1.5% agarose gel in 1x Tris-borate-EDTA buffer (TBE) at 100 v for 40 min. The gel was stained with ethidium bromide and viewed under ultraviolet light to detect the presence and size of the amplified DNA product. The PCR products were purified using the EXO-SAP (Exonuclease and Shrimp Alkaline phosphatase) stored at -20°C. The EXO removes any single DNA strand or primer from the product, while the SAP removes the unconsumed dNTPs that may interfere with the sequencing. Both EXO and SAP utilize hydrolytic enzymes to remove unwanted area from the DNA fragments. Each purification reaction contained 10 µL of Exo-SAP mix (0.025 µL ExoI; 0.25 µL SAP and 9.75 µL water) and 5µL of PCR products. The reaction requires two incubation holds in the thermal cycle at 37°C for 60 minutes and 95°C for 5 minutes to deactivate the enzymes.

DNA sequencing reaction

After the purification of the PCR products, the sequencing was carried out with Applied Biosystems v3.1 Big Dye Cycle sequencing kit where both forward and reverse reactions were conducted separately. Each sequencing reaction contained 2µL of purified PCR product, 2 µL of primer, 1.5 µL of free-nuclease water, 2 µL of Q solution, 0.5 µL of Big Dye® Terminator v3.1 5X sequencing buffer, 2 µL of dye terminator 5X cycle sequencing. The sequencing reactions were run in the Bio-Rad Thermal Cycler with thermal conditions of 96°C for 1 min, followed by 25 cycles at 96°C for 10 seconds, 54°C for 5 seconds, and 60°C for 4 minutes. The sequences were purified using DyeEx® 2.0 spin kit (250) for dye terminator removal (QIAGEN). After purification, the product was sequenced using the 3130x/Genetic Analyzer (Applied Biosystems). With the help of Bioedit program, the resulted ITS sequences were compared with the sequences of fungal isolate at the National Center for Biotechnology Information (NCBI) using BLAST search.

Effect of sodium hypochlorite on fungal spores

For testing the effect of sodium hypochlorite (NaOCl) on the fungal spore germination, 10 fungal species were selected, namely *Aspergillus arborescens, A. clavatus, A. flavus, A. niger, Cladosporium cladosporioides, Drechslera australiensis, Erysiphe pisi, Fusarium oxysporum, Penicillium marneffi,* and *Mycosphearella graminicola*. From 7-day old fungal cultures grown on PDA, 5 loops full inocula were added to 9 ml sterile distilled water, thoroughly mixed and serial dilutions of up to 10^{-3} were prepared. Different concentrations of sodium hypochlorite (0.3, 0.7, 1.3., 2.6, 5.2%) were prepared. From the spore suspension of each fungus, 1 ml was added to sterile petri dish and mixed with 1 ml of different concentrations of sodium hypochlorite and 10 ml of molten cool Malt Extract Agar (MEA). As a control, similar set was prepared using sterile distilled water instead of sodium hypochlorite. Three replicates were incubated at room temperature for 10 days.

Effect of sodium hypochlorite fumigation on fungal contamination of books

To investigate the effect of sodium hypochlorite vapor on naturally contaminated books, 6 pages from contaminated books were placed inside a clean fish tank (29.5 × 22 × 44 cm) lined with filter papers. The tank was sprayed with 20 ml of sodium hypochlorite (5.2%), covered and sealed with Vaseline. As a control similar tank was sprayed with 20 ml of water. The tanks were incubated for 48 hours. The presence of the fungi was tested by the swab method and the observations were reported.

RESULTS AND DISCUSSION

The morphological and molecular identification of fungi from archives and books showed the high contamination of these documents with different fungal species (Table 1, Figure 1). The fungal contamination of the archives and books was investigated using morphological and molecular identification methods. For the molecular identification, Internal Transcribed Spacer region (ITS) which of high degree of variation than other genic regions of rDNA was used. The size of the fragment within this region is between 600-750 bp; and the resulted fragments in our results fall in this range (Figure 1). In the present study, 102 swab samples collected from different books and manuscripts show evident fungal contamination (100%). Twenty-two species which belong to 11 genera of fungi were identified (Table 1). Of these fungi the genus *Penicillium* (46.8%) is the most prevalent, followed by *Aspergillus* (30.7%), *Cladosporium* (7%), *Rhizopus* (4%), and *Chaetomium* (3.5%) whereas the remaining 6 genera

represent only 8% of the isolated fungi. In similar study, some of these fungi were isolated from archives and documents (Fassatiová et al. 1987; Maggi et al. 2000; Nittérus 2000; Gutarowska et al. 2012; Roussel et al. 2012). Filamentous fungi of 84 genera, represented by 234 species, were isolated in the period 1911-1977 from books, paper and other materials (Zyska 1997). In Prague, of the fungi isolated from atmosphere, archive documents, walls and shelves fungi, *Aspergillus niger, A. versicolor, Cladosporium herbarum, C. cladosporioides, Alternaria alternata, Rhizopus arrhizus*, and *Penicillium chrysogenum* were the most frequent isolates followed by *A. flavus, A. fumigatus, A. nidulans, Aureobasidium pullulans, Chaetomium* sp., *Fusarium* sp., *Rhizopus stolonifer, Ulocladium* and many *Penicillium* species (Fassatiová et al. 1987). More than fifty fungal species were isolated and identified from air and dust samples of 10 French archives associated with occupational symptoms (Roussel et al. 2012). These fungi include *Cladosporium sphaerospermum, C. herbarum, C. cladosporioides, A. fumigatus, A. ochraceus, A. sydowii, A. niger, A. versicolor, Penicillium chrysogenum, P. crustosum, P. citrinum, Rhizopus* sp., *Alternaria alternata, Trichoderma, Ulocladium* and *Verticillium* species. In Warsaw, Poland many species of fungi were isolated from three libraries such as *Alternaria alternata, Aspergillus candidus, A. fumigatus, A. niger, Aspergillus ochraceus, Chaetomium indicum, Penicillium chrysogenum* and *Cladosporium herbarum* (Gutarowska et al. 2012). The most frequent species are *Cladosporium indicum, Alternaria alternata, Fusarium* sp., *Penicillium* sp., *Chaetomium* sp., *Aspergillus* sp. (Abdel-Mallek 1994; Rakotonirainy et al. 1999; Nittérus 2000; Rakotonirainy and Lavédrine 2005). Inside six repositories of the National Archives of the Republic of Cuba, *Aspergillus*, *Cladosporium*, *Curvularia*, *Mucor*, *Neurospora*, and *Penicillium* genera were isolated (Borrego and Perdomom 2012). Of the 28 genera and 31 species identified in a range of public and private buildings including libraries, *A. flavus*, *A. niger*, *P. citrinum*, *C. cladosporioides*, *C. sphaerospermum* were the most prevalent species (Rojas and Aira 2012). In the present study, eleven species of the isolated fungi were previously encountered from archives and library documents, whereas eleven species and one genus were considered new to the mycoflora of archives and books (Table 1).

In order for any fungus to grow on the paper surface, they must produce some types of enzymes such as cellulases which attack the fibers and lead to the cleavage of carbohydrates (Abdel-Mallek 1994; Area and Cheradame 2011). These enzymes are species dependent where each species produces different enzyme such as highly efficient cellulose degrading enzymes of *Trichoderma reesei* (Martinez et al. 2008; Kubicek et al. 2011; Van den Brink and de Vries 2011), and pectin degradation enzymes by *Aspergillus* and *Rhizopus* species (Benoit et al. 2012). The genera of *Aspergillus*, *Alternaria*, *Cladosporium*, *Curvularia*, *Fusarium*, *Mucor*, *Neurospora*, and *Penicillium* were capable of degrading cellulose and excreting pigments and acids (Borrego and Perdomo 2012). In the present study, some of these fungi were recovered from books and archives such as the species of *Aspergillus niger, A. flavus, Alternaria alternata, Penicillium* sp., *Rhizopus* sp., and *Chaetomium globosum*, *Cladosporium* spp., *Erysiphe pisi*, *Phycomyces* sp., and *Puccinia striiformis* which have a strong cellulolytic activity and high capability to secrete different enzyme as biodegradation agent. Of the isolated fungi, the fungus *A. flavus* is one of the serious pathogenic and aflatoxin producers in food and feed products (El-Nagerabi et al. 2012, 2013a,b,c). Most of the isolated fungi produce large numbers of spores which may be hazardous to the workers and the visitors of the libraries. Many of these fungi are associated with various human diseases such as aspergillosis of *Aspergillus fumigatus* (Abdel-Mallek 1994; Gutarowska et al. 2012). Other studies showed that direct contact with contaminated documents with fungi was linked to headache, fatigue, eye irritation, throat irritation and coughing (Roussel et al. 2012). From cultural institutions at Havana University, the genera of *Aspergillus*

Table 1. Incidence of fungi isolated from Omani books and archives collected from different countries.

Document source	CFU/plate $\times 10^3$	Fugal isolates
USA, India	5.5	*Alternaria alternate**
Kenya	8	*Alternaria arborescens* **
Kenya, Oman, Tanzania	6.6	*Aspergillus clavatus* **
Oman, UK	3.5	*Aspergillus flavus**
Oman, Kenya	4.3	*Aspergillus nidulans**
East Africa, Kenya, Oman, Tanzania	12.6	*Aspergillus niger**
USA	15	*Aspergillus ochraceus**
East Africa	4	*Aspergillus versicolor**
Tanzania	8.5	*Chaetomium globosum**
East Africa, Kenya	10	*Cladosporium caryigenum***
Oman, USA	3.5	*Cladosporium cladosporioides**
Kenya	5	*Cladosporium sphaerospermum**
Oman	12	*Erysiphe pisi***
Oman	3	*Mycosphearella graminicola***
Oman	5	*Paecillomyces lilacinus***
Oman, Tanzania, UK, USA	9.6	*Penicillium chrysogenum**
Kenya, Oman, Tanzania, UK, USA	17.3	*Penicillium digitatum***
India, Oman	4	*Penicillium marneffi***
India, Oman, Tanzania	8.6	*Penicillium paxilli***
Oman	8	*Penicillium stipitatus***
Oman	3.5	*Phycomyces* sp.**
UK	3	*Puccinia striiformis***
Oman, Tanzania, UK	5	*Rhizopus arrhizus**

Note: *: Previously isolated from library documents. **: New records

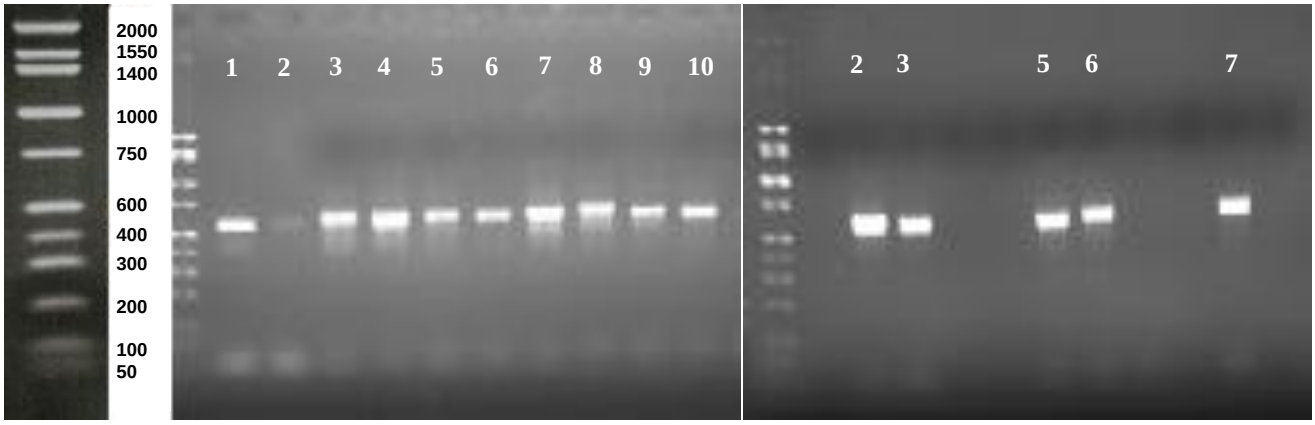

Figure 1. Electrophoresis of the PCR products of the fungal isolates in 1.5% agarose gel with ladder of 200bp with fragments fall between 500-750bp.

(*A. flavus, A. niger, A. terreus, A. fumigatus*), *Penicillium*, *Cladosporium*, *Fusarium*, and *Monilia* were identified as airborne mycoflora (Rojas et al. 2002). More than fifty fungal species were isolated and identified from air and dust samples of 10 French archives associated with occupational symptoms (Roussel et al. 2012). These fungi include *Cladosporium sphaerospermum, C. herbarum, C. cladosporioides, A. fumigatus, A. ochraceus, A. sydowii, A. niger, A. versicolor, Penicillium chrysogenum, P. crustosum, P. citrinum, Rhizopus* sp., *Alternaria alternata*, *Trichoderma*, *Ulocladium* and *Verticillium* species. In Warsaw, Poland from three libraries many species of fungi were isolated such as *Alternaria alternata, Aspergillus candidus, A. fumigatus, A. niger, A. ochraceus, Chaetomium indicum, P. chrysogenum* and *Cladosporium herbarum* (Gutarowska et al. 2012). The concentration of filamentous fungi in archives is associated with health risk for workers (Krysinska-Traczyk 1994). In the present study, some of these fungi were isolated and may cause similar health hazards to the workers and visitors. This could be avoided by controlling the fungal infestation of archives and books in libraries.

Different chemicals were commonly used to compact mold growth and contamination of different substrates. However, there are numerous difficulties that prevent the disinfection of books such as gaseous penetration inability, and steam injuries of the books (Beebe 1911). Thermal fogging with alkyl dimethyl benzyl ammonium chloride solution has been employed for cleaning of the libraries atmosphere contaminating by fungi (Rakotonirainy et al. 1999). Several researchers have pointed out the possibility of using gamma radiation for paper disinfection (ex. Barkai et al. 1969; Pavon 1975). Thiabendazole (Thiazol-4) - 2benzimidazole at 10% was effective sanitation of atmosphere (Rakotonirainy et al. 1999). Ethylene oxide has been widely used as powerful sterilant in museum fumigation (Nittérus 2002). However, the high toxicity and carcinogenic properties prevent its application in paper conservation practice. The vapor of nine essential oils and their components showed the potential use of linalool as an alternative to chemical fungicides to disinfect is difficult to assess, but may be useful in preventing fungal contamination in the storage area of cultural properties (Rakotonirainy and Lavédrine 2005). The vapors of thymol have been extensively used in fumigation cabinets for books and archives, but is no longer used because of its health hazard and deleterious effects on the object (Byers 1983; Isbell 1997; Rakotonirainy and Lavédrine 2005).On the other hand, sodium hypochlorite (NaOCl, household bleach) is known to be one of the most effective antimicrobial chemicals which is useful against fungal and bacterial contamination (Okungbowa and Usifo 2010; Reynolds et al. 2012). It is evidently safe and effective chemical compared to other chemical disinfectants (Ebling 2007; Yoo et al. 2011). It is widely used in food industries, despite the increasing availability of other disinfectants (Fukuzaki 2006). At 1-5.7%, NaOCl caused a 100% reduction in spore's viability of *P. brevicompactum* (Ebling 2007). The minimum inhibitory concentration of NaOCl against *P. expansum* (apple blue mold), was 50 mg/liter (Cerioni et al. 2013). It is effective against bacterial contamination of kitchenware and labware items (Feliciano et al. 2012). Sitara and Akhter (2007) reported that 10% NaOCl was effective against seed has borne mycoflora of maize including *A. flavus, A. wentii*, *Chaetomium*, *Drechslera*, *Rhizopus*, and *Fusarium* species. All fungal spores on grains were completely inhibited by 1-5% NaOCl (Sauer and Burroughs 1986). The use of low concentration of NaOCl (2.4%) was effective and recommended for controlling indoor mold (Reynolds et al. 2012).

In the present study, it is evident that the use of different concentrations of sodium hypochlorite (0.3-5.2%) significantly inhibited the fungal growth of the tested fungal species namely *Aspergillus arborescens, A. flavus, A. niger, Cladosporium cladosporioides, Penicillium marneffi* (Figure 2), and *A. clavatus, Drechslera australiensis, Erysiphe pisi, Fusarium oxysporum, Mycosphearella graminicola* (Figure 3). The minimum inhibitory concentration was recorded at 0.7% for all fungi. In previous studies, the minimum inhibitory concentration against the growth of spores was reported at 0.4% sodium hypochlorite (Cerioni et al. 2013). On the other hand,

fumigation of pages from contaminated books with 5.2% sodium hypochlorite completely inhibited the fungal growth without evident effect on the paper quality comparable to spray with water. Therefore, it can be recommended as a safe control measure against fungal contamination of books and archives without adverse effect on the quality of paper text discoloration as suggested by many researchers.

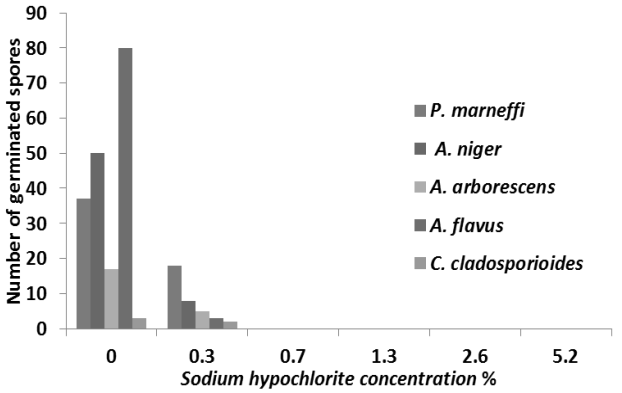

Figure 2. The inhibitory effect of different concentrations sodium hypochlorite using three replicates on *P. marneffi, A. niger, Alternaria arborescens, A. flavus, and C. cladosporioides.*

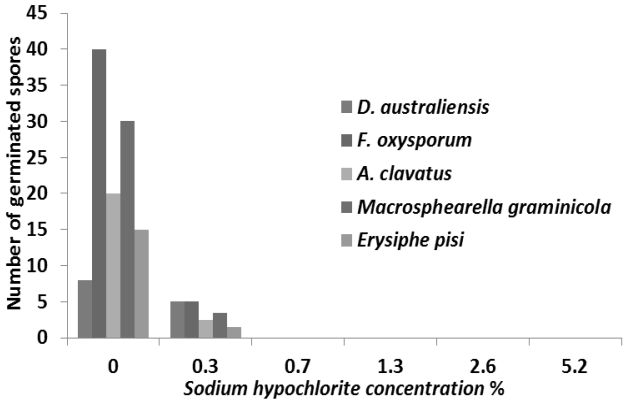

Figure 3. The effect of different concentrations of sodium hypochlorite using three replicates on *A. clavatus, F. oxysporum, Erysiphe pisi, M. graminicola and D. australiensis.*

CONCLUSION

It is evident that the collected archives and books by the National Records and Archives Authority of Oman from different sources are subject to high invasion with numerous molds. Twenty-two species which belong to 11 genera of fungi were recovered from these documents. Of these recovered fungi, 11 species were previously isolated from similar archives and library materials, whereas 11 species and one genus are new records. Some of these fungi are of cellulolytic activity which degrades papers and associated with many health hazards. Sodium hypochlorite was found effective against mold growth with minimum inhibition concentration (MIC) of 0.7%. Therefore, NaOCl can be used as eco-friendly fumigant against mold growth on archives, books and other library materials without apparent damage to paper.

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