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SCIENTIFIC EVALUATION OF USING HABAT ALBARAKA (*NIGELLA SATIVA L.*) AGAINST MICROORGANISMS FOR THE PRESERVATION OF HISTORICAL PARCHMENT AND LEATHER ARTIFACTS

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ABSTRACT

In Egypt, parchment manuscripts and tanned leathers artifacts in public and private libraries, archives, museums and store houses of museums are susceptible for the growth of fungi, and this may due to the improper factors such as extreme temperature, relative humidity, high light levels and etc. this study aims to isolate and identify fungi from different locations in Egypt; and to evaluate the efficiency of Habat Albaraka (*Nigella sativa L.*) as a natural extract of oil fungicide used against microorganisms which isolated from historical parchment and leather samples and follows the improvements or changes of selected parchment and leather properties. As it is the first time to use it in the field of conservation of the historical manuscripts.
Radial growth, change of color by spectrophotometer, mechanical properties (tensile strength and elongation) and Fourier Transform Infrared Spectroscopy (FTIR) were used for the evaluation of efficiency of Habat Albaraka used as a natural extract of oil- less toxic antifungal agent. The results showed that the most dominant fungi were *Aspergillus niger* and *Aspergillus sulphureus*. The results proved that Habat Albaraka used prevents the growth of identified fungi, but the third concentration is the best. The loss of color changes and mechanical properties decreased with increasing the concentration of fungicide used. This study confirms that the application of Habat Albaraka natural extract of oil as fungicide is necessary and useful in the preservation of parchment manuscripts and leather artifacts besides using Habat Albaraka as a natural antifungal essential oil improved the mechanical properties of the historical manuscripts.

KEYWORDS: Parchment, Leather, Fungal ageing, Habat Albaraka, Radial growth, color change, mechanical properties, natural extract of oils, essential oil.

1. INTRODUCTION

Parchment and leather are composed of collagen, some amounts of keratin, elastin and minimal amounts of albumin and globulin (Kowalik, 1977). It can be added that the degree of susceptibility of parchment and leather to the attack by microorganisms depends on the raw material, and its method of preparation (Valentine, 1996).

In Egypt the state of preservation of parchment manuscripts and leather artifacts in most locations (museums, storage rooms, libraries, archives and excavation areas) is unfavorable conditions, which are far from the international standards (rapid fluctuation in relative humidity and temperature, excess light etc.) (Abdel-Maksoud, 2000). These conditions are suitable for the growth of different types of fungi in historical manuscripts generally (Caneva et al., 1991; Zyska, 1997, 2002; Nol et al., 2001; Corte et al., 2003; Adelantado et al., 2005; Cappitelli and Sorlini, 2005; Michaelsen et al., 2006, 2009; Rakotonirainy et al., 2007; Ricelli et al., 1999; Zotti and Ferroni, 2008; Mesquita et al., 2009). Fungi infestation could be happened when conidia dispersed in the air to fallon damp and dirty manuscripts (Florian, 1997; Maggi et al., 2000; Lugauskas and Krikstaponis, 2004). Some forms of deterioration are resulted such as different colored stains (Szczepanowska and Lovett, 1992; Aranyanak, 1995; Arai, 2000), discoloration of pigments and inks, and changes in the chemical and physical properties of the manuscript (Caneva et al., 1991; Nitte´ rus, 2000). The most common fungi which cause degradation in parchment manuscripts are generally Penicillium, Aspergillus, Alternaria, Fusarium, and Trichoderma (Sahib et al., 2003). Also, fugal strains (Aspergillus niger, Penicillium funiculosum, Alternaria alternate and Trichderma viride) which are commonly found in archaeological vegetable-tanned leather artifacts (Abel-Maksoud, 2006; Orlita, 1968a, 1968b).

Controlling and avoiding fungal damage are considered one of the most important necessities that should be done by the conservators in the museum artifacts, particularly organic materials due to their extreme sensitivity to this type of damage. Consequently, it is thought that it would be appropriate to use fungicides to prevent manuscripts from biodeterioration. Traditionally, conservators used fungicidal treatments that involved toxic and potentially damaging chemicals to inhibit fungus growth on manuscripts. Recent studies propose replacing these traditional chemical toxic fungicides with others alternative that are natural and less toxic against the environment also the human healthy which called natural extracts of oils; this study selected Habat Albaraka because its important antifungal properties which not studied before in historical manuscripts conservation procedures (Dersarkissian and Goodberry, 1980; Fabbri et al., 1997; Bubiniene, 1999; Silva et al., 2006).

Natural extracts obtained from plants have been widely used since many centuries ago in order to treat many pathologic processes. According to their antimicrobial properties, which are mainly attributed to some of the compounds such as terpens, essential oils (volatile), resins, alkaloid glycosides and fixed oils, coumarines and flavonoids. Essential oils are products of complex general composition that contain volatile principles present in plants, more or less modified during their preparation. Essential oils are mainly found in superior plants, obtained by distillation (Eos).

The exact mechanism of action of many natural extracts is not well known, but it has been proved that owe their antimicrobial activity to the overload that the microorganism cellular wall undergoes. This fact determines the loss of control and integrity of the wall for the action over the enzymatic system, thus improving appetite and optimizing nutrient absorption (Calvo et al., 2006; Rhayour et al., 2003; Vardar-Ünlü et al., 2003; Kamel, 2002; Peris, 2002). The solubility in water of essential oil constituents is directly related to their ability to penetrate the cell walls of a bacterium or fungus. The antimicrobial activity of essential oils is due to their solubility in the phospholipid bilayer of cell membranes. Terpenoids which are characterized by their lability have been found to interfere with the enzymatic reactions of energy metabolism (Karl, 2011). Also the studies have made use of these fungicides to see the extent of their impact on the damage caused by fungi without studying the impact of determination of fungicides residues and their half-life impact (Rastogi, 2000; Perlovich et al., 2005; Xu et al., 2004; Reynolds, 1996; Doron et al., 2001; Soni et al., 2005).

Habat Albaraka was selected as the study aims to evaluate the efficiency of Habat Albaraka (Nigella sativa L.) as a natural extract of oil fungicide used against microorganisms which isolated from historical parchment and leather samples and follows the improvements or changes of selected parchment and leather properties as it is the first time to use it in the field of conservation of the historical manuscripts (El Menshawy et al., 2018).

Color change measurement is a vital in the conservation process to evaluate the conditions or to follow the changes in this property with ageing time during experimental studies. The parchment and leather samples passed through microbiological ageing cycle for months; the effect of the ageing on the samples properties has been studied. Many authors (Herascu, et al., 2008; Hulubei, 2014; Kite and Thomson, 2006;

and Abdel-Maksoud, 2009) used different analytical techniques for evaluating the properties of parchment and leather (color change and mechanical properties).

This study aims to evaluate the efficiency of Habat Albaraka against microorganisms by determination of inhibition zone at different times; follow the change of color, mechanical properties and determine the degree of degradation of collagen during ageing time and concentrations used.

2. MATERIALS AND METHODS

2.1 Samples collection

Parchment manuscripts and leather artifacts samples were collected from the following places:

1- The Egyptian Museum (separated piece of leather excavated in 1966; registered under No. JE 90809 SR 5/13677, 32cm x 45 cm dimensions , written on it unknown lines with the black and red inks)
2- Different separate historical parchment manuscripts form National Library and Archives dates back to 11th century AD.

The samples preparation of parchment and leather was applied according to the international references in this field (Reed1972; EL-Moselhy, 2012).

The samples taken for the isolation and identification of fungi were in accordance with Abdel-Maksoud (2011) with some modifications. Sterile cotton swabs were wiped along the most damaged margins of the verso and recto of each piece of the historical parchment document to obtain samples for fungal culturing and identification. Loose dust and powder were also collected from the document pages with a soft brush. Fungi isolated were identified according to Raper and Fennell; Barnett and Hunter; Domsch *et al*., Stevens and Carlile *et al*. (Raper and Fennell, 1965; Barnett and Hunter, 1972; Domsch *et al*., 1980; Stevens, 1981; and Carlile *et al*., 2001).

2.2 Fungicide used (natural extract of essential oil of Habat Albaraka), classification, analysis, separation

Nigella sativa is an annual flowering plant. It grows to 20–30 cm (7.9–11.8 inch) tall and has linear lanceolate leaves. This plant is known by numerous names, for example black cumin (English), black caraway seeds (USA), Shonaiz (Persian) and Kalajira (Bangali). *N. sativa* is an annually flowering plant which grows from 20 cm to 90 cm in height, with finely divided leaf. The seeds of nigella (black cumin) (Nigella sativa L.) have been widely used as a natural remedy, either alone or in combination with bee products, for the treatment of many acute as well as chronic conditions for centuries, especially in the Middle East and Southeast Asia (Salih et al., 2009). Also studies on the Egyptian (Nigella sativa L.) some pharmacological properties of the seeds' active principle in comparison to its dihydro compound and its polymer (el-Dakhakhny M., 1965).

The Scientific Classification is as follows:

Scientific Name: *Nigella sativa* L.

Local Name(s): Alhabah Alsodaa, Habat Albaraka.

Arabic Name(s): Alhabah Alsodaa, Habat Albaraka; Cumon Aswad, Showneez (Per.)

Common Name(s): Black seed, Black Cumin

Family: Ranunculaceae

The used part was Seeds and oil.

Fig.1: Habat Albaraka different parts ordered as: Seeds, Flowers, Leaves & Fruit

Extensive studies were done to identify the composition of the black cumin seed, the ingredients of *N. sativa* seed includes: fixed oil, proteins, alkaloid, saponin and essential oil. The fixed oil (32-40 %) contains: unsaturated fatty acids. The volatile oil (0.4-0.45%) contains saturated fatty acids. Black cumin seed have two different forms of alkaloids: isoquinoline alkaloid that includes: nigellicimine, nigellicimine n-oxide and pyrazol alkaloid that includes: nigellidine and nigellicine. The nutritional compositions of *N. sativa* are vitamins, carbohydrates, mineral elements, fats and proteins that include eight or nine essential amino acids. Black cumin seeds also have saponin and alpha hederine and in trace amount has carvone, limonene and citronellol, as well as provide relatively good amounts of different vitamins and minerals such as Fe, Ca, K, Zn, P, Cu.

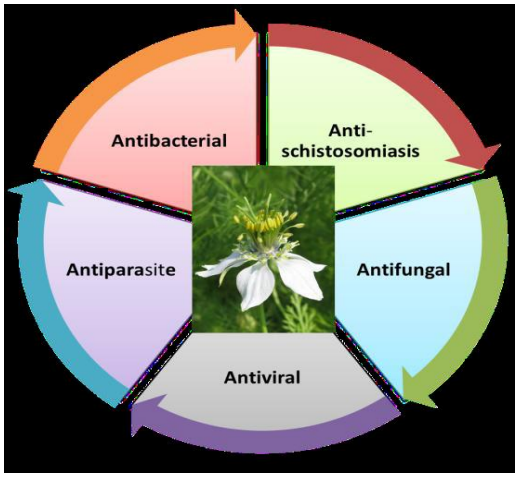

Fig.2: Different effects of Nigella sativa against microorganisms

The natural extract of essential oil of Habat Albaraka preparation was as follows:

Extraction: The seeds of *Nigella sativa* are crushed to course powder using electric grinder. The extraction is done using four different solvents namely Petroleum Ether, Chloroform, Methanol, Hydroalcoholic solution (20% DM Water in Methanol). Equipment used are:

1. Speed Extractor: Grounded Powder is loaded in the four chambers along with purified sand. The lead taking solvent is put in pure covered solvent. After three cycles of automatic extraction the equipment the process continues for all four solvents. The extracts are stored in clean dried container.

2. Aspirator: Dried grounded powders are put in closed glass aspirator. Solvent in same order is poured over the powder so that all powder is in direct contact with solvent. The aspirator is kept overnight undisturbed with solvent. Next day the extract is collected and stored.

All the extracts are subjected to concentration using rota vapor equipment until only concentrated oil or sticky solid is left. The hydro alcoholic portion is fractionated against Petroleum ether, ethyl acetate and chloroform. The organic portion is collected and dried until minimum drop of oily substance is collected. They are mixed with parent extract and stored in cool and dry place. The only left water part is dried to form solid (Samajdar, 2017).

2.3 Soxhlet extraction and fractionation of N. sativa extracts by Silica gel column

Solvent-extraction was performed using approximately 20 g of finely ground powder of *N. sativa* was placed in a thimble and extracted in an all glass Soxhlet extractor for 8h using hexane and methanol as solvents. Solvent was removed by rotary evaporation at 40°C under vacuum; the organic phase was then concentrated under vacuum and dried for 5 min in an oven at 103± 2 °C. And the last traces of solvent in the extract were removed under a stream of nitrogen. The extract was stored at 18°C until protected from sunlight prior analysis.

The extract obtained by Soxhlet extraction technique was subjected to fractionation on silica gel. A 250 g of activated silica gel was loaded to a column and cleaned with about 100 ml of hexane. About 10 g of the extract was loaded on to the column. The compounds were eluted successively with 500 mL each of hexane, 15% diethyl ether in hexane, diethyl ether, and methanol. Solvent in the fractions was removed by rotary evaporation at 40°C under vacuum. A known quantity of each fractionation (100 mg) was made up to 10 mL with methanol (Said *et al.,* 2013).

2.4 Accelerated ageing of parchment and leather samples (micro ageing technique)

The most commonly used methods in the laboratory for their simplicity and quickness are:

- Agar diffusion disk technique: used to obtain qualitative results.
- Dilution in broth and agar: used to obtain quantitative results. This method is used in order to evaluate the antimicrobial activity of natural extracts and organic acids against bacteria and fungi.

Methodology for fungi

The used culture medium was Sabouraud Dextrose Agar (SDA). The medium was poured onto 90mm diameter Petri plates until the thickness of the agar was 4mm so that possible problems of diffusion of the tested products could be prevented.

0.1ml of each fungal solution was inoculated onto the plates and by means of sterile swabs was uniformly distributed. Plates were allowed to stand for 15 minutes. At the same time, 6mm diameter disks (Schleicher & Schuell) were soaked with the products to be tested at different concentrations. After discarding the excess of product, the disks were symmetrically placed onto the medium by means of sterile tweezers. One of the disks was soaked with sterile distilled water as a control. The plates were incubated for 48±2h at 28°C under aerobic conditions. The results were evaluated by measuring the areas with no fungal growth.

Inhibition value = Inhibition diameter in mm – Disk diameter (6mm) / 2

These experiments were carried out in triplicates and control cultures were prepared for all the strains (Hammer, 1999).

Microbiological ageing will be used in different time periods. To evaluate the clear zone in in Petri dishes and to evaluate other properties after the ap-

plication of Habat Albaraka against *Aspergillus niger* and *Aspergillus sulphureus*, the technique of Hasan *et al*. (2009) was modified and employed for measurement of clear zone.

The Petri dishes were incubated for 3months. After the incubation period, every month clear zone was measured and the parchment and leather samples were picked out and cleaned mechanically by brush to remove mycelia to evaluate other properties. The plates were incubated at 21+ 2° C and 65% relative humidity (RH) before the investigation and study the effect of ageing on the samples in comparison with the control samples and evaluate the efficiency of the Habat Albaraka on the treated samples. Data was statistically analyzed using a T-TEST program with the factorial experiment as the completely randomized block design (CRD) for all the tests carried out in this study. Each set of treatment was replicated 3 times and results were analyzed by analysis of variance (T-TEST).

The New parchment and leather samples were sterilized with U.V. beams. The tests were performed in 15cm diameter Petri dishes on solid medium (PDA) medium without the Carbone source. The medium was inoculated with mould spores (The spores of *Aspergillus niger* and *Aspergillus sulphureus*) and the various parchment and leather samples control/treated samples with Habat Albaraka fungicide in different concentrations were placed in the centre of Petri dishes (Abdel-Maksoud, 2006).

2.5 Testing for growth Media and Conditions, antifungal screening

MIC (Minimum Inhibitory Concentration) of the antifungal screening was determinate as each plate was observed (checked) each day for presence of growth.

The activity was determined after 72 h of incubation at room temperature (32°C). When the fungus had grown on control plates close to the margin of plate, the radial growth of *Aspergillus niger* and *Aspergillus sulphureus* was measured for each plate.

Each sample was used in triplicate for the determination of antifungal activity.

The Inhibition zone (%) of the treatment samples of parchment and leather of the Habat Albaraka fungicide. The percentage of fungal growth inhibition was calculated according to the Pandey *et al*. (1982) formula: Growth inhibition% = [(growth in the control –– growth in the sample)/growth in the control] × 100.

After calculating the percent of growth inhibition, the EC50 of the fungicide was determined. By means of the Probit analysis program, the effect of the Habat Albaraka fungicide at its EC50 concentration data was statistically analyzed employing a completely randomized design (CRD).

3. DATA ANALYSIS

Each set of treatment was replicated 3 times. The growth inhibition percentage was recorded, and data were analyzed by the log-probit method of Finney (1971) using the EPA probit analysis program version 1.5, Florida to calculate the EC50, EC90 and slope values. The standard deviation and standard error of mean was calculated using the Microcal origin soft- ware version 5.

The Color changes were measured using CIE L*a*b* system. The total color difference (ΔE*) was calculated according to the this equation: ΔE* = [(ΔL*)2+ (Δa*)2 +(Δb*)2]1/2. The measurement was made using Macbeth color eye 7000 (U.S.A.) UV spectrophotometer at the Textile Testing Lab., Division of Chemical Metrology, National Institute for Standards, Egypt.

The respective mechanical properties (tensile strength – elongation) of the aged and blank samples were tested using tensile testing machine of model H5KT, Tinius Olsen Co. SDL-UK of capacity 5kN (1,000 lbf). In the Textile Testing Lab., Division of Chemical Metrology, National Institute for Standards, Egypt. Each set of treatment was replicated 3 times and results were analyzed.

Fourier Transform Infrared Spectroscopy (FTIR) was applied. This method of analysis gives information on the composition of the material and at the same time gives an indication of the behavior of the protein materials degradation. Infra-red spectra were obtained using a FTIR spectroscopy (JASCO-ATR-FT/IR-6100). Infrared spectra were obtained using a Fourier transform infrared spectroscopy (JASCO-FT/IR-6100) in Laser Technology Unit (LTU), Center of Excellence for Advanced Sciences (CEAS), National Research Center (NRC).

4. RESULTS AND DISCUSSION

The results revealed that the identified fungi were *Aspergillus niger* and *Aspergillus sulphureus*. Valentin (1996) reported that fungi cause loss of an object's strength and even its integrity. Szczepanowska and Cavaliere (2000) proved that *Aspergillus sp*. is considering one of the most occurrence found on documents. Tiano (2002) reported that As a result of biodeterioration, parchment loses its original properties and becomes hard and brittle, often with deformation of the structure. The microbial attack also causes variegated spots, white films and fading of the texts. He also reported that fungi attack tanned leather and uti-

lize the fats present in leather as a source of carbon. In this case the proteins are not directly affected, but can be damaged by organic acids released as a metabolic end products and the artifact becomes stained and stiff. The principal effects of microbial deterioration on protein materials are the presence of different stained spots, and loss in tensile strength. Strzelezyk (2004) found that during decay of the manuscripts by microorganisms (such as fungi) it becomes thinner and weaker and also loose color, she also said that acids secreted by microorganisms can bring about a weakening in structure of the manuscript. Orlita (1993) reported that isolated fungi from parchment and tanned skin belonged to genera *Aspergillus*. Sterflinger (2010) confirmed that fungi cause change of color for cul- tural heritage. Thomson (2006) said that the fungal deterioration is now considered to be due to hydro- lytic chemical deterioration. Abdel-Maksoud (2011) mentioned that parchment and leather materials are highly susceptible to aerobic fungal growth, which can cause some white, green or dark colored colonies.

4.1 Radial Growth

Results of the present study (Table 1) revealed that growth in the *Aspergillus niger* and *Aspergillus sulphureus* were prohibited by all the Habat Albaraka fungicide concentrations tested. Mycelia diameter of control was 14.9cm after 90 day's incubation. The efficiency of Habat Albaraka fungicide concentration varied on the samples. There was significant difference in growth of *Aspergillus niger* and *Aspergillus sulphureus* in the control and at 20 µl concentration. The maximum mycelia spread for *Aspergillus niger* in the Habat Albaraka-treated medium was 12cm at 6.25 µl concentration. High concentration (20 µl) of the Habat Albaraka fungicide inhibited growth completely in both of *Aspergillus niger* and *Aspergillus sulphureus*. Above 20 µl *Aspergillus niger* and *Aspergillus sulphureus* did not grow in Habat Albaraka incorporated PDA. The concentration of 12.5 µl and 20 µl was more effective than the concentration of 6.25 µl in radial growth spread in *Aspegillus niger* and *Aspergillus sulphureus*. The concentration of 20 µl proved to be the best fungicide concentration among those tested concentration in this study, but the concentration of 6.25 µl was the least effective on the fungi. The Habat Albaraka fungicide efficiency is due to the active ingredients of the fungicide (Alpha-phellandrene, Benzen, 1 methyl – 4 – (1- ethyl ethyl), Junipene, Hexadecanoic acid). This interference inhibits a range of essential key fungal development processes like spore germination, germ tube growth, appressorium development and mycelial growth.

Table 1: The Inhibition zone (%) and statistic analysis of the treatment parchment and leather samples with different concentrations of the Habat Albaraka fungicide.

Month of micro ageing cycle	Habat Albaraka Fungicide concentrations used	*Aspergillus niger*			*Aspergillus sulphureus*		
		Inhibition zone (%)	Statistical analysis		Inhibition zone (%)	Statistical analysis	
			Mean ± SD	Sum		Mean ± SD	Sum
1	Control	0%	13.8 ± 0.1	33.6	0%	13.8 ± 0.1	33.6
	6.25 µl (Parchment)	30%	8.2 ± 0.4	27	50%	5.3 ± 0.4	18.2
	6.25 µl (Leather)	10%	11.3 ± 0.5	26.2	35.6%	7.8 ± 0.2	25.7
	12.5 µl (P)	43.2%	6.6 ± 0.7	23.1	62.2%	3.6 ± 0.3	13
	12.5 µl (L)	23.2%	9.3 ± 1.0	21.1	55.6%	4.3 ± 0.3	15.1
	20 µl (P)	50%	4.8 ± 0.1	16.5	70%	2.1 ± 0.3	8.6
	20 µl (L)	63.2%	3.7 ± 0.2	13.3	62.2%	3.2 ± 0.5	11.7
2	Control	0%	13.8 ± 0.1	43.6	0%	13.8 ± 0.1	33.6
	6.25 µl (Parchment)	43.2%	6.5 ± 0.5	21.8	55.6%	4.1 ± 0.3	14.4
	6.25 µl (Leather)	23.2%	8.6 ± 0.2	28	42.2%	6.1 ± 0.4	10.5
	12.5 µl (P)	56.6%	3.8 ± 0.7	13.6	62.2%	3.2 ± 0.5	11.8
	12.5 µl (L)	36.6%	6.7 ± 0.2	22.4	62.2%	3.4 ± 0.5	12.3
	20 µl (P)	63.2%	3.2 ± 0.3	11.7	75.6%	1.2 ± 0.5	5.8
	20 µl (L)	70%	3.3 ± 0.5	9.3	75.6%	1.4 ± 0.5	6.4
3	Control	0%	13.8 ± 0.1	43.6	0%	13.8 ± 0.1	33.6
	6.25 µl (Parchment)	63.2%	3.3 ± 0.4	12.2	70%	2.1 ± 0.5	8.4
	6.25 µl (Leather)	43.2%	5.8 ± 0.27	19.9	55.5%	4.1 ± 0.5	14.4
	12.5 µl (P)	76.5%	1 ± 0.1	5	75.5%	1.4 ± 0.1	6.4
	12.5 µl (L)	56.5%	4 ± 0.1	14	70%	2.4 ± 0.3	9.3
	20 µl (P)	70%	2.1 ± 0.1	8.5	82.2%	1.1 ± 0.3	2.6
	20 µl (L)	76.5%	1.1 ± 0.1	5.5	82.2%	1.1 ± 0.3	2.5

The calculation of Habat Albaraka fungicide EC_{50} is given in Table 2.

Table 2: Effective Concentration (EC_{50}, EC_{90}), and slope of Habat Albaraka against Aspergillus niger and Aspergillus sulphureus

Tested material		EC_{50} (%)	EC_{90} (%)	%95 Confidence Limits	Chi-Square		Slope ±se
					Cal.	Tab.	
Parchment	***Aspergillus niger***	1.53	18.41	1.02-3.51	1.23	4.88	1.36±0.41
	Aspergillus sulphureus	1.53	8.48	3.4-1.33	1.38	4.88	1.56±0.44
Leather	***Aspergillus niger***	4.43	17.28	2.83-1.075	1.68	4.88	1.35± 0.55
	Aspergillus sulphureus	1.33	8.76	1.48-2.73	0.54	4.88	1.14±0.38

EC_{50}: the fungicide concentration for 50% inhibition
EC_{90}: the fungicide concentration for 90% inhibition
Cal.: calculated Chi-square for heterogeneity
Tab.: tabular Chi-square for heterogeneity at 0.05 levels
Slope (±standard error).

Regarding color change, the exact cause of the stains produced by mold growth or in dead or dormant colonies is difficult to determine. Stains may be caused by metabolic processes, such as acids produced during the hydrolysis of the proteins or other nutrient matter; chemicals produced during the digestive process and excreted by-products; or simply by pigments present in the fungal structure itself. Certain molds are known to produce pigments such as *Aspergillus niger*, and may cause extensive color changes in the substrate, even though their growth is limited (Beckwith, 1940).

Belyakova identified numerous *genera* which produce stains on paper due to the production of pigments. The color of the stain is not an accurate guide to the specific mold which caused it, since the nature of the substrate affects the morphology of the organism. Belyakova noted that *Penicillium frequentas* produced yellow stains in some instances, pink stains in others. There is some evidence that staining is most prevalent in mature colonies that have been allowed prolonged growth and development, and is most pronounced in those areas where the older hyphae have deteriorated. Staining seldom occurs when the growth is removed during the vegetative stages, or before the mature organism begins to deteriorate. Staining may also result from attack on the organism, including adverse environmental conditions designed to retard its growth, or even fumigation (Belyakova, 1967).

Rakotonirainy *et al.* (1999) said that the deterioration of parchment materials is mainly due to the degradation of proteins caused by chemical or biological factors as microorganisms. The most common source of contamination of old parchment is the fungal spores which are identified by discolored spots or particles resembling dust giving undesirable aesthetic aspects and leading to irreversible degradation.

Sterflinger and Piñar (2013) parchment provides good conditions for the development of proteolytic fungi, among which numerous representatives of fungi in the genera *Aspergillus*. They also confirmed that fungi cause the color change of parchment.

Concerning the Lightness (L*value) it was clear, from the data obtained (Table 3) that there is a slight increase in L* value after one month of ageing compared with the control samples, but the results have increased gradually with increasing ageing time. The variation in L* value between the control sample and aged sample in the first month of micro ageing cycle was 1.56 in aged parchment sample but 12.84 in aged leather sample for *Aspergillus niger*. But it was 0.71 in aged parchment sample and 5.35 in aged leather sample for *Aspergillus sulphruses*.

It should be noticed that the results varied in the third month of micro ageing cycle as the results decreased from the control sample. The variation in L* value between the control sample and aged sample was 41.88 in aged parchment sample and12.83 in aged leather sample for *Aspergillus niger*. Also it was 17.36 in aged parchment sample and 26.57 in aged leather sample for *Aspergillus sulphruses*.

For the Red-green color (a* value), it is observed that the color got redder with the increase of all of the ageing time samples from the first month of micro ageing cycle till the third month of micro ageing cycle. The variation between samples in a* values in the first month of micro ageing cycle compared with the control sample was 0.12 in the aged parchment sample and 1.16 in the aged leather for *Aspergillus niger*. It was 1.31 in aged parchment sample and 3.06 in aged leather sample for *Aspergillus sulphruses*.

It was clear that the results varied in the third month of micro ageing cycle. The variation of a* val-

ue compared with the control sample was 0.21 in aged parchment sample and 1.34 in aged leather sample for *Aspergillus niger*. Also it was 2.05 in aged parchment sample and 10.14 in aged leather sample for *Aspergillus sulphruses*.

Regarding, Yellow- blue color (b* value) it was clear that the colour was more yellow and there is a little increase in the results, from the first month of micro ageing cycle till the third month of micro ageing cycle.

Table 3: Change of color of treated parchment and leather samples with Habat Albaraka fungicide after fungal ageing

Month of micro ageing cycle	Habat Albaraka Fungicide concentrations used	*Aspergillus niger*				*Aspergillus sulphrues*			
		Color values			Total color difference	Color values			Total color difference
		L	a	b	ΔE	L	a	b	ΔE
1	Control (P)	33.87	0.33	9.85	4.41	24.72	10.11	11.76	1.62
	6.25 µl	38.21	0.57	11.01	5.52	25.26	11.13	12.68	1.72
	12.5 µl	40.37	0.56	14.26	8.23	25.41	11.43	13.60	7.67
	20 µl	41.87	3.65	16.37	11.25	25.54	11.77	14.52	12.16
	Control (L)	3.03	0.13	0.28	2.38	0.43	0.01	1.73	2.16
	6.25 µl	1.16	1.27	4.16	2.16	0.82	3.08	1.81	2.46
	12.5 µl	6.54	2.62	5.41	3.11	6.00	1.11	1.22	5.86
	20 µl	16.88	5.00	11.73	4.31	11.58	5.00	2.25	8.68
2	Control (P)	44.17	0.47	8.81	1.00	27.23	8.42	12.64	0.88
	6.25 µl	42.73	0.50	10.68	1.13	31.37	11.24	14.74	1.26
	12.5 µl	45.47	1.06	11.15	2.75	27.74	13.48	16.54	2.65
	20 µl	48.21	0.88	10.44	12.87	24.87	10.71	11.44	4.16
	Control (L)	7.17	6.12	7.71	3.24	1.78	0.12	1.30	2.23
	6.25 µl	1.02	0.14	0.31	3.10	1.33	0.01	1.77	5.17
	12.5 µl	0.12	0.23	0.82	4.27	4.11	0.13	1.15	6.44
	20 µl	2.71	0.32	0.25	5.14	0.38	0.01	0.75	10.11
3	Control (P)	2.33	1.30	1.42	1.31	16.46	1.66	2.62	1.50
	6.25 µl	0.04	0.52	1.15	3.10	0.31	1.73	1.11	1.81
	12.5 µl	36.15	1.80	10.18	12.10	16.82	2.26	4.31	2.38
	20 µl	41.41	1.87	14.05	14.24	0.10	1.14	1.17	17.15
	Control (L)	17.64	2.10	4.55	3.83	41.45	1.21	11.47	4.80
	6.25 µl	5.16	1.16	2.35	6.16	8.65	2.57	0.58	10.34
	12.5 µl	33.27	1.26	10.40	6.35	31.75	5.67	13.16	11.60
	20 µl	3.66	0.42	1.17	15.52	4.00	1.21	1.11	15.51

The results were approximately very close compared with the control samples. The variation in the first month of micro ageing cycle between them was 1.10 in aged parchment sample and 10.34 in aged leather sample for *Aspergillus niger*. It was 1.72 in aged parchment sample and 1.33 in aged leather sample for *Aspergillus sulphruses*. It was noticed that the variation in the third month of micro ageing cycle between them was 10.51 in aged parchment sample and 0.74 in aged leather sample for *Aspergillus niger*. It was 2.42 in aged parchment sample and 2.22 in aged leather sample for *Aspergillus sulphruses*.

4.2 Total color differences (ΔE value)

It was noticed from the obtained data that there are differences between the aged samples compared with the control samples. It was clear in the parchment aged samples after one month was 5.52 against *Aspergillus niger* and 2.16 against *Aspergillus sulphruses* compared to the control sample 4.41. The results are different in the leather aged samples after one month was 2.16 against *Aspergillus niger* and 4.31 against *Aspergillus sulphruses* compared to the control sample 2.38. The variation differences in the total color increased with increasing the ageing time from the first month of micro ageing cycle till the third month of micro ageing cycle.

The difference between control sample and the highest concentration was 10.42 in parchment aged samples and 4.51 in the leather aged samples in the first month of micro ageing cycle against *Aspergillus sulphruses*. The difference between control sample and the highest concentration was 15.74 in parchment aged samples and 1.82 in the leather aged samples in the first month of micro ageing cycle against *Aspergillus niger*.

In the third month of micro ageing cycle the difference between control sample and the highest con-

centration was 11.8 in parchment aged samples and 19.34 in the leather aged samples. The difference between control sample and the highest concentration was 14.13 in parchment aged samples and 17.61 in the leather aged samples in the first month of micro ageing cycle against *Aspergillus niger*.

5. MECHANICAL PROPERTIES

5.1 Elongation

It was clear from the data obtained (Fig.3) that the elongation of parchment and leather in the control sample (infected sample without treatment by Habat Albaraka fungicide) was 8.75 for parchment with *Aspergillus niger*, and was 7.65 with *Aspergillus sulphruses*, for leather, the control samples was 10.5 with *Aspergillus niger*, and was 12.60 with *Aspergillus sulphruses*. It was noticed that there is a slight increasing in the elongation with treated samples with Habat Albaraka fungicide at the concentrations used. The results showed that fungal ageing of the control samples and treated samples decreased elongation with increasing the ageing time. The reduction in elongation was clear after the third months of fungal ageing. It was also noticed that the effect of *Aspergillus sulphruses* on parchment and leather elongation was higher that the effect of *Aspergillus niger*.

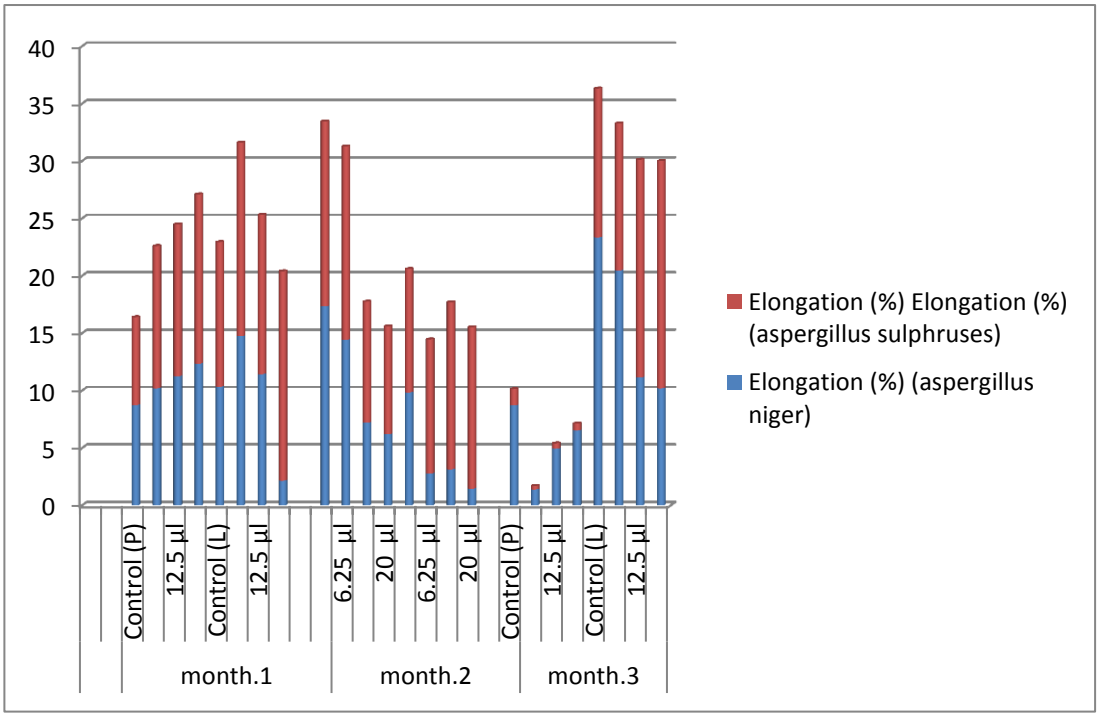

Fig.3: Elongation (%) of treated parchment and leather samples with different concentrations of Habat Albaraka fungicide (6.25, 12.5, and 20 µl) against two types of fungi at different ageing times (three months) compared to the control samples.

5.2 Tensile strength

The tensile strength (Fig.4) of the control sample infected with *Aspergillus niger* was 17.32 and was 22.64 with *Aspergillus sulphruses*. Tensile strength of leather in the control samples infected with *Aspergillus niger* was 21.30 and was 23.20 with *Aspergillus sulphruses*. It was clear that the effectiveness of Habat Albaraka fungicide was good after the first and the second months of fungal ageing. The reduction in tensile strength increased after the third months of fungal ageing.

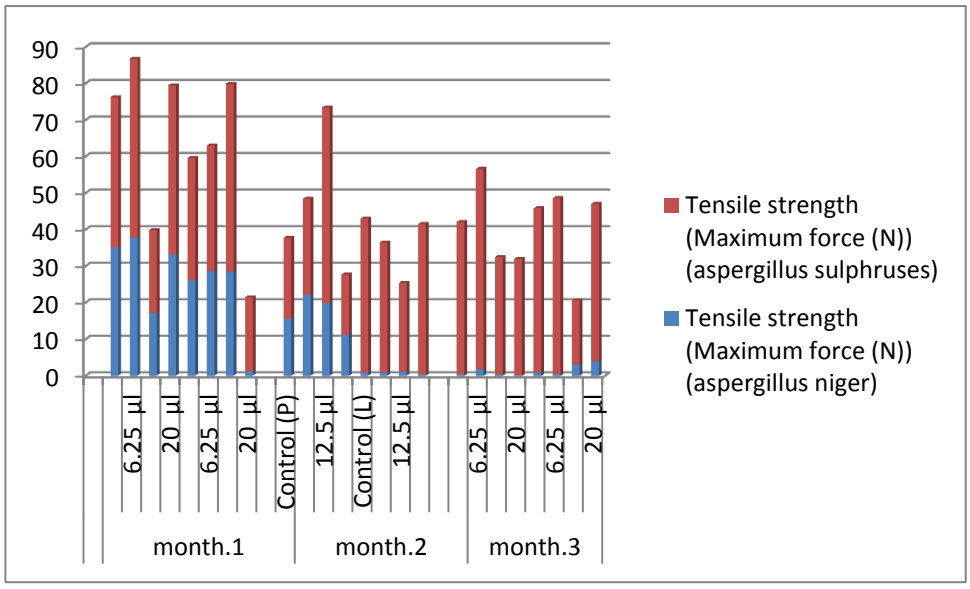

Fig.4: Tensile strength of treated parchment and leather samples with different concentrations of Habat Albaraka fungicide (6.25, 12.5, and 20 µl) against two types of fungi at different ageing times (three months) compared to the control samples.

5.3 Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra of control and aged samples treated with the Habat Albaraka fungicide in different concentrations (Fig. 5) were recorded from 4000-400 cm^{-1}. The spectra of amide I and the amide II bands were examined. The results showed that there are changes between the aged samples compared to the control samples.

It was noticed that after one month that the band at 3650.59 cm^{-1} in the control sample of parchment, also at 3646.73 cm^{-1} in aged parchment sample treated with fungicide (6.25 µl concentration), at 3632.27 cm^{-1} (12.5 µl concentration) and at 3667.94 cm^{-1} (20 µl concentration) against *aspergillus niger* assigned to a broad band represents (OH) hydroxyl stretching due to intermolecular hydrogen bonding of the hydroxyl group. This band shifted to a higher position in after two months at 3680.78 cm^{-1} and after three months at 3657.34 cm^{-1} in the control sample of parchment

After one month, the band at 3653.48 cm^{-1} in the control sample of parchment, also at 3648.66 cm^{-1} in aged parchment sample treated with fungicide (6.25 µl concentration), at 3639.02 cm^{-1} (12.5 µl concentration) and at 3651.55 cm^{-1} (20 µl concentration) against *Aspergillus sulphureus* assigned to a broad band represents (OH) hydroxyl stretching. This band shifted to a higher position after two months at 3675.15 cm^{-1} and after three months at 3659.27 cm^{-1} in the control sample of parchment.

It was noticed after one month that the band at 3629.37 cm^{-1} in the control sample of leather, also at 3648.66 cm^{-1} in aged leather sample treated with Habat Albaraka fungicide (6.25 µl concentration), at 3622.63 cm^{-1} (12.5 µl concentration) and at 3647.7 cm^{-1} (20 µl concentration) against *aspergillus niger* assigned to a broad band represents (OH) hydroxyl stretching due to intermolecular hydrogen bonding of the hydroxyl group. This band shifted to a higher position after two months at 3660.16 cm^{-1} and after three months at 3639.02 cm^{-1} in the control sample of leather.

After one month, the band at 3640.95 cm^{-1} in the control sample of leather, also at 3645.77 cm^{-1} in aged leather sample treated with Habat Albaraka fungicide (6.25 µl concentration), at 3649.02 cm^{-1} (12.5 µl concentration) and at 3649.62 cm^{-1} (20 µl concentration) against *Aspergillus sulphureus* assigned to a broad band represents (OH) hydroxyl stretching. This band shifted to a higher position after two months at 3660.10 cm^{-1} and after three months at 3659.27 cm^{-1} in the control sample of parchment.

This band includes multiple bands made up of multiple N-H groups (its primary amides) these bands are shifted to about (3114.47 cm^{-1} in the control sample of parchment and 3267.79 cm^{-1} in the aged treated sample of 20 µl concentrate of fungicide) in the molecule, which represent a symmetric N-H stretching.

This band includes multiple bands made up of multiple N-H groups (its primary amides) these bands are shifted to about (3114.47 cm^{-1} in the control sample of leather and 3175.22 cm^{-1} in the aged treated sample of 20 µl concentrate of fungicide) in the molecule, which represent a symmetric N-H stretching.

The C-H stretching vibrations occur in the region of 2957.3 cm^{-1} stretching of aliphatic groups in the

control parchment sample, and they were found in the aged leather samples that were studied at 2968.87 cm^{-1}. The position of the band in the control sample was very close to that of the bands of the treated samples with the Habat Albaraka fungicide. The bands become 2997.8 cm^{-1} after four months in the parchment samples.

Collagen exhibits a series of absorptions from 1600-1690 cm^{-1} is assigned to amide I (C=O stretching) and from 1480-1575 cm^{-1} is assigned to amide II (CN stretching, NH bending) and from 1229-1301 cm^{-1} is assigned to amide III (CN stretching, NH bending). The increasing or decreasing of C=O is dependent on the state of the sample. The presence of hydrogen bonding is an important contributing factor to this decrease in frequency.

The bands in the aged parchment samples changed after one month till after the third month at 1699.94 cm^{-1} of the control sample which assigned to Amide I. The band of amide I of 1691.27 cm^{-1} changed to 1700.91 cm-1 in the concentration of 12.5 µl, from 1696.09-1698.98 cm^{-1} in the concentration of 5% and 1694.63-1699.94 cm^{-1} in the concentration of 20 µl.

The band at 1563.02 cm^{-1} is assigned to amide II in the control parchment sample. But the amide II disappeared after 90 days of microbiological ageing cycle in the parchment samples treated with the fungicide 6.25 µl concentrations after three months.

The band at 1478.17 cm^{-1} is assigned to amide II in the control leather sample. But the amide II disappeared in after 30 day of microbiological ageing cycle in the leather samples treated with the fungicide 20 µl concentration after one month.

The bands in the aged leather samples changed after one month till after three months at 1697.05 cm^{-1} of the control sample which assigned to Amide I. The band of amide I of 1694.16 cm^{-1} changed to 1639.95 cm^{-1} in the concentration of 6.25 µl, from 1695.12-1691.27 cm^{-1} in the concentration of 12.5 µl and 1698.02-1684.52 cm^{-1} in the concentration of 20 µl.

The band at 1478.17 cm^{-1} is assigned to amide II in the control leather sample. But the amide II disappeared after 90 days of microbiological ageing cycle in the leather samples treated with the fungicide 20 µl concentration after three months.

But the amide II disappeared in after 60 days of microbiological ageing cycle in the leather samples treated with the fungicide 20 µl concentrations after two months.

Collins *et al*. (2002) reported that the rate of collagen loss will be accelerated according to the ageing cycle.

According to Götherstrom *et al*. (2002) and Abdel Maksoud (2010), the collagen was influenced by the presence or absence of water.

Centeno *et al*. (2004) classified the changes that resulted from FTIR spectra into two groups: the first group resulted from the influence of the hydrogen bonding of water on hydration sites such as C=O and N-H, resulting in the intensities and positions of the corresponding IR bonds (IR spectra look quite complex because the bond vibrations create absorption bands.

The results obtained from the first till the third month revealed also another types of degradation in the aged parchment and leather samples compared to the control sample. It was noticed that there is a hydrolytic cleavage of the collagen which indicated by the increase of OH group at 3600 cm^{-1} which indicated the degradation of hydrolytic breakdown mechanism in the ages samples compared to the control one. Also it was clear that there is an oxidation of the polypeptide chains which appeared in position 1750 cm-1 that indicated oxidation degradation.

Fungi can cause proteolysis of collagen in collagen materials but there are a number of factors that facilitate the process, such as the storage conditions and the presence of substances that reside in the original skin (other proteins, lipids, carbohydrates, mineral constituents and impurities) which can be available to be used in fungi metabolism and thus facilitate their colonization (Arroyo, 2009; Abdel-Maksoud, 2011).

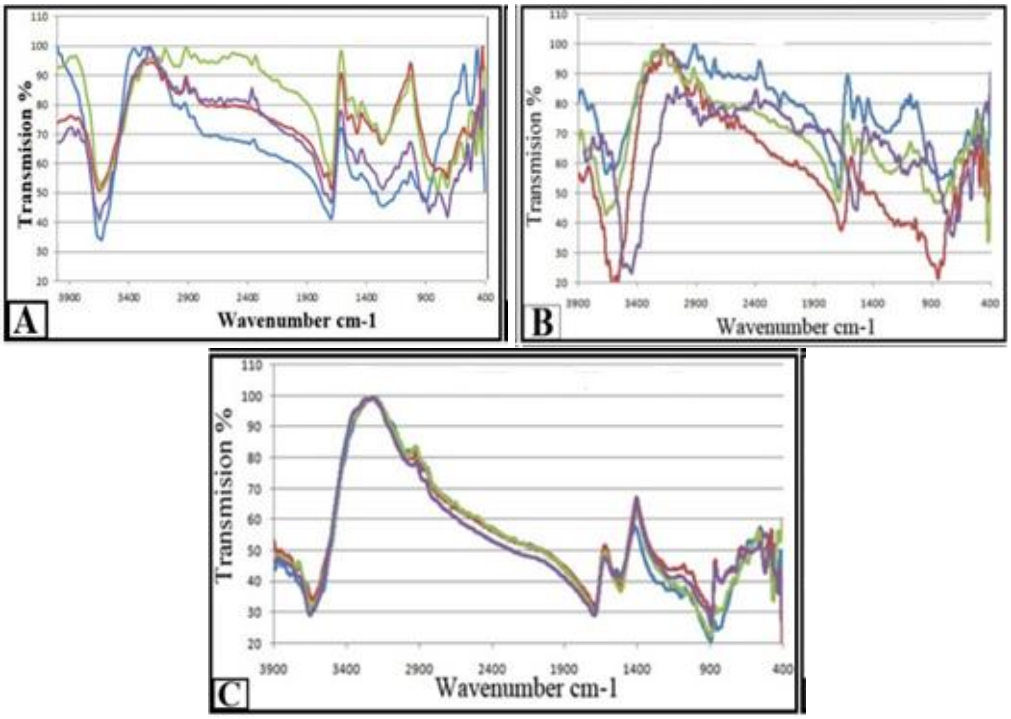

Fig. 5: Fourier transform infrared spectroscopy (ftir) of the aged samples of parchment and leather control/treated with different concentrations 6.25, 12.5, and 20 µl of habat albaraka fungicide against two types of fungi after one, two and three months of microbiological ageing cycle.

6. CONCLUSION

There is a hydrolytic cleavage of the collagen which indicated the degradation of hydrolytic breakdown mechanism and there is an oxidation of the polypeptide chains. It was revealed that growth in the *Aspergillus niger* and *Aspergillus sulphureus* were prohibited by all the Habat Albaraka fungicide concentrations tested. The Habat Albaraka fungicide efficiency is due to the active ingredients of the fungicide (Alpha-phellandrene, Benzen, 1 methyl – 4 – (1- ethyl ethyl), Junipene, Hexadecanoic acid).

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