# Impact of Some Plant Source Additives on Enhancing the Properties and Antifungal Activities of Pulp Made from Linen Fibers

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The effects of some pulp additives on mechanical, optical, and antifungal (Aspergillus terreus Ate456, A. niger Ani245, and Fusarium culmorum Fcu761) properties of papersheets produced from linen fiber pulp were evaluated. The ground materials (80 mesh) of Pinus rigida wood (PRW), Costus speciosus rhizomes (CSR), and Senegalia catechu rhizomes (SCR) were used as pulp additives at the concentrations of 0, 1, 2, and 4%. Papersheets with PRW at 2 and 4% as pulp additives had significant effects on tensile index with 26.41 and 30.22 N.m/g; burst index with 2.91 and 2.92 kPa.m<sup>2</sup>/g; and tear index with 2.52 and 2.53 mN.m<sup>2</sup>/g, respectively. The highest (64.36%) and lowest (62.90%) percent of brightness was observed in paper sheets produced from pulp without additives and CSR at 4%, respectively. Scanning electron microscopy showed that the produced papersheets with plant source additives had different degrees of decaying patterns, except for papersheets with PRW as additive, which showed some inhibition against the fungal growth. In conclusion, the mechanical properties of papersheets were significantly enhanced by the addition of the three pulp additives.

Keywords: Antifungal activity; Linen fibers; Pulp additives; Papersheets

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# INTRODUCTION

Nonwoody plant materials such as kenaf, straws (wheat straw, rice straw), bamboo, hemp, jute, sisal, abaca, cotton linters, linen, tobacco, sugar cane bagasse, elephant grass, giant reed fibers, switch grass, Napier grass, date palm midribs, and reeds have been extensively used as fiber sources for pulp and paper production in Asia, Africa, Eastern Europe, and Latin America (Agarwal 1992; Kaldor 1992; Fu-Wang *et al.* 1996; Pande and Roy 1999; Girouard and Samson 2000; Ashori 2006; Khristova *et al.* 2006; Jimenez *et al.* 2008; Madakadze *et al.* 2010; Zhang *et al.* 2011; Obi Reddy *et al.* 2014; Nassar *et al.* 2015; Nasser *et al.* 2015).

Several materials have been added to the pulp as additives to improve the properties of the product; for example, the physical properties of handsheets are improved by the addition of cationic and carboxymethyl hemicelluloses in the sulfate kraft pulp of spruce wood (Ren et al. 2009). Strength properties of sheets made from old corrugated carton furnishes are enhanced by the addition of chitosan, cationic starch, and poly vinyl alcohol as additives to the pulp (Hamzeh et al. 2013a). Hamzeh et al. (2013b) showed that the strength properties of paper made from bagasse fibers are enhanced by the addition of chitosan and cationic starch as pulp additives. In addition, the structural, optical, and strength properties of handsheet paper made from soda bagasse pulp are enhanced by the addition cellulose nanofibrils in combination with a high degree of substitution cationic starch as bio-additives (Tajik et al. 2018). Handsheets strength properties made from the mixture of bleached hardwood and softwood pulp (50/50%) are enhanced by more than 15% with the addition of starch-coated filler (Yan et al. 2005). However, the strength, optical, and surface properties of the manufactured paper with about 10 to 15% of wood pulp replaced by kenaf pulp are not affected (Mohta 2001; Liu 2002). Mixtures of hemp and linen fiber with higher concentrations of hemp (*i.e.*, 75%) were been used to produce writing paper specimens made in Europe between 1400 and 1800 (Collings and Milner 1984).

In the present study, *Pinus rigida* wood and rhizomes from *Costus speciosus* and *Senegalia catechu* (*Acacia catechu*) in the form of powder were used as additives for pulp of linen fiber. Several studies showed that the extracted chemical compounds present in the bio-additives powdered materials could act as antimicrobial agents. For example, *C. speciosus* rhizomes extract shows activity against *Chaetomium globosum* and *Fusarium subglutinans*, but it is not active against *Alternaria alternata*, *Aspergillus niger*, or *Trichoderma viride* (Salem *et al.* 2016a). In addition, some compounds in the *C. speciosus* extract, such as saponin and *p*-coumaric acid methyl ester, have antifungal activities (Bandara *et al.* 1988; Atap *et al.* 1992); polyphenolic compounds in *C. speciosus* rhizomes extracts also have antifungal activity (Abirami *et al.* 2014). In contrast, Saraf (2010) did not observe antimicrobial activity in *C. specious* rhizome extracts. Some antimicrobial activity has been observed in *S. catechu* extracts (Negi and Dave 2010; Prabhat and Navneet 2010). The extract and essential oil from *P. rigida* wood have remarkable activity against the growth of *A. alternata*, *F. subglutinans*, *C. globosum*, *A. niger*, and *T. viride* (Salem *et al.* 2016a,b).

Fungal spores are linked to different disorders such as allergy, asthma, and various pulmonary illnesses. Fungal decay is a real concern to either the manufacturers or the users of certain grades of paper or paperboard. During paper manufacture, they have a negative economic impact on the pulp produced (Jerusik 2010). Paper and paperboard packaging materials can be infested with mold, especially when used in humid conditions; this impacts the safety of the packaged product (Szczepanowska and Lovett 1992; Alwaeli 2010; Torres *et al.* 2011; Guzińska *et al.* 2012).

Therefore, this study evaluated the effects three powder additives (*P. rigida* wood and the rhizomes of *C. speciosus* and *S. catechu*) on the mechanical and optical properties of papersheets of pulp linen fibers. Furthermore, the decaying patterns of the manufactured papersheets were evaluated using three isolated and molecularly identified molds.

## EXPERIMENTAL

## Materials

#### Fiber material

Linen (*Linum usitatissimum* L., Fam. Linaceae) fibers (Fig. 1) of 50 cm in length and 3 mm in thickness were obtained from Rakta Paper Manufacturing Company (Alexandria, Egypt) and used to produce the pulp.

#### Additives

Three additives—*Pinus rigida* wood (PRW), *Costus speciosus* rhizomes (CSR), and *Senegalia catechu* rhizomes (SCR)—were air-dried at room temperature, ground to powders, and sieved to obtain 80 mesh.



A: PRW; B: SCR; C: CSR

Fig. 1. Morphological photo for linen fibers and the manufactured laboratory papersheets from pulp with three additives.

# Methods

#### Pulp production process

Linen fiber (360 g) was cut into small sizes (3 cm), soaked in water for 24 h, and beaten in mechanical treatment beater (Jokro-Muhle beater) for 2 h. The beating elements and the pulp suspension were maintained at a temperature of  $20 \pm 5$  °C. The speed of the beater was 150 rpm. At the end of beating, the pulp was transferred to a 2-L measuring cylinder. The stock was diluted to 2000 mL and processed in a disintegrator for 2 min at 3000 rpm, and the degree of the Schopper Reigler freeness (°SR) was measured with 50 °SR (T 220 sp01 2001). The pulp production was carried out according to T 200 sp-01 (2001).

The three powdered materials at the amount of 0, 5, 10, and 20 g were added to the

pulp (500 mL of homogenized linen fiber with water) to reach the concentrations of 0, 1, 2, and 4%, respectively. Each treatment was repeated in three replicates.

## Forming of papersheets for physical testing

A measured quantity of diluted pulp suspension containing 12 g of oven-dry pulp was diluted to 10 L. From the well-mixed stock in disintegrator, 1000 mL portions (1.6 g moisture free-pulp) were withdrawn, and each was placed in a handsheet cylinder to make a standard sheet (80 g/m<sup>2</sup>) with area of 200 mm<sup>2</sup>. The wet pulp sheets were pressed for 5 min (first pressing), and the order of sheets was reversed and repressed for 2 min (second pressing) at 50 lb/in<sup>2</sup> pressure. The mirror plates were put on the sheets, which the attached test sheets fitted into a set of drying rings and kept in an atmosphere conditioned at (23 ± 1 °C temperature and 50 ± 2% relative humidity), until an equilibrium moisture was achieved allowing the sheets to become fully dried in position in the ring before removing them from the plates (TAPPI T 402 sp-08 2013).

#### Testing of papersheets

Tensile index (TAPPI T 404 cm-92 1997), burst index (TAPPI T 403 om-97 1997), tear index (TAPPI T 414 om-88), and the brightness (ISO standard 2469 2004) of the manufactured paper sheets were tested.

#### Isolation and identification of fungal strains

Three cultures were swabbed from an old manuscript that showed symptoms of fungi defects at Manuscript library in Cairo (named "Tuhfet Elmulouk fe alferouh". This manuscript is a book on meanings interpretation for the revelations, and it was listed under Public No. 84811 and Special No. 5007 in the stores of Al-Azhar library in Cairo, Egypt), were grown over PDA media.

#### DNA extraction protocol

For DNA extraction, each isolate was grown in potato dextrose broth for 3 to 4 days. The mycelia of each isolate were harvested and processed for genomic DNA extraction using a protocol published by Saitoh *et al.* (2006).

#### Analysis of DNA sequences of partial ITS gene

Three representative isolates of *Aspergillus niger, A. terreus*, and *Fusarium culmorum* were selected for DNA sequence analysis. A partial ITS gene sequence was amplified with primer pairs ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) to form a PCR product of 600 bp. PCR amplifications were performed by a 3 Prime Techne Prime thermal cyclers (Seoul, Korea) in a 25- $\mu$ L volume containing 20 ng of fungal genomic DNA, 0.2  $\mu$ mol/L of each primer, 0.2 mmol/L of each dNTP, 2.0 mmol/L of MgCl<sub>2</sub>, 1× Promega Taq polymerase buffer (10 mmol/L of Tris-HCl, pH 9.0, 50 mmol/L of KCl and 0.1% Triton X-100) and 1.5 units of Taq polymerase (Promega, Madison, WI, USA). The following PCR parameters were used: an initial preheat for 2 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing for 1 min at 55 °C and extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The PCR products were separated on 1% agarose gels pre stained with red safe in Tris-acetate (TAE) buffer and photographed. The PCR products were purified with QIAquick Gel Extraction Kit (Qiagen, USA) and sequenced by Macrogen. Co. Ltd. (Seoul, Korea).

#### In vitro antifungal evaluation

The produced paper sheets with or without bio-additives were subjected to antifungal evaluation against the growth of three molds (*A. niger, A. terreus*, and *F. culmorum*). A 15-day-old PDA culture of each fungus was prepared. Discs of 9 mm in diameter from the produced handsheets were inoculated with each fungus-disc (5 mm diameter) in a Petri dish that contained 15 mL of PDA-culture and then incubated for 14 days at  $25 \pm 1^{\circ}$ C. Three replicates were used for each fungus. The inhibition zones (IZs, mm) around the treated and untreated paper discs against each fungus and the growth of each fungus on paper discs were measured and recorded using recommendations of previously published works (Reinprecht and Kizlink 2007; Rrinprecht 2010; Mansour and Salem 2015; Mansour *et al.* 2015; Taha *et al.* 2019).

#### Scanning electron microscope (SEM) examination of produced papersheets

Scanning electron microscopy was used to show the distribution of the additives intertexture with linen fiber. Discs of the paper sheets with or without pulp bio-additives inoculated with each of the three molds were examined with Scanning Electron Microscope (SEM, JFC-1100E; Ion sputtering device model JSM–5300, JEOL Co., Tokyo, Japan) 8 kV to observe the fungal growth on paper (Salem 2016; Hassan and Mansour 2018).

#### Fourier transform infrared spectroscopy

The three powdered materials were studied for their functional groups using Fourier transform infrared spectroscopy on a (Perkin Elmer- FTIR system- spectrum BX, Waltham, MA 02451 USA) spectrometer at the Institute of Graduate Studies and Research - Alexandria University. All spectra were obtained using the KBr pellet method at a resolution of 4 cm<sup>-1</sup> ranging from 400 to 4000 cm<sup>-1</sup>. Standard Ø13 mm diameter pellets were prepared by mixing and pressing 10 mg of each dried sample in 300 mg of KBr for 5 min under 200 bar pressure. Three parallel measurements were performed. The obtained FTIR spectra were further processed using the Spectrum One software (ver. 5.0.1, Perkin Elmer, Waltham, MA 02451 USA) (Miklečić *et al.* 2012; Salem *et al.* 2016).

#### Statistical analysis

Data for tensile index, burst index, tear index, and brightness were statistically analyzed with two factors (additives and their concentrations) using SAS system software (SAS Institute, Release 8.02, Cary, NC, USA). Comparisons among means were recorded using LSD<sub>0.05</sub>.

# **RESULTS AND DISCUSSION**

#### **Mechanical and Optical Properties of Papersheets**

Table 1 presents the mechanical and optical properties of papersheets made from linen pulp fibers with three additives *Pinus rigida* wood (PRW), *Costus speciosus* rhizomes (CSR), and *Senegalia catechu* rhizomes (SCR).

For mechanical properties, the paper sheets manufactured with PRW additive at 4% and 2% to the pulp showed the best tensile index (TI, N.m/g) with 30.2 and 26.4, respectively, followed by 4% of CSR (25.8 N.m/g), which were higher than those reported from the control (TI, 21.9 N.m/g). The TI was increased by increasing the additive concentration (Nassar *et al.* 2015).

With significant effects (P < 0.0001), the highest values of burst index (BI) were observed from the papersheets manufactured with pulp additive of PRW at 4% (BI, 2.9 kPa.m<sup>2</sup>/g) and 2% (BI, 2.9 kPa.m<sup>2</sup>/g), followed by CSR at 4% (BI 2.84 kPa.m<sup>2</sup>/g), which were higher than those from the control (2 kPa.m<sup>2</sup>/g). These values were higher than those from papersheets made from pulp of alfa (1.3 kPa.m<sup>2</sup>/g), bamboo (2 kPa.m<sup>2</sup>/g), giant reed (0.5 kPa.m<sup>2</sup>/g), and miscanthus (1.2 kPa.m<sup>2</sup>/g), as reported by Marrakchi *et al.* (2011), Anapanurak *et al.* (2009), Shatalov and Pereira (2002), and Barba *et al.* (2002), respectively. However, they were lower than of 4, 5.3, and 4.9 kPa.m<sup>2</sup>/g, as reported from paper sheets manufactured from the pulp of reed cannery (Finell *et al.* 2002), switch grass (Law *et al.* 2001), and Napier grass (Obi Reddy *et al.* 2014), respectively.

For tear index (TI), papersheets produced from pulp with PRW additive at 4%, 2%, and 1% showed the best TI values of 2.5, 2.5, and 2.2 mN.m<sup>2</sup>/g, respectively.

The brightness (%) of the manufactured paper sheets was higher in the control treatment (64.3%), but not significantly different from those manufactured from pulp additives of 1% and 2% of PRW, which observed brightness percentages of 64.1% and 64.2%, respectively. In contrast, the lowest value (62.9%) was reported with the examined paper sheets manufactured from pulp with CSR additive at 4%. These values were higher than those from hand-made papers produced from pulp of [palm midribs (14.1%), wheat straw (37.3%), and *Juniperus procera* wood (18.1%)] (Nasser *et al.* 2015), alfa (47.3%) (Marrakchi *et al.* 2011), bamboo (39.9%) (Anapanurak *et al.* 2009), giant reed (22.8%) (Shatalov and Pereira 2002), and switch grass (30.1%) (Law *et al.* 2001). These values were lower than the value reported from papersheets made from Napier grass (74.6%) (Obi Reddy *et al.* 2014).

The grammage  $(g/m^2)$  of the examined papersheets from all the treatments ranged from 80.8 to 83.8 g/m<sup>2</sup>, which was significantly higher than the control (80.6 g/m<sup>2</sup>).

The PRW powder additive obviously improved the mechanical properties of paper sheets compared with the control sample without any additive. However, the percentage of brightness was decreased by the addition of all additives. Previously, the physical strength properties of paper sheets made from banana stems pulp have been improved with the additive of wheat straw and bagasse prior to pulping up to a level of 5 to 20% (Tripathi *et al.* 2013).

#### Morphology of the Manufactured Papersheets

Figure 2 shows the SEM images of the manufactured paper sheets with or without the pulp bio-additives. As shown in the Fig. 2A, the paper sheet produced from pulp without any additives exhibited the formation appearance, the degree of bonding, and the presence of interlocking among the linen fibrils (Hills 1988). Figures 2B, 2C, and 2D present the good distribution of the additives at 4%, which filling the interspaces among linen fibers. Therefore, the enhancing combination among additives and linen fibers resulted in the increment in the physical properties of handsheets.

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**Fig. 2.** SEM images of the manufactured linen paper without fungal inoculation. A) Linen paper without any additives; B) with 4% PRW; C) with 4% SCR; D) with 4% CSR. Arrows refer to good interferences between the fibers, and the fibers with the additives. The two columns indicates different places in the same sample with the same magnification.

**Table 1.** Effect Additives and their Concentrations on the Mechanical and Optical Properties of Papersheets Made from Linen

 Fibers

		Me	echanical Propertie	Optical Properties	Pagia Waight		
Treatment	Concentration	Tensile Index (N•m/g)	Burst Index (kPa•m²/g)	Tear Index (mN•m²/g)	Brightness (%)	(g/m <sup>2</sup> )	
Control	0%	21.90 ± 0.10	2.04 ± 0.005	1.61 ± 0.005	64.36 ± 0.25	80.61 ± 0.49	
P. rigida	1%	22.23 ± 0.04	2.53 ± 0.05	2.19 ± 0.02	64.10 ± 0.20	80.87 ± 0.49	
wood	2%	26.41 ± 0.02	2.91 ± 0.005	2.52 ± 0.01	64.23 ± 0.31	81.65 ± 0.49	
powder	4%	$30.22 \pm 0.04$	2.92 ± 0.01	2.53 ± 0.02	63.68 ± 0.58	83.32 ± 0.49	
S. catechu	1%	21.68 ± 0.21	$2.23 \pm 0.02$	1.93 ± 0.02	63.10 ± 0.10	80.86 ± 0.49	
rhizomes	2%	21.91 ± 0.04	2.67 ± 0.04	1.91 ± 0.03	63.00 ± 0.10	81.64 ± 0.49	
powder	4%	23.58 ± 0.03	2.82 ± 0.01	$2.03 \pm 0.03$	63.00 ± 0.10	83.81 ± 0.49	
C. specious	1%	22.71 ± 0.06	2.64 ± 0.005	1.81 ± 0.02	63.20 ± 0.10	80.84 ± 0.49	
rhizomes	2%	22.86 ± 0.04	$2.73 \pm 0.02$	1.93 ± 0.02	63.10 ± 0.10	81.63 ± 0.49	
powder	4%	25.84 ± 0.04	2.84 ± 0.02	1.96 ± 0.03	62.90 ± 0.10	83.30 ± 0.49	
P-Value		<0.0001	<0.0001	<0.0001	0.0102	<0.0001	

Values are presented as mean ± SD

## Table 2. DNA Sequences and Organisms Identified by Blast on NCBI

Isolate codes	Length of query NCBI Best Match		Sources of isolate	Accession
	sequences (bp)	(Identified organisms)		number
Ani245	583	Aspergillus niger strain FC24771	Archaeological Manuscripts	MH355955
Ate456	590	Aspergillus terreus strain Y.H. Yeh V0103	Museum archaeological tissue	MH355953
Fcu761	517	Fusarium culmorum strain CBS 128537	Museum organic materials	MH355954

# **Table 3.** Screenings of the Antifungal Activity of Paper Discs Made from Linen Pulp with Three Additives with Different Concentrations

Pulp additive		Aspergillus niger				Fusarium culmorum				Aspergillus terreus			
		Inhibiti	Inhibition zone Growth or		on paper	Inhibition zone		Growth on paper		Inhibition zone		Growth on paper	
		(mm)		(mm)		(mm)		(mm)		(mm)		(mm)	
		7 <sup>th</sup> day	14 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day	7 <sup>th</sup> day	14 <sup>th</sup> day
Contro	bl	0	0	8-9	4-5	0	0	10	10 0 0		0	2-3	8-10
	1%	0	3-4	0	0	0	0	10	10	0- 1	3	0	0
P. rigida	2%	1-2	3-5	0	0	0	0	9-10	10	4- 5	3- 5	0	0
(wood)	4%	2-3	3	0	0	0	0	8-10	10	2-3	3-6	0	0
S.	1%	0	0	0	0	0	0	9-10	10	0-2	0- 1	0	0
catechu	2%	8-9	2	0	0	0	0	6-8	10	0-3	0-2	0	0
(rhizomes)	4%	8-9	0	0	6-8	0	0	7-8	10	0- 5	1- 4	0	0
C.	1%	2-3	0	0	0	0	0	10	10	0	0	4- 8	10
specious	2%	0	0	10	10	0	0	10	10	0	0	1- 6	6-8
(rhizomes)	4%	0	0	3-4	2-4	0	0	10	10	0	0	1-9	8-10

Note: Inhibition zones were recorded without adding the disc diameter. Each value in the table corresponds to the arithmetic mean of three treated discs situated in three Petri dishes (Reinprecht and Kizlink 2007).

#### PCR Amplification and DNA Sequences Results

Polymerase Chain Reaction (PCR) amplification of ITS region of rRNA genes with ITS1 and ITS 4 primers yielded distinct DNA bands for all representative isolates investigated (Fig. 3).



**Fig. 3.** 1% agarose gel electrophoresis of PCR amplicons of the Internal Transcribed Spacer (ITS) region genes of the fungal isolates, Key: Lane M- 100bp DNA marker; Lane 1, 2, and 4 - Isolates from museum archaeological tissue, museum organic materials and archaeological manuscripts; Lane 3-water (negative control)

DNA sequences of the ITS regions of three Aspergillus and fusarium isolates in the GenBank database yielded Aspergillus terreus, A. niger, and Fusarium culmorum from museum archaeological tissue, manuscripts, and organic materials (Table 2). The same strains of Aspergillus were isolated from museum textiles in the museum of Jordanian heritage (Abdel-Kareem 2010). Notable is the predominance of Aspergillus species in museum samples (Abdel-Kareem 2010; Osman et al. 2014). In Abdel-Kareem's study, all of the isolated fungi were identified by morphological characteristics and confirmed by scanning electron microscope. It is noticed that textile collections in some museums are displayed and storied in inappropriate environmental conditions. Most of textiles in museums are natural organic substances. Hence, these textiles are liable to deterioration. The conditions in museums can be very different from standard international regulations concerning the environment required in storage rooms, display showcases, windows, and halls. As in the present results, it is thought that these conditions may encourage the deterioration of textile objects by fungi. Although a few studies have been done for monitoring and controlling of airborne pollutants in the museum of Jordanian heritage (Al-Saad and Khasawneh 2006), to our knowledge, this is the first study on molecular identification of archaeological tissue fungi in Egypt. This is also the first report of F. culmorum from archaeological material from museum sources.

#### Evaluation of the Antifungal Activity of Papersheets

The antifungal activities of paper discs made from linen pulp with different concentrations of additives are presented in Table 3. Compared with control treatment, nearly no growth of *A. terreus* was found in paper discs with 1, 2, and 4% of PRW and SCR with 1 and 2% after 14 days of incubation (Fig. 4a). *F. culmorum* growth covered all the paper discs manufactured with the three pulp additives at all the concentrations, which reached 10 mm. Furthermore, no growth was observed of *A. niger* after 14 days over the paper discs manufactured with the pulp additive of PRW and SCR at 1, 2, and 4%. Paper discs manufactured with pulp additive of CSR did not show any bioactivity against *A. niger*, where the growth on paper discs ranged from 6 to 10 mm.



a) *A. terreus*; b) *F. culmorum*; c) *A. niger* Disc of linen paper manufactured with pulp additive of PRW (1), SCR (2) and CSR (3).

**Fig. 4.** Fungal growth after 14 days from the inoculation and incubated with paper discs manufactured with different three additives. Arrows refer to the inhibition zones around the paper discs.

Figure 5 shows the decay patterns of linen papersheets produced with/without pulp additives and inoculated with *A. terreus*. Figure 5a,b shows the huge growth of *A. terreus*. The hyphae colonization penetrated the fiber bundle, and the mycelium covered the fibers. The fungal growth decreased as the paper manufactured from pulp additive of 1% PRW (Fig. 5c). Figures 4c, 4d, and 4e show growth intensities of *A. terreus* among and over the fiber of linen with different degrees.



**Fig. 5.** SEM images of linen paper manufactured with/without pulp bio-additives and inoculated with *A. terreus.* a,b) Linen paper produced from pulp without any bio-additives; c) with 1% PRW; d) with 1% SCR; e) with 2% CSR. Arrows refer to dense growth of the fungus mycelium in a & b, while growth intensity varied with different proportion of pulp with bio-additives in c, d, and e.

Figure 6 presents the SEM examination of inoculated linen paper with *F*. *culmorum*. Figures 6a,b present the huge growth of *F*. *culmorum* over the fibers as well as the decaying patterns with interference of fungal hyphae with the linen fibers.

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**Fig. 6.** SEM images of linen paper manufactured with/without pulp bio-additives and inoculated with *F. culmorum*. a, b) linen paper without any bio-additives; c) with 1% PRW; d) with 1% SCR; e) with 2% CSR. Arrows refer to dense growth of the fungus mycelium in a and b; while growth intensity varies with different proportion of pulp with bio-additives in c, d and e.

Erosion in linen paper fibers produced without any additives was caused by spores and fungal mycelium of *A. niger* (Fig. 7a,b). Some decreases in mycelial growth were found when the linen paper manufactured with pulp additive of 1% PRW (Fig. 7c).

The results of SEM and fungal infestation experiments showed that pulp treated with the additive of PRW at all the concentrations had positive effects against the growth of *A. terreus* and *A. niger*. Whatman paper, cotton paper, and chemical pulp show different decaying patterns as colonized by *Trichoderma harzianum* and *Paecilomyces variotii* (Hassan and Mansour 2018). Recently, papyrus strips pre-treated with some nanomaterials and natural extracts were enhanced in terms of the technological (mechanical and optical) and antifungal (against *A. flavus*, *A. niger*, and *C. gloeosporioides*) properties of produced papyrus sheets, respectively (Taha *et al.* 2019).



**Fig. 7.** SEM images of linen paper manufactured with/without pulp bio-additives and inoculated with *A. niger.* a, b) linen paper without any pulp bio-additives; c) with 1% PRW; d) with 1% SCR; e) with 2% SCR; f) with 2% CSR; g) with 4% CSR. Note: Arrows refer to dense growth of the fungus mycelium in a and b; while growth intensity varies with different proportion of pulp with bio-additives in c, d, e, f, and g.

#### FTIR spectra of additives

FTIR spectroscopy is an established technique for determining the chemical composition of various biological and chemical samples (Naumann *et al.* 2007). The FTIR spectra of the three bio-additives (PRW, CSR, and SCR) are presented in Fig. 8. The intensities of the functional chemical groups are reported in Table 4. The bands at 3429, 3427, and 3429 cm<sup>-1</sup> in PRW, SCR, and CSR respectively were characteristics of OH stretching in alcohols and phenols of cellulose, lignin, and hemicellulose. Lignin bands with syringyl < guaiacyl were found at 1512 cm<sup>-1</sup> and 1508 cm<sup>-1</sup> in PRW and CSR. For C=C stretching of the aromatic ring, there were lignin and cellulose bands at 877 cm<sup>-1</sup> and 918 cm<sup>-1</sup> in PRW and at 874 cm<sup>-1</sup> and 933 cm<sup>-1</sup> in SCR and at 858 cm<sup>-1</sup>, 930 cm<sup>-1</sup> in CSR, respectively (Shearer 1989; Evans *et al.* 1992; Chen *et al.* 2010; Herrera *et al.* 2014). The band at 1738 cm<sup>-1</sup> in PRW and SCR refers to unconjugated C=O stretching as a shoulder, which is typical for xylan and other hemicellulose (Zhou *et al.* 2015; Gandolfo *et al.* 2016).

Crystallized and amorphous cellulose bands were found at 1437, 1425, and 1423 cm<sup>-1</sup> for CH<sub>2</sub> bending in PRW, SCR, and CSR respectively. Typical bands assigned to cellulose were located at 1162 cm<sup>-1</sup>, 1147 cm<sup>-1</sup>, and 1157 cm<sup>-1</sup> for C-O-C bridge oxygen stretching at PRW, SCR, and CSR, respectively, and assigned to cellulose and hemicellulose at 1111 cm<sup>-1</sup>, 1121 cm<sup>-1</sup>, and 1018 cm<sup>-1</sup> for C-O stretching at PRW, SCR, and CSR, respectively, and at 2929 cm<sup>-1</sup> in CSR for C-H<sub>2</sub> asymmetric stretching (McCann *et al.* 1992; Colom and Carrillo 2005; Labbe *et al.* 2006; Popescu *et al.* 2007; Mohebby 2008; Zhang *et al.* 2010). The FTIR results were consistent with the fact that the additives led to high improvement in the mechanical properties of paper samples, especially the treatment of PRW (Ren *et al.* 2009; Hamzeh *et al.* 2013a,b; Tajik *et al.* 2018).



Fig. 8. FTIR spectra of the studied three powdered bio-additives (see Table 3)

Finally, increasing the amount of bio-additives had a greater positive effect on the strength of the produced papersheets from pulp of linen fibers. Therefore, the utilization of fine powders should be used based on end-product quality requirements and the cost effectiveness of the process. Further studies are recommended for the effect of natural extracts as bioadditives to pulp during the manufacturing process to show their antifungal activity.

No.	Ref	erenced data	*	Pinus	S.	Canadiaua	
	Functional group bands	Frequency range (cm <sup>-1</sup> )	Compound Type	<i>rigida</i> (wood) (cm⁻¹)	<i>catechu</i> (rhizomes) (cm <sup>-1</sup> )	(rhizomes) (cm <sup>-1</sup> )	Assignment**
А	OH stretching	3160 - 3640	Alcohols, Phenols	3429	3427	3429	Cellulose, Lignin and hemicellulose
В	C-H <sub>2</sub> asymmetric stretching	2850 - 3000	Alkanes	2931	2931	2929	Cellulose, Lignin and hemicellulose
С	Unconjugated C=O stretching as a shoulder	1670 - 1760	Carboxylic acids, Esters	1738	1738		Xylan and hemicellulose
D	Conjugated C=O stretching + H-O-H absorption	1500 - 1700	amides, esters	1622	1635	1657	Due to oxidation of cellulose
E	C=C stretching of the aromatic ring	1500 - 1600	Aromatic rings	1512	1508		Lignin (Syringyl < Guaiacyl)
F	CH <sub>2</sub> bending	1290 - 1430	Alkans	1437	1425	1423	Cellulose (crystal & amorphous)
G	C-O-C bridge oxygen stretching	1000 -	Alcohols, Ethers, Carboxylic	1162	1147	1157	Cellulose
	C-O stretching	1200	acids, Esters	1111	1121	1018	Cellulose and hemicellulose
	C-H aromatic		Alkenes.	877	874	858	
Н	bending out of plan + C-H rocking	675 - 1000	Phenyl ring	918	933	930	Lignin and cellulose

Table 4.	FTIR	Data	of the	Studied	Three	Additives
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\* Data according to previous published works (Chow 1971; Ajuong and Breese 1998; Nuopponen *et al.* 2003; Aluong and Redington 2004; Miklečić *et al.* 2012; Sulakshana and Rani 2014; Salem *et al.* 2016a; Traore *et al.* 2018).

\*\* Data according to previous published works (Shearer 1989; Evans *et al.* 1992; Faix and Böttcher 1992; McCann *et al.* 1992; Colom and Carrillo 2005; Labbé *et al.* 2006; Popescu *et al.* 2007; Mohebby 2008; Chen *et al.* 2010; Zhang *et al.* 2010; Herrera *et al.* 2014; Zhou *et al.* 2015; Gandolfo *et al.* 2016).

# CONCLUSIONS

1. Linen fibers paper sheets were manufactured from pulp treated with three powdered additives, *Pinus rigida* wood, *Costus speciosus* rhizomes, and *Senegalia catechu* rhizomes. The mechanical properties (tensile strength, burst resistance, and tear resistance) of paper were enhanced by the addition of powdered material, especially 2 and 4% of *P. rigida* wood.

- 2. The brightness percentages appeared to be decreased with the addition of 1% and 2% of *P. rigida* wood powder, but the differences were not significant compared with the control treatment. The lowest value was observed with *C. speciosus* rhizomes at 4%.
- 3. It was shown for the first time that paper with antifungal tendencies can be prepared with 1 to 4% of natural powder. Therefore, future researchers or entrepreneurs might be able to implement products in the future based on this approach.

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