14 *Egypt. J. Bot.*, **Vol. 54,** No.2, pp.219-246 (2014)

Control of Fungal Paper Deterioration by Antifungal Drugs, Essential Oils, Gamma and Laser Irradiation

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THIS STUDY aimed to control two selective fungi (*Aspergillus parasiticus* and *Penicillium commune*, causing biodeterioration of books and documents in storage areas of libraries) using antifungal drugs, essential oils (added singly and in combination), gamma irradiation and laser radiation. The results obtained indicated that fluconazole and clotrimazole were effective against *A. parasiticus* and *P. commune*, while nystatin was of lesser effect .*P. commune* showed more resistance than *A.* parasiticus to the antifungals (MIC_{60} determination). Cinnamon and clove oils were efficient antifungal agents, while garlic and thyme oils have no reasonable activities. The combination treatment revealed some synergestic interaction between garlic and thyme oils with antifungal drugs. Generally the highest control of both fungal species was shown by single treatment of clotrimazole. It was found that the two fungi responded differently against γ- irradiation .*A. parasiticus* was more tolerant than *P .commune* in dry biomass, while they were equal in γ- irradiation tolerance in the cfu / plate. The D_{10} determination revealed more tolerance of *P. commune* than *A. parasiticus.* The two fungal species showed resistance in their growth parameters (cfu and dry biomass) with laser beam treatment for 1-5 min. Reductions of 75% to 95% of control was detected after 10 and 15 min exposure to laser beam. The lethal effect, however, was observed after 20 min for both fungi.

Keywords: Control paper biodeterioration, *Aspergillus parasiticus* and *Penicillium commune*, Antifungal drugs, Essential oils, Gamma irradiation, Laser radiation

Biodeterioration of library materials is a worldwide problem due to the vast numbers of libraries spread allover the world and the number of volumes stored in the library buildings (Zyska, 1997). Biodeterioration can be defined as a change in material properties due to the vital activities of the organisms (Hueck, 1965) and biofouling is the accumulation of biological deposit on a surface (Beech and Sunner, 2004). Biodeterioration and biofouling are due to microbiological, biological and physicochemical processes (Beech and Sunner, 2004).

A wide range of materials can be affected and among them are the following: metals, paints, paper, paper-board, rocks, photos, textiles, leather, plastics, etc. These materials, depending on the microclimatic conditions (temperature and relative humidity), can suffer from physical, chemical and esthetic damage caused by insects, algae, lichens, fungi and bacteria (Villalba *et al*., 2004; Borrego *et al*., 2010). The purpose of libraries, archives and museums is to preserve the items collected, enabling access to the knowledge contained in books and documents and enjoyment of the artworks contained on paper. Long-term preservation of the collections of historic, artistic and cultural heritage of these repositories involves controlling the environment in which they are kept and exhibited (Reis-Menezes *et al*., 2011).

The microbial degradation of documents is one of the most serious and unappreciated sources of damage to library and archived materials (Cappitelli and Sorlini, 2005). The presence of spores or vegetative cells of microorganisms on the surface of documents may indicate a possible degradation in the future (Mesquita *et al*., 2009). Microorganisms, particularly fungi, because of their cellulolytic activity are potential candidates responsible for paper deterioration (Coughian *et al*., 1993). Microorganisms, because of their rapid reproduction and physiological activity, generally are highly adaptive to environmental conditions. Thus, they can rapidly reproduce on various substrates, causing them to discolour or decompose (Kowalik, 1980). They often cause degradation of objects such as paintings, stone, wood, paper, masonry, leather, parchment, glass, metal, and cinematographic films (Cappitelli and Sorlini, 2005; Abrusci *et al*., 2005).

The antifungal properties of different compounds designed to control the presence of filamentous fungi on industrial paper or archives have been evaluated (Gilbert and Brown, 1995; Rakotonirainy *et al*., 1999; Clausen, 2000). Parks and Casey, 1996 reported that there are three major groups of antifungal agents which inhibit synthesis of or direct interact with ergosterol (the predominant component of the fungal cell membrane), Azoles (such as miconazole, econazole, ketoconazole, fluconazole and itraconazole), polyenes (such as amphotericin B) and allylamine (such as terbinafine and naftifine).

Azoles are compounds used in dermatology as antifungal drugs (Harvey, 1985) and in plant pathology against fungal diseases. They act by selectively impairing the cytochrome P.450 dependant 14 ὰ-demethylase, a key enzyme of ergosterol biosynthesis (Vanden- Bossche, 1990). Polyene compounds lead to breakdown of cell wall constituents, so it altered permeability and leakage of vital cytoplasmic components and death of microorganisms. Chemicals that specially inhibit chitin (main component of fungal wall) and ergosterol (main component of fungal membranes) are used to prevent fungal infections and contaminations.

Since antiquity, volatile oils from herbs, spices and plants have been recognised as having biological activities. In recent years, there has been

renewed interest by scientists in the use of these natural substances for their antibacterial and antifungal properties. However, among several studies reported, only a few mention their eventual use in the field of conservation of cultural properties (Chingduang *et al*., 1995; Dhawan, 1995; Perumal and Wheeler, 1997; Gatenby and Townley, 2003). In Asia, some traditional practices have already been applied in museums and libraries. Several conservators are beginning to use this kind of product in Western countries. Published results on the antifungal activity of essential oils have been mainly concerned with fungi studied in an individual way (Hitokoto *et al*., 1980; Thompson, 1989; Mahmoud, 1994; Pattnaik *et al*., 1996; Adams *et al*., 1996; Bishop and Thornton, 1996; Montes-Belmont and Carvajal, 1998; Guynot *et al*., 2003). However, in the environment fungal populations are often a combination of many species. Many factors affect the constituents of essential oils. Depending on the methods of extraction, ecological factors and biological parameters, the same species of plant can give rise to essential oils with different composition and different type of action (Pellecuer *et al*., 1976; Arras and Grella, 1992; McGimpey *et al*., 1994; Echeverrigaray *et al*., 2003). Conversely, there are sometimes more similarities between actions of oils produced by different species of plants than oils produced by the same species. It is worth noting that essential oils are very expensive. Therefore, it appears more judicious to use a pure component (more stable) than an essential oil itself.

Gamma irradiation as sterilizing treatment causes direct damage to cell DNA through ionization inducing mutation and killing the cell. It also has an indirect effect as a result of radiolysis of cellular water and formation of active oxygen species, free radicals and peroxides causing single and double strand DNA breakages (McNamara *et al*., 2003).

Gamma rays, electromagnetic waves with high penetrating power, pass through materials without leaving any residue, an advantage comparing to other disinfection treatments, since the handling of books and documents may be done safely just after irradiation (Adamo *et al*., 1998 and 2001). Studies demonstrated that the damage in mechanical–physical properties caused by gamma rays on paper was not significant (Adamo *et al*., 1998 and 2001; Gonzalez *et al*., 2002). These studies were extended to the color of printing inks on paper and the result was that they are resistant to gamma radiation (Rocchetti *et al*., 2002).

Today radiation process for books and documents preservation is a continuous controversy, but many countries are trying to fix doses and conditions to be suggested and used as a suitable option.

Fungi have been successfully inactivated from different materials, such as paper, wood and soil with radiation doses ranging from 6 to 15 kGy (Hanus, 1985; Jorg *et al*., 1992; Pointing *et al*., 1998; McNamara *et al*., 2003). However, in a Brazilian study some fungi from books could not be completely eliminated after irradiation with doses of 20 kGy (Tomazello and Wiendl, 1995). Thus,

recent studies on the irradiation dose required to inactivate fungi were conducted on axenic cultures and on naturally contaminated books and documents, taking into account the tropical climatic conditions present in Brazil and its rich fungal diversity, resulting in many different fungal species as potential biodeteriorating agents.

Laser irradiation is used as a successful control to human pathogenic bacteria and fungi (Burns *et al*., 1993; Smijs and Schuitmaker, 2003; Vural *et al*., 2007; Manevitch, *et al*., 2010). Nowadays, portable laser systems can remove contaminants from substrates with no surface damage, thus being a great solution for conservation of documents and art objects (Speranza *et al*., 2012).

This study aimed to determine whether the traditional antifungal drugs or essential oils could be used to control fungal deterioration of documents in storage areas of libraries. Furthermore, the role of gamma and laser irradiation in fungal control and some enzymes activities were also studied.

Material and Methods

Selected organisms

Two fungal species (*Aspergillus parasiticus & Penicillium commune*) were previously isolated from fungal deteriorated books located in Cairo University old library and were identified by phenotypic and molecular technique. These two species were used as models to control the paper biodeteriorating fungi as they were the most frequent fungal species in the isolation experiment. They were tested to show how far they will be affected by some antifungal agents, essential oils, gamma and laser irradiation.

Effect of some antifungal drugs on fungal growth

a. Tested antifungal drugs from local market

b. Concentrations used of antifungal drugs

Serial dilutions of fluconazole were made by ethanol 50 % to obtain the following concentrations: 3.75, 7.5, 15, 30 and 60mg ml^{-1}. Nystatin was diluted by sterile distilled water to obtain 25000, 50000 and 100000 $I.Uml^{-1}$ concentrations. Clotrimazole concentrations were diluted to 5 and 10 mg $ml^{-1}$by sterile distilled water (Table 1).

TABLE 1. list of tested antifungal drugs .

Drug	Active component	Concentration	Manufactured by
Fungican	Fluconazole	Each capsule contains 150 mg	Amoun Pharmaceutical Co., Cairo, Egypt.
Nystatin	Nystatin	100,000 I.U ml^{-1}	E.I.P.I.Co., Egypt.
Dermatin	Clotrimazole	10 $mgml^{-1}$	PHARCO Pharmaceuticals, Alex, Egypt.

c-Antifungal susceptibility test using diffusion agar plate method

Spore suspensions of *Aspergillus parasiticus* and *Penicillium commune* were prepared. 250 µl of each spore suspension were seeded into 250 ml Malt Extract Peptone Agar (MEPA) medium (Merk, 1982) containing flasks. Media were poured immediately in sterile Petridishes and left to solidify. Wells of 5 mm diameter were made in the agar plates by sterile cork borers. Each well received 100 µl of each antifungal drug. Control plates contained sterile distilled water. Plates were incubated at 27 ±2°C for 3-5 days.

After the end of the incubation period, the sensitivity of fungi to the tested drugs was determined by measuring the mean diameter of the growth inhibition zones in mm. The relative activity (RA) percent was also calculated. The minimum inhibitory concentration (MIC_{60}) was determined (if achieved) as the least concentration of antifungal that inhibit 60 % of fungal growth.

Effect of Essential oils on fungal growth

The activity of the tested essential oils was estimated by using diffusion plate method described above (Table 2).

TABLE 2. List of tested essential oils.

Essential oil	Active component	Manufactured by
Clove oil	Eugenol	Elcaptaincompany, Cairo, Egypt.
Thyme oil	Thymol	Elcaptain company, Cairo, Egypt.
Cinnamon oil	Cinnamon aldehyde	Elcaptain company, Cairo, Egypt.
Garlic oil	Allicin	Elcaptain company, Cairo, Egypt.

Effect of combinations between antifungal drugs and essential oils on fungal growth

Combinations between the highest concentration of antifungal drugs and essential oils had been also tested and the interaction between them was expressed as functional inhibitory concentration index (FICI) (Mukherjee *et al*., 2005).

$$FICI = \frac{\text{\% of inhibition of combined mixture}}{\text{\% of inhibition of compound (a)}} + \frac{\text{\% of inhibition of combined mixture}}{\text{\% of inhibition of compound (b)}}$$

FICI < 0.5 means antagonism.
FICI = 0.5-4.0 means indifference.
FICI > 4.0 means synergism.

Effect of gamma radiation on fungal growth

a. Source and doses of gamma radiation

Spore suspensions from 7-day old cultures of each of the two selected fungi (*Aspergillus parasiticus* and *Penicillium commune*) were irradiated in a CO^{60} unit Gamma cell 220 Excel in the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt, at dose rate 2.48KGy/h. Spore suspensions were exposed to increasing doses of gamma radiation; 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 2.0 KGy, for studying the effect of irradiation on fungal growth.

b. Determination of decimal reduction dose (D_{10}-value)

Immediately after irradiation, 10^{-1} dilution of spore suspensions was made from each dose. Then, 100 µl from each dilution were seeded into 100 ml of MEPA medium containing flasks. The media were poured in sterile Petri dishes and left to solidify. Controls were made of spore suspensions not exposed to gamma radiation. Plates were incubated at 27 ± 2°C for 3-5 days, then the number of colonies was manually counted in each plate.

The radiation sensitivity of two test fungi was expressed in terms of D_{10} values. The D_{10}-valuewas determined by calculating the negative reciprocal of the slope by linear regression after fitting the survival data versus irradiation doses(Choi *et al.*,2012).TheD_{10}-value for each fungus was calculated using the regression line:

$Y = a+bx$

$D_{10} = - 1/b$

$B = \sum xy - nx'y' / \sum x^2 - n'^2$

Where

a = log of microbial count when x equal zero.

b = regression factor.

x = dose level (KGy).

y = microbial count (log count).

n = number of treatments.

$x' = \sum x/n$and$y' = \sum y / n$

c. Effect of different doses of gamma radiation on the fungal mycelial dry biomasses

For each treatment of both fungi, three conical flasks (250 ml volume), each containing 100 ml Czapek-Doxs liquid medium were inoculated, each with 1 ml of the previously irradiated spore suspension. Non-irradiated spore suspensions were used as controls. The flasks were incubated at 27 ±2°C for 7 days, after which the produced mycelial mats were collected by filteration, oven-dried at 60°C (until constant weight) then weighed to determine the final dry biomass as a measure of fungal growth.

d. Effect of different doses of gamma radiation on the fungal proteolytic, cellulolytic and amylolytic activities

Amylase and cellulase activities were determined by measuring the release of reducing sugars from starch and cellulose, respectively using the dinitrosalicylic acid (DNS) method (Ghose, 1987). Proteolytic activity was determined by measuring the release of amino acids using ninhydrin reagent (Jones *et al*., 2002).

Control of paper deteriorating fungi by laser radiation

Spore suspensions from *Aspergillus parasiticus* and *Penicillium commune* were exposed to the green light (532 nm) continuous wave (CW) from a Diode Pumped Solid State (DPSS) laser [LSR-PS-II] in the National Institute of Laser Enhanced Science (NILES), Cairo University, Egypt. For stability, the laser was turned on 10 min before starting the irradiation. Spore suspensions of the two fungal species were exposed for different exposure times to laser radiation; 0, 1, 2, 3, 4, 5, 10, 15 and 20 minutes. Then, the number of viable colonies and the dry biomass were also determined as described previously in gamma irradiation experiments.

Statistical analyses

The results were expressed as the mean ± standard deviations (mean±SD). Data were analyzed by one-way analysis of variance (ANOVAs) using SPSS statistical program, version, 16. The differences among the mean values of some experiments were compared by the Duncan's Multiple Range Test (DMRT) with the significance set at $p \leq 0.05$.Statistical analysis of other experiments was performed using Student's test, where p-values ≤ 0.05were considered significant.

Results and Discussion

Effect of some antifungal drugs on the growth of Aspergillus parasiticus and Penicillium commune

In Fig. 1, it was found that *P. commune* was more resistant to the three tested antifungal drugs, fluconazole (a), clotrimazole (c) (Azole group) and nyastatin (b) (polyene group) than *A. parasiticus* which showed high susceptibility especially against fluconazole. In all cases the sensitivity of the two fungal species was a function of antifungal concentrations. The MIC_{60} was achieved in the presence of 60.0 mg ml^{-1} fluconazole and 10.0 mg ml^{-1} clotrimazole against *A. parasiticus*, while it was not achieved against *P. commune* in any cases.

It is worthy to mention that Fabbri *et al*. (1997) reported the inhibition of some paper deteriorating fungi by antifungals such as antioxidants (butylated hydroxyl toluene and butylated hydroxyanisole); azole antifungals (econazole, miconazole, ketoconazole) and chitin synthase inhibitors (vridine, 5- fluorouridin-2-deoxyuridine) against *Penicillium chrysogenum*, *Aspergillus terreus*, *Stachybotrys atra* and *Chaetomiumelatum*. The most evident inhibitory effect on fungal growth has been obtained with miconazole and econazole at 10^{-3} M and to lesser extent butylated hydroxytoluene and butylated hydroxyanisole.

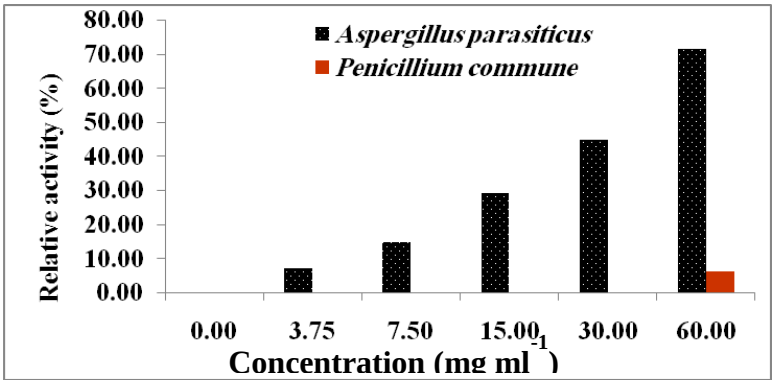

Fig. 1 (a). Effect of different concentrations of fluconazole on the growth of *Aspergillus parasiticus* and *Penicillium commune*.

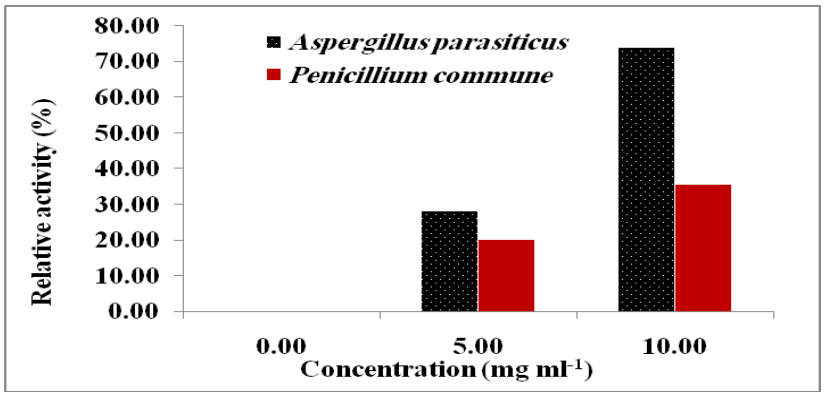

Fig. 1 (b). Effect of different concentrations of nystain on the growth of *Aspergillus parasiticus* and *Penicillium commune*.

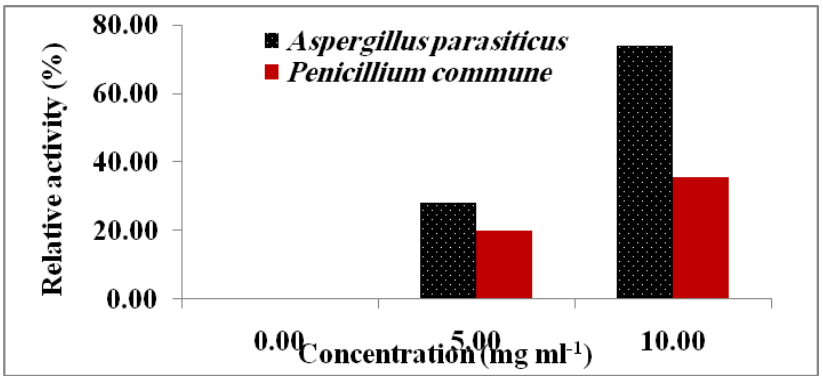

Fig. 1 (c). Effect of different concentrations of clotrimazole on the growth of *Aspergillus parasiticus* and *Penicillium commune*.

In this connection Neves *et al*. (2009) demonstrated that the minimum inhibitory concentration (MIC) of the antifungal methyl and propyleparabens against *Cladosporium* species and *Penicillium corylophilum* isolated from paper artistic works was 5% and 1%, respectively. Calcium propionate 5% was added to ethanolic mixture of both compounds to produce a multipurpose formulation for deacidification and decontamination of paper documents. This treatment shows only minor increase in fiber strength, while raising the pH cause alkaline reserve with minimum paper deformation.

The antifungal activity of essential oils against paper fungal deteriorating fungal growth

The bioassay of the antifungal activities of some essential oils (cinnamon, clove, garlic and thyme) indicating that cinnamon and, to lesser extent, clove oils exerted reasonable antifungal activities against the paper deteriorating fungi *Aspergillus parasiticus* and *Penicillium commune*. Garlic and thyme oils, however, had no detectable antifungal activities against both fungal species (Table, 3).

TABLE 3. The antifungal activity of essential oils against *Aspergillus parasiticus* and *Penicillium commune* growth.

Treatment	*Aspergillus parasiticus*		*Penicillium commune*	
	Inhibition zone diameter (mm) (Mean ± SD)	**Relative activity (%) (Mean ± SD)**	**Inhibition zone diameter (mm) (Mean ± SD)**	**Relative activity (%) (Mean ± SD**
Control	0.00 ± 0.00 [a]	0.00± 0.00 [a]	0.00 ± 0.00 [a]	0.00 ± 0.00 [a]
Cinnamon oil	13.83 ± 2.32 [c]	15.37 ± 2.58 [c]	16.00 ± 1.79 [d]	17.78 ± 1.99 [d]
Clove oil	9.33 ± 1.21 [b]	10.37 ± 1.34 [b]	9.33 ± 0.52 [c]	10.37 ± 0.57 [c]
Garlic oil	0.09 ± 0.00 [a]	0.01 ± 0.00 [a]	0.09± 0.00 [a]	0.01± 0.00 [a]
Thyme oil	0.09 ± 0.00 [a]	0.01 ± 0.00 [a]	2.00 ± 1.26 [b]	2.22 ± 1.40 [b]

Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test.

Combinations activities of antifungal drugs and essential oils against fungal growth

Combination between antifungal drugs and essential oils had been tested and the interaction between them (antagonism, synergism or indifference) was determined by the functional inhibitory concentration index (FICI) as mentioned in materials and methods. The data represented in Fig. 2 - 5 revealed indifference interaction between the single treatments and combination treatments against both fungi tested in case of cinnamon and clove containing mixtures. However, garlic and thyme oils interacted synergistically with antifungals increasing their efficacy in almost all mixtures. Antagonistic interaction was recorded in mixtures of garlic oil + fluconazole and thyme + fluconazole where they decreasing their efficacy. Generally the highest control of both fungal species was showed by single treatment of clotrimazole.

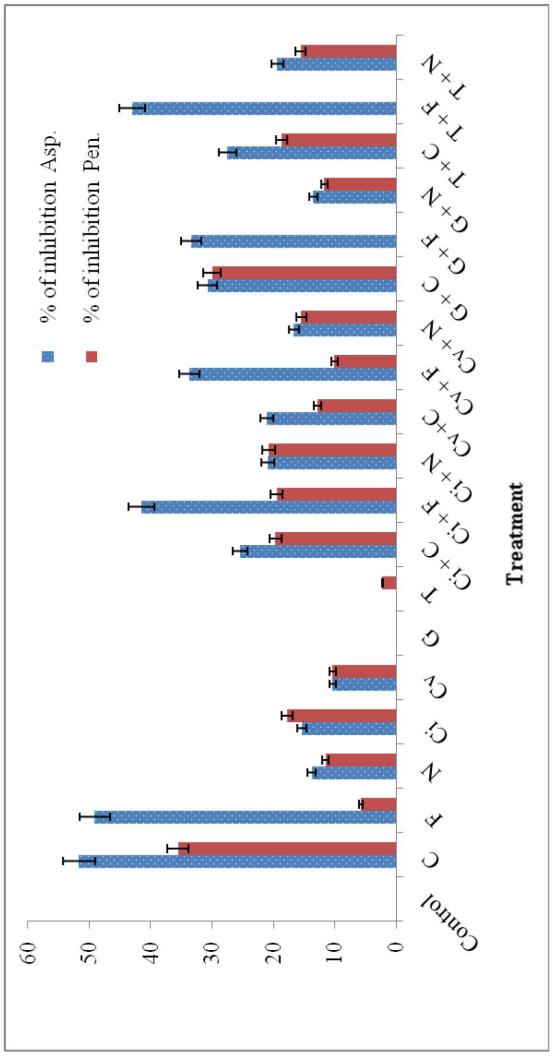

Figure (2): Effect of antifungal drugs and essential oils tested individually and in combination mixtures on fungal growth. C = Clotrimazole, F = Fluconazole, N = Nystatin, Ci = Cinnamon oil, G = Garlic oil, Cv = Clove oil and T =Thyme oil.

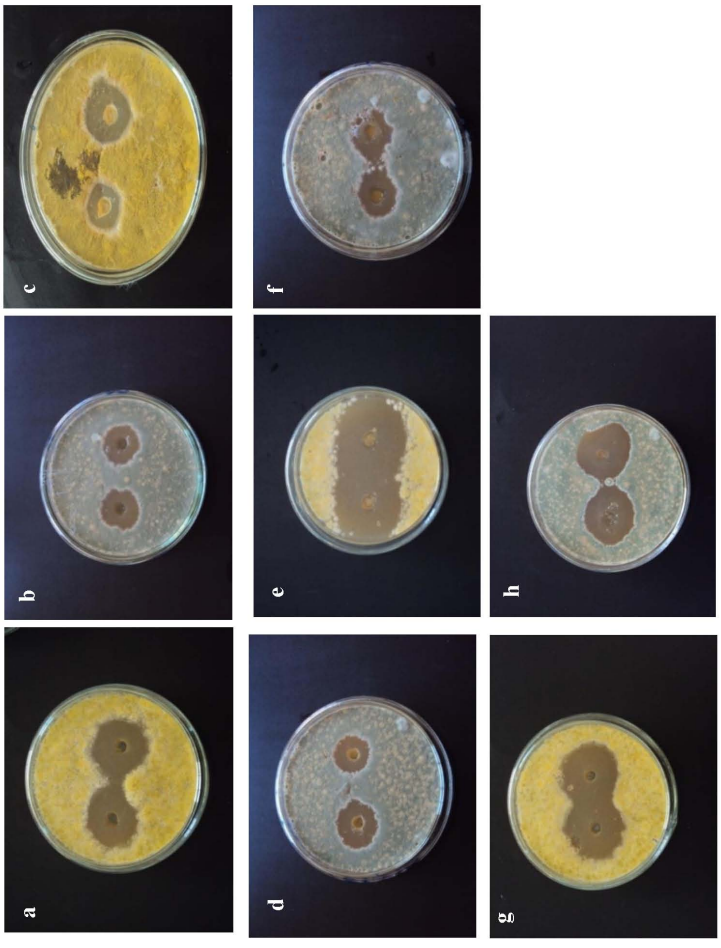

Fig. 3. Combination activities of antifungal drugs and essential oils against fungal growth. a) Cinnamon oil + Clotrimazole on *Aspergillusparasiticus*. b) Cinnamon oil + Clotrimazole on *Penicillium commune*. c) Cinnamon oil + Nystatin on *Aspergillus parasiticus*. d) Cinnamon oil + Nystatin on *Penicillium commune*. e) Cinnamon oil + Fluconazole on *Aspergillus parasiticus*. f) Cinnamon oil + Fluconazole on *Penicillium commune*. g) Garlic oil + Clotrimazole on *Aspergillus parasiticus*. h) Garlic oil + Clotrimazole on *Penicillium commune*.

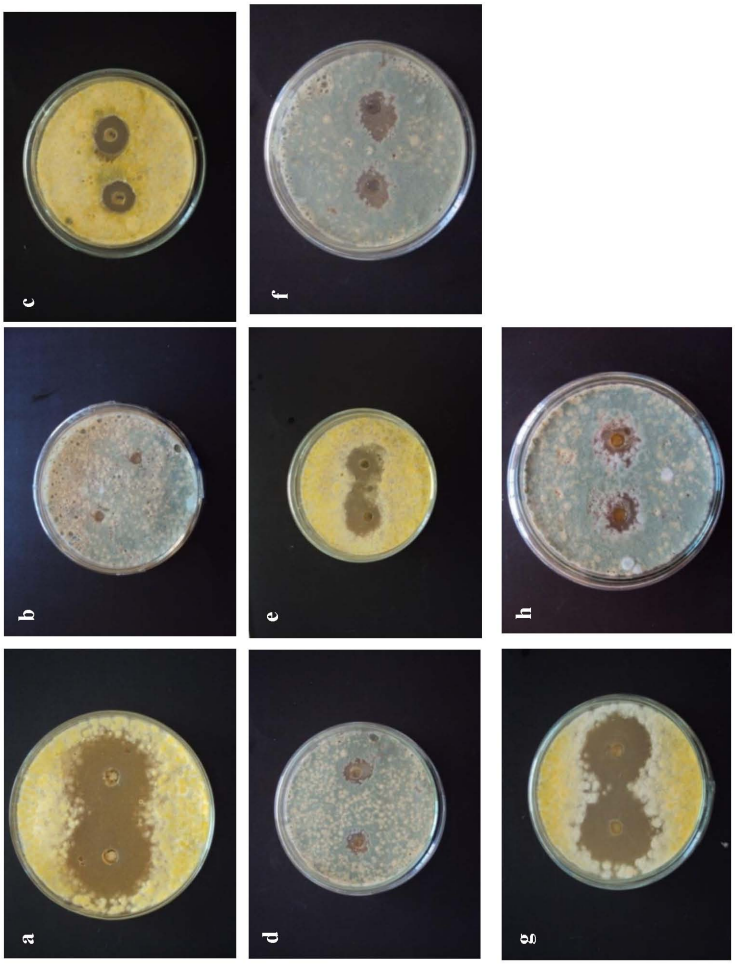

Fig. 4. Combination activities of antifungal drugs and essential oils against fungal growth. a) Garlic oil + Fluconazole on *Aspergillus parasiticus*. b) Garlic oil + Fluconazole on *Penicillium commune*. c) Garlic oil + Nystatin on *Aspergillus parasiticus*. d) Garlic oil + Nystatin on *Penicillium commune*. e) Clove oil + Clotrimazole on *Aspergillus parasiticus* . f) Clove oil + Clotrimazole on *Penicillium commune*. g) Clove oil + Fluconazole on *Aspergillus parasiticus*. h) Clove oil + Fluconazole on *Penicillium commune*.

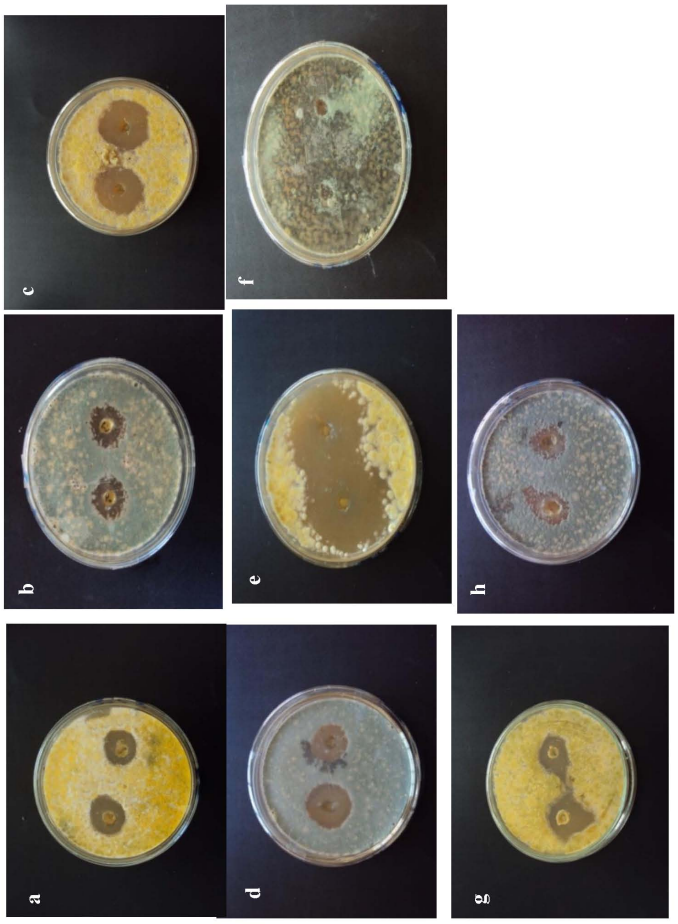

Fig. 5. Combinations activities of antifungal drugs and essential oils against fungal growth. a) Clove oil + Nystatin on *Aspergillus parasiticus* . b) Clove oil + Nystatin on *Penicillium commune*. c) Thyme oil + Clotrimazole on *Aspergillu sparasiticus* . d) Thyme oil + Clotrimazole on *Penicillium commune*. e) Thyme oil+ Fluconazole on *Aspergillus parasiticus*. f) Thyme oil + Fluconazole on *Penicillium commune*. g) Thyme oil + Nystatin on *Aspergillus parasiticus*. h) Thyme oil + Nystatin on *Penicillium commune*.

It could be concluded that the action of single treatment with either antifungal drugs or essential oils on paper deteriorating fungi are fungistatic rather than fungicidal. Combination of both increase efficaciesin some mixtures but the action is still fungistatic. Their use may be beneficial as a complement to treat environment in libraries to prevent new combination in stored books and other archive materials.

The awareness of microbial deterioration of library materials came only after investigating the Ludwig Pasteur and Robert Koch. While cedar oils and lemon flavor extracts were used in ancient libraries to protect papyrus from the insect attack.

Interestingly, Rakotoniraing and Lavedrine (2005) investigated the inhibitory action of vapour phase of essential oils of armoise, clove, boldo, eucalyptus, ravensare, lavender, tea tree, thuya, wormseed and their main components against mould species commonly found on library and archive materials. Among oils, wormseed oil showed the strongest antifungal activity. Among the chemical components linalool was the most efficient. At low concentration linalool exhibited higher activity than wormseed oil. Among fungi that resist all treatments *Penicillium variotii* and *Aspergillus niger*. Linalool vapour did not affect the brightness of papers tested or the degree of cellulose polymerization, but it reduces the pH of the paper which makes it susceptible to fungal degradation.

However, many factors affect the constituents of essential oils. Depending on the methods of extraction, ecological factors and biological parameters, the same species of plant can give rise to essential oils with different composition and different types of action (Pellecuer *et al*., 1976; Arras and Grella, 1992; McGimpey *et al*., 1994 and Echeverrigaray *et al*., 2003).

Effect of gamma radiation on fungal growth

The effect of different doses of gamma radiation on the fungal growth revealed that γ-irradiation proved to be effective treatment to paper deteriorating fungi at relatively low doses. Data in Table 4 indicated that the survival cfu / plate of the two tested fungal species decreased linearly by increasing the irradiation doses. *A. parasiticu*s was more tolerant to some extent to γ-irradiation than *P. commune* where complete inhibitions were recorded in exposure to1.0KGy, and 0.8KGy, respectively.

TABLE 4. Effect of different doses of gamma radiation on the fungal colony forming units after 5 days incubation.

Dose (KGy)	*Aspergillus parasiticus* cfu / plate (Mean ± SD)	*Penicillium commune* cfu / plate (Mean ± SD)
0.00	196.00 ± 11.11^{d}	276.89 ± 18.12^{e}
0.20	131.30 ± 8.21^{c}	152.30 ± 9.11^{d}
0.40	49.33 ± 2.52^{b}	77.33 ± 2.11^{c}
0.60	9.33 ± 0.91^{a}	27.33 ± 1.12^{b}
0.80	1.00 ± 0.00^{a}	0.00 ± 0.00^{a}
1.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
2.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test. **cfu**: colony forming unit.

The results in (Table 5) showed that the mycelial dry biomasses decreased by increasing the irradiation doses up to 1 KGy in the two tested fungi after incubation for ten days. There was no growth at 2 KGy. In case of *A. parasiticus* dry biomasses, there was no significant difference between the doses 0.2, 0.4and 0.6 KGy. At the doses 0.8 and 1.0KGy, significant decrease in dry biomass of *A. parasiticus* was obtained. The data also revealed a remarkable reduction in the dry biomasses of *P.commune* by increasing the irradiation doses starting from 0.2 KGy. The dose 2.0 KGy was lethal to the growth and biomasses gain in both fungal species.

TABLE 5. Effect of different doses of gamma radiation on the fungal mycelial dry biomasses after 10 days incubation.

Dose (KGy)	*A. parasiticus*	*P. commune*
	DB (mg/flask)	DB (mg/flask)
	(Mean ± SD)	(Mean ± SD)
0.0	132.0 ± 10.1^{b}	230.0 ± 11.2^{c}
0.2	125.0 ± 11.2^{ab}	140.0 ± 29.3^{b}
0.4	122.0 ± 12.1^{ab}	64.0 ± 11.4^{b}
0.6	108.0 ± 11.4^{ab}	63.0 ± 9.1^{b}
0.8	64.0 ± 7.3^{b}	62.0 ± 8.2^{b}
1.0	54.0 ± 5.5^{b}	54.0 ± 6.1^{b}
2.0	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}

Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test.

DB: Dry Biomass

Determination of decimal reduction dose (D_{10}-value)

The D_{10}-value was determined by calculating the negative reciprocal of the slope by linear regression after plotting the survival data versus irradiation doses (Choi *et al*., 2012). The D_{10}-value is defined as the dose of ionizing radiation required to reduce a given microbial population by 90% or by one logarithmic cycle.

The data in Table 6 indicated that the value of D_{10}(0.46) which reduced 90 of the growth of *A. parasiticus* was lower than that of *P. commune*(0.60). This indicating the higher sensitivity of *A. parasiticus* to γ-irradiation than *P. commune.*

TABLE 6. Calculation of D_{10} value of *A. parasiticus* and *P. commune*.

	A. parasiticus				*P. commune*			
	X	**Y**	**Xy**	**X^2**	**X**	**Y**	**Xy**	**X^2**
	0.00	2.29	0.00	0.00	0.00	2.44	0.00	0.00
	0.20	2.12	0.42	0.04	0.20	2.18	0.44	0.04
	0.40	1.69	0.68	0.16	0.40	1.89	0.76	0.16
	0.60	0.97	0.58	0.36	0.60	1.44	0.86	0.36
summation	$\sum x=1.20$	$\sum y=7.07$	$\sum xy=1.68$	$\sum x^2=0.56$	$\sum x=1.20$	$\sum y=7.95$	$\sum xy=2.05$	$\sum x^2=0.56$
Sum. square	$(\sum x)^2=1.44$				$(\sum x)^2=1.44$			
Average	$x'=0.30$	$y'= 1.77$			$x'= 0.30$	$y'= 1.99$		
Avg. square	$x'^2= 0.09$	$y'^2= 3.13$			$x'^2= 0.09$	$y'^2= 3.95$		
b	b= -2.20				b= -1.66			
D_{10}	**D_{10}=0.46**				**D_{10}= 0.60**			

x = dose level (KGy), y = microbial count (log count) and b = regression factor.

From the growth parameters data in Fig. 6, it was found that the two fungi responded differently against γ- irradiation. *A. parasiticus* was more tolerant than *P. commune* in its biomasses, while they were equal in γ- irradiation tolerance in the cfu / plate. The D_{10} determination revealed more tolerance of *P. commune* than *A. parasiticus.*

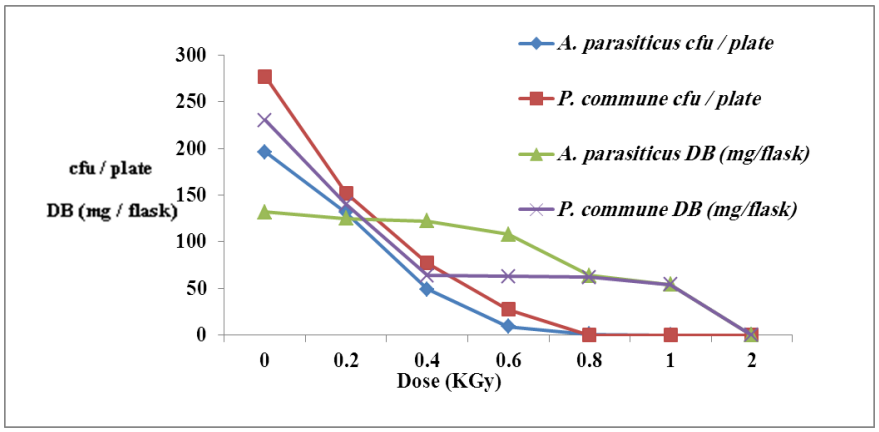

Fig. 6. Effect of gamma irradiation on fungal growth parameters.
-DB: dry biomass.

Gamma- irradiation is the most effective physical method for killing fungi and their spores. Many authors report that the dose ranged from 10 to 20 KGy is needed for complete inhibition of fungal growth (Nitterus, 2000). However, such high doses of γ-irradiation significantly affect the chemical composition of cellulose fibers and thus the application dose has to be thoroughly considered. In similar studies lower doses of γ-irradiation were also reported by D'Almeida *et al*. (2009) who found that γ- doses from 3-15 KGy at dose rate of 0.817 GY/S is successfully applied to books decontaminated by microorganisms under safety conditions. They recommended thinking about lowest radiation dose as possible and also verifying the dose rate for the process. Mangaudda (2004) proved that using γ-radiation is an efficient treatment (roughly 0.2-0.5KGy for fungi and 3-8KGy for insects). No significant harmful effect has occurred on the mechanical and physical properties of pure cellulose and paper or in printing ink.

Gamma -irradiation dose of 2.8 Gy/ h reduce the degree of cellulose fiber polymerization that is because the irradiation was long enough to allow oxidative degradation (Adamo *et al*.,1998).

The handling of books after γ-irradiation may be done safely because γ- rays pass through materials without leaving any residue (Adamo *et al*., 1998, 2001). It also causes no change in the physic-chemical characters of papers (Gonzales, 2002) or in printing compounds (Rocchetti *et al*., 2002). Superizingly, Da Silva *et al.* (2006) isolated and identified several fungi from contaminated books in a Brazilian public

library and from the environment. Those were *Acremonium*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Trichospora*. They were treated with γ-irradiation doses ranging from 14.5 to 25 KGy. The minimum lethal dose was 16 KGy. After the treatment no living fungi were detected from the irradiated material even the books were maintained in favorable condition for new fungal growth.

Effect of different doses of gamma radiation on the fungal caseinolytic, cellulolytic and amylolytic activities

Under control condition, the data in Tables 7 & 8 revealed that the caseinolytic activities of *A. parasiticus* and *P. commune* were higher than cellulolytic activities while amylolytic activities were of minor values. Comparing the two fungal species, it was found that *A. parasiticus* produces higher extracellular caseinolytic, amylolytic and cellulolytic activities than *P. commune*. Under control condition, γ-irradiation significantly reduced the activities of the three assayed enzymes, in dose dependent manner, in both fungal species. The dose 2 KGy completely inactivated the three enzymes activities in both fungal species.

These results are promising and demonstrate the effectiveness of γ-irradiation for recovery of severely damaged books and old documents leading to preservation of our cultural heritage and prevention of human diseases caused by molds in libraries and archives. The used lethal dose (2KGy) exerts no harmful effect on paper material.

Gamma irradiation causes direct damage to cell DNA through ionization inducing mutation and death of the cells. It also exerts indirect effect through radiolysis of cellular water and formation of active oxygen species, free radicals and peroxides causing single and double strand DNA breakage (McNamara *et al*., 2003).

Because of the tremendous diversity of exoenzymes produced by fungi, cellulases, gluconases, laccases, phenolases, keratinases, mono-oxyginases, proteases, lipases and many more; the preservation of libraries and museum books and documents is inevitably connected with prevention of moulds by inhibiting these or most of these enzymes (Sterflinger, 2010).

Borrego *et al*. (2010) evaluated the microbial prevalence inside library (in Cuba) and museum archive(in Argentina)and reported that various fungal species produce cellulases, proteases and amylases and could degrade cellulose, proteins, starch and also excrete acids and pigments.

TABLE 7. Effect of different doses of γ-irradiation on some enzymatic activities of *Aspergillus parasiticus*.

Dose (KGy)	Amylase (U/ml)			Reduction of control (%)	Cellulase (U/ml)			Reduction of control (%)	Caseinase (U/ml)			Reduction of control (%)
	(Mean	±	SD)		(Mean	±	SD)		(Mean	±	SD)	
0.00	432.0	±	21.6^{e}	0.00	961.9	±	21.5^{f}	0.00	6561.3	±	85.5^{g}	0.00
0.20	138.2	±	3.0^{d}	68.01	827.2	±	7.9^{e}	14.00	5839.5	±	91.9^{f}	11.00
0.40	136.4	±	6.0^{d}	68.43	799.6	±	10.8^{e}	16.87	4412.8	±	98.2^{e}	32.75
0.60	101.8	±	7.8^{c}	76.44	525.0	±	49.2^{d}	45.42	2456.5	±	45.1^{d}	62.56
0.80	50.9	±	2.2^{b}	88.22	212.0	±	2.7^{c}	77.96	756.5	±	20.8^{c}	88.47
1.00	9.8	±	0.3^{a}	97.73	30.2	±	1.1^{b}	96.86	104.4	±	11.1^{b}	98.41
2.00	0.00	±	0.00^{a}	100.00	0.00	±	0.00^{a}	100.00	0.00	±	0.00^{a}	100.00

Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test.

TABLE 8. Effect of different doses of γ-irradiation on some enzymatic activities of *Penicillium commune*.

Dose (KGy)	Amylase(U/ml) (Mean	±	SD)	% reduction of control	Cellulase(U/ml) (Mean	±	SD)	% reduction of control	Casinase(U/ml) (Mean	±	SD)	% reduction of control
0.00	214.17	±	7.0^{f}	0.00	718.5	±	3.0^{g}	0.00	4876.7	±	158.2^{f}	0.00
0.20	186.5	±	5.2^{e}	12.92	531.9	±	5.9^{f}	25.97	2912.1	±	5.8^{e}	40.29
0.40	183.0	±	3.0^{e}	14.55	454.2	±	29.5^{e}	36.78	2606.8	±	23.7^{e}	46.55
0.60	102.3	±	3.0^{d}	52.23	321.7	±	10.8^{d}	55.23	1171.9	±	11.6^{d}	75.97
0.80	30.4	±	1.2^{b}	85.81	109.3	±	1.3^{c}	84.79	332.3	±	12.3^{c}	93.19
1.00	6.7	±	0.5^{a}	96.87	18.2	±	0.8^{b}	97.47	88.2	±	5.6^{b}	98.19
2.00	0.00	±	0.00^{a}	100.00	0.00	±	0.00^{a}	100.00	0.00	±	0.00^{a}	100.00

Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test.

Guiamet *et al*. (2011) found that most fungi isolated from biodeteriorated photos and maps stored at Historical Archive of Museum La Plata, Argentina, degraded cellulose and produce extracellular cellulases, pigments and acids. *Talaromyces helices* (*Penicillium* teleomorph) has been reported from documents surfaces. It has cellulolytic activity (Moloney *et al*., 2004; Chacon & Waliszewski, 2005) so its presence involves a high risk for document conservation. Cellulolytic fungi can destroy paper materials in short time (Adamo *et al*., 2003) hence it uses cellulose as carbon source.

Fungi degrade materials and thus affect objects substantially by enzymatic degradation of paper objects. Fungi penetrate cracks and migrate underneath upper layer and causes detachment. In paper conservation, considerable damage can be caused by cellulases and lipases (Bech-Andersen and Elborne, 2004).

Control of paper deteriorating fungi by laser radiation

The data in Fig. 7 revealed general reduction in the viable colonies (cfu) and dry biomass of both fungal species when exposed to laser beam as compared to control. Both parameters, however, showed persistence to extended exposure period to laser beam from 1 to 5 min with no significant decrease. After 10 and 15 min, a reduction of 75% and 95% was recorded in the two growth parameters, respectively. Lethal laser effect had been detected after 20 min exposure in both fungal species.

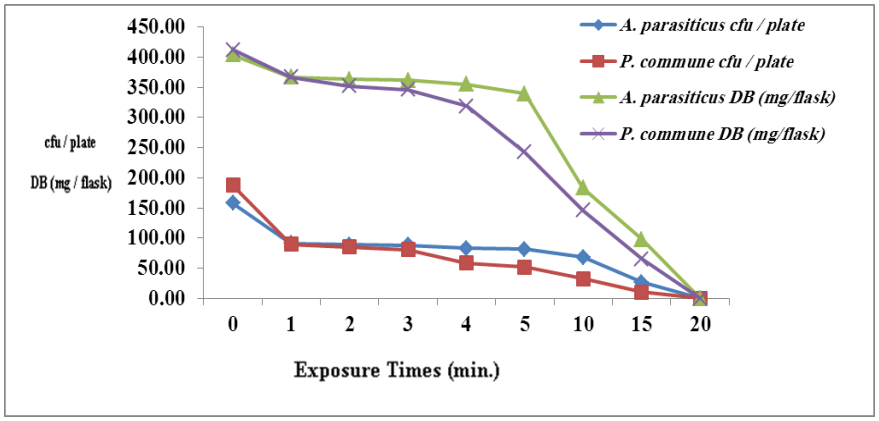

Fig. 7. Effect of laser irradiation on fungal growth parameters.
-DB: dry biomass.

Kolar *et al*. (2003) found no immediate effects on paper irradiated with laser running at 532 nm or on solid cellulose. Also, Kolar *et al*. (2000a) used contactless cleaning of paper surfaces by application of short laser pulses to remove foreign material, such as dirt which serves as a culture medium for microorganisms. Laser at 532 nm and fluencies below 0.86 J/ cm^2 showed no degradation of paper.

It has been suggested that laser cleaning of ancient parchment manuscripts from the 15^{th} and 16^{th} century and printed paper from 19^{th} century to remove dirt and microorganisms was maintained by Kautek *et al*., 1998. This method avoids the toxic cleaning fluids and has potential to accelerate conservation work offering high quality results and save costs. Ochocinska *et al*. (2003) reported that the penetration depth as well as the dose of laser energy delivered can be controlled, and therefore the risk of possible damage to the treated old documents and works of arts can be markedly reduced during restoration and cleaning from microbial spores and dirts. The less destructive wavelength (532 nm) and energy fluencies (< 1 J/cm^2) are recommended by (Kolar *et al*., 2000b).

It is worth mentioning that Speranza *et al*. (2012) controlled stone biodeterioration in heritage buildings by endolithic algae, fungi and the lichen *Verrucaria nigrescence* by using pulsed laser of 1064 nm from Nd:YAG laser. The fungal and algal cells were completely destroyed or presented a high plasmolysis degree resulting from heating microenvironment.

Finally, an advanced knowledge and thorough understanding of the materials from which the paper made together with identification and characterization of any original damage are fundamental prior to carry out any restoration work. Furthermore the precise identification of the microbial species which responsible for the damage is also an important require.

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(*Received* 7 /11/2013;
accepted 25 /11/2013)

التحكم فى الفطريات التى تسبب تلف المطبوعات الورقية بواسطة العقاقيـر المضـادة للفطـريات ، الزيوت الطيارة ، الاشعـاع الجامى و أشعة الليزر

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تهدف هذه الدراسة الى السيطرة على فطرتين مختارتين (الاسبيرجيللس بارازيتيكس و البنيسليوم كميونى واللتان يسببان تلف الكتب و الوثائق فى أماكن تخزين الكتب فى المكتبات) باستخدام العقاقيرالمضادة للفطريات ، الزيوت الطيارة(تضاف منفردة أو متجمعة) ، الاشعاع الجامى و أشعة الليزر. أوضحت النتائج المتحصل عليها أن فلوكونازول و كلوتريمازول كانت فعالة ضد كلا من الاسبيرجيللس بارازيتيكس و البنيسليوم كميونى بينما كان النيستاتين أقل تأثيرا. أظهرت فطرةالاسبيرجيللس بارازيتيكسمقاومة أكثر من فطرة البنيسليوم كميونى لمضادات الفطريات (عن طريق تعيين تركيز الحد الأدنى المثبط لنمو ٦٠ ٪ من الفطريات). كانت زيوت القرفة والقرنفل فعالين كعوامل مضادة للفطريات فى حين أن زيوت الثوم والزعتر ليس لديهما أنشطة ملموسة. كشفت المعاملات المدمجة لزيوت الثوم والزعترمع العقاقيرالمضادة للفطريات عن زيادة الفاعلية فى بعض الأحيان و عموما فان أعلى سيطرة على الفطرتين قد ظهرت باستخدام كلوتريمازول منفردا. وقد وجد أن الفطرتين اختلفا فى الاستجابه لأشعة جاما. كانت فطرة الاسبيرجيللس بارازيتيكس أكثر تحملا من فطرة البنيسليوم كميونى فى الكتلة الحيوية الجافة فى حين أنهما كانا متساويين فى الاستجابة لوحدات تكوين المستعمرات الفطرية. كما كشف تعيين قيمة ال D_{10} أن فطرة البنيسليوم كميونى أكثر تحملا من فطرةالاسبيرجيللس بارازيتيكس للاشعاع الجامى. وأظهرت الفطرتين مقاومة فى معاملات النمو (وحدات تكوين المستعمرات الفطرية و الكتلة الحيوية الجافة) عند تعريضهما لأشعة الليزر لمدة ١-٥ دقائق وعند التعريض لأشعة الليزر لمدة ١٠-١٥ دقيقة ظهر انخفاض بنسبة ٧٥٪ و ٩٥٪ عن التجربة الحاكمة على التوالى. ومع ذلك لوحظ التأثير المميت للفطرتين بعد التعريض لأشعة الليزر لمدة ٢٠ دقيقة.