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ASSESSMENT OF SOME GREEN FUNGICIDES AGAINST FUNGI ISOLATED FROM DIFFERENT HERITAGE SITES AND MUSEUMS IN EGYPT

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ABSTRACT

Fungal biodeterioration represents one of the most crucial risks that threaten the cultural heritage resulting in irrevocable damages. Surveillance studies of the deteriorating fungi at archaeological sites are of great importance to update our knowledge of their diversity and distribution and help in finding appropriate conservation technology. Green conservation using plant essential oils (EOs) may offer an eco-friendly, effective, and economical approach to control these fungi in recent years. In this study, one hundred and ten swaps were collected from ten archaeological sites and museums in five Egyptian governorates (Cairo, Giza, Alexandria, Luxor, and Aswan). Eighteen fungal species belonging to seven genera were isolated and identified using the plate method and molecular identification, and a phylogenetic tree was generated by the maximum likelihood method. Among the identified fungi, *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceopetaliformis, Cladosporium halotolerans*, and *Neocamarosporium goegapense* were the most prevalent, while the most diverse genera were *Penicillium* and *Cladosporium.* Fifty EOs were screened for their antifungal activity against the five most prevalent fungi. The methods used, such as microscopic morphological characterization and growth inhibition (%) of the studied EOs revealed the variant antifungal activity depending on the type of EO and the fungus studied. The maximum antifungal activity was observed for EO of black pepper. This EO highly inhibited the fungal growth of *N. goegapense, A. flavus*, *A. ochraceopetaliformis, C. halotolerans,* and *A. niger* recording an inhibition of 100, 94.1, 90.7, 89.9, and 87.8%, respectively. Based on these results, we can conclude that the EO of black pepper, ginger, camphor, red pepper, and cinnamon are potential candidates for use in controlling deteriorating fungi in different materials of cultural heritage, although further investigations are required on simulated archaeological samples before application.

KEYWORDS: Bio-deterioration, green conservation, essential oils, fungi, cultural heritage, Aspergillus

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1. INTRODUCTION

Egypt has a rich and diverse cultural heritage that records thousands of years of history including prehistoric, Pharaonic, Hellenic, Christian and Islamic cultures. Biological deterioration, induced by diverse microbial communities such as fungi, bacteria and lichens, is one of the most important threats to cultural heritage and leads to irrevocable damages. Damage from biodeterioration ranges from discoloration to complete destruction. Fungi have a wide range of enzymatic activities and the ability to grow at a relatively low water activity level enabling them to inhabit, alter and/or degrade various organic and inorganic materials used for cultural heritage purposes (Branysova et al., 2022, Hamed and Mansour, 2018). They may cause serious damage and/or undesirable staining of artefacts. In addition, fungi can enzymatically degrade the organic paint binders leading to reduction or separation of the paint layers (Sterflinger, 2010, Fidanza and Caneva, 2019). Moreover, fungi are able to release spores, hyphal fragments, toxins and allergens in the aerosol of indoor cultural heritage that affect human health and cause serious respiratory infections such as bronchial irritation and allergy (Di Carlo et al., 2016, Afifi et al., 2020). Fungi commonly occurring on the historical art objects in museum mostly belong to *Alternaria* sp., *Aspergillus* sp., *Aureobasidium* sp., *Botrytis* sp., *Chaetomium* sp., *Cladosporium* sp., *Eurotium* sp., *Fusariu*m sp. *Mucor* sp. *Penicillium* sp., *Rhizopus* sp., *Stemphilium* sp. *Trichoderma* sp., and *Ulocladium* sp. (Pangallo et al., 2009, Abdelmoniem et al., 2020).

Many methods are available to disinfect and prevent the biodeterioration of the cultural heritage. Various physical, chemical and biological techniques have been studied to control the deteriorating fungi affecting plant-origin artefacts such as manuscripts, textiles and wood or animal-origin artefacts such as painting and mummies and stone artefacts (Shin and Bianucci, 2021, Elsayed et al., 2023). Chemical methods include alcohols, alkylating agents, azole antifungals, phenol derivatives, photocatalysts, quaternary ammonium compounds, salts and esters of acids, and green methods of nanomaterials and essential oils (EOs). However, the green chemicals used for the preservation of cultural heritage materials must be non-toxic and non-destructive (Stupar et al., 2014, Sequeira et al., 2012). Physical methods include dehydration, high frequency current, low-oxygen environments, temperature extremes, ultraviolet radiation, gamma irradiation and dielectric barrier discharge (DBD) plasma are considered the most common and effective physical methods. However, this method has to be thoroughly considered owing to their significant effects on the chemical composition of the historical art objects (Drábková et al., 2018, Sequeira et al., 2012, Sakr et al., 2015).

EOs have been known for a long time, and they are used in many fields, e.g. medicine, aromatherapy, food, pharmaceutical and cosmetics industries, etc. Recently, EOs have been used to control the biodeterioration of archaeological materials, representing a powerful resource in green conservation of cultural heritage (Elsayed and Shabana, 2018). EOs provide an eco-friendly, effective, and economical approach for the control of different types of microbial colonization on heritage materials (Palla et al., 2020, Taha et al., 2022). Their constituents, which are rich in various bioactive phytocompounds such as quinines, phenols, tannins, and flavonoids provide potent multifunctional antimicrobial activity (Baka and Rashad, 2016). However, reports on the implementation of green conservation of cultural heritage are extremely scarce. In this regards, some studies reported 23 EOs among 61 natural substances of plant origin and other compounds can be used in controlling biological deterioration (Fidanza and Caneva, 2019), others focused on the antifungal activity of *Origanum vulgare, Rosmarinus officinalis,* and *Lavandula angustifolia* against *Bipolaris spicifera, Epicoccum nigrum, A. niger, A. ochraceus, Penicillium* sp. and *T. viride* isolated from cultural heritage objects (Stupar et al., 2014). In the present work, different fungal strains were isolated and identified from archaeological samples in different museums and archaeological sites in Cairo, Giza, Alexandria, Luxor, and Aswan governorates. Various EOs were screened for their antifungal activity against the most frequent fungi.

In accordance with the global tendency to using green materials and methods, the present study focused on using some EOs as a green method of heritage conservation. Green materials are completely safe for heritage objects, the conservators, the visitors, the heritage staff, and the environment as well. Among the fifty used EOs, the study tried using some novel EOs, that have never been tried in heritage conservation, in addition to other common EOs used before in different applications in heritage conservation. Firstly, it aimed at isolating and identifying the deteriorating fungi in different archaeological sites and museums in North, Middle and South Egypt, secondly, assaying the most patent EOs against the most common fungi isolated from the case study archaeological sites and museums.

2. MATERIALS AND METHODS

2.1. *Sampling of the study area*

One hundred and ten samples were collected from different Egyptian archaeological sites and museums. The survey area lied between latitudes 24°01'N and 31°21'N, and longitudes 29°89'E and 32°53'E, as illustrated in the sampling map (Fig. 1), which was generated using ArcGIS software, version 10.1 (Environmental Systems Research Institute, ESRI, 2012). The study area included ten archaeological sites representing the main historical and archaeological sites and museums in Egypt, namely the Alexandria National Museum, Catacombs of Kom El-Shoqafa, El-Shatby Hellenistic Necropolis, the Saqqara Necropolis, the Great Pyramid of Cheops (Khufu), the Agricultural Museum in Giza, Karnak and Luxor temples in Luxor, Philae temple in Aswan, and Satet temple in Elephantine island. According to the microbial deterioration in each site, at least five different swap samples in each site were collected.

At the Alexandria National Museum, samples were collected from Hathour status, offering table, head of king Akhenaton, and the underground Pharonic tomb. At the Greek-Roman Catacomb of Kom El-Shoqafa, samples were collected from the ceilings, walls, and mural paintings. At El-Shatby Hellenistic Necropolis, samples were collected from walls, rocks, and soil. At Saqqara Necropolis, samples were collected from 1) the wall paintings in the tomb of Ankh Mahur (a doctor and priest during the reign of King Titi of the 6th dynasty), 2) the stone sarcophagi in the Serapeum. At Giza pyramids, samples were collected from the walls of burial chamber of king Cheops (Khufu). At the Agricultural Museum in Giza, samples were collected from 1) woolen Coptic embroidered textile fragment No. 347/2, woolen embroidered Coptic textile fragment No. 329/4, cotton embroidered prayer carpet No. 24 from 18th century, cotton embroidered Indian textile No. 26 from 18th century, cotton embroidered Jilbab (robe) No. 224/2 from 17th, 2) "WELSH PONEY" oil canvas painting by the Egyptian painter Mohammed Hassan in 1934, "FRUIT TREES" oil painting on canvas by the Italian painter Amelia Casonato in ~1930, "FLOWERS PAINT" oil canvas painting by by the Egyptian painter Ali Eldeeb in 1939, "Cows and Buffalos" oil painting on canvas by the Turkish painter Hedyat Shirazi in ~1930. At Karnak temple, samples were collected from the White Chapel of Senusret I, the Middle Kingdom Court, the Sacred Lake, and the second edifice built by Horemheb and Ramses II. At Luxor temple, samples were collected from the entrance, the edifice built by Ramses II, and the chapels of Thutmose III. At Philae temples (Ptolemaic era) in Aswan, samples were collected from the Mamezi (birthing room), the cabin of Nectanebo I, and the cabin of Trajan. At the Satet temple (Ptolemy VI) in Elephantine island, samples were collected from the main sanctuary, the small room, and the Nilometer.

Samples from the aforementioned archaeological objects and museums showing signs of bio-deterioration were aseptically collected using sterile cotton swaps or sterile scalpels, transferred to the lab in sterile tubes, and stored at 4°C until use. For each sampling site, field information was recorded, and the location was georeferenced using the Global Positioning System (GPS).

2.2. *Isolation of the deteriorating fungi*

Isolation of the fungal strains was done from the collected samples exhibiting fungal biofilm on potato dextrose agar (PDA) plates (Difco Laboratories, Detroit, WI, USA) supplemented with antibacterial agents (5 μgml^{-1} of L-chloramphenicol and 5 μgml^{-1} of streptomycin sulphate). Purification of the isolated fungi was done using the hyphal tip and/or single spore isolation techniques. Pure cultures of the isolated fungi were transferred into PDA slants and kept at 4°C for further studies.

2.3. *Extraction of essential oils (EOs)*

EOs of fifty plant species (Table 1) were separately extracted from different plant parts (seeds, leaves, fruits, or stems). The extraction was done by hydrodistillation of 200 g from plant materials for 150 min using a Clevenger apparatus. The plant material was placed in a 2-liter round bottomed flask with distilled water (400 ml per 200 g fresh material). The distillation period was 1 h and the purified extracted EOs were then stored in clean dark glass bottles at 4°C until use (Charles and Simon, 1990, Unlu et al., 2010). The extracted EOs were screened for their potential to inhibit mycelial growth of the five most common fungi identified in samples collected from heritage sites and museums, using the agar plate technique.

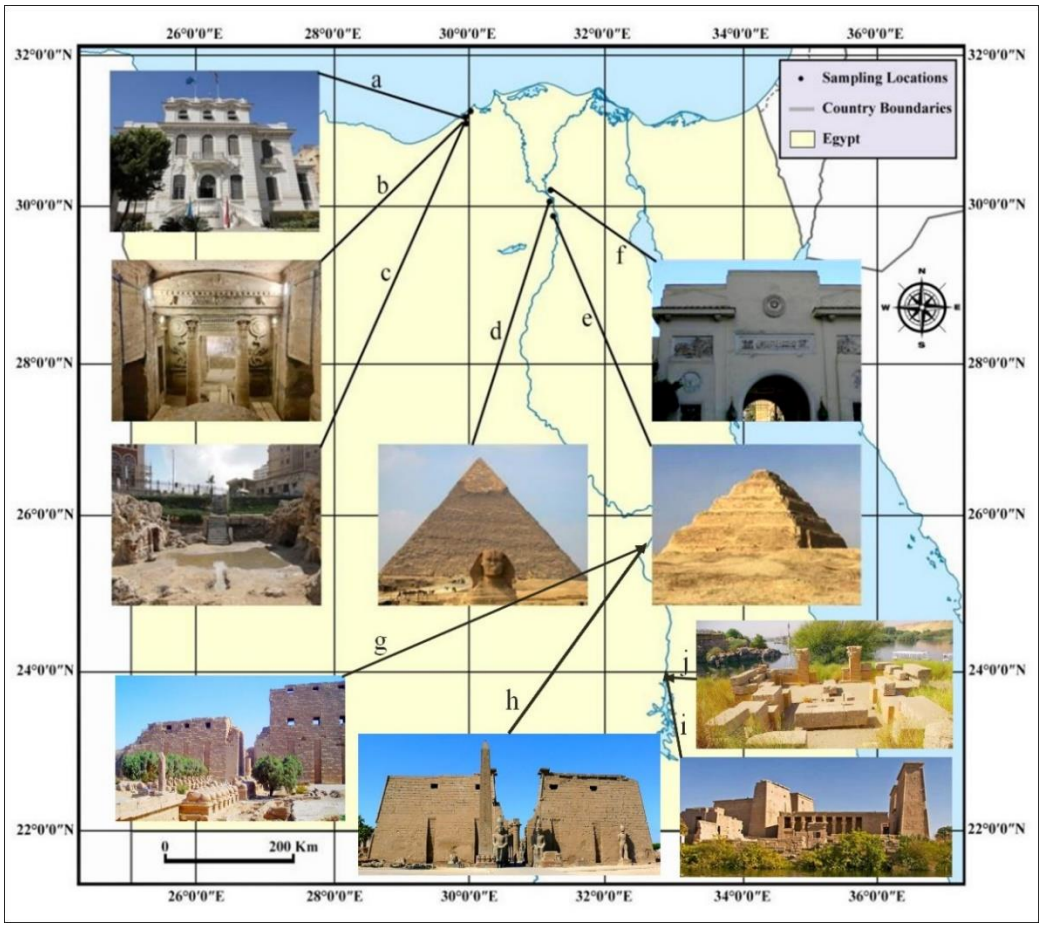

Figure 1. A sampling map showing the archaeological sites and museums surveyed in this study; a) Alexandria National Museum, b) Catacombs of Kom El-Shoqafa, c) El-Shatby Hellenistic Necropolis, d) the Great Pyramid of Cheops, e) Saqqara Necropolis, f) the Agricultural Museums in Doqqi, g) Karnak temple, h) Luxor temple,, i) Philae temple, j) Satet temple in Elephantine

Table 1. The studied essential oils

No.	English name	Scientific name	No.	English name	Scientific name
1	Almond	*Prunus dulcis* (Mill.) D. A. Webb	26	Grapes	*Vitis vinifera* L.
2	Anise	*Pimpinella anisum* L.	27	Hibiscus	*Hibiscus sabdariffa* L.
3	Argan	*Argania spinose* (L.) Skeels	28	Jasmine	*Jasminum officinale* L.
4	Avocado	*Persea Americana* Mill.	29	Jojoba	*Simmondsia chinensis* (Link) Schneid.
5	Basil	*Ocimum basilicum* L.	30	Juniper	*Juniperus communis* L.
6	Black Pepper	*Piper nigrum* L.	31	Lavender	*Lavandula angustifolia* P. Mill.
7	Black seed	*Nigella sativa* L.	32	Linseed	*Linum usitatissimum* L.
8	Cactus	*Opuntia ficus-indica* (L.) Mill.	33	Lupine	*Lupinus albus* L.
9	Camomile	*Matricaria chamomilla* L.	34	Marjoram	*Origanum majorana* L.
10	Camphor	*Cinnamomum camphora* (L.) J.Presl.	35	Mint	*Mentha spicata* L.
11	Caraway	*Carum carvi* L.	36	Mustard	*Brassica juncea* (L.) Czern.
12	Cardamom	*Elettaria cardamomum* (L.) Maton	37	Nutmeg	*Myristica fragrans* Houtt.
13	Castor	*Ricinus communis* L.	38	Olibanum	*Boswellia sacra* Flueck.
14	Celery	*Apium graveolens* L.	39	Parsley	*Petroselinum crispum* (Mill.) Fuss
15	Cinnamon	*Cinnamomum verum* J. Presl.	40	Radish	*Raphanus sativus* L.
16	Coconut	*Cocos nucifera L.*	41	Red pepper	*Capsicum annuum* L.
17	Cumin	*Cuminum cyminum* L.	42	Rosemary	*Rosmarinus officinalis* L.
18	Cyperus	*Cyperus papyrus* L.	43	Sage	*Salvia officinalis* L.
19	Dill (A)	*Anethum graveolens* L.	44	Saussurea costus	*Saussurea costus* (Falc.) Lipsch.
20	Fennel	*Foeniculum vulgare* Mill.	45	Sesame	*Sesamum indicum* L.
21	Fenugreek	*Trigonella foenum-graecum* L.	46	Sider	*Ziziphus spina-christi* (L.) Desf.
22	Garden cress	*Lepidium sativum* L.	47	Thyme	*Thymus vulgaris* L.
23	Ginger	*Zingiber zingiber* (L.) H. Karst.	48	Tilia	*Tilia cordata* Mill.
24	Ginseng	*Panax ginseng* C.A.Mey.	49	Turmeric	*Curcuma longa* L.
25	Gooseberries	*Phyllanthus emblica* Linn.	50	Watercress	*Nasturtium officinale* W.T.Aiton

2.4. Screening of EOs for their antifungal activity

Fifty EOs were screened for their antifungal activity against the five most frequent fungi, namely *A. niger*, *A. flavus*, *A. ochraceopetaliformis, C. halotolerans,* and *N. goegapense.* The EO was added to sterilized PDA medium before solidification to obtain a final concentration of 10% (v/v) of each EO. PDA plates treated with sterile water (instead of EO) was used as negative controls. The PDA plates were then inoculated with 8 mm-diameter discs of a 5-d-old culture of the fungal isolate. Plates were then incubated at 25±2°C for 72 h. The diameter of each fungal colony was measured and the average growth reduction relative to the negative controls was calculated. The tests were performed in triplicate.

2.5. Statistical analyses

Comparison of means was performed with Tukey's HSD test at $P \leq 0.05$ (on one-way ANOVA) using the statistical analysis software "CoStat 6.4" (Stat, 2005).

2.6. Transmission electron microscopy (TEM)

The TEM (L120C TEM for life science, Thermofisher, USA in Damietta University) was used to examine the samples, which were fixed in phosphate-buffered 3% glutaraldehyde at pH 6.8, post-fixed in phosphate buffered 1% Osmium tetroxide and dehydrated in graded series of ethanol. Mycelial plugs were embedded in plastic resin. Ultra-thin sections were cut with Reichert ultra-microtome, stained with uranyl acetate and lead citrate and examined with TEM.

3. RESULTS

3.1. Identified fungi

Eighteen fungal species belonging to 7 genera were isolated from the collected samples. Distribution of the isolated fungi according to the sampling sites and museums are presented in Table 2. Among the identified fungi, *A. niger, A. flavus*, *A. ochraceopetaliformis*, *C. halotolerans*, and *N. goegapense* were the most prevalent fungi found in most of the archaeological sites and museums. In addition, El-Shatby Hellenistic Necropolis and the Agricultural Museums recorded the highest species richness (8 fungal species each). While, the lowest species richness was observed in Luxor temple (2 fungal species).

Table 2. Distribution of isolated fungi according to sampling sites

	Fungus	Sampling site									
		Alex. National Museum	Kom ElShoqafa	El-Shatby	Saqqara	Great Pyramid	Agri. Museum	Karnak temple	Luxor temple	Philae temple	Satet temple
1	*Alternaria tenuissima*			■	■					■	■
2	*A. flavus*			■	■		■	■	■		■
3	*A. niger*				■		■	■	■	■	■
4	*A. ochraceopetaliformis*	■	■	■				■			
5	*A. sydowii*					■					■
6	*Byssochlamys spectabilis*					■					
7	*Cladosporium halotolerans*			■		■	■			■	■
8	*C. parahalotolerans*	■								■	■
9	*C. ramotenellum*	■					■				
10	*C. sphaerospermum*			■				■		■	
11	*C. tenellum*			■							
12	*Neocamarosporium goegapense*		■	■		■	■				■
13	*Penicillium chrysogenum*		■								
14	*P. gravinicasei*			■							
15	*P. roqueforti*		■								
16	*P. steckii*	■								■	
17	*P. thomii*						■				
18	*Pyronema domesticum*						■				

Genetic DNA of twenty-six decay fungus isolates, isolated from surveyed archaeological sites and museums, was subjected to PCR to amplify the rRNA-ITS gene. The PCR-amplified amplicones were then subjected to DNA sequencing. The nucleotide sequences were aligned using the ClustalW algorithm and identified against available sequences in the GenBank database using the NCBI search tool BLAST. Based on the molecular data of the isolated fungi, a phylogenetic tree was generated by the maximum likelihood method, using MEGA X software version 10.2.4 to identify their clustering structure and to investigate their phylogenetic relationships (Fig. 2). The

results showed that all isolates came from one ancestor and are divided into two main clusters, the first cluster includes sixteen fungal isolates, while the other contains two isolates. The first main cluster contains two sub-clusters, one of them contains one fungal species (*Pyronema domesticum*), while the other sub-cluster includes two sub-sub-clusters, the first sub-sub-cluster includes five species from the genus *Cladosporium*. While, the other sub-sub-cluster includes three groups, one contains five species of the genus *Penicillium*, the second contains four species of the genus *Aspergillus*, and the third contains one species (*Byssochlamys spectabilis*). The other main cluster includes two fungal species (*A. tenuissima* and *Neocamarosporium goegapense*). DNA can also provide valuable insights into the identity of archaeobotanical remains, early human-environment interactions and an understanding of social and religious activities (Liritzis et al., 2021).

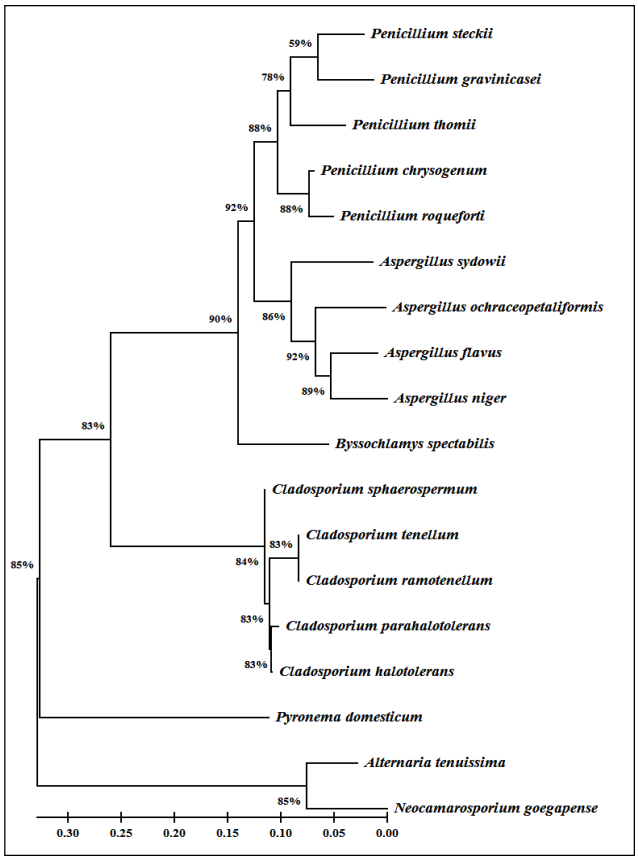

Figure 2. The phylogenetic tree of the identified decay fungi, which was generated using the neighbor-joining method with 500 bootstrap replicates. Bootstrap values ≥ 59% are illustrated on the nodes. The scale bar represents number of nucleotide substitutions per site

3.2. *Antifungal activity of EOs*

The average reduction in the fungal growth in response to treatment with EOs at 10% concentration is shown in Table 3. Most of the tested EOs showed inhibitory activity to varying extents, compared to the EO-free control. Some EOs completely inhibited the fungal growth. Some EOs did not significantly affect fungal growth. It was noted that the five most powerful antifungal EOs were black pepper, ginger, camphor, red pepper, and cinnamon. Black pepper oil proved optimal efficacy as an antifungal, inhibiting fungal growth of *N. goegapense, A. flavus*, *A. ochraceopetaliformis, C. halotolerans,* and *A. niger* recording 100, 94.1, 90.7, 89.9, and 87.8% inhibition, respectively (Fig. 3). EO of ginger ranked second, followed by camphor, red pepper, and cinnamon. Garden cress (watercress) EO had the lowest antifungal activity against the tested fungi; it inhibited the fungal growth of *N. goegapense, A. flavus*, *C. halotolerans, A. ochraceopetaliformis,* and *A. niger* recording 11.6, 9.4, 2.4, 2.4, and 1.1% inhibition, respectively (Fig. 3). However, the antifungal activity of the same EO differed according to the tested fungus. In contrast, some EO recorded no antifungal effect against some of the tested fungi, and very low activity against other fungi.

Table 3. Mean growth reduction (%) of the fungal isolates tested when exposed to the essential oils of various medicinal plants at 10%.

No.	Essential oil	Growth Inhibition (%)					Mean growth inhibition (%)
		A. niger	*A. flavus*	*A. ochraceopetaliformis*	*C. halotolerans*	*N. goegapense*	
0	Control	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	0
1	Almond	27.1^{f}	34.5^{ef}	22.7^{f}	31.4^{ef}	36.1^{ef}	30.36
2	Anise	6.7^{g}	0^{g}	25.9^{f}	3.5^{g}	0^{g}	7.22
3	Argan	8.2^{fg}	0^{g}	30.1^{ef}	15.7^{fg}	9.1^{g}	12.62
4	Avocado	44.3^{e}	40.1^{e}	0^{g}	32.9^{ef}	6.3^{g}	24.72
5	Basil	0^{g}	0^{g}	18.9^{fg}	33.8^{ef}	0^{g}	10.54
6	Black Pepper	87.8^{ab}	94.1^{a}	90.7^{a}	89.9^{a}	100^{a}	92.5
7	Black seed	33.8^{ef}	19.8^{fg}	35.9^{ef}	39.7^{ef}	65.8^{d}	39
8	Cactus	14.8^{fg}	8.4^{g}	31.3^{ef}	43.0^{e}	16.9^{fg}	22.88
9	Camomile	31.6^{ef}	59.1^{de}	20.3^{f}	18.6^{fg}	28.7^{f}	31.66
10	Camphor	90.7^{a}	80.2^{ab}	55.7^{de}	81.9^{ab}	6.3^{g}	62.96
11	Caraway	4.2^{g}	0^{g}	11.4^{fg}	26.6^{f}	0^{g}	8.44
12	Cardamom	10.2^{fg}	0^{g}	29.4^{f}	17.6^{fg}	87.1^{ab}	28.86
13	Castor	53.6^{de}	10.5^{fg}	1.1^{g}	13.9^{fg}	0^{g}	15.82
14	Celery	5.9^{g}	5.9^{g}	29.2^{f}	52.9^{de}	8.2^{g}	20.42
15	Cinnamon	84.8^{b}	19.8^{fg}	24.5^{f}	48.9^{e}	62.0^{d}	48
16	Coconut	36.4^{ef}	13.7^{fg}	18.8^{fg}	16.5^{fg}	21.6^{f}	21.4
17	Cumin	13.7^{fg}	1.4^{g}	24.7^{f}	42.3^{e}	20^{f}	20.42
18	Cyperus	46.4^{e}	53.6^{de}	48.9^{e}	21.1^{f}	9.7^{g}	35.94
19	Dill (A)	6.8^{g}	3.4^{g}	15.2^{fg}	38.8^{ef}	0.8^{g}	13
20	Fennel	11.7^{fg}	20^{f}	23.1^{f}	22.4 ^{f}	39.2^{ef}	23.28
21	Fenugreek	21.6^{f}	8.2^{g}	0^{g}	1.2^{g}	12.9^{fg}	8.78
22	Garden cress	2.4^{g}	9.4^{g}	1.1^{g}	2.4^{g}	11.6^{fg}	5.38
23	Ginger	92.4^{a}	61.2^{d}	70.0^{cd}	84.4^{ab}	78.5^{cd}	77.3
24	Ginseng	4.7^{g}	1.1^{g}	30.5^{ef}	20.8^{f}	0^{g}	11.42
25	Gooseberries	27.1^{f}	21.2^{f}	28.2^{f}	17.4^{fg}	12.9^{fg}	21.36
26	Grapes	5.4^{g}	0^{g}	61.9^{d}	9.8^{g}	2.4^{g}	15.9
27	Hibiscus	5.9 ^{g}	1.4^{g}	62.3^{d}	64.7^{d}	27.1^{f}	32.28
28	Jasmine	9.4^{fg}	4.7^{g}	20.4^{f}	14.1^{fg}	25.4^{f}	14.8
29	Jojoba	37.9^{ef}	31.6^{ef}	61.2^{d}	59.9^{de}	46.4^{e}	47.4
30	Juniper	44.3^{e}	35.9^{ef}	7.8^{g}	16.0^{fg}	15.6^{fg}	23.92
31	Lavender	6.3 ^{g}	1.7^{g}	14.8^{fg}	28.3^{f}	3.4^{g}	10.9
32	Linseed	6.3^{g}	0^{g}	21.9^{f}	12.7^{fg}	7.6^{g}	9.7
33	Lupine	62.9^{d}	62.9^{d}	0^{g}	40.5^{e}	6.3^{g}	34.52
34	Marjoram	8.2^{fg}	12.9^{fg}	0^{g}	9.4^{g}	11.2^{fg}	8.34
35	Mint	11.8^{fg}	12.1^{fg}	70.6^{d}	74.1^{cd}	18.8^{fg}	37.48
36	Mustard	54.9^{de}	44.3^{e}	0^{g}	12.7^{fg}	21.1^{f}	26.6
37	Nutmeg	38.4^{ef}	0^{g}	25.5^{f}	36.5^{ef}	8.2^{g}	21.72
38	Olibanum	11.4^{fg}	3.9^{g}	0^{g}	6.7^{g}	10.6^{fg}	6.52
39	Parsley	52.7^{de}	35.0^{ef}	1.7^{g}	39.2^{ef}	6.3^{g}	26.98
40	Radish	46.4^{e}	6.3^{g}	14.8^{fg}	36.3^{ef}	40.9^{e}	28.94
41	Red pepper	78.1^{cd}	82.7^{ab}	48.4^{e}	61.6^{d}	37.9^{ef}	61.74
42	Rosemary	2.1^{g}	21.1^{f}	48.1^{e}	53.2^{de}	37.6^{ef}	32.42
43	Sage	9.5^{fg}	35.9^{ef}	51.5^{de}	56.1^{de}	25.3^{f}	35.66
44	Saussurea costus	4.2^{g}	0^{g}	1.0^{g}	18.6^{fg}	19.8^{fg}	8.72
45	Sesame	7.8^{g}	1.1^{g}	41.1^{e}	14.1^{fg}	0^{g}	12.82
46	Sider	5.5^{g}	0^{g}	29.7^{f}	55.7^{de}	32.9^{ef}	24.76
47	Thyme	69.6^{d}	2.1^{g}	32.5^{ef}	57.8^{de}	39.2^{ef}	40.24
48	Tilia	2.1^{g}	6.3^{g}	12.7^{fg}	21.5^{f}	9.3^{g}	10.38
49	Turmeric	7.5^{g}	0^{g}	25.8^{f}	11.8^{fg}	0^{g}	9.02
50	Watercress	0^{g}	2.4^{g}	35.2^{ef}	23.5^{f}	14.1^{fg}	15.04

* In each column, values followed by different letter(s) are significantly different according to Tukey's HSD test ($P \leq 0.05$), each value is the mean of three replicates.

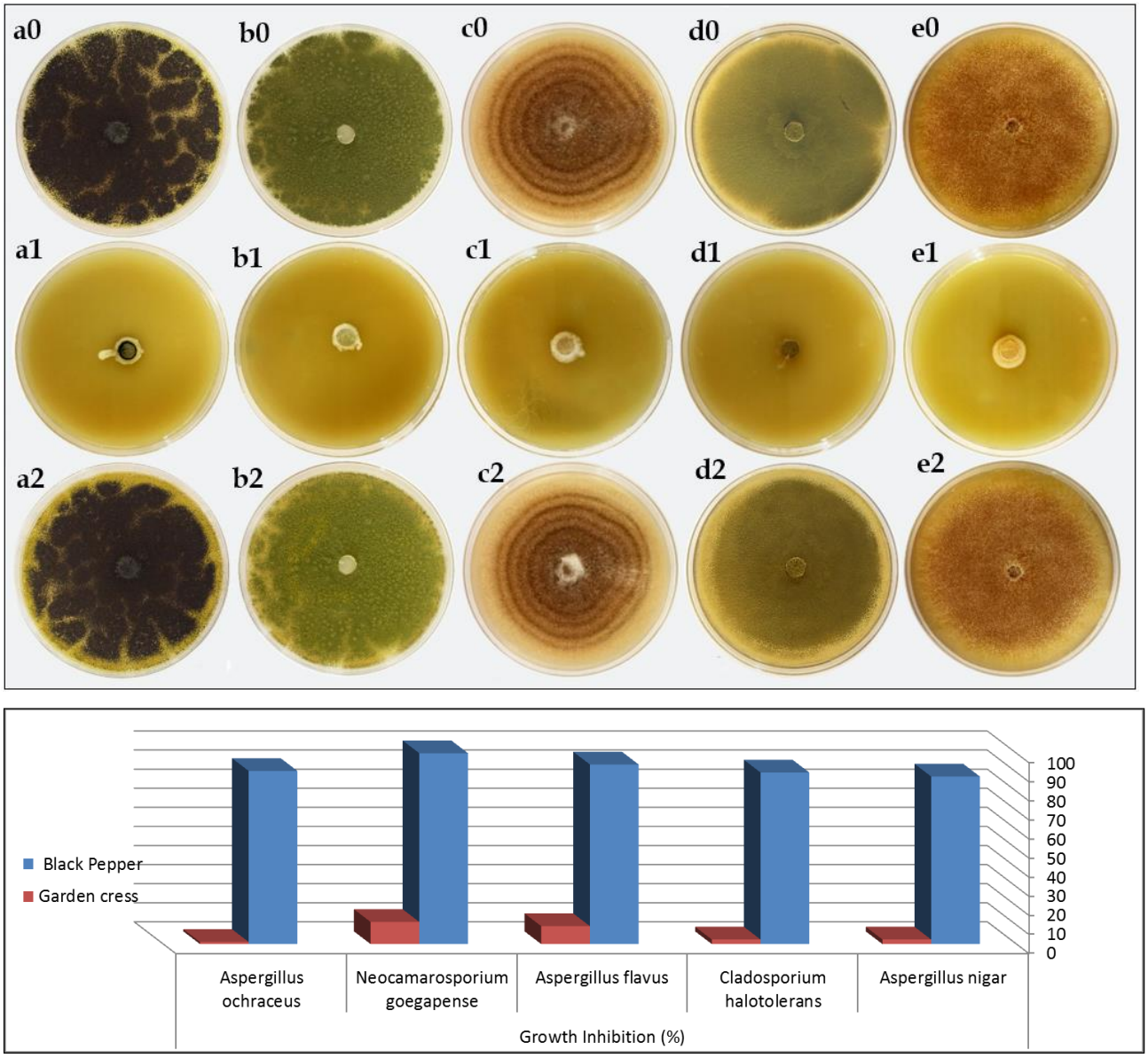

Figure 3. Antifungal activity of black pepper EO (the most effective) and garden cress EO (the least effective) against the tested fungi; a. A. niger, b. A. flavus, c. A. ochraceopetaliformis, d. C. halotolerans, e. N. goegapense. 0. control, 1. treated with black pepper EO, 2. treated with garden cress EO.

3.3. TEM results

The TEM micrographs for the control samples of *A. niger, A. flavus*, *A. ochraceopetaliformis, C. halotolerans*, and *N. goegapense*, which are the most prevalent fungi found in most sampling sites and museums, and the samples treated with different EOs revealed that the maximum antifungal activity attributed to black pepper EO, where the garden cress EO had the minimum effect (Fig. 4). The untreated (EO-free control) samples (Fig. 4a0-e0) revealed a granulated hyphal cytoplasm (CY), which contained numerous lipid bodies (L). Thin cell walls (W), plasma membrane (PM), mitochondria (M), small vacuoles (V), vesicles (VS), nucleus (N), and some unknown electron-dense bodies (B) were observed. The garden cress-EO-treated samples (Fig. 4a2-e2) showed little collapsed organelles, the vacuoles increased in number and sometimes some of them coalesced into large vacuoles. Some mitochondria disappeared or disintegrated. In some cases, the walls became thicker than the control sample, and the organelles aggregated and became part of the wall. On the contrary, the black pepper-EO-treated samples (Fig. 4a1-e1) showed completely collapsed organelles, the vacuoles fused into one large vacuole. No signs to the presence of mitochondria, they are completely disappeared, and the nucleus has also disintegrated (DN). Vesicles almost disintegrated, rarely appeared.

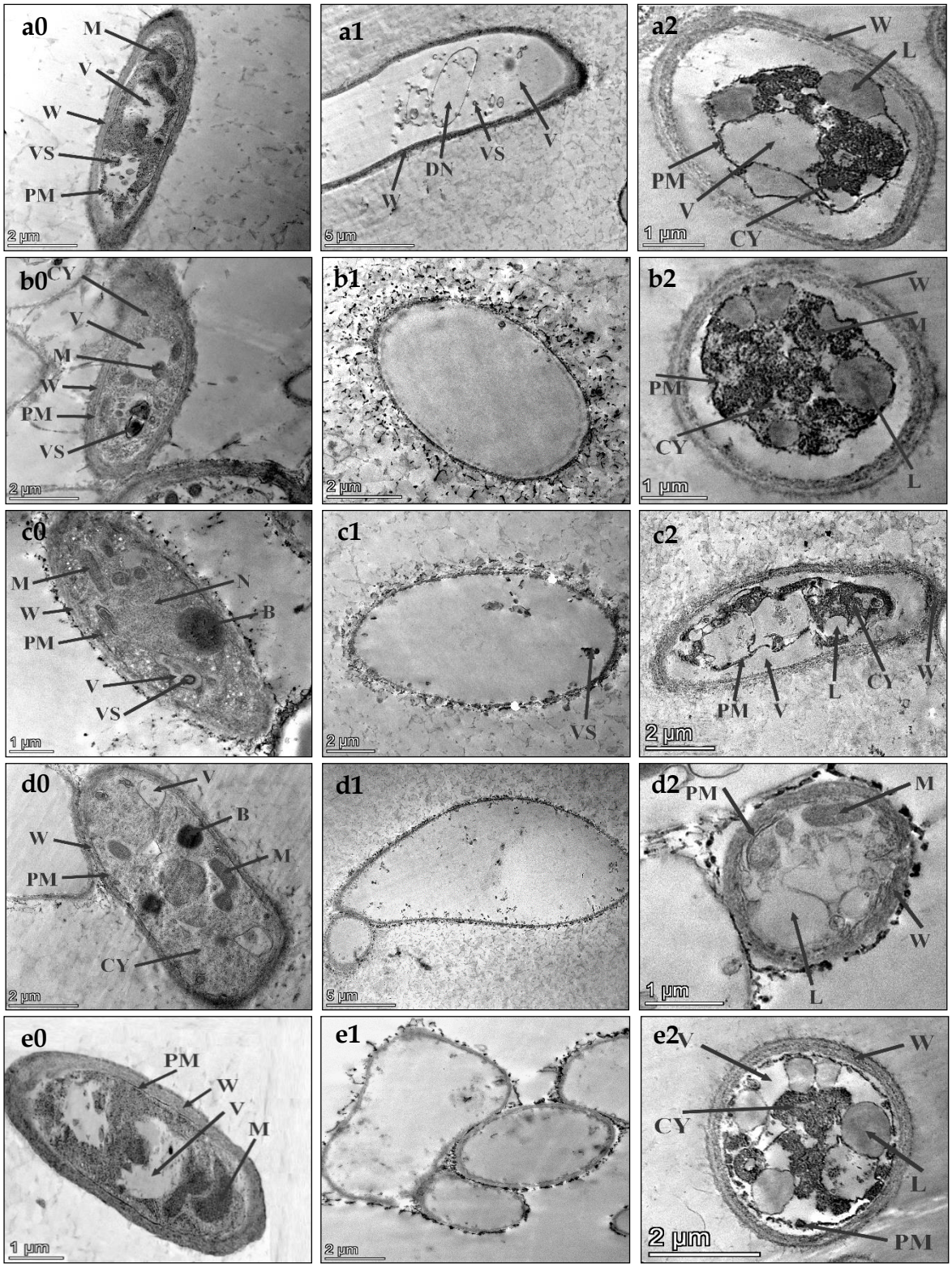

Figure 4. TEM micrographs of the antifungal activity of black pepper EO (the most effective) and garden cress (the least effective) against the tested fungi; a. A. niger, b. A. flavus, c. A. ochraceopetaliformis, d. C. halotolerans, e. N. goegapense. 0. control, 1. treated with black pepper EO, 2. treated with garden cress EO.

4. DISCUSSION

Periodic monitoring of the deteriorating fungi at the archaeological sites and museums is of great importance to update our knowledge about their diversity and distribution in order to detect the specific risks to cultural heritage materials. The results obtained should lead to timely intervention by implementing appropriate conservation procedures and employing correct risk models. The obtained

results showed the presence of a diverse of deteriorating fungi (18 fungal species belonging to 7 genera) that varied in occurrence and frequency according to the behavior of the cultural heritage objects and the environmental conditions in the archaeological sites and museums. Beside their historical importance, the surveyed sites are varied in their ecological conditions and represent different climatic conditions from hot dry to open humid conditions (Fidanza and Caneva, 2019). The results revealed that *A. niger, A. flavus, A. ochraceopetaliformis, C. halotolerans*, and *N. goegapense* were the most prevalent fungi. Occurrence of these fungi in five/six sampling sites among the ten surveyed sites (different climatic conditions) indicates their capability to grow well in variant conditions and their potential role in deterioration of cultural heritage materials, in addition to their health risks to the operators and visitors as well. This finding is consistent with the literature that reporting a predominance of *A. niger*, *A. flavus* over other fungi isolated from different archaeological artefacts in the surveyed heritage objects (Omar et al., 2018, Noshyutta et al., 2016). Due to their extensive sporulation, wide distribution, capability to inhabit diverse habitats, and great enzymatic capacity, *Aspergillus* spp. have a great potential for biological degradation, which threatens different cultural heritage objects (Romero et al., 2021, Borrego et al., 2016). However, the fungus *C. halotolerans* can considerably tolerate more moisture dynamics than *Aspergillus* spp. and *Penicillium* spp. This survival strategy could explain the dominance of this fungus (Segers et al., 2016).

Essential oils are eco-friendly green fungicide, effective and safe alternatives to control the fungal deterioration of the artefacts (Geweely, 2023). In this regard, fifty EOs were screened for their antifungal activity in this study. The results obtained indicated that the EO of black pepper exhibited the highest fungitoxic potential. This result is in agreement with the other findings which reported an effective growth inhibition in *A. flavus* when treated with black pepper EO (Zhang et al., 2021). The obtained results showed impaired cell membrane permeability and mitochondrial disruption in the studied fungi due to the high antifungal activity of the active components in EOs, such as β-caryophyllene, 3-carene, D-limonene, β-pinene, and α-pinene (Liu et al., 2007, Selestino Neta et al., 2017, Nikitina et al., 2012, Elsayed and Shabana, 2018). The mechanisms discussed were perturbing the permeability and integrity of the cell membrane and disrupting the metabolic processes in the cell. In addition, the antifungal activity of β-pinene against eleven studied fungi was also reported (Feng et al., 2020). Based on these results, we can conclude that all these fungitoxic components synergistically contribute to the antifungal behavior of the black pepper EO. This may provide a multifunctional antifungal activity, making it more difficult for fungi to overcome or improve resistance against this EO. This makes the black pepper EO a potential candidate for use in controlling the deteriorating fungi in the cultural heritage, although further testing on simulated archaeological samples is necessary before any recommendation for use.

5. CONCLUSION

Green treatment using plant essential oils (EOs) offer an eco-friendly, effective, and economical approach to control the fungi colonizing the heritage materials. Eighteen fungal species belonging to 7 genera were isolated from five Egyptian governorates (Cairo, Giza, Alexandria, Luxor, and Aswan) study areas. *A. niger, A. flavus, A. ochraceopetaliformis, C. halotolerans*, and *N. goegapense* were the most prevalent. Fifty used EOs showed antifungal activity to varying degrees depending on the type of EO and the fungus studied. The maximum antifungal activity was observed for EO of black pepper, it highly inhibited the fungal growth of *N. goegapense, A. flavus*, *A. ochraceopetaliformis, C. halotolerans,* and *A. niger*. Moreover, ginger, camphor, red pepper, and cinnamon are potential candidates for use in controlling the studied fungi. The present study presented a huge number of EOs, that have variant effects on variant fungi. So far, no study tried similar materials and technique, especially using the EO of black pepper, which is considered a pioneer application to the common fungi colonizing the heritage materials, it showed better results than other common EOs tried in other studies. Further researches are required to apply these potent EOs on simulated mock-ups before application heritage objects in museums. Potent EOs confirmed high efficacy in controlling the tested fungi, but they are required to confirm that they are completely safe to heritage martials as well.

Authors contributions: N.M., Y.E: Collecting samples, microbial isolation, and oil preparation; Y.S., P.S.: Microbial purification and identification; Y.R.: antifungal assessment; H.E., Y.S.: writing and reviewing the manuscript.

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