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THE EFFICIENCY OF MICROBIAL CULTURE EXTRACTS AS GREEN ANTIMICROBIAL PRODUCTS AGAINST SOME MICROORGANISMS COLONIZING THE HISTORIC OIL PAINTINGS

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ABSTRACT

The aim of this study is to evaluate the antimicrobial efficacy of three microbial culture extracts (MCEs) and their mixtures as green and eco-friendly biocides against microbes colonizing historic oil paintings (MCPs). Two case study historic paintings in the Agricultural museums in Egypt were swabbed, many microbes were identified in both objects. Colored and aged painting mock-ups (PMs) similar to the historic paintings were used to assay the antimicrobial efficacy of the MCEs, and their effects on mock-ups contaminated with the three common microbes *Escherichia coli (E. Coli), Staphylococcus aureus (Staph.),* and *Saccharomyces cerevisiae (Sacch.)* identified in the case study objects. The study focused on: 1) identifying the biodegradation agents of the historic paintings in the Agricultural museum; 2) the producing three MCEs from *Streptomyces griseus, Bacillus subtilis*, *Penicillium* sp., and their mixtures; 3) Cultivation of microbes and evaluation of biocidal activity of MCEs by the agar-well-plate diffusion method; 4) Application the most effective biocides on PMs colonized with *Escherichia coli, Staphylococcus aureus, and Saccharomyces cerevisiae*.; 5) Evaluating the antimicrobial activities of MCEs using different methods, that confirmed the antimicrobial efficacy of crude MCEs, and the better efficacy of their mixtures. Finally, these green and eco-friendly biocides proved promising future in preventing biodegradation of historic paintings.

KEYWORDS: Historic, Paintings, Biodegradation, Microorganisms, Eco-friendly, Green antimicrobial

1. INTRODUCTION

Historic oil paintings are commonly affected by microbial contamination because of their organic ingredients, e.g., proteinaceous materials and oils, as well as supporting materials, like wood, fabric, and paper, helping micro-organisms in the biodeterioration of paintings (Gatti et al., 2020, Caselli et al., 2018, Elsayed, 2015). Bacteria and fungi colonizing the observe and the reverse sides of paintings cause decay in biological technique, resulting in different aesthetic and structural alterations in painting and other heritage materials as well (Elsayed, 2019b; Delegou et al., 2022; Omar et al., 2022). Furthermore, fungi are the most established detrimental microbes among all the microbial communities (Pinna, 2021, Biswas et al., 2013, Ciferri, 1999). Bacteria, yeasts, algae, and filamentous fungi colonizing the observe and the reverse sides of historic paintings are the main biological agents affecting artworks, whose development and metabolic activities are closely correlated with their state of alteration (Salvador et al., 2016). The most common bacteria and fungi colonizing the historic oil paintings in museums mainly belong to *Bacillus* sp., *Escherichia coli, Pseudomonas* sp., *Serratia* sp., *Streptomyces* sp., *Alternaria* sp., *Aspergillus* sp., *Aureobasidium* sp., *Rhizopus* sp., etc. These different types of microbes have their different deteriorative effect on different heritage materials and artworks (Darwish, 2010, Pangallo et al., 2009, López-Miras et al., 2013a, Ogbulie and Obiajuru, 2004, Pinna, 2021, Torralba et al., 2021, Liritzis et al., 2021). Environmental yeasts belonging to the order *Saccharomycetales* are also identified in oil paintings (Caselli et al., 2018; López-Miras et al., 2013b).

Direct and indirect methods are used to control microbial growth in artworks. Indirect methods are used to inhibit the growth of fungi and bacteria by modifying the microclimatic conditions around the artworks. Direct methods are used to eradicate the existing colonization of microorganisms through the use of traditional methods and appropriate biocides (Mishra et al., 1995; Knut and Westphal, 1999; Hamed, 2019). Traditional methods, e.g. scalpels, scrapers, soft brushes, etc, maybe used in cleaning microbial hypha. Unfortunately, these methods aren't effective enough in the long term, as only mycelium on the surfaces is cleaned, not the inner parts of the microbes. Biocidal treatments, based on chemical toxic compounds, aim to prevent and/or control microbial growth. These treatments have been used to mitigate the effects of this biodegradation (Fonseca et al., 2010; Elserogy et al., 2016). However, in the past decades, many of the most effective biocides have been banned due to their environmental and health hazards (Buffet-Bataillon et al., 2012).

In recent years, treatments using green biocides are considered the practical approaches for bioremediation and conservation of artworks, as they produce a wide variety of antimicrobial substances (Caldeira, 2021; Young et al., 2008). Innovative researches are needed to replace the biocides currently used in conservation and restoration of art works by green solutions that are eco-friendly and do not present negative effects on the environment or human beings (Ashraf et al., 2014). Although microbes play an important role in degrading the historic painting component, they may help treat them as well. Traditional methods of biocidal treatment have a more negative impact on treated objects than positive ones. In this case, fungi or bacteria play a role as organisms, not as enzymes or antibiotics (Helmi et al., 2011). Microorganisms are not only considered as an eventual danger for paintings but also as potential tools for restoration (Salvador et al., 2022).

In current conservation practices, conservators found alternative solutions that are effective against bio-agents and, at the same time, considered eco-friendly and safe for humans because of their less impact on human health, historic objects, and ecosystems (Salvador et al., 2022, Gatti et al., 2020). The study of new compounds produced by natural means is an effective green solution against microbes that promote the biodeterioration of oil paintings (Silva et al., 2018). Green biocidal standards are set according to the 12 Principles of Green Chemistry (Anastas and Eghbali, 2010). Biocidal treatments are usually employed for controlling microbial growth and their efficacy. Specific microorganisms belonging to the *Bacillus* genus have been used as a decontaminating approach against several bacteria and mycetes (Soffritti et al., 2019, Caselli et al., 2016b). They are able to produce antifungal peptides, lipopeptides, and antimicrobial polypeptides (Slimene et al., 2012). Thus, this study was carried out to explore the efficiency of crude culture extracts by two bacteria (*Streptomyces griseus, Bacillus subtilis*) and one fungus *Penicillium* sp., as green antimicrobial products against the most common microorganisms identified in the case study historic paintings. This study gave insight into the possibility of extending the use of Crude culture extracts in the conservation of and biodegradation of historic oil paintings by innovative and eco-friendly cleaning methods for painting control procedures.

2. MATERIALS AND METHODS

2.1. Case study museum objects

The case study objects (Fig. 1) are two oil paintings on canvas, dated back to the beginning of the 20th century, and displayed in the Heritage Collection Mu-

seum in the Agricultural Museums in Egypt. Both objects are suffering physic-chemical and biodegradation, due to the inappropriate display environment. Object 1 (Fig. 1a) is named "BANANA TREES" by Amelia Da Forno Casonato (Italian painter, 1878 - 1969), size 156 x 116 cm. Object 2 (Fig. 1b) is named "LA FEMME A LA CHEMISE ROSE" by Mahmoud Said (1897-1964) an Egyptian judge and modern painter. In spit that both paintings are dated back to the 20th century, but both were painted in the same materials and technique used in ancient oil painting, authenticated and displayed in a governmental museum, suffering many deterioration forms and need treatment and conservation.

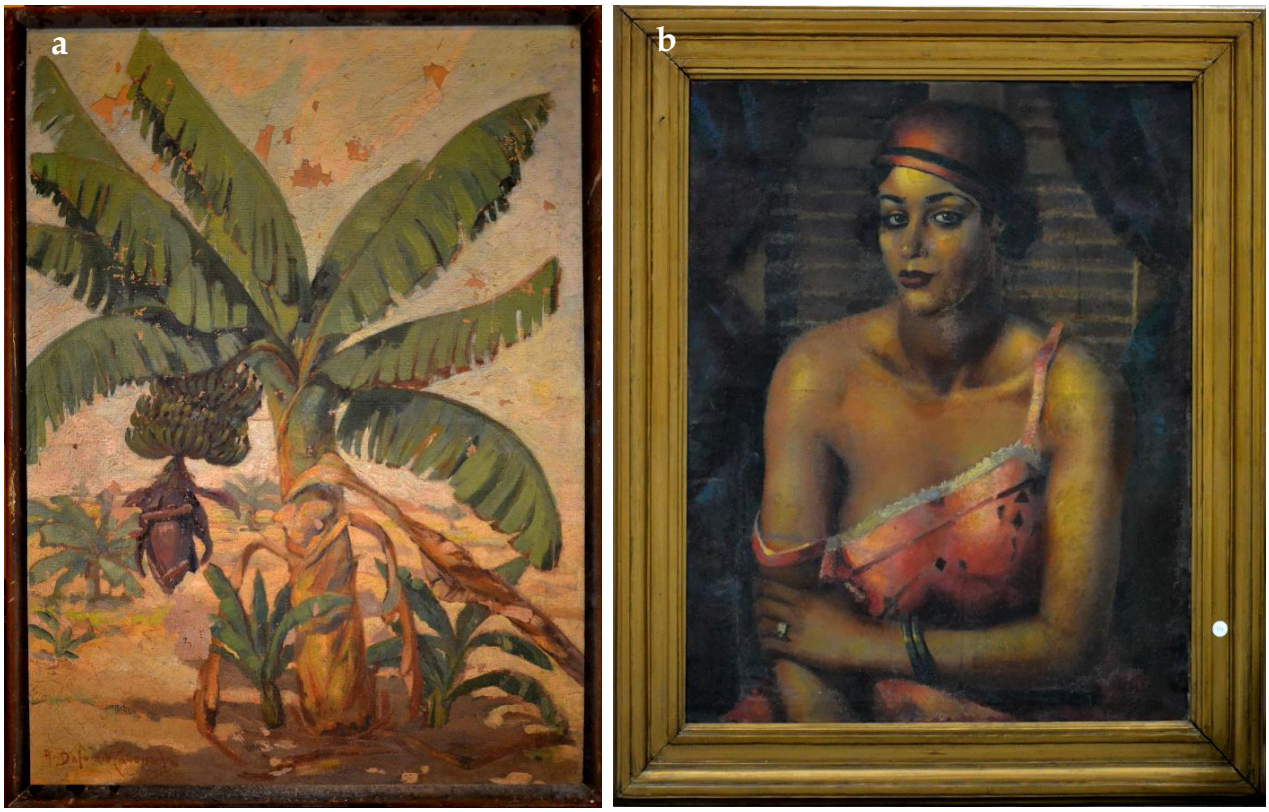


Figure 1. Case study historic painting; a) BANANA TREES painting, b) LA FEMME A LA CHEMISE ROSE

2.1.1. Isolation of microbial strains

Isolation of the fungal strains was done from the collected swab samples exhibiting fungal biofilm on potato dextrose agar (PDA) plates (Difco Laboratories, Detroit, WI, USA) according to (Ellis, 1971). For bacterial strains, isolation from the collected samples showing bacterial biofilm was done on nutrient agar (NA) plates supplemented with an antifungal agent (25µgml-1 of nystatin) to limit the fungal development (Akhter et al., 2012).

2.1.2. Purification of microbial strains

Purification of the isolated fungi was done using the hyphal tip and/or single spore techniques. Pure cultures of the isolated fungi were transferred into PDA slants and kept at 4°C for further studies (Domsch et al., 1980). Purification of the isolated bacterial was done using the single colony techniques. Pure cultures of the isolated bacteria were transferred into NA slants and kept at 4°C for further studies (Bhaduri et al., 2016).

2.1.3. Morphological identification of microbial strains

The isolated fungi were identified according to the cultural properties, macro-and microscopic characteristics of each fungus according to (Ellis, 1971, Booth and Commonwealth Mycological, 1971), (Summerell et al., 2003), (Raper and Fennell, 1965), (Domsch et al., 1980). The bacterial isolated were identified according to Simple staining, Gram and spore staining were done according to (Akhter et al., 2012). Capsule staining (Bhaduri et al., 2016) and motility (Bisen, 2014) were microscopically studied. Catalase test and hydrolysis of starch (Akhter et al., 2012), liquefaction of casein (Atlas, 2010) were carried out. Results (Table 1) revealed that 17 microbes were identified colonizing the case study objects.

Table 1. The identified 17 microbes colonizing the case study paintings

	Type	Name	BANANA TREES	LA FEMME A LA CHEMISE ROSE
1	Gram-negative Bacteria	*Escherichia coli*	√	√
2		*Salmonella*	√	--
3		*Enterococcus*	--	√
4		*Pseudomonas*	√	√
5		*Pseudomonas*	√	--
6		*Azotobacter*	--	√
7		*Serratia mar-*	√	√
8	Gram-positive Bacteria	*Staphylococcus*	√	√
9		*Streptomyces*	√	√
10		*Streptomyces*	√	--
11		*Bacillus cereus*	√	√
12		*Bacillus mega-*	√	√
13	Fungi	*Aspergillus fla-*	--	√
14		*Aspergillus ni-*	√	√
15		*Rhizopus* sp.	√	--
16	Yeasts	*Candida albi-*	√	√
17		*Saccharomyces*	√	√

2.2. *Preparation of painting mock-ups (PMs)*

The effectiveness of the MCEs was evaluated on prepared canvas painting mock-ups PMs to simulate the historic paintings according to the most common and traditional recipes of historic oil paintings detailed in some pieces of literature (Luke, 1988, Elsayed, 2019a, Walter and de Viguerie, 2018) (Fig. 2). A linen fabric support (Egyptian Company for Textile Industry, Egypt), was coated by a white ground layer of animal glue and white lead (local market, Egypt). The ground layer was brushed by a paint layer of color pigments in line seed and oil (Winsor & Newton, USA). Three basic pigments (red ocher, yellow ochre, and ultramarine) were used in coloring the white ground layer in straight-1cm-wide lines (Caselli et al., 2018, Luke, 1988, Blažek et al., 2014). After normal drying at room temperature at 25±2 °C for a month, the PMs were conditioned in the dark in a fan oven at 105±1 °C in the absence of light for 357 h. The elevated temperature accelerated the oil drying/aging process. It was low enough to allow thermal aging of the canvas painting (Hot air oven, Binder, ED115, Germany) (Barbera et al., 2022, Elsayed and Shabana, 2018). The experiments were carried out at the Experimental Lab, Faculty of Archaeology, Damietta University.

Figure 2. Painting mock-ups (PMs)

2.3. *Microbial strains and maintenance*

According to the results of the case study historic paintings, which match up with the literature review of painting biodegradation (López-Miras et al., 2013a, Knut and Westphal, 1999, Salvador et al., 2022, Sterflinger, 2010, Koul and Upadhyay, 2018), 17 microbes colonizing historic paintings (MCP) were selected for this study (Table 1), including gram-negative bacteria (7), gram-positive bacteria (5), fungi (3), and yeast (2) listed in table1. These isolates were taken from the Agricultural Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt. The bacterial and fungal strains were maintained on nutrient agar (NA) and potato dextrose agar (PDA) media slants, respectively, at 5°C till use. NA, nutrient broth (NB), PDA, and potato dextrose broth (PDB) media were used for bacterial and fungal growth (Atlas, 2010). These ready media were purchased (Arena BioScien, Egypt) and sterilized in an autoclave (Tommy SX500, Japan) at 121°C for 15 min. The experiments were carried out at Bio-Lab., Faculty of Agriculture, Damietta University.

2.4. *Microbial isolates and their sensitivity to streptoquin antibiotic*

The 17 microbes listed above in table 1 were tested for their sensitivity to antibiotics. This test aimed at clarifying the sensitivity of the MCP to the hereafter used bioactive compounds intended to be extracted from the different microbes. It was performed by agar plate methods using the streptoquin antibiotic (Arena BioScien, Egypt) at a concentration of 1g /L. The inhibition zones were carefully measured after one day (El-Fadaly et al., 2018, Chenikher et al., 2010, Silva et al., 2015, Caldeira et al., 2011). The sensitivity of tested 17 microbes to the streptoquin was measured as follows: not sensitive (-) for zone diameters (Z.D.) ≤8mm; sensitive (+) for Z.D. 8:≤14mm; very sensitive (++) for Z.D. 14:≤20mm and extremely sensitive (+++) for Z.D. >20mm (El-Fadaly et al., 2018).

2.5. *Production of bioactive compounds of MCEs*

For producing the MCEs' bioactive compounds, NB and PDB media were used for bacterial and fungal antimicrobial agents, respectively (Atlas, 2010). *Streptomyces griseus (Strept.) Bacillus subtilis (Bacil.),* and *Penicillium* sp. *(Penic.)* were used as antimicrobial microorganisms. The cultures were incubated in a shaker incubator (SKIR-602L SH-Incubator) at 37°C and 25°C for 2 and 5 days, respectively. After the growth period, the cultures were filtered and centrifuged (PLC-012 centrifuge, Gemmy Industrial Corp., Taiwan) at 2000 rpm for 10 min. to separate the biomass of microorganisms. The cultures filtrate was sterilized by a microfilter (Flow pore D 0.2µm, Germany) and used as an antimicrobial agent. Seven treatments (No. 1 – 7) using different extracted antimicrobial compounds (*Streptomycin. Subtilin. and Penicillin*) were applied in both separate and mixed formulas (Table 2).

Table 2. Seven used MCEs treatments, crude, and mixed formulas

Microbial culture extracts (MCEs)		
No.	**Name/mixture**	**Antibiotic agent**
1	*Streptomyces griseus*	*Streptomycin, Tetracycline*
2	*Bacillus subtilis*	*Subtilin*
3	*Penicillium* sp.	*Penicillin*
4	No.1 + No.2	*Streptomycin, Tetracycline + Subtilin*
5	No.1 + No.3	*Streptomycin, Tetracycline + Penicillin*
6	No.2 + No.3	*Subtilin + Penicillin*
7	No.1 + No.2 + No.3	*Streptomycin, Tetracycline + Subtilin + Penicillin*

2.6. *Contactless Test; antimicrobial assay of MCP treated by MCEs*

Based on the results of using the antibiotic streptoquin in assaying the sensitivity of the 17 tested MCP, the top three extremely sensitive MCP (*E. Coli, Staph.* and *Sacch.*) were selected for the next step. The seven MCEs formula detailed in Table 2 (3 crudes of *Strept., Bacil., Penic.,* and four mixtures of the same three crudes) were tested as green biocides against *E. Coli, Staph.,* and *Sacch.* to assay the effect of these selected MCEs on the three tested MCP. In order to develop an application method capable of demonstrating the potential biocontrol effect of MCEs on the MCP, the contactless test was performed to assay the antimicrobial activity of MCEs against the MCP.

2.6.1. Cultivation methods on agar plates

All microbial strains (*E. Coli, Staph.* and *Sacch.*) were grown on NA and PDA slant at 37°C and 25°C for 2 and 5 days, respectively. Five ml of sterilized saline solution (0.09% NaCl) was added to each slant. The bacterial cells/yeast cells were loosened by gentle brushing with a sterile inoculating loop. A vortex mixer (VM-300, Gemmy Industrial Corp., Taiwan) was used for 1 min. to remove all cells from the slant. The antimicrobial activity was determined by well diffusion methods on Petri dishes containing about 20 ml of NA and PDA media for bacteria and fungi, respectively (El-Kadi et al., 2018). All plates were inoculated with suitable microbial strains using a sterile cotton swab. Subsequently, one small well of 6.3 mm in diameter was done by a sterilized cork borer. Each well was filled up with 100 µl of the MCEs mentioned (1, 2, 3, 4, 5, 6, and 7). All plates were incubated at 37°C and 25°C for bacteria and yeast, respectively (El-Fadaly et al., 2018, Chenikher et al., 2010, Silva et al., 2015, Caldeira et al., 2011).

2.6.2. Antimicrobial assay

After the growth period, inhibition zones around the well were carefully measured after 2-6 days according to the microbes using a digital vernier caliper**.** The average value of three replicates was calculated. The assessment of MCEs = A-B (mm), where (A) is the diameter of the complete clear zone (mm), and (B) is the diameter of the cork borer (6.35mm) (Azzaz et al., 2017). (*E. Coli, Staph.* and *Sacch.*) were tested for their sensitivity to MCEs (El-Fadaly et al., 2018).

2.7. *Contact Test; antimicrobial assay of MCP+PMs treated by MCEs*

Based on the results of the previous experiment of contactless tests (*E. Coli, Staph.* and *Sacch.* treated with the 7 MCEs formula of *Strept., Bacil., Penic.*), the most efficient MCEs treatments against the MCP (treatments No. 4, 5, and 7) were selected for further experiments of contact tests as green biocides against *E. Coli, Staph.* and *Sacch.* to assay the effect of these three treatments on the infested PMs. This step aimed to clarify the antimicrobial activity of these three treatments and their probable effects on the PMs properties as previously described.

To simulate the in-situ historic painting, groups of 2×3 cm-PMs were dipped into the broth culture of MCP for 30s (Salvador et al., 2017). These pre-infested PMs were placed on a sterile glass slide in the center of 9-cm sterile Petri dishes lined with three sterile filter papers moistened with sterile distilled water, then 0.5 ml of MCEs at concentrations of 50% and 100% (v/v) in sterile distilled water were sprayed onto PMs. A control sample of PMs, with neither MCP nor MCEs, was kept to check if there was any effect on the physical and/or chemical characteristics of PMs due to only MCEs (Silva et al., 2015). A reference sample of PMs, dipped in MCP with no MCEs, was kept in Petri dishes to assay the antimicrobial activity of the MCEs used. Three replicas were used for each treatment. All plates were then incubated in the dark at 30±2°C for one week**.** Sterile distilled water was periodically added to keep the filter paper moistened (Elsayed and Shabana, 2018) (Fig. 3).

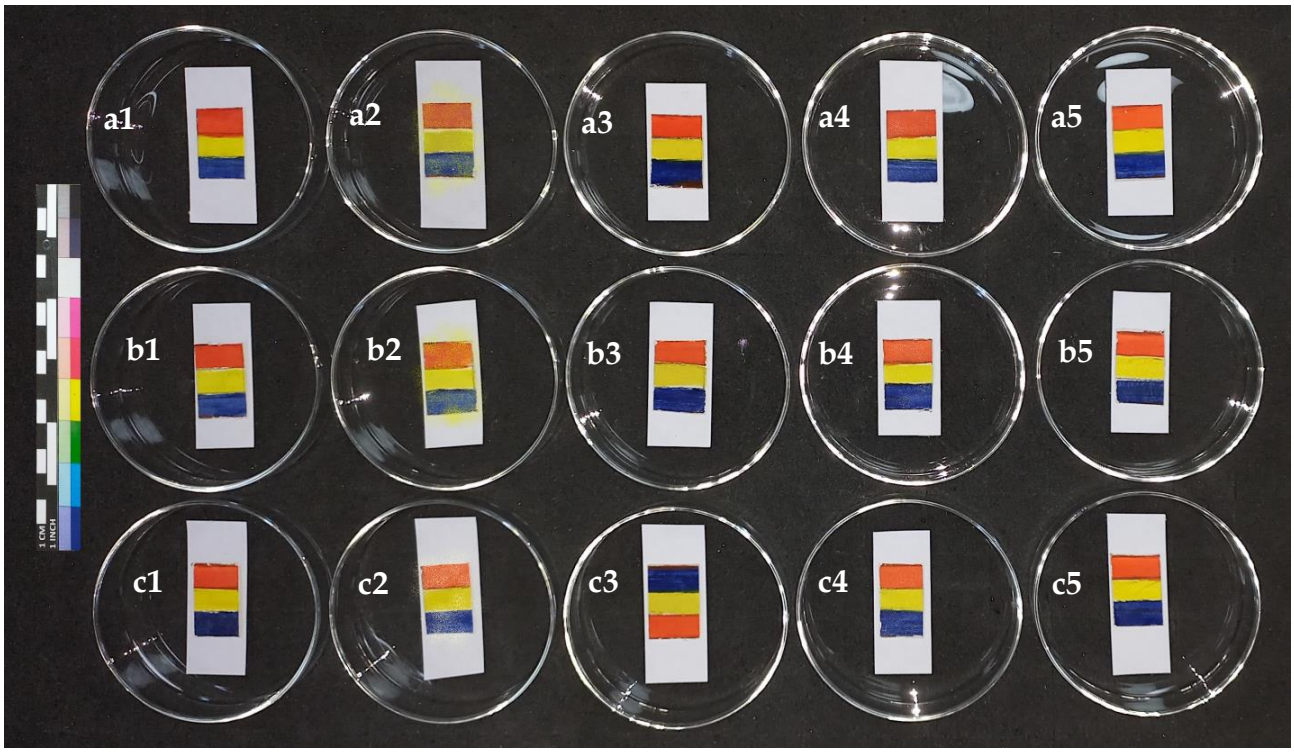


Figure 3. a), b), and c); Treatments No. 4, 5, and 7 against E. Cloi, Staph., and Sacch. Respectively. 1, 2, 3, 4, and 5; Controls, references, 100% MCEs only, 50% MCEs + MCPs, 100% MCEs + MCPs Respectively

2.8. Stereo microscope (SM)

A stereo microscope (Olympus CX31 stereo microscope, Japan) was used to examine the surfaces of all controls, references, and treated PMs with MCEs, and to identify the precise color nuances and the different overlapped pictorial layers (Elsayed and Shabana, 2018).

2.9. Scanning Electron microscope (SEM)

The SEM (JEOL JSM-6510 LV, JEOL Ltd., Japan, AC-CEL_20KV, MAG 4300, SIGNAL SEI, WD10mm) was used to observe the microbiological alterations in the surface morphology of the infected and treated PMs, the effects of bio-deterioration on these materials, and the evaluation of the growth of microorganisms (Poyatos Jiménez, 2018). Mycelial plugs (1×1×3 mm) were fixed in phosphate-buffered 3% glutaraldehyde at pH 6.8 and dehydrated in graded series of acetone (25, 50, 75, and 100% for 15 min. each). Later, samples were at a critical point dried with CO2 using acetone as an intermediate fluid. The pieces of agar were sputter-coated with plutonium by JFC-1600 auto-fine coater (Elsayed and Shabana, 2018).

2.10. Colorimetric measurements

Colorimetric measurements were performed using a spectrophotometer (PCE-CSM 2, Germany, color space: CIE Lab, observer angle: 10°, light source: D65, measuring aperture: Φ4mm, wavelength range: 400-700nm, measurement mode: SCI). The appearance of the PMs samples was characterized by means of the L*, a* and b* uniform color space (CIELAB) (Ruyter et al., 1987). The data reported were based on an average of three measurements for each color and calculated for the CIE Lab 1976 color space (Mecklenburg et al., 2013). $\Delta L*$ is the difference in luminosity system for detecting (lightness, achromatic coordinate, and ranging from black to white), Δa is the difference in greenness - redness parameter (- $a*$ = green, +$a*$ = red), and $\Delta b*$ is the difference in yellow – blue (−$b*$ = blue +$b*$ =yellow (Prodan et al., 2015). The color stability of the

MPs was determined by (ΔE) according to the equation: $\Delta E*=[(\Delta L*)^2+ (\Delta a*)^2+ (\Delta b*)^2]^{1/2}$ (Schanda, 2007, Bratitsi et al., 2018). The interpretation of every (Δ) value is as follows: 0–1 (chromatic difference not detectable by the human eye), 1–3 (small chromatic difference), 3–6 (detectable difference), and > 6 (large difference) (Drzewinska, 2002).

3. RESULTS

3.1. Results of MCPs' sensitivity to streptoquin:

The antimicrobial activities of the streptoquin against the 17 tested MCP (Table 1) were determined. The results of the inhibition assays (Fig. 4), corresponding to the stationary phase of microbes growth, proved that *E. coli, Staph aureus,* and *Sacch.* were extremely sensitive +++, (Z.D. 23-25mm); *Salmo. Typhy., chroc., faecalis, Serr.,* and *Cand.* were very sensitive ++, (Z.D. (15-18mm); *Bacil., Pseud., Pseud., Bacil.,* and *Strept.* were sensitive+ (Z.D. 9-12mm); *Strept. albus, Asper. flavus, Asper. niger and Rhizo.* sp. were non-sensitive–, (Z.D. 7mm).

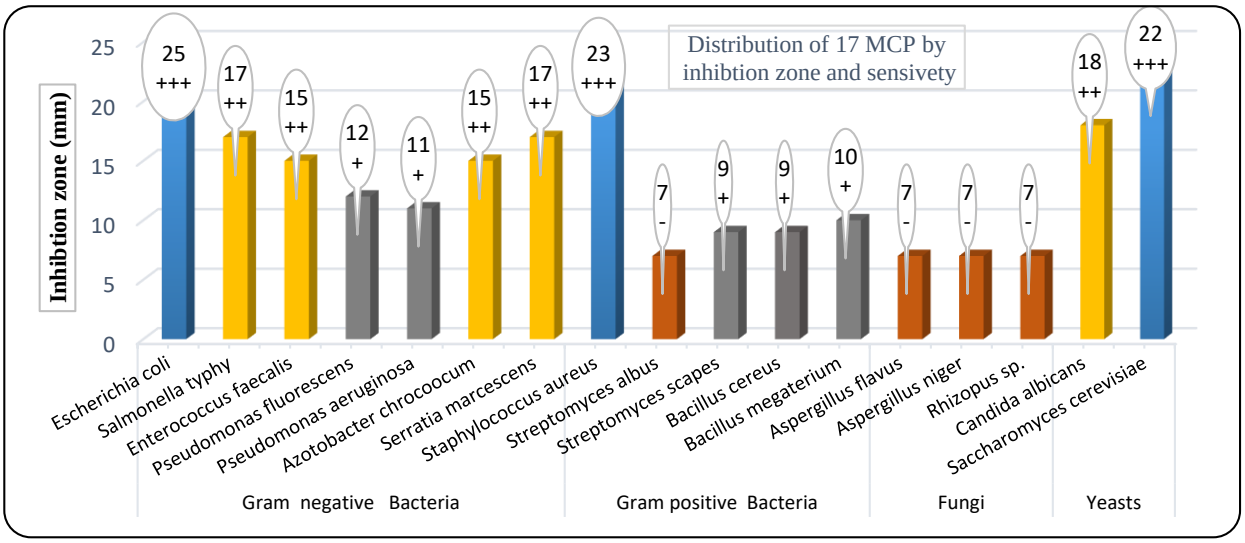


Figure 4. Characteristics of the antimicrobial activity contactless test; Sensitivity of the 17 MCP against streptoquin according to the diameter of the inhibition zone (mm) by the agar-well-plate diffusion method against E. Coli, Staph., and Sacch.

3.2. Results of contactless tests

The results (Fig. 5) revealed that the 7 treatments showed a variant positive effect against the tested MCPs, especially treatments No. 4, 5, and 7. They revealed that the optimum efficiency against *E. Coli.* was attributed to treatment No.4, which showed D.Z. 39 mm growth inhibition. The optimum efficiency against *Sacch.* was attributed to treatment No.5. It showed D.Z. 15 mm growth inhibition. The optimum efficiency against *Staph.* was attributed to treatment No.7. It showed D.Z. 21 mm growth inhibition. Due to the optimum obtained results being attributed to treatments No. 4, 5, and 7, these three treatments were only chosen for further experiments of contact tests.

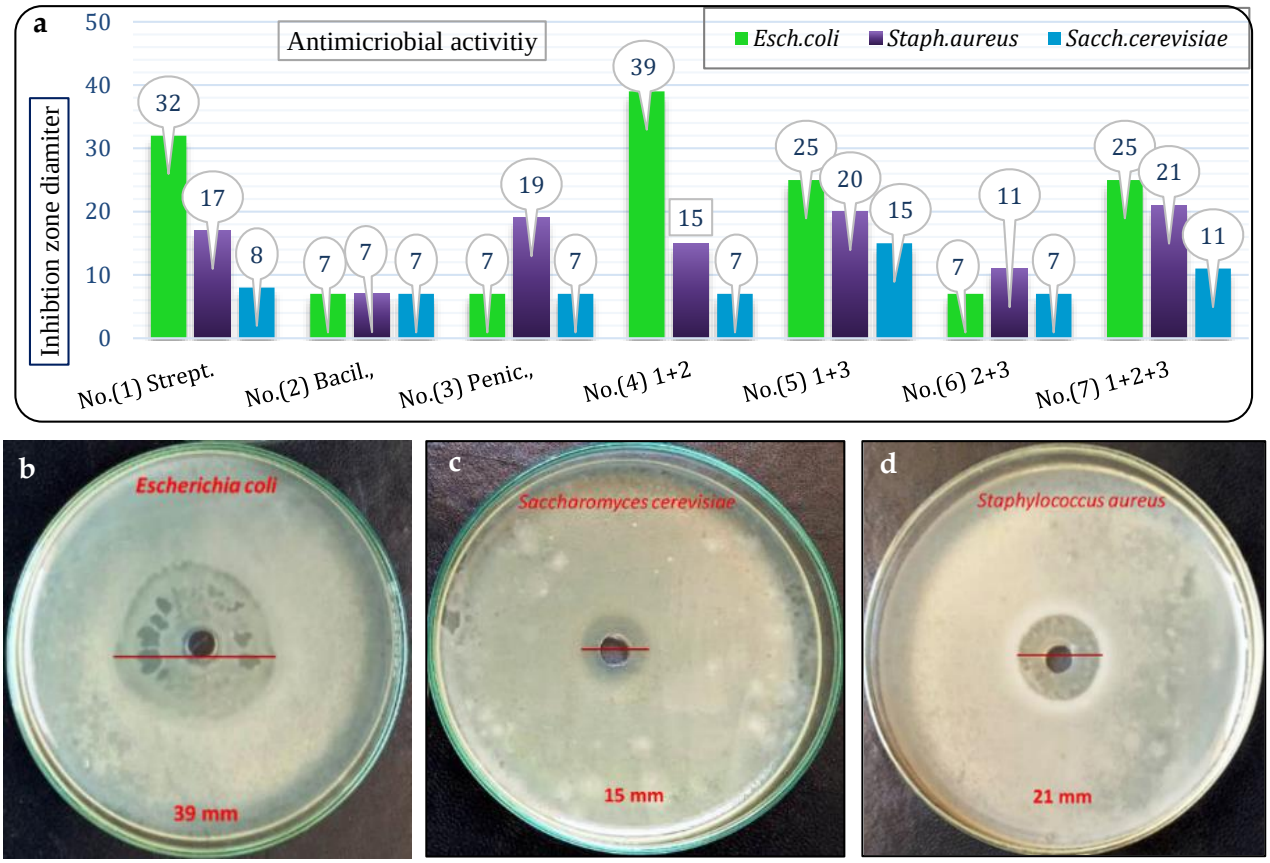


Figure 5. Antimicrobial activity of MCEs in Contactless Tests database; a) Distribution of tested 7 treatments of MCEs based on the inhibition zone (mm) in antibiotic plate's diffusion assays against Staph., E. Coli, and Sacch.; b) treatment No. 4 against E. Coli; c) treatment No. 5 against Sacch., d) treatment No. 7 against Staph.

3.3. *Results of the contact test (MCP+PMs treated by MCEs)*

The results of the antimicrobial activity of MCEs in the contact test of treatments No. 4, 5, and 7 (Table 3, Fig. 6) revealed the optimum efficient results of the tested MCEs. Treatment No. 4 resulted in an inhibition zone area of 2655 mm² against *E. coli*. (Fig. 6a). Treatment No.5 resulted in an inhibition zone area of 682 mm² against *Sacch. cerevisiae* (Fig. 6b). Additionally, treatment No.7 resulted in an inhibition zone area of 1176 mm² against *Staph. aureus* (Fig. 6c).

Table 3. Antimicrobial activity contact test by inhibition zone evaluated by plate agar

Treat No.	Antimicrobial activity by inhibition zone								
	Escherichia coli			*Staphylococcus aureus*			*Saccharomyces cerevisiae*		
	Dimension (mm)	Area (mm²)	Inhibition diameter (100%)	Dimension (mm)	Area (mm²)	Inhibition diameter (100%)	Dimension (mm)	Area (mm²)	Inhibition diameter (100%)
4	59*45	2655	41.7	ND	ND	---	ND	ND	---
5	ND	ND	---	ND	ND	---	31*22	682	15.7
7	ND	ND	---	42*28	1176	18.5	ND	ND	---

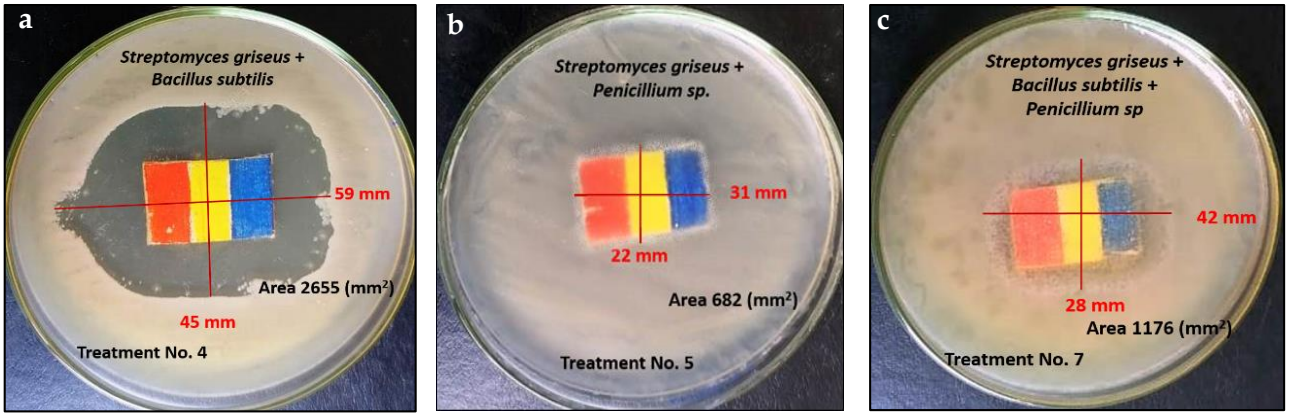


Figure 6. Antimicrobial activity contact test by inhibition zone evaluated by plate agar; a) treatment No. 4 against E. Coli; b) treatment No. 5 against Sacch.; c) treatment No. 7 against Staph

3.4. SM results

The SM results of treatments No.4, 5, and 7 (Fig. 7) showed variant alteration ratios in the PMs surfaces after colonizing and treatment. Regarding the reference samples of *E. Coli only, Sacch. cerevisiae only,* and *Staph. aureus only* (Fig. 7 a2, b2, c2), the surfaces were partially collapsed and deformed. PMs treated with con. 100% MCEs only with no MCPs (Fig. 7 a3, b3, c3) showed no observed negative effect correlated to using MCEs. PMs infected with *E. Coli, Staph. aureus* and *Sacch. cerevisiae* and treated with con. 50% MCEs (Fig. 7 a4, b4, c4) showed some degradation in colored surfaces with little microbial growth, whereas, with con. 100% MCEs (Fig. 7 a5, b5, c5), neither degradation nor microbial growth was observed, except for little growth in the case of *Sacch. cerevisiae*.

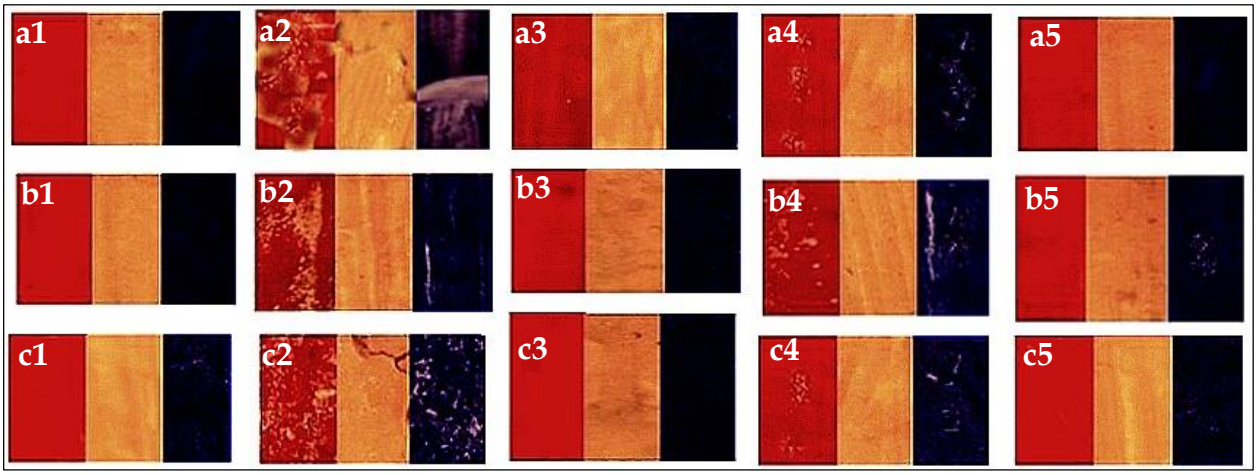


Figure 7. SM micrographs; a), b), and c) treatments No. 4, 5, and 7 against E. Cloi, Sacch. and Staph., respectively. 1, 2, 3, 4, and 5; Controls, references, 100% MCEs only, 50% MCEs + MCPs, 100% MCEs + MCPs, respectively

3.5. SEM results

In order to clarify the previous results, SEM was used to observe microbial alterations on PMs surface and evaluate the microbial growth in different PMs (Fig. 8). In the reference samples of *E. Coli.* only, *Sacch.* only, and *Staph.* only (Fig. 8 a2, b2, c2), the results showed the super microbial growth, deformation, and detachment of some areas. High microbial contamination in the surface samples was observed. Light spots and small pits were noticed on the surface of the samples, especially in the case of *Sacch.* (Fig. 8 b2). No morphological alteration was noticed in the case of using 100% MCEs only with no MCPs (Fig. 8 a3, b3, c3). PMs treated with con.100% (Fig. 8 a5, b5, c5) showed no microbial growth in comparison to the PMs treated with con. 50% (Fig. 8 a4, b4, c4), which showed little observed growth residue. The small pits were clearly observed on the surface of *Sacch.* sample, which disappeared under microbial growth but clearly appeared after treatment.

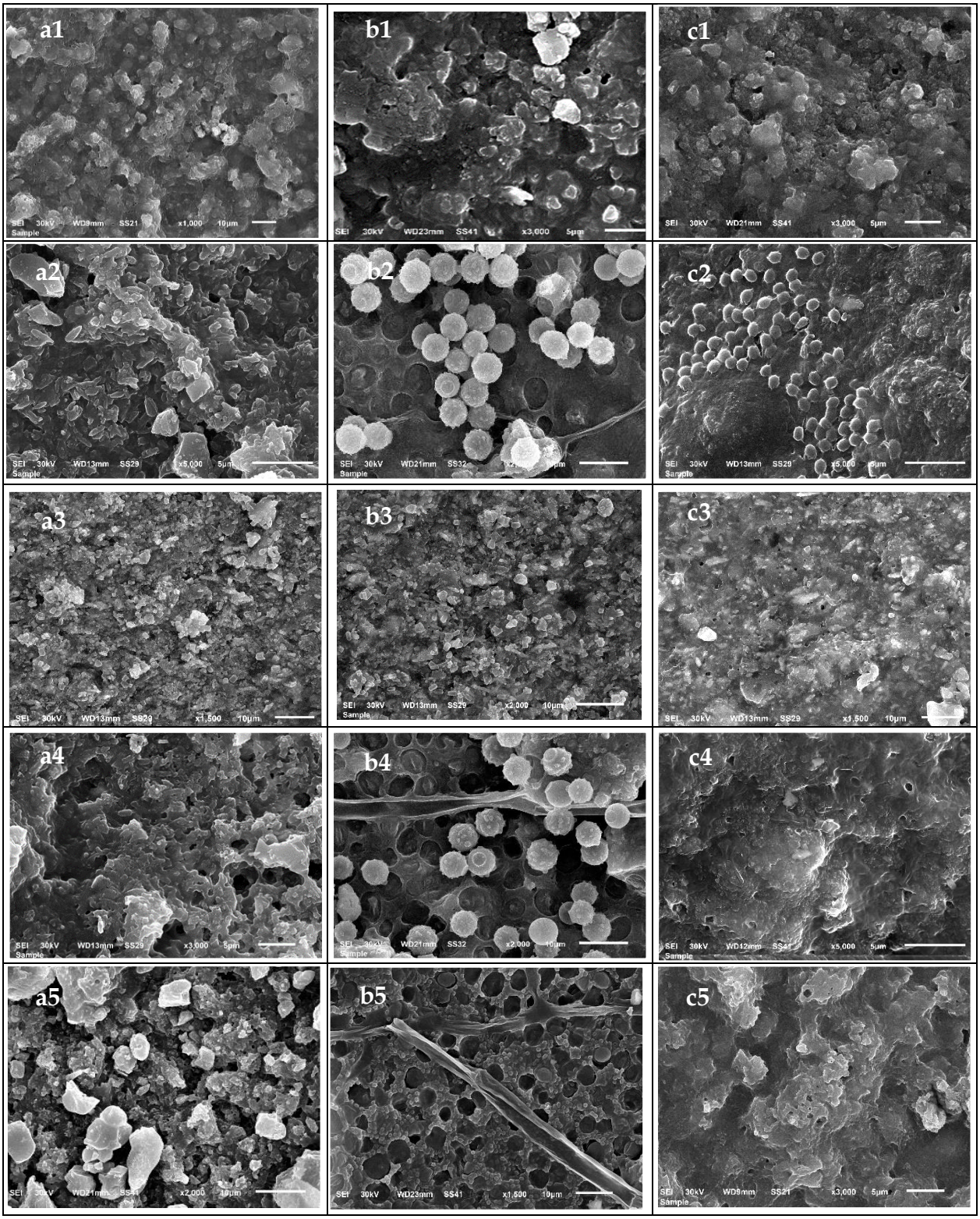
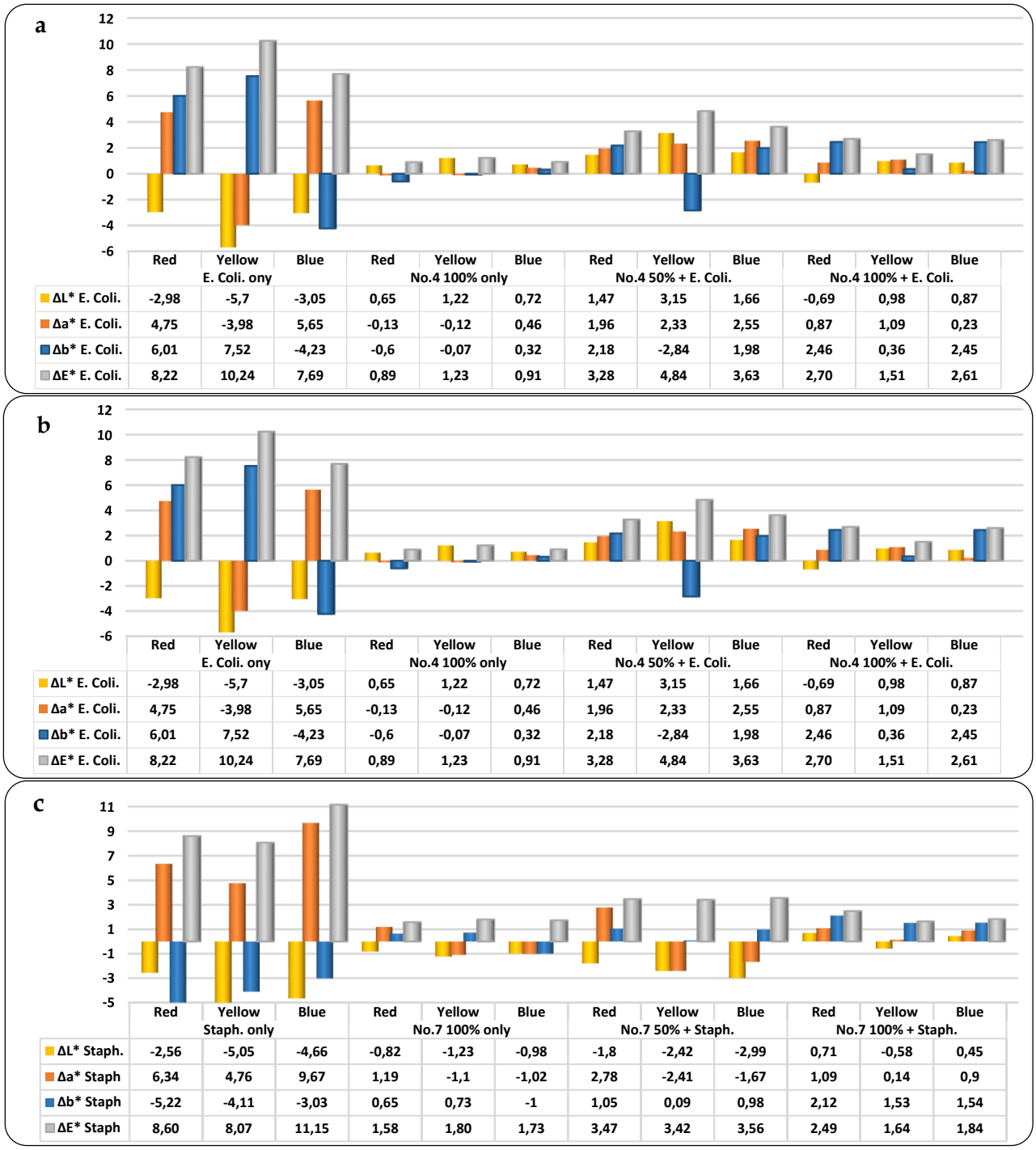


Figure 8. SEM micrographs; a), b), and c) treatments No. 4, 5, and 7 against E. Cloi, Sacch. and Staph., respectively. 1, 2, 3, 4, and 5; Controls, references, 100% MCEs only, 50% MCEs + MCPs, 100% MCEs + MCPs, respectively

3.6. Colorimetric results

The obtained results of chromatic changes in treatments No. 4, 5, and 7 against *E. Cloi*, *Sacch.*, and *Staph.*, respectively (Fig. 9) revealed variant changes in the samples after treatment using MCEs. The obtained ΔE of the reference samples (MCPs with no MCEs) compared to the control one (no MCPs, no MCEs) showed high values (from 7.69 in *E. Coli.* blue color to 11.15 in *staph.* blue color also). Treating PMs using MCEs only

(with no MCPs) showed no notable changes in all PMs (ΔE <2). Treating PMs infested with MCPs using con. 100% MCEs almost showed little changes (from 1.51 in *E. Coli.* yellow color to 2.70 in *E. Coli.* red one).



	Red	Yellow	Blue	Red	Yellow	Blue	Red	Yellow	Blue	Red	Yellow	Blue
	E. Coli. ony			No.4 100% only			No.4 50% + E. Coli.			No.4 100% + E. Coli.		
ΔL* E. Coli.	-2,98	-5,7	-3,05	0,65	1,22	0,72	1,47	3,15	1,66	-0,69	0,98	0,87
Δa* E. Coli.	4,75	-3,98	5,65	-0,13	-0,12	0,46	1,96	2,33	2,55	0,87	1,09	0,23
Δb* E. Coli.	6,01	7,52	-4,23	-0,6	-0,07	0,32	2,18	-2,84	1,98	2,46	0,36	2,45
ΔE* E. Coli.	8,22	10,24	7,69	0,89	1,23	0,91	3,28	4,84	3,63	2,70	1,51	2,61

	Red	Yellow	Blue	Red	Yellow	Blue	Red	Yellow	Blue	Red	Yellow	Blue
	Staph. only			No.7 100% only			No.7 50% + Staph.			No.7 100% + Staph.		
ΔL* Staph.	-2,56	-5,05	-4,66	-0,82	-1,23	-0,98	-1,8	-2,42	-2,99	0,71	-0,58	0,45
Δa* Staph	6,34	4,76	9,67	1,19	-1,1	-1,02	2,78	-2,41	-1,67	1,09	0,14	0,9
Δb* Staph	-5,22	-4,11	-3,03	0,65	0,73	-1	1,05	0,09	0,98	2,12	1,53	1,54
ΔE* Staph	8,60	8,07	11,15	1,58	1,80	1,73	3,47	3,42	3,56	2,49	1,64	1,84

Figure 9. Colorimetric measurements of the PMs before and after treatments at 50% and 100% concentrations with MCEs infested by a) E. Coli, b) Staph. aureus and c) Sacch.

4. DISCUSSION

Antimicrobial activities; are based on the identified microbes in the case study historic paintings. The results of sensitivity (contact and contactless tests) showed the efficiency of all MCEs and their mixtures in inhibiting the growth of different MCPs in variant ratios, depending on the type of microbe, the type of the extract, and the concentration of the extract (Kaleem et al., 2021). The reasons for the variant ratios of MCPs' sensitivity may be attributed to different components in the metabolite pool or variation in the antimicrobial activity, which may reflect differences in the abilities to detoxify the metabolites (Caldeira et

al., 2007, Kowall et al., 1998). Different microorganisms that produce lipopeptides seem to be directly linked to their protection and can influence the growth of other microorganisms in their habitat (Moffitt and Neilan, 2000).

Sensitivity test, according to (El-Fadaly et al., 2018), of *E. Coli*, *Staph. aureus*, and *Sacch. cevisiae* revealed that *E. Coli* was extremely sensitive to treatments No. 1, 4, 5, and 7, especially No. 4, due to the efficiency of the produced products, such as *streptomycin*, *Tetracycline*, and *Subtilin*, but not sensitive to treatment No. 2, 3, and 6 (Procópio et al., 2012, Ishigaki et al., 2017). *Tetracycline* antibiotics inhibited the growth of gram-negative bacteria and certain gram-positive bacteria (Badger-Emeka, 2013). *Staph. aureus* proved not sensitive to treatment No. 2 but extremely sensitive to treatment No. 7 *(Streptomycin, Tetracycline, Subtilin, Penicillin).* This efficacy, in addition to *Streptomycin, Tetracycline, Subtilin*, is attributed to the presence of *Penicillin,* which prevents gram-positive bacteria from forming peptidoglycan, which leads to swelling and bursting the bacterium. *Penic.*has been recognized as a rich source of bioactive metabolites. Moreover, *Penicillin* is considered the family of antibiotics, which includes *penicillin F, penicillin G, and penicillin X*, as well as *ampicillin, amoxicillin,* and *nafcillin* (Badger-Emeka, 2013, Fleming, 2001). *Sacch. cerevisiae* was not sensitive to treatments No. 2, 3, 4, and 6, but very sensitive to treatment No. 5. This efficacy is attributed to the presence of *Strept.,* and *Penic.*, which produce bioactive antifungal and antibacterial products (Wei et al., 2021, Badger-Emeka, 2013). Results generally proved that mixing two or more MCEs showed better impact and higher positive efficacy in the growth inhibition of the tested MCPs. This effect is attributed to the multi-positive impact of the joint bioactive agents.

Microscopy results (SM and SEM); the microscopy micrographs showed a variant bio-degradation in the reference samples (MCPs only), correlated with the high acid production and the extracellular enzymes production leading to the formation of acidic products that might cause chemical alteration of the infected colors (Li et al., 2013). The discoloration increased in the reference samples due to the microbial growth, which covered the PMs' surfaces but decreased after treating the PMs with MCEs concentration of 50% and disappeared after treating the PMs with MCEs concentration of 100% due to the appearance of the normal color of PMs' surface and disappearance of the microbial growth (Ilieş et al., 2021, Pavić et al., 2015). The inhibition of microbial growth returned the treated surfaces to their original features (Poyatos and Hurst, 2007) (Elsayed and Shabana, 2018). Bacteria and yeasts colonize paint films, especially in case of high moisture and prevail pink, cream, or yellow discoloration often correlated with yeast growth on the interior painted subject to high condensation. A distinct pattern of microorganisms colonizing the samples related to specific dark or light-colored zones and suggesting specific metabolisms depending on the chemical nature of the pictorial substrate was observed due to the presence of *bacteria*, *Staphylococcus* and *Saccharomyces* that colonialized and contaminated the samples (Zhao et al., 2010) and the sustainability of some colored surfaces to the microbial effects. A similar study proved that *Sacch. cerevisiae* strains were the most effective in fading azurite blue in laboratory cultures (SAKR et al., 2012). A similar result proved that the red and yellow ochers in several artistic materials could sustain the bacterial growth of diverse microorganisms (Pavić et al., 2015).

Samples treated with 100% MCEs only with no MCPs showed neither degradation nor visual discoloration in all treatments. This proved that the MCEs were safe and had no negative effects on the colored surfaces (Bravery, 1988). The application of MCEs to the PMs did not promote notable surface alterations or damages. Somewhat alteration was significant in the case of PMs treated with MCEs con. 50%, but it disappeared in the case of PMs treated with MCEs con. 100% (Ye et al., 2012),(Poyatos Jiménez, 2018). The small pits were clearly observed on the surface of the samples using SEM, especially in the case of *Sacch.* It disappeared under microbial growth but clearly appeared after treatment. This might be attributed to the effect of *Sacch.* on the surface (Pavić et al., 2015).

The colorimetric results; the chromatic changes in ΔE, mainly attributed to ΔL, Δa, and Δb values, reflected the darkness and discoloration of the reference samples infested with MCPs with no MCEs, and somewhat in the MCPs treated with MCEs con. 50% (Drubi-Filho et al., 2012). These changes were mainly attributed to the activities of the microbes, which played a significant role in the darkening and discoloration of the red, blue, and yellow colors at different rates due to microbial growth and the fine deposits of microbes on the surface of the PMs. The highest ΔE change (11.5) was noted in *Sacch. cerevisiae* blue color. This might be attributed to the difference in color between the microbes (almost beige or creamy) and the PMs surface (blue color). When using MCEs, con. 50%, the ΔE changes decreased (3.56) and mostly disappeared (1.84) after treatment with MCEs con. 100% because the PMs surface returned to its original color after the growth inhibition (Othman et al., 2020, Refaat et al., 2020). The PMs treated with MCEs only (with no MCPs) showed no notable changes (ΔE 0.83 in *Sacch.* Yellow color to 1.8 in *Staph.* Yellow one). This result reflects the chromatic suitability of the used MCEs to the prepared PMs. Treating PMs infested

with MCPs using con. 100% MCEs almost showed little changes (from 1.51 in *E. Coli*. yellow color to 2.70 in *E. Coli.* red one); an acceptable chromatic change (Drubi-Filho et al., 2012). The tested antibiotic agents generally showed no notable chromatic changes in addition to the growth inhibition of the tested microbes, not only at the surface of the paintings but also inside their pores due to the death of infection spores (Helmi et al., 2011, Caselli et al., 2016a).

5. CONCLUSION

The present study proved the efficiency of tested materials (crude extracts of *streptomycin, Tetracycline, subtilin, and Penicillin*) extracted from (*Streptomyces griseus*, *Bacillus subtilis,* and *Penicillium* sp.), respectively, against the tested microbes. The scientific assessment of the painting mock-ups confirmed the optimum efficiency of *Streptomycin*, *Tetracycline,* and *Subtilin* biocidal mixture against *E. coli*. *Streptomycin, Tetracycline, Subtilin, and Penicillin* mixture is recommended as an optimum antibiotic against *Staphylococcus aureus*. *Streptomycin, Tetracycline, and Penicillin* mixture is recommended as an optimum antibiotic against *Saccharomyces cerevisiae*. In spite of using a single component being efficient, the mixture was more efficient without notable negative effects. The use of MCEs con. 100% is recommended in similar cases due to its deadly effect on *E. Coli, Staph. aureus* and *Sacch. Cerevisiae* colonizing oil paintings. Moreover, it had no significant negative effect on painting properties as a green, safe, and cost-effective material compared to chemical, conventional, harmful, and expensive materials. So, the tested antibiotics proved promising features in treating the tested painting mock-ups. They are highly recommended to be applied in similar cases. More studies on using microbial culture extracts in bio-restoration are recommended.

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