



Isolated essential oils as antifungal compounds for organic materials

Ayman S. Taha¹ · Wael A. A. Abo-Elgat² · Yahia G. D. Fares³ · Mohamed Z. M. Salem⁴

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Abstract

Organic industrial materials are exposed to fungal deterioration; to prevent this, several additives can be used. In the present work, Egyptian cotton linters, linen textile, and parchment (goat skin) provided from industrial zones in Egypt were used. The application of eco-friendly essential oils (EOs) isolated from *Pinus rigida* wood and *Origanum majorana* green leaves to cotton linter paper pulp (CLP), linen textile, and parchment as bio-fungicides to protect against the growth of *Aspergillus terreus*, *Aspergillus flavus*, and *Aspergillus niger* was evaluated using the fungal growth inhibition (FGI) assay and examined under SEM to show the extent of fungal infestation. By gas chromatography-mass spectrometry (GC-MS) analysis, the abundant compounds in *P. rigida* EO were determined to be 2-methylisoborneol (29.52%), and 4-isopropyl-5-methylhex-2-yne-1,4-diol (16.53%); in *O. majorana* EO, they were determined to be *cis*- β -terpineol (15.4%), terpinen-4-ol (14.39%), oleic acid (10.75%), and *D*-limonene (8.49%). CLP treated at a level of 500 μ L/L with *O. majorana* EO showed a higher FGI against *A. niger* (47.66%), while *P. rigida* EO showed a higher FGI against *A. flavus* (74%) and *A. terreus* (100%). Parchment treated with 500 μ L/L of *O. majorana* EO showed an FGI of 49% against the growth of *A. niger*, while *P. rigida* EO treated at a level of 500 μ L/L showed FGIs of 78% and 100% against *A. flavus* and *A. terreus*, respectively. Linen textile treated with *O. majorana* EO at a level of 500 μ L/L showed a higher FGI (49%) against *A. niger*, while *P. rigida* EO showed a higher activity against *A. flavus* (FGI 77.3%) and *A. terreus* (FGI 100%). The examined SEM images of materials treated with the EOs confirmed how these EOs suppressed or prevented the growth of molds compared with the control treatments. The findings indicate that the EOs from *P. rigida* and *O. majorana* considerably enhanced the performance of CLP, linen textile, and parchment materials; therefore, they can be recommended as promising antifungal agents with which to extend the shelf-life of these materials. This study shows the high effectiveness of the addition of natural oils that contain bioactive compounds to natural raw materials (CLP, linen textile, and parchment) in protecting against the growth of fungi. Subsequently, it is possible to protect these raw materials from deterioration and damage and prolong their lives as long as possible while maintaining the natural and mechanical specifications of the raw materials, especially in atmospheric conditions with a high humidity.

Keywords Essential oils · Cotton linters pulp paper · Linen textile · Parchment · Antifungal activity · SEM examination

✉ Mohamed Z. M. Salem
mohamed-salem@alexu.edu.eg

Ayman S. Taha
AymanSalah@arc.aswu.edu.eg

Wael A. A. Abo-Elgat
watsat20@yahoo.com

Yahia G. D. Fares
yahyagml@yahoo.com

² Restoration Department, High Institute of Tourism, Hotel Management and Restoration, Abukir 21526, Alexandria, Egypt

³ Laboratory and Research, Misr Edfu Pulp Writing and Printing Paper Co. (MEPPCO), Aswan 81656, Egypt

⁴ Forestry and Wood Technology Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt

¹ Conservation Department, Faculty of Archaeology, Aswan University, Aswan 81528, Egypt

1 Introduction

Different materials, including paper, wood, cellulose, and leather, that are widely used in libraries and archives, are suffering from biodeterioration caused by fungi [1]. Fungi are the main deterioration agents that affect wood, historical manuscripts, leathers, wood products, paper, and other heritage artifacts. They consume and degrade the polysaccharide components and cause the degradation of the cell walls or the discoloration of organic materials [2–4]. They have a remarkable ability to produce extracellular hydrolytic enzymes, which cause severe damage to valuable documents, and produce pigments or weak acids, resulting in the discoloration, disfigurement, and staining of these materials [5, 6].

Paper, which is a source of organic carbon for many microorganisms, is used to manufacture objects such as books, prints, and documents [7, 8]. It contains several organic substances such as inks, vegetable or animal glues, fillers, and pigments, which are sources of nutrition to various microorganisms and can augment its biodegradability [7–10].

Cotton linters (CLs), a byproduct from cotton seeds after cotton fiber harvesting, are short, thick-walled, curly, much less costly [11, 12], and an alternative and renewable source for cellulosic materials for paper manufacturing worldwide [13]. Some varieties of Egyptian cotton have long fibrous linters with a length average varying from 10 to 40 mm contaminated with higher amounts of impurities such as mineral matter (12–16% based on dry weight) [14, 15].

CLs contain α -cellulose as the main compound with the proteinaceous matter, pectin, waxes, and ash [16]. CL fibers are composed of pure cellulose and, therefore, could provide higher-quality regenerated cellulose than wood fibers [17]. When using CLs as raw materials for regenerated cellulose, milder pulping and bleaching processes are recommended over wood. These processes are very important for removing impurities and increasing paper brightness, as well as for adjusting the polymerization degree of cellulose [17].

Furthermore, fungi are responsible for the enzymatic degradation of cellulosic textile substrates such as linen and flax [18, 19]. Enzymes produced by fungi are functionalized to decrease the degree of polymerization while damaging the structure of the fibers and leading to loss of strength [20]. The degradation rate also depends on other parameters, such as the degree of orientation or substitution and the presence of non-cellulosic substances [21].

Parchment, the thin material made from animal skin (e.g., sheep and goat), has widely been used for writing

from the second century BC until the end of the Middle Ages until it was replaced by paper in Europe [22, 23]. Parchment is treated with lime, while leather is mostly tanned and, therefore, is more sensitive to fluctuations in relative humidity and, subsequently, deteriorated by fungi [24]. Collagen fibers and proteinaceous structures of parchment can be hydrolyzed, and their inorganic components can be modified by microorganisms, resulting in parchment discolored by pigments and organic acids and indirectly damaged [25, 26]. Proteolytic fungi can easily develop on ancient parchment and hydrolyze collagen fibers [27]. Under high humidity conditions, microorganisms can easily develop on leather and furs; for this reason, they are particularly vulnerable to deterioration [28–30].

To maintain and store these valuable materials, it is urgent to control the active fungal growth and remove or reduce the number of fungal ascospores and conidia [31–34]. Thus, using appropriate treatment measures for contaminated objects is challenging for restorers, curators, and scientists [35]. Therefore, to prevent the biodeterioration caused by fungi, materials must first be disinfected. The requirements for a disinfectant include the ability to inhibit the growth and metabolic activity of microorganisms without adversely affecting the material. Currently, fumigation with ethylene oxide is the most popular method for disinfecting fabrics, papers, and leather [36, 37]. However, this gas is an irritant and a dangerous human carcinogen, and its use should be avoided [38].

Recently, in the manufacturing of industrial materials, several additives have been used to improve the quality of the product or increase its durability against fungal infestation. Eco-friendly additives such as natural plant extracts, including phenolic and flavonoid compounds [39–41] and essential oils (EOs), are used to increase the resistance of these materials against mold infestations [42].

Bioactive compounds are used against microorganisms that grow on papers and textiles and have become highly important for bio-functionalization with safe, non-toxic, and environment-friendly properties [3, 43–46]. EOs from medicinal and aromatic plants have shown different biological activities when applied to different materials such as wood, paper, and textiles [34, 41, 47, 48]. EOs have been applied to combat the biodeterioration process in cultural heritage as an eco-friendly solution [49].

Furthermore, the disinfection process using EOs requires a long time, so this work aimed to find a way to accelerate this.

Therefore, this work was carried out to use and evaluate two essential oils from *Pinus rigida* wood and *Origanum majorana* leaves as eco-friendly additives to some

industrial materials (cotton linter paper pulp, linen textile, and parchment) against fungal infestation.

2 Materials and methods

2.1 Cotton linters paper

2.1.1 Preparation of cotton linters samples and their chemical characterizations

The Egyptian cotton linter fibers (CLs) used in this work were provided by the Holding Company for Spinning and Weaving, El-Mahalla El-Kubra, Egypt. The CL samples were supplied free of seeds as the local company typically separates the linters from the seeds. Linters were chopped, fractionated using a knife mill, screened, cut to between 2 and 3 cm long, and homogenized in single lots.

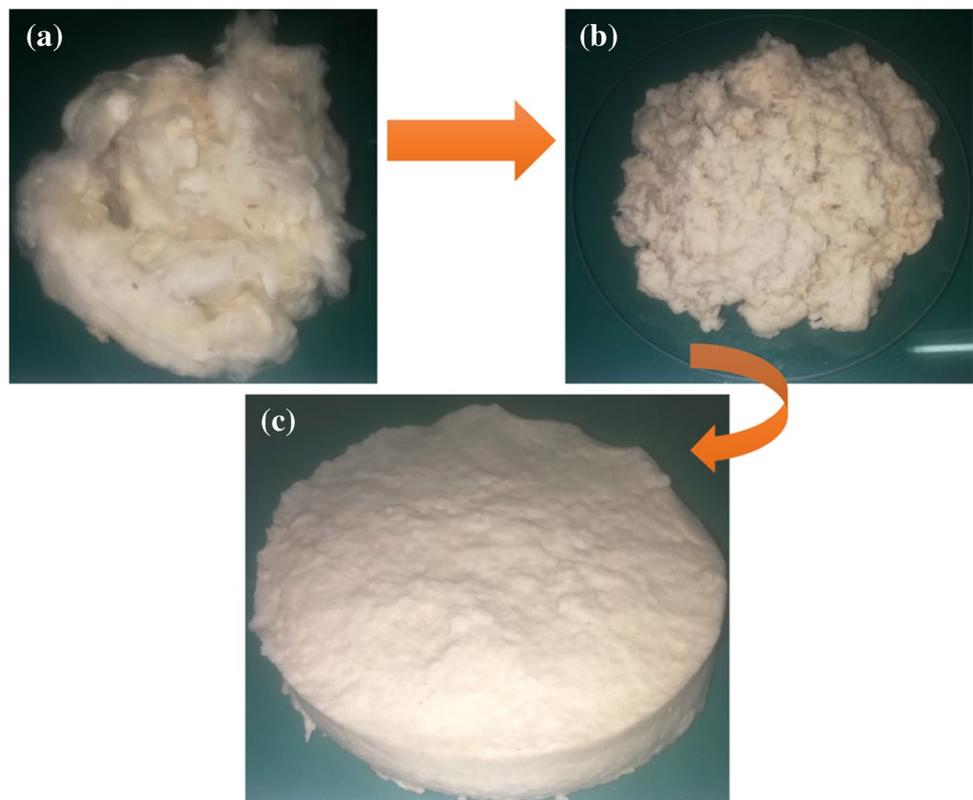
Approximately 20 g of CLs were ground into a powder in a Culatti micro-impact mill type grinder (Model MFC, CZ13; ZENITH, Zurich, Germany) with a 1 mm screen, and the fraction passing through a 40-mesh size but retained on a + 60-mesh size was used for chemical analysis. The chemical characterizations of CLs were conducted as per TAPPI standard test methods. The tests that were carried out included: solubility in alcohol/benzene

(1:2 v/v) solvent extraction (TAPPI T 204 cm-07) [50], acid-insoluble lignin (TAPPI T 222 om-21) [51], and pentosans as per TAPPI T 223 cm-10 [52]. For α -cellulose (gravimetric method), holocelluloses, and ash content, the following methods were used TAPPI T 203 cm-09 [53], TAPPI T 249 cm-21 [54], and TAPPI T 211 om-16 [55], respectively. Four samples were used for each chemical characterization. All results were reported as percentages of the initial weight of CLs.

2.1.2 Kraft pulping process of cotton linters

Samples of 200 g of oven-dried CLs (Fig. 1) were soaked in water for one day, followed by cooking under the Kraft pulping process, conducted in a stainless-steel vessel with a 3 L capacity, equipped with a rotating and heating oil bath. The pulping conditions were: 6% active alkalinity charge relative to the dry weight of the raw material, 20% sulfidity, cooking temperature of 140 °C, cooking time of 120 min, and a liquid ratio (liquid to CLs ratio) of 12:1. The solid residue was defibrated and washed with hot water (70 °C) until reaching a neutral (pH = 7). The resultant pulp was screened on a valley flat screen with 0.25 mm slots. Samples of CLs were pulped in triplicate. The bleaching was carried out the traditional way, using sodium hypochlorite with 8% active chlorine for 3 h at 35 °C and pH 10. The bleaching process was carried

Fig. 1 Kraft pulping process of Cotton linters (a), bleached pulp (b,c)



out in compliance with safety controls and industrial safety requirements. After the bleaching process was completed, the samples were washed thoroughly with running water, given an antichlor treatment of 0.5% sodium sulfite for 30 min, and washed with distilled water. The samples were air-dried and weighed accurately to obtain the percentage of total pulp yield from CLs and then screened using valley type laboratory equipment (Iron Work Corp, Appleton, WI) with a slot size of 0.25 mm. Subsequently, the pulp was beaten to 40°SR with type VOIT valley laboratory equipment (Voit Inc., Appleton, WI).

The yield, kappa number, freeness (Schopper Riegler, SR⁰), and brightness of the pulp were determined according to TAPPI standard methods T 210 cm-93 [56], T 236 om-13 [57], ISO 5267-1, and T 452 om-92 [58], respectively. The physical strength properties of cotton linter paper pulp (CLP), tensile index, internal tearing index, and burst index were measured according to TAPPI T 220 sp-16 [59], TAPPI T 404 wd-03 [60], TAPPI T 414 om-12 [61], and TAPPI T403 om-15 [62], respectively.

2.2 Source of parchment and textile fibers

Goat parchment was provided by Leather City in Al-Rubiki, Cairo, Egypt, while the Textile fibers were obtained from Golden Tex Company at AL-Asher Men Ramadan City, Cairo, Egypt (Fig. 2). For sterilization of the materials, CLPs and linen textile samples were autoclaved at 121 °C for 20 min, but parchment samples were exposed to a UV lamp at a wavelength of 256 nm for 1.5 h.

2.3 Essential oils extraction

The essential oils (EOs) from *Pinus rigida* wood and *Origanum majorana* green leaves were extracted via the hydrodistillation method, in which ~ 150 g of small pieces of each material were put in a 2 L flask containing 1500 mL of distilled

water then connected to a Clevenger unit and heated for 3 h under refluxing [63]. The EOs were collected and dried over anhydrous sodium sulfate; the EO amount was calculated based on sample weight as 0.66 mL/100 fresh leaves of *O. majorana* and 0.44 mL/100 g of wood from *P. rigida*. The obtained EOs were kept dry in sealed Eppendorf tubes and stored at 4 °C in the refrigerator until subsequent analysis.

2.4 Chemical analysis of the essential oils

EOs from *P. rigida* wood and *O. majorana* green leaves were analyzed for their chemical constituents with a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX) with a direct capillary column TG-5MS (30 m × 0.5 mm × 0.25 μm film thickness) apparatus. The column oven temperatures and chemical separation and identification conditions can be found in the previous study [64]. Xcalibur 3.0 data system in the GC-MS with its threshold values was used to confirm that all the mass spectra of the identified compounds were attached to the library. Furthermore, the measurement indices of Standard Index (SI) and Reverse Standard Index (RSI) with values ≥ 650 were used to confirm the identified compounds [44, 63, 65, 66].

2.5 Antifungal activity

2.5.1 Vapor treatment with the essential oils

The extracted EOs were prepared at the concentrations of 500, 250, and 125 μL/L. The respective amount of EO was diluted in 10% DMSO, and 0.5 mL of Tween 40 was added. Tween 40 was added to increase the spread of the EO, which supports the homogeneity of evaporation. Samples of CLPs, linen textile, and parchment, with the dimensions of 2 × 2 cm, were vapor treated with the prepared concentrations of the EOs using the



Fig. 2 Samples of Cotton linters Paper (a), Linen Textile (b) and Parchment (c)

evaporation method [42, 66–68]. Briefly, the tested samples were placed in Petri dishes containing 8 layers of filter papers (Whatman No. 1) overlaid by a mesh (polyethylene spacer). The Petri dishes were autoclaved and left to cool. Then, the EOs with respective concentrations were impregnated over the filter papers and kept for 48 h to allow the evaporation of the EOs, which the fumigants (EOs) subsequently absorbed by the tested samples (CLP, linen textile, and parchment).

2.5.2 Inhibition growth of fungi by the essential oils

The three fungi, *Aspergillus terreus* Ate456, *Aspergillus flavus* AF1375, and *Aspergillus niger* Ani245, listed in GenBank under accession numbers MH355953, MH355958, and MH355955, respectively [3, 69], were used in the bioassay. Tests of inhibition of microorganisms were performed in 9 cm Petri dishes with PDA with or without EOs. For comparisons, samples treated with 10% DMSO with Tween40 were used as controls. Each treatment was evaluated in triplicate. A seven-day-old colony from each fungus with a 9 mm diameter was placed in the center of the treated PDA dishes, and the controls were then incubated at 26 ± 1 °C for 14 days. When the mycelial growth filled the Petri dish in the control treatment (negative), the fungal growth inhibition (FGI) percentage was calculated as follows:

$$\text{FGI}\% = [(A_C - A_T)/A_C] \times 100$$

where A_C and A_T represent the average diameters of the fungal growth of control and treatment, respectively.

2.6 SEM examination

At the end of the incubation period of CLP, the symptoms or inhibition of fungal infestation over the linen textiles and parchment samples treated with the tested EOs and inoculated with the three fungi were examined using a scanning electron microscope (SEM). The samples were finely coated with gold and examined via SEM-JEOL (JFC-1100E Ion sputtering device, model JSM- 5300, JEOL Co., Tokyo, Japan) at 8 kV.

2.7 Statistical analysis

The fungal growth inhibition was statistically analyzed for two factors (EO type and EO concentration) applied to each material using analysis of variance and the Statistical Analysis Software (SAS, Release 8.02, Cary, NC, USA) system [70]. Differences among means were measured using Duncan's multiple range test at a 0.05 level of probability.

3 Results and discussion

3.1 Chemical analysis of cotton linters and the pulp paper properties

The summative chemical analysis of Egyptian cotton linters (CLs) is shown in Table 1, where the holocellulose was 88%, or specifically, the α -cellulose was 82%. Previous research showed that the CLP possessed a higher α -cellulose content (98.79%) and lower ash content (0.22%) [71]. The cellulose content dry weight of CLs typically reaches 80% [72, 73]. Another study showed that the fibers of CL contain α -cellulose over 99% and a small amount of residual waxes and oils [74].

Pulp yield, Kappa number, and brightness values of 85%, 3.5, and 75%, respectively, were found for the bleached cotton linter pulp (CLP). Bleached pulp hand sheets made from CLs with a Grammage value of 60 g/m² showed the following mechanical properties: tear index 11.76 mN·m²/g, burst index 4.2 kPa·m²/g, and tensile index 51 Nm/g. These values were higher than those reported from paper sheets made from some hardwoods pulps such as *E. camaldulensis* [2, 75, 76]. Other studies showed that papers made from CLP are characterized by a higher curl value of fiber, mostly due to their softness [77].

3.2 Chemical composition of the essential oils and their antifungal activities

The chemical compounds of the essential oil (EO) from *Pinus rigida* wood are shown in Table 2 and Fig. 3a. The abundant bioactive compounds were 2-methylisoborneol (29.52%), 4-isopropyl-5-methylhex-2-yne-1,4-diol (16.53%), 1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene (12.06%), 2-methyl-2-bornanol (11.86%), (E)-3,4,4-trimethyl-5-oxo-2-hexenoic acid (5.93%), 2,5-dimethyl-3-hexyne-2,5-diol (4.10%), 1-vinyl cyclohexanol (2.87%) and terpinen-4-ol (2.82%).

The chemical compounds of *Origanum majorana* green leaf EO are shown in Table 3 and Fig. 3b. The

Table 1 Chemical composition of Egyptian cotton linters

Component	Value (%)
Holocellulose	88
α -Cellulose	82
Alcohol/benzene solubility	2.5
Pentosans	1.28
Acid-insoluble lignin	0.85
Ash	1.75
Moisture content	6

Table 2 Phytochemicals of essential oil from *Pinus rigida* analyzed via GC–MS

R.T. (min)	Compound	Percentage (%)	SI ¹	RSI ²
6.77	<i>P</i> -Cymene	1.24	904	925
7.43	Thujanol	0.28	868	902
8.25	<i>trans</i> -para-2-Menthen-1-ol	1.47	894	900
9.15	5-Methyl-1,5-hexanediol	0.43	829	965
9.94	Terpinen-4-ol	2.82	900	908
10.16	Δ -3-Carene	0.15	761	803
10.35	α -Terpineol	0.32	883	912
10.95	4-Isopropylbenzyl alcohol	0.52	868	921
11.14	<i>cis</i> -Hexahydro-7a-methyl-3-methylene-2(3H)-benzofuranone	0.06	688	699
11.44	1-Vinyl cyclohexanol	2.87	771	783
12.82	4-Isopropyl-5-methylhex-2-yne-1,4-diol	16.53	666	685
13.16	2,5-Dimethyl-3-hexyne-2,5-diol	4.10	735	795
13.39	7-Hydroxy-6-methyl-oct-3-enoic acid	0.17	667	673
13.54	4,4-Dimethylpent-2-enal	1.59	722	801
13.77	7-Methyl- <i>Z</i> -tetradecen-1-ol acetate	0.14	715	715
14.49	<i>trans</i> -1,10-Dimethyl- <i>trans</i> -9-decalinol	0.89	752	774
14.79	2-Isopropyl-5-methyl-6-oxabicyclo[3.1.0]hexane-1-carboxaldehyde	0.15	745	759
15.03	Isocaulalol	0.98	702	723
15.25	2,4,7,9-Tetramethyldec-5-yne-4,7-diol	0.40	690	726
15.76	1,2- <i>cis</i> -1,5- <i>trans</i> -2,5-Dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene	12.06	741	792
16.13	1-Benzoxirene-2,5-dione, 4-(3-oxobutyl)hexahydro-3,3,4-trimethyl	0.65	725	794
16.57	(<i>E</i>)-3,4,4-Trimethyl-5-oxo-2-hexenoic acid	5.93	730	786
17.45	2-Methylisoborneol	29.52	632	671
17.83	2-Methyl-2-bornanol	11.86	732	744
18.02	<i>p</i> -Menthane-1,2,3-triol	0.49	681	702
18.19	2,3-Bornanediol	1.12	676	680
18.30	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	0.20	663	664
18.68	4-(4-Hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)butan-2-one	0.14	729	771
18.85	Corymbolone	0.32	705	705
19.26	2-Pentanone,4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-	0.55	719	733
19.63	3,7-Dimethyl- 6-nonenal	1.00	671	672
19.87	6-Oxododecanedioic acid	0.14	657	666
20.47	6-(3-Hydroxy-but-1-enyl)-1,5,5-trimethyl-7-oxabicyclo[4.1.0]heptan-2-ol	0.34	674	678
25.45	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butyric acid	0.10	666	771

Note: R.T.: Retention time (min); SI = Standard Index; and RSI = Reverse Standard index

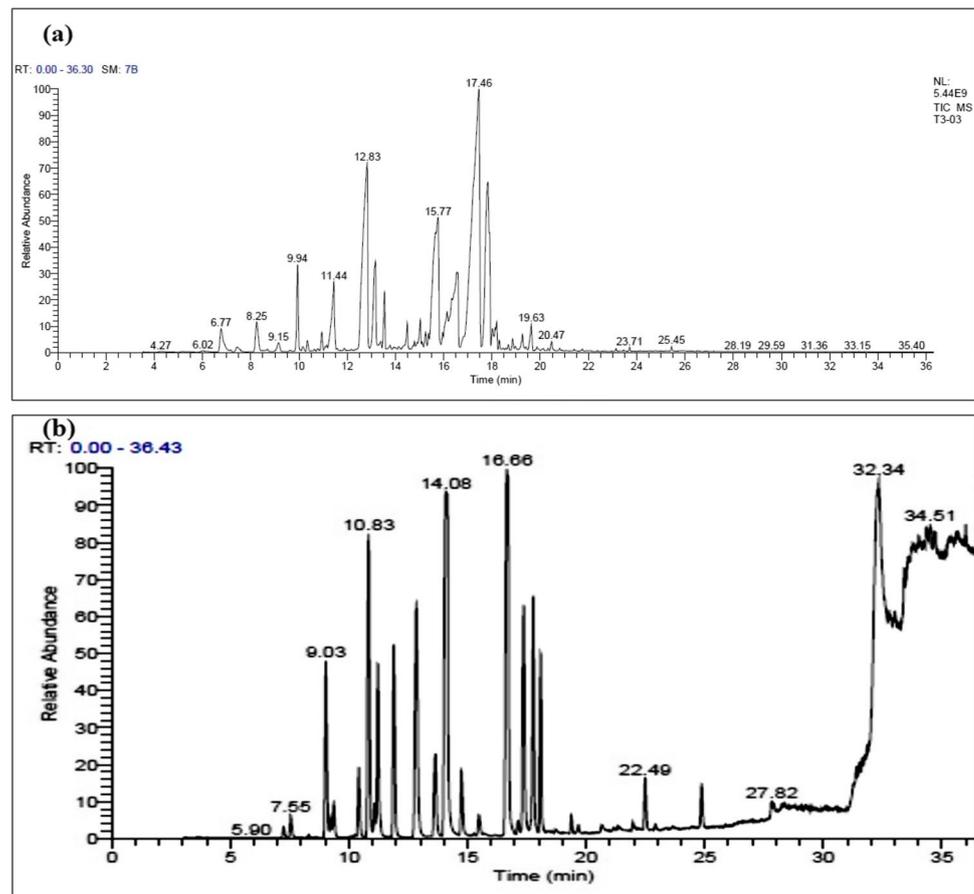
main chemical compounds were *cis*- β -terpineol (15.4%), terpinen-4-ol (14.39%), oleic acid (10.75%), *D*-limonene (8.49%), thujanol (7.1%), α -terpineol (6.41%), linalyl anthranilate (5.49%), γ -terpinene (5.06%), sabinene (4.5%), *cis*-sabinene hydrate acetate (4%), *o*-cymene (3.9%), and linalool (2.51%).

Samples of cotton linter pulp paper (CLP), linen textile, and parchment treated with *P. rigida* and *O.*

majorana EOs using the vapor method at 125, 250, and 500 μ L/L and compared with the control are shown in Fig. 4a,b,c. Generally, with an increase in the concentration of the tested EOs applied to the tested materials, the fungal radial growth decreased compared to the control samples (10% DMSO).

CLP treated with *O. majorana* EO showed the fungal growth inhibition (FGI) percentages of 47.6% and

Fig. 3 GC–MS chromatograms of the essential oils analysis from *Pinus rigida* wood (a) and *Origanum majorana* green leaves (b)



46.3%, at 500 $\mu\text{L/L}$ and 250 $\mu\text{L/L}$, respectively, followed by *P. rigida* EO (500 $\mu\text{L/L}$) with FGI of 46% against *A. niger* growth (Table 4). The treated CLP with *P. rigida* EO at 500 $\mu\text{L/L}$ showed an FGI of 74%, followed by *O. majorana* EO with an FGI of 48.6% at the same concentration against the growth of *A. flavus*. *P. rigida* and *O. majorana* EOs at 500 $\mu\text{L/L}$ showed FGI values of 100% and 94.6%, respectively, against the growth of *A. terreus*.

Table 5 shows the antifungal activity of the tested EOs when applied to parchment samples. At 500 $\mu\text{L/L}$, *O. majorana* and *P. rigida* EOs observed 49% and 46% of FGI, respectively. *O. majorana* EO at 250 $\mu\text{L/L}$ observed FGI values of 46.6% against the growth of *A. niger*. The higher activity (FGI 78%) against the growth of *A. flavus* was observed in parchment samples treated with *P. rigida* EO at 500 $\mu\text{L/L}$ followed by *O. majorana* EO (FGI 54.3%). Potent activity against the growth of *A. terreus* was reported as parchment samples treated with *P. rigida* EO (500 $\mu\text{L/L}$), *O. majorana* EO (500 $\mu\text{L/L}$), and *O. majorana* EO (250 $\mu\text{L/L}$) with FGI values of 100%, 97.3%, and 94.6%, respectively.

Table 6 presents the antifungal activity of treated linen textiles with the EOs. Linen textiles vaped with *O. majorana* and *P. rigida* EOs at 500 $\mu\text{L/L}$ had higher activity against the

growth of *A. niger* with an FGI of 49 and 47%, respectively. The *P. rigida* EOs at 500 $\mu\text{L/L}$ showed higher activity against *A. flavus* with an FGI of 77.3%, followed by *O. majorana* EO with an FGI of 53.3%. Potent antifungal activity against *A. terreus* was found in linen textile vaped with *P. rigida* EO at 500 $\mu\text{L/L}$ (FGI of 100%), followed by *O. majorana* EO at 500 and 250 $\mu\text{L/L}$ with FGI of 97.3 and 96%, respectively.

CLP, linen textile, and parchment samples treated with the two EOs showed promising bioactivity against the mold infestation of *Aspergillus terreus*, *A. flavus*, and *A. niger*. These bioactivities could be related to the presence of bioactive compounds in the EOs.

The previous work showed that α -terpineol, borneol, and fenchyl alcohol were the most abundant compounds in *P. rigida* wood oil [42]. Ethyl ether extract of *P. rigida* wood identified terpinen-4-ol, *cis*-4-thujanol, α -terpineol, γ -terpinene, sabinene, fenchol, 14- β -H-pregna, and α -terpinene as the main compounds [78]. Meanwhile, α -terpineol, terpin hydrate, borneol, D-fenchyl alcohol glycol, and limonene were found in the methanol extract from *P. rigida* wood [41]. The extract from *P. rigida* wood applied to wood samples showed no changes to their structure and gave some protection

Table 3 Phytochemicals of the essential oil from *Origanum majorana* green leaves analyzed via GC–MS

R.T. (min.)	Compound	Percentage in the Oil (%)	SI ¹	RSI ²
7.55	<i>cis</i> -Ocimene	0.56	939	946
9.03	Sabinene	4.50	966	974
9.36	Myrcene	0.79	949	957
10.42	α -Terpinene	1.75	950	957
10.83	D-Limonene	8.49	937	938
11.22	<i>o</i> -Cymene	3.90	943	952
11.89	γ -Terpinene	5.06	943	945
12.84	Thujanol	7.10	949	952
13.64	Linalool	2.51	962	971
14.08	<i>cis</i> - β -Terpineol	15.40	929	941
14.75	<i>p</i> -Menth-3-en-1-ol	1.59	911	915
15.48	<i>trans</i> - <i>para</i> -2-Menthen-1-ol	0.59	912	929
16.66	Terpinen-4-ol	14.39	944	948
17.36	α -Terpineol	6.41	947	947
17.78	Linalyl anthranilate	5.49	953	273
18.08	<i>cis</i> -Sabinene hydrate acetate	4.00	871	932
19.38	α -Fenchyl acetate	0.39	870	911
22.49	Caryophyllene	1.32	893	909
24.87	γ -Elemene	1.10	853	904
31.39	Linoleic acid	0.45	818	842
32.34	Oleic acid	10.75	873	874
33.41	1-Heptatriacotanol	0.60	801	818

Retention time (min); SI=Standard Index; and RSI=Reverse Standard index

against the fungal infestations of *Trichoderma harzianum* and *A. niger* [78]. The most abundant chemicals generated from *P. rigida* wood at the standard emission rate (temperature of 30 °C) were α -pinene, β -pinene, myrcene, and α -terpinene [79]. Caryophyllene, thunbergol, 3-carene, cembrene, α -thujene, and terpinolene were found in the EO from *P. roxburghii* wood with some bioactivity against bacterial growth [80].

O. majorana EO showed the presence of *cis*- β -terpineol, terpinen-4-ol, oleic acid, D-limonene, thujanol, α -terpineol, sabinene, linalyl anthranilate, γ -terpinene, and *cis*-sabinene hydrate acetate as the main abundant compounds. The main component of marjoram EO was terpinen-4-ol [81–84]. Terpinen-4-ol (30.4%) with *cis*-sabinene hydrate, α -terpinene, γ -terpinene, α -terpineol, and sabinene were identified as major compounds in marjoram EO [85] with antimicrobial activity. Other compounds

such as linalool (32.68%) and terpinen-4-ol (32.30%) were found in the EO of *O. majorana*, with promising activity against some strains of yeasts, molds, and bacteria [86]. The EO showed a broad range of fungitoxicity against *A. niger*, *A. fumigatus*, *A. luchuensis*, *P. chrysogenum*, *Penicillium italicum*, *Cladosporium cladosporioides*, *Fusarium poae*, and *Alternaria alternata* [83].

Terpineol, terpinen-4-ol, 4-thujanol, α -terpineol, cymene, and sabinene were the main EO compounds from *O. majorana* with effectivity against some fungi (*Fusarium verticillioides*, *F. graminearum*, *Bipolaris oryzae*, and *Curvularia lunata*) [87]. *O. majorana* EOs, with their main compounds, terpinen-4-ol, and *p*-cymene, inhibited mycelia growth of *A. alternata* [88]. *O. majorana* EO applied to some wood species had good bio-fungicides against *T. harzianum* and *A. niger* without changing their structures [78]. The main compounds in *O. majorana* EO were 4-carvomenthenol, γ -terpinene, α -terpinene, *trans*-sabinene hydrate, *p*-cymene, β -fenchol, limonene, β -caryophyllene, sabinene, myrcene, *cis*-4-thujanol, terpinolene, α -pinene, linalyl acetate, and γ -elemene [78].

3.3 Scanning electron microscopy observation

3.3.1 SEM images of CLP samples inoculated by molds

The SEM images of the treated CLP samples with the EOs and inoculated with *A. terreus* are shown in Fig. 5. Extensive mycelial growth of *A. terreus* over the untreated CLP samples was observed (Fig. 5a). The fungal growth decreased as the CLP was treated with *O. majorana* EO at 125 μ L/L (Fig. 5b). The structure of CLP fibers was clearly shown, and the fungal growth was suppressed when treated with *P. rigida* EO at 250 μ L/L (Fig. 5c) and *P. rigida* EO at 125 μ L/L (Fig. 5d).

Excessive growth of *A. flavus* was observed in untreated CLP samples (Fig. 5e), where conidiophores are often seen under SEM examination. Even in the CLP treated with either 125 μ L/L of *O. majorana* EO (Fig. 5f), 125 μ L/L of *P. rigida* EO (Fig. 5g), or 250 μ L/L of *P. rigida* EO (Fig. 5h), fungal growth was still observed.

SEM images of CLP samples inoculated with *A. niger* showed extensive fungal mycelial growth over the control samples (Fig. 5i) and the vaped samples with 125 and 250 μ L/L of *P. rigida* EO (Fig. 5j,k). Growth of *A. niger* decreased as CLP was treated with 500 μ L/L of *P. rigida* EO (Fig. 5l), 125 μ L/L of *O. majorana* EO (Fig. 5m), and 250 μ L/L of *O. majorana* (Fig. 5n). The growth of *A. niger* was vaped with 500 μ L/L *O. majorana* EO (Fig. 5o).

Fig. 4 Visual observation of antifungal activity of essential oils applied to cotton linters paper, linen textile and parchment by vapor method at 500, 250 and 125 $\mu\text{L/L}$. (P): cotton linters paper, (L): linen textile and (T): parchment; against (a) *A. terreus*; (b) *A. flavus*; (c) *A. niger*

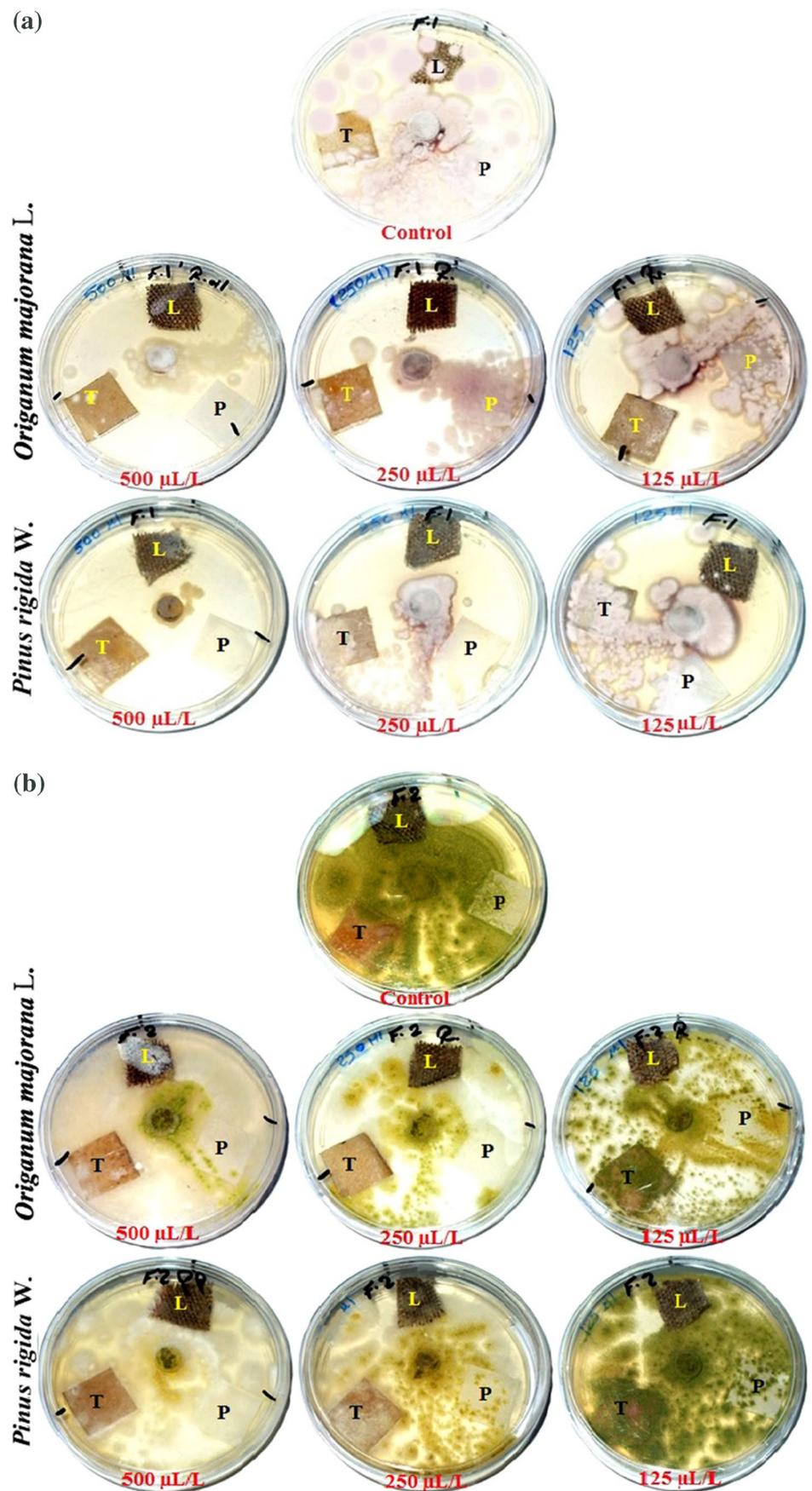


Fig. 4 (continued)

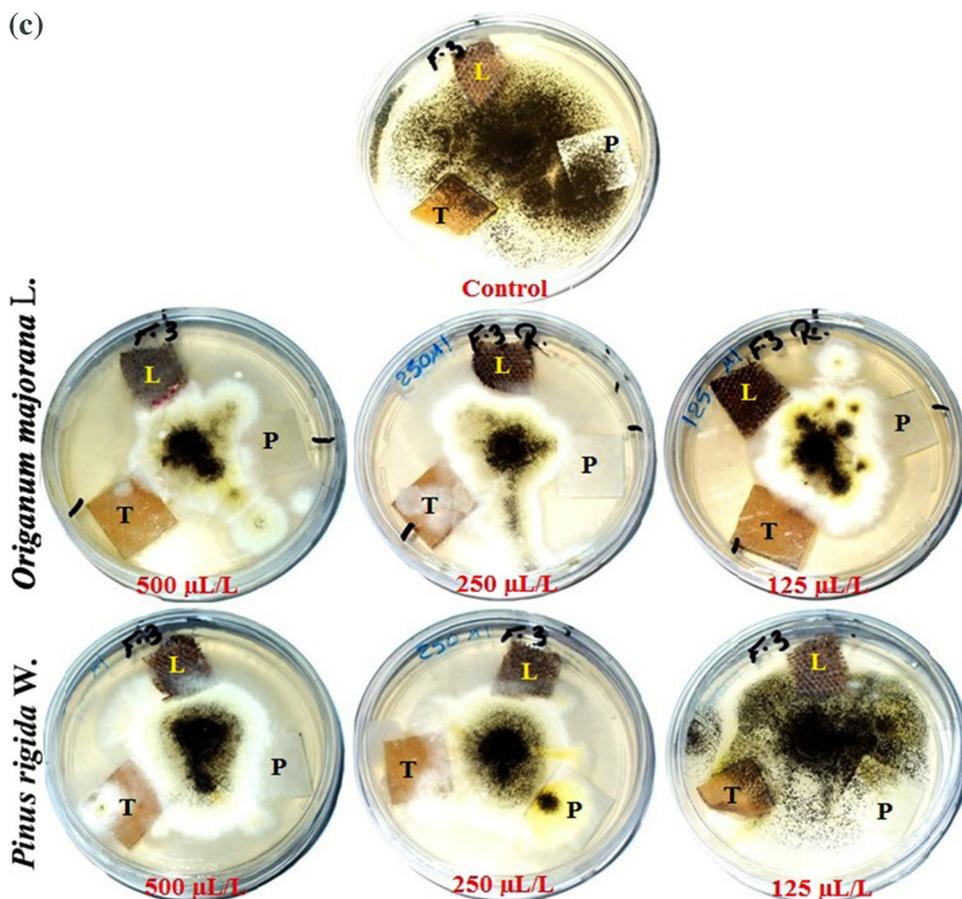


Table 4 Antifungal activity of treated cotton linter pulp paper with *O. majorana* and *P. rigida* EOs

Treatment	Conc. ($\mu\text{L/L}$)	Fungal growth inhibition (%)		
		<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
Control	10% DMSO + 0.5 mL of Tween 40	0.00	0.00	0.00
<i>Origanum majorana</i> green leaves EO	125	43.6 \pm 4.04	0.00	6.6 \pm 5.85
	250	46.3 \pm 0.57	2.6 \pm 1.52	6.6 \pm 5.77
	500	47.6 \pm 1.15	48.6 \pm 1.52	94.6 \pm 0.57
<i>Pinus rigida</i> wood EO	125	2.3 \pm 1.15	0.00	13.6 \pm 5.51
	250	40.3 \pm 0.57	0.3 \pm 0.57	27 \pm 6.55
	500	46 \pm 2	74 \pm 1	100
p value		<0.0001	<0.0001	0.0049

3.3.2 SEM images of parchment samples inoculated with molds

Figure 6 shows SEM images of the inoculated parchment samples with the three molds. The growth of *A. terreus* is clearly shown over the control samples (Fig. 6a). Insignificant decreases in the fungal growth were observed as parchment vaped with *P. rigida* EO at 125 $\mu\text{L/L}$ (Fig. 6b) and *O. majorana* EO at 125 $\mu\text{L/L}$ (Fig. 6c).

The same trend of *A. flavus* fungal growth was observed in untreated parchment samples (control) (Fig. 6d), and the vapor was treated with 125 $\mu\text{L/L}$ of *P. rigida* EO (Fig. 6e) and with 125 $\mu\text{L/L}$ of *O. majorana* EO (Fig. 6f). *A. niger* mycelial growth was shown with extensive distribution over untreated parchment samples (Fig. 6g) and the vaped samples with 125 $\mu\text{L/L}$ of *P. rigida* EO (Fig. 6h) or 125 $\mu\text{L/L}$ of *O. majorana* EO (Fig. 6i).

Table 5 Antifungal activity of treated parchment with the EOs from *O. majorana* and *P. rigida*

Treatment	Conc (μL/L)	Fungal growth inhibition (%)		
		<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
Control	10% DMSO + 0.5 mL of Tween 40	0.00	0.00	0.00
<i>Origanum majorana</i> green leaves EO	125	41 ± 1.73	0.3 ± 0.57	71.6 ± 10.41
	250	46.6 ± 0.57	1.6 ± 1.52	94.6 ± 1.15
	500	49 ± 1	54.3 ± 2.51	97.3 ± 0.57
<i>Pinus rigida</i> wood EO	125	3.3 ± 0.57	0.00	5 ± 5
	250	41 ± 1	0.6 ± 0.57	39.6 ± 17.89
	500	46 ± 2	78 ± 2.64	100
p value		< 0.0001	< 0.0001	< 0.0001

Table 6 Antifungal activity of treated linen textile with *O. majorana* and *Pinus rigida* EOs

Treatment	Conc (μL/L)	Fungal growth inhibition (%)		
		<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
Control	10% DMSO + 0.5 mL of Tween 40	0.00	0.00	0.00
<i>Origanum majorana</i> green leaves EO	125	41.3 ± 2.51	0.6 ± 1.15	45 ± 1
	250	46.66 ± 0.57	3.3 ± 1.52	96 ± 1
	500	49 ± 1	53.3 ± 2.51	97.3 ± 0.57
<i>Pinus rigida</i> wood EO	125	4.3 ± 0.57	0.00	46.3 ± 1.52
	250	43.6 ± 2.31	2 ± 1	57.6 ± 3.05
	500	47 ± 2	77.3 ± 2.51	100
p value		< 0.0001	< 0.0001	< 0.0001

3.3.3 SEM images of linen textile samples inoculated with molds

Figure 7 shows the SEM images of linen textile vaped with the EOs and inoculated with the three molds. The growth of *A. terreus* is shown in the control samples (Fig. 7a), and this growth was significantly decreased as the linen textile was vaped with *P. rigida* EO at 125 μL/L (Fig. 7b) and with 125 μL/L of *O. majorana* EO (Fig. 7c).

The extensive growth of *A. flavus* conidia was observed over the control samples of the linen textile (Fig. 7d). The growth decreased as the samples were vaped with 125 μL/L of *O. majorana* EO (Fig. 7e) or 125 μL/L of *P. rigida* EO (Fig. 7f), but the conidia are still shown. With the increased concentration level of *P. rigida* EO to 250 μL/L, the fungal growth decreased, and the mycelia and conidia were destroyed (Fig. 7g).

Conidia of *A. niger* were observed over the untreated or control linen textile (Fig. 7h) and decreased as the linen textile was treated with 125 μL/L of *O. majorana* EO (Fig. 7i),

with 125 μL/L of *P. rigida* EO (Fig. 7j) and 250 μL/L of *P. rigida* EO (Fig. 7k). The anatomical features of fibers were observed, and the fungal growth was suppressed as the linen textile was vaped with 250 μL/L of *O. majorana* EO (Fig. 7l).

From the previous SEM results of the studied materials, the mode of action of the studied EOs in *A. terreus*, *A. flavus*, and *A. niger* was measured by SEM. The EO treatments led to the distortion and thinning of the hyphal wall, the absence of conidiophores, and a reduction in hyphal diameter [89]. The damage and decay in both fibers and dyes caused by fungi resulted in a reduction in the hardness of fibers; loss of parts; weakness in fibers and dye; and the separation of parts, stain, and dust [90].

Pulp additives were used to improve the quality of the produced paper sheets, i.e., a significant decrease in *A. niger* mycelial growth over Papyrus strips treated with *S. babylonica* leaf extract (2%) or *E. camaldulensis* bark extract (2%) was observed [44]. For the protection from and prevention of the growth of microorganisms such as fungi and bacteria, pulp papers were treated with extracts and EOs from ornamental and woody plants [3, 4, 45, 46,

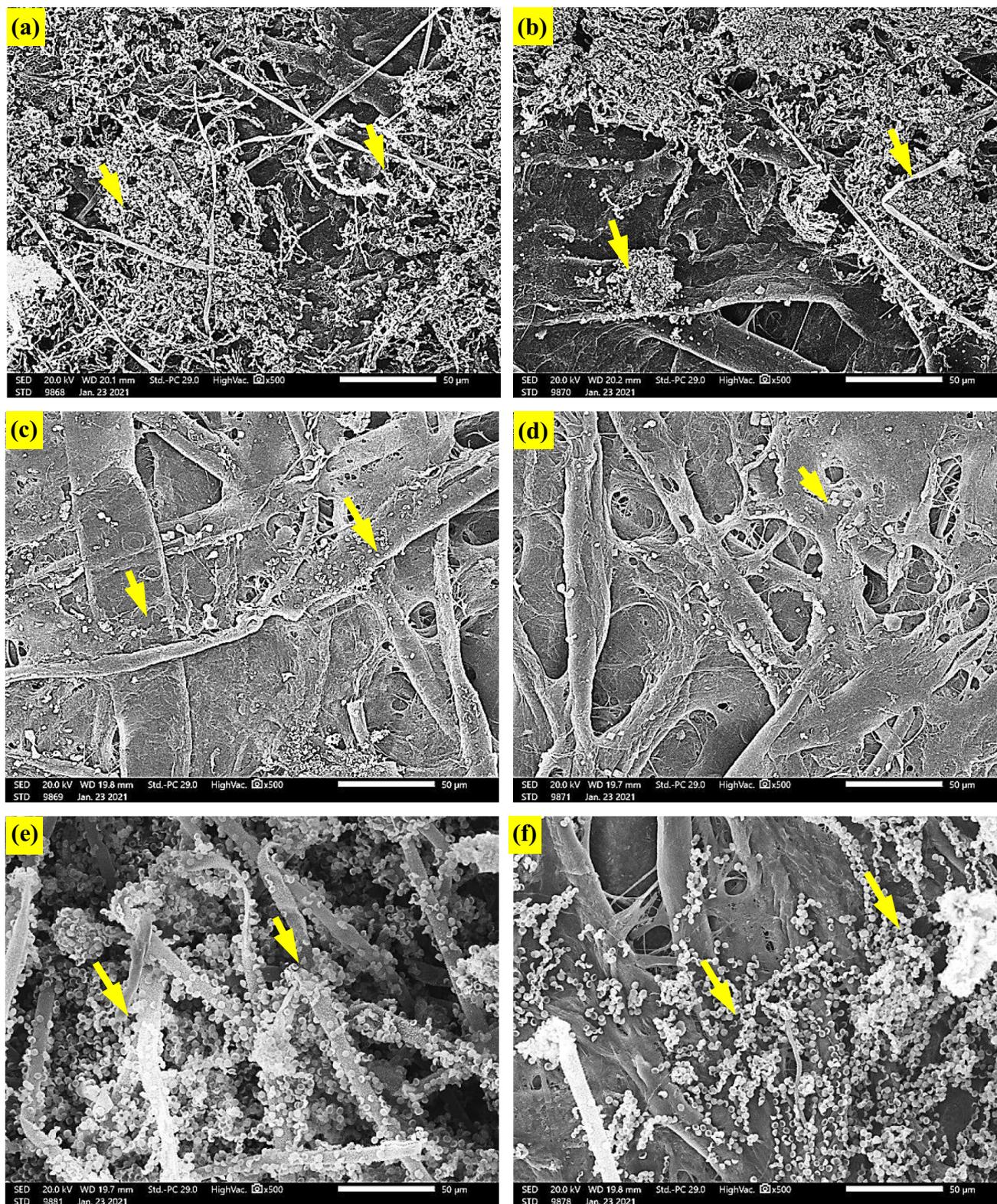


Fig. 5 SEM images of cotton linters paper samples: (a–d) inoculated with *A. terreus*: (a) without treatment, (b) with *O. majorana* EO 125 $\mu\text{L/L}$, (c) with *P. rigida* EO 250 $\mu\text{L/L}$, and (d) with *P. rigida* 125 $\mu\text{L/L}$. (e–h) inoculated with *A. flavus*: (e) without treatment, (f) with 125 $\mu\text{L/L}$ *O. majorana* EO, (g) with 125 $\mu\text{L/L}$ *P. rigida* EO, and (h) with 250 $\mu\text{L/L}$ *P. rigida* EO. (i–o) inoculated with *A. niger*: (i) with-

out treatment, (j) with 125 $\mu\text{L/L}$ *P. rigida* EO, (k) with 250 $\mu\text{L/L}$ *P. rigida* EO, (l) with 500 $\mu\text{L/L}$ *P. rigida* EO, (m) with 125 $\mu\text{L/L}$ *O. majorana* EO, (n) with 250 $\mu\text{L/L}$ *O. majorana* EO and (o) with 500 $\mu\text{L/L}$ *O. majorana* EO. Arrows refer to growth of fungal mycelia based on concentrations of oil treatments

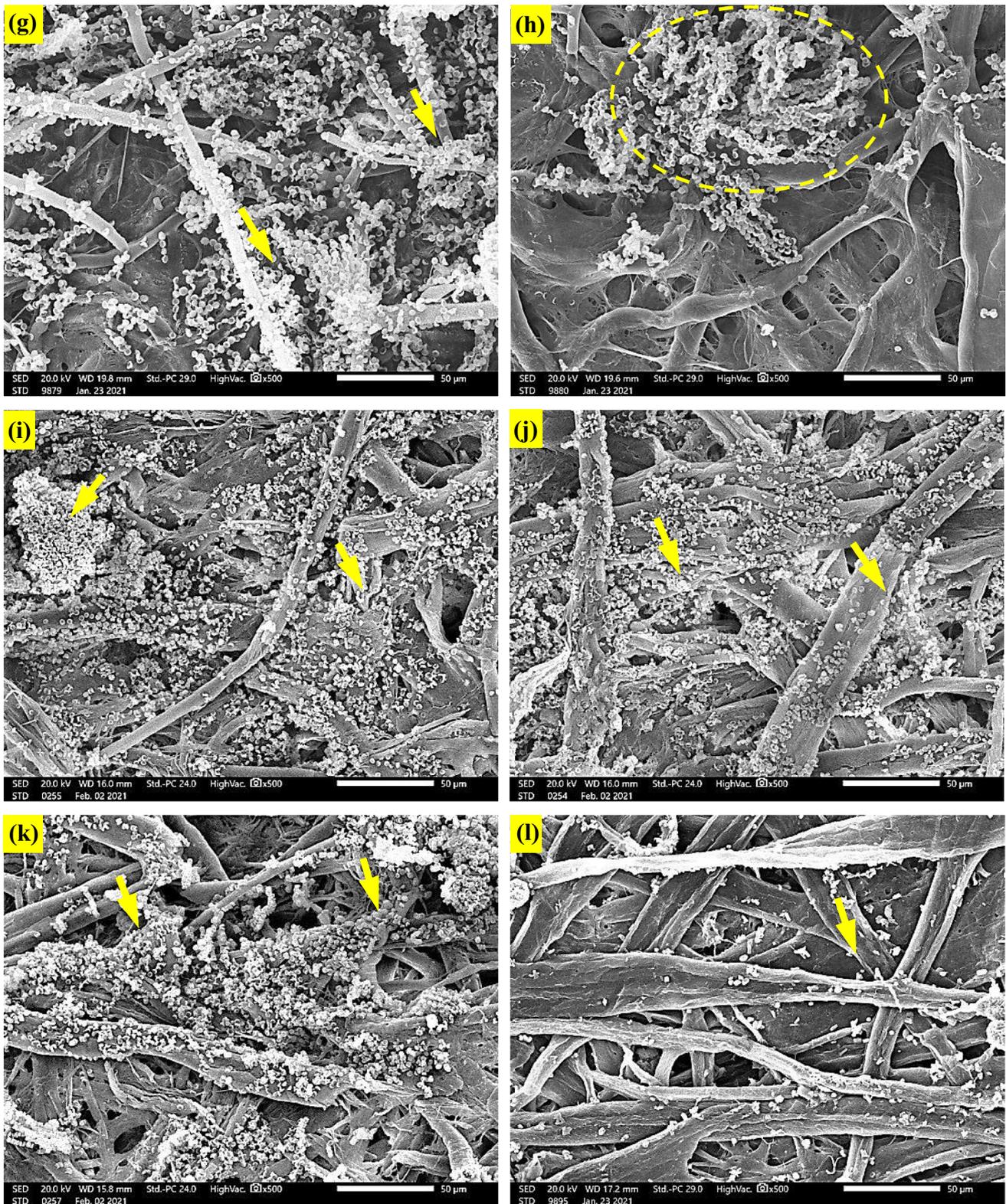


Fig. 5 (continued)

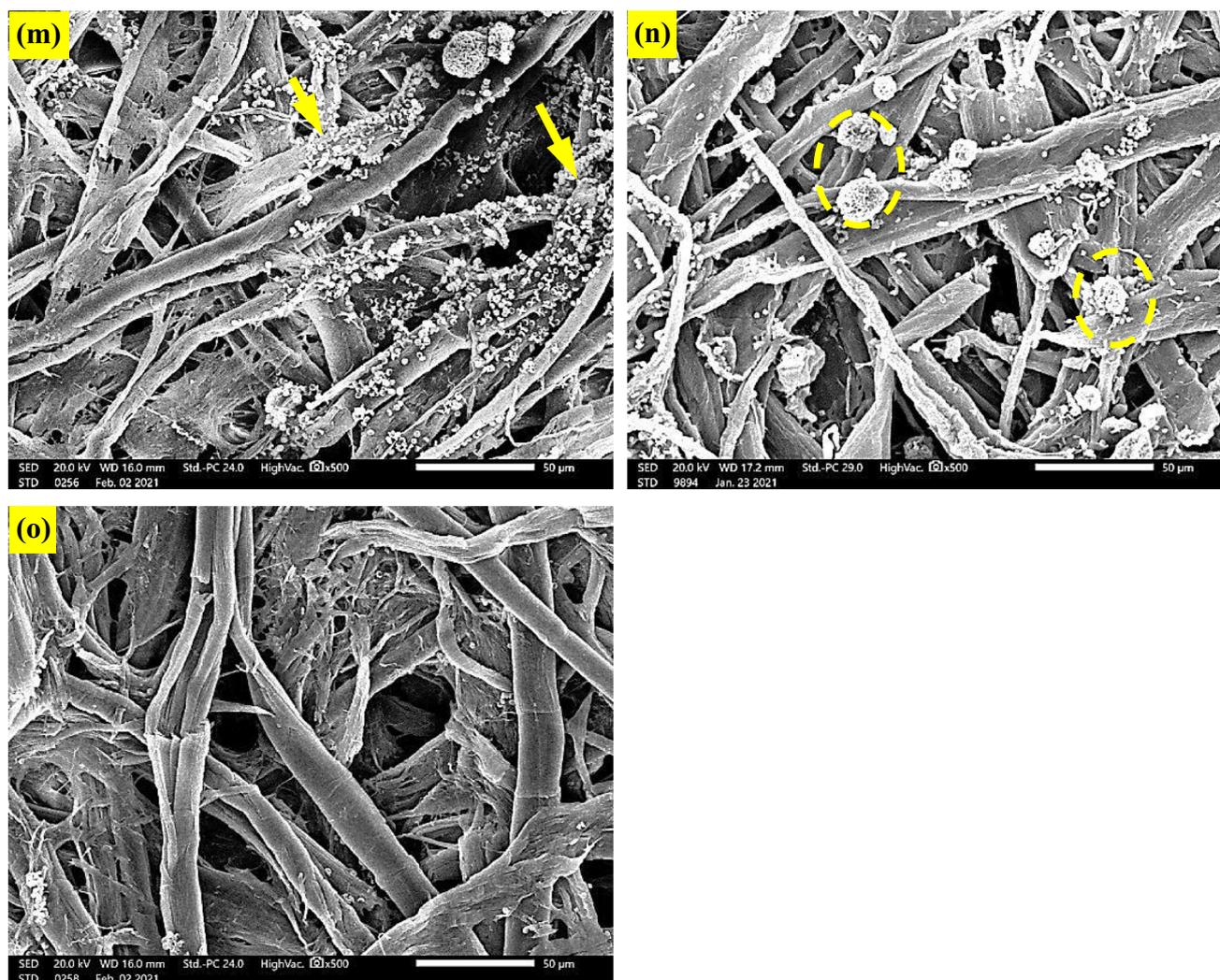


Fig. 5 (continued)

69, 91]. No fungal growth of *A. terreus* and *A. flavus* was observed in pulp paper treated with oils at levels of 3% and 5% from *S. alba* seeds, *M. azedarach* fruits, and *M. grandiflora* leaves, probably related to the presence of bioactive compounds [31]. The growths of *A. niger*, *Penicillium roqueforti*, and *Eurotium chevalieri* were inhibited at the concentration of 1000 mg/mL of *Lemna gibba* extract when impregnated with interleaving papers [92].

The obtained results show that the application of EOs to some industrial materials can prevent or stop the spread of mold growths that may be caused during their handling or service. We suggest reusing these oils after a certain period of time, the length of which should be determined in future studies.

4 Conclusions

P. rigida and *O. majorana* essential oils were used for controlling the growth of *Aspergillus terreus*, *A. flavus*, and *A. niger*, with beneficial effects on organic materials. When applied to industrial materials (cotton linter pulp paper, linen textile, and parchment), the two essential oils possessed promising antifungal properties against *A. terreus*, *A. flavus*, and *A. niger*. These two essential oils could be used to control fungal infestations in various organic materials.

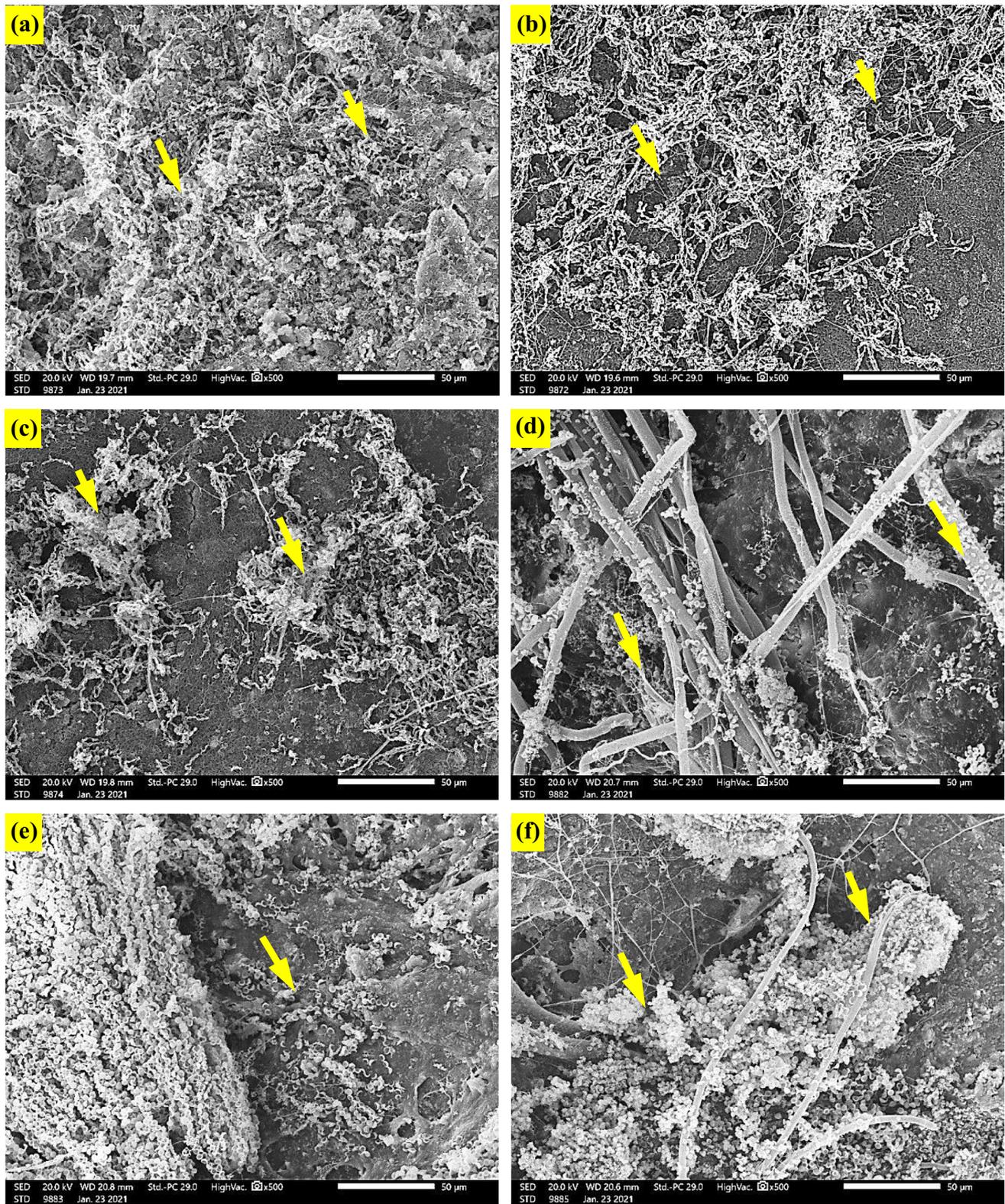


Fig. 6 SEM images of Parchment samples: (a-c) inoculated with *A. terreus*: (a) without treatment, (b) with 125 µL/L *P. rigida* EO, and (c) with 125 µL/L *O. majorana* EO. (d-f) inoculated with *A. flavus*: (d) without treatment, (e) with 125 µL/L *P. rigida* EO, and (f) with

125 µL/L *O. majorana* EO. (g-i) inoculated with *A. niger*: (g) without treatment, (h) with 125 µL/L *P. rigida* EO, and (i) with 125 µL/L *O. majorana* EO. Arrows refer to growth of fungal mycelia based on concentrations of oil treatments

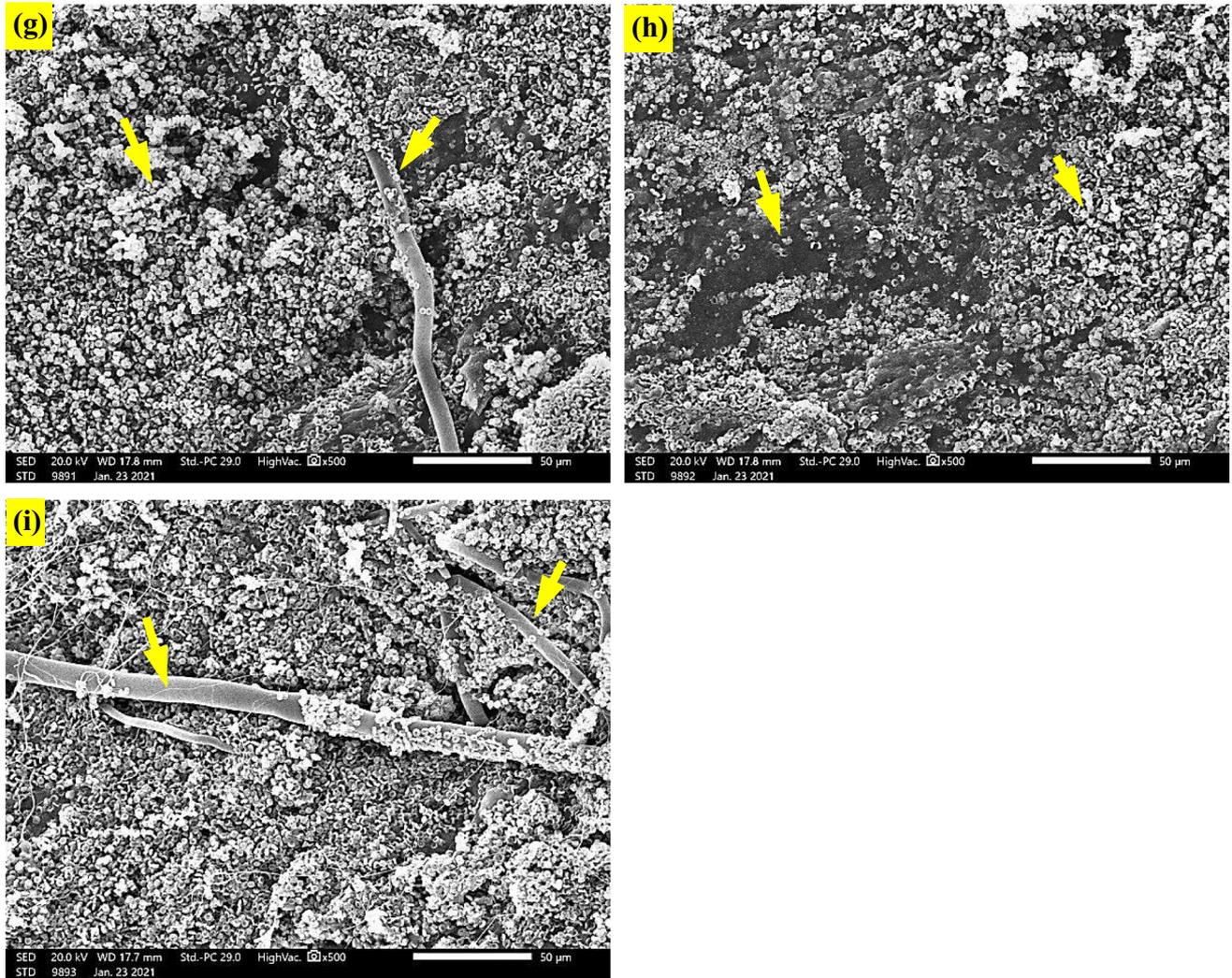


Fig. 6 (continued)

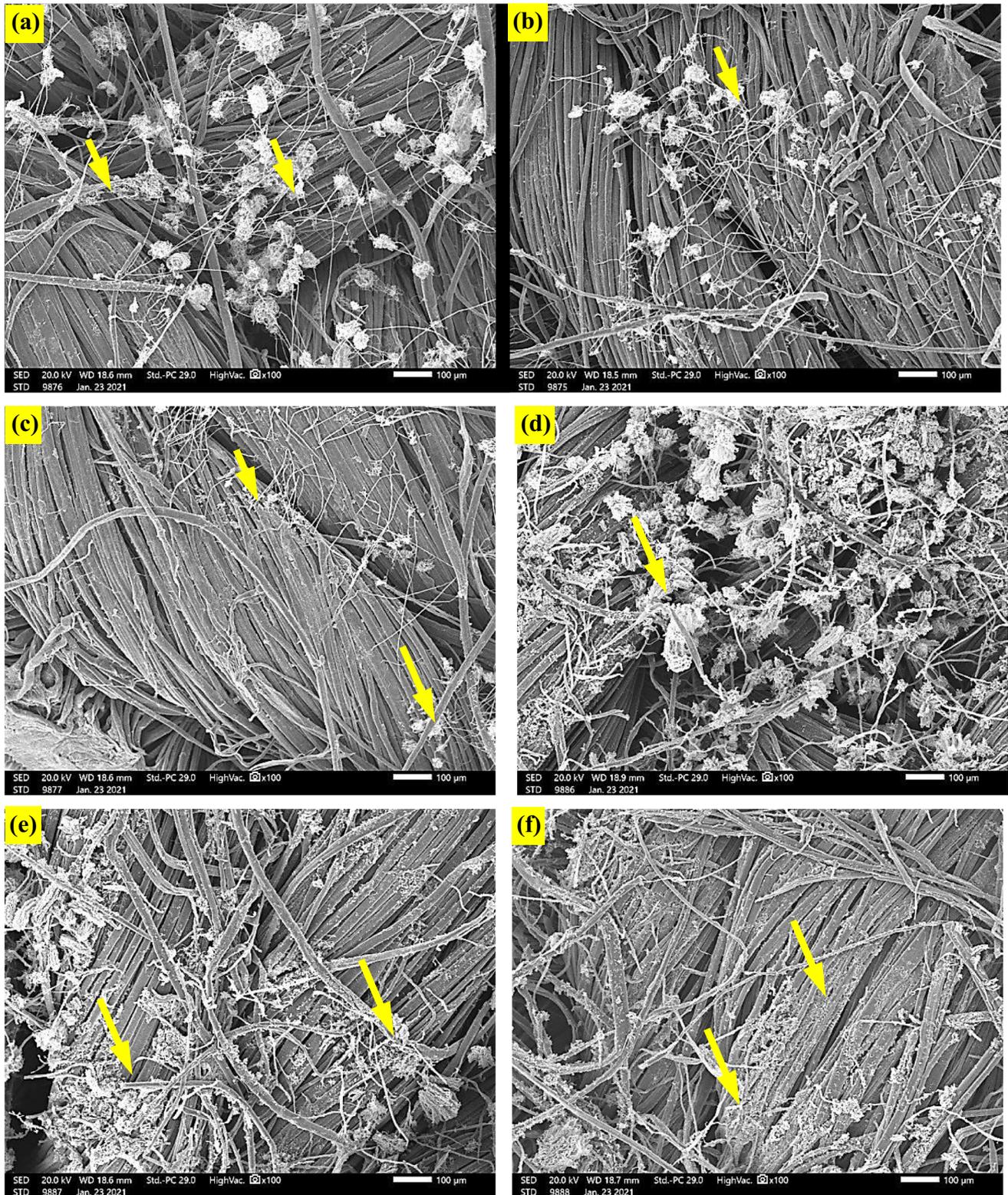


Fig. 7 SEM images of Linen textile samples: (a–c) inoculated with *A. terreus*: (a) without treatment, (b) with 125 $\mu\text{L/L}$ *P. rigida* EO, and (c) with 125 $\mu\text{L/L}$ *O. majorana* EO. (d–g) inoculated with *A. flavus*: (d) without treatment, (e) with 125 $\mu\text{L/L}$ *O. majorana* EO, (f) with 125 $\mu\text{L/L}$ *P. rigida* EO, and (g) with 250 $\mu\text{L/L}$ *P. rigida* EO. (h–l)

inoculated with *A. niger*: (h) without treatment, (i) with 125 $\mu\text{L/L}$ *O. majorana* EO, (j) with 125 $\mu\text{L/L}$ *P. rigida* EO, (k) with 250 $\mu\text{L/L}$ *P. rigida* EO, and (l) with 250 $\mu\text{L/L}$ *O. majorana* EO. Arrows refer to growth of fungal mycelia based on concentrations of oil treatments

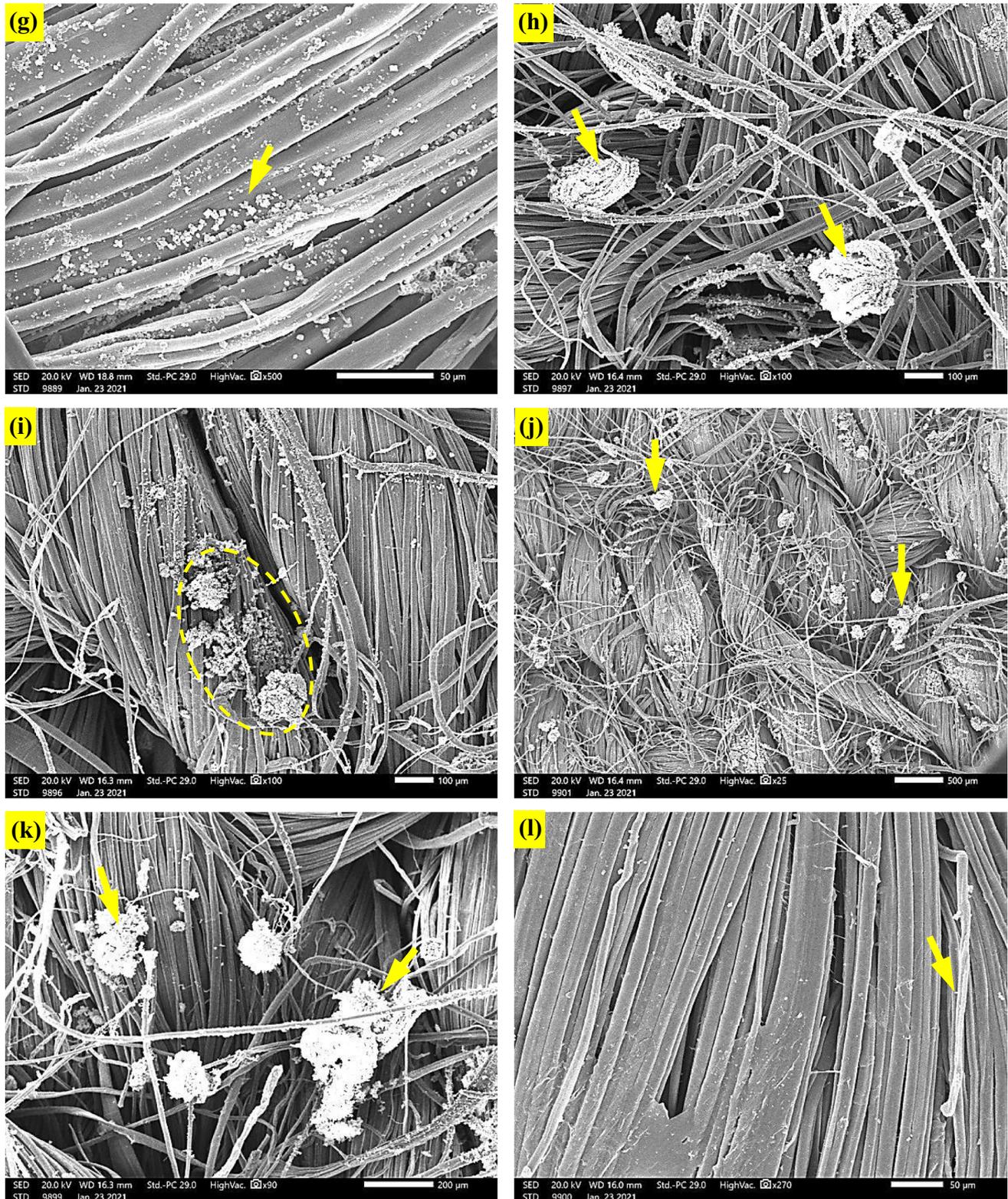


Fig. 7 (continued)

Author contributions Conceptualization was contributed by M.Z.M.S.; methodology was contributed by A.S.T., W.A.A.A.-E., Y.G.D.F., and M.Z.M.S.; formal analysis and investigation were contributed by A.S.T., W.A.A.A.-E., Y.G.D.F., and M.Z.M.S.; data curation was contributed by A.S.T., W.A.A.A.-E., and M.Z.M.S.; writing—original draft preparation, was contributed by A.S.T., W.A.A.A.-E., Y.G.D.F., and M.Z.M.S.; writing—review and editing, was contributed by A.S.T., W.A.A.A.-E., and M.Z.M.S. All authors have read and agreed to the published version of the manuscript.

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Data availability Supported by authors declaration.

Declarations

Conflict of interest The authors declare no competing interests.

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