Study of Mold Invasion on the Surface of Wood/Polypropylene Composites Produced from Aqueous Pretreated Wood Particles, Part 2: *Juniperus procera* Wood-Branch

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Mold invasion by *Trichoderma harzianum* T6776 over the surface of wood-plastic composites (WPCs) made from *Juniperus procera* wood-branch and polypropylene with a melt-blending technique was examined using scanning electron microscopy (SEM) and electron dispersive X-ray spectroscopy (EDX). Before the addition of coupling agent, the WPC samples were made from untreated and pretreated wood-branch particles of *J. procera* with either cold or hot water and then mixed with polypropylene to produce panels. The surfaces of WPC samples were inoculated by a mold suspension of *T. harzianum*. SEM-EDX measurements of WPCs made from *J. procera* particles showed little or no growth of *T. harzianum*, irrespective of treatment with cold or hot water. The results suggest that WPCs made from the particles of *J. procera* wood-branch pretreated with either cold or hot water could be useable in wet conditions. In addition, using of *J. procera* as durable wood for manufacturing of WPCs had good effects on the prevention of the mold infestation over the surfaces of the produced panels.

Keywords: SEM-EDX; Wood-plastic composites; Surface characterization; Juniperus procera; Trichoderma harzianum

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INTRODUCTION

Several biodegrading agents, such as fungi, insects, and marine borers, can attack wood or wood-based products by using them either as a food source (primarily wood) or as shelter (both wood and plastic) (Morrell *et al.* 2010). Molds colonize the surface of wood and wood-based products, but they do not necessarily degrade the product. Wood colonization by molds involves the formation of a dense hyphal network on the surface of wood blocks with enzyme and organic acid release on the inside (de Boer *et al.* 2010; An *et al.* 2016).

Enzymes like cellulase and hemicellulases are produced by different species of *Trichoderma* (Shafique *et al.* 2009). *Trichoderma* species have been reported to degrade cellulose and other carbohydrates and to cause large losses to the wooden frames in

museums (Garg *et al.* 1995; Osono and Takeda 2001). Other strains of *Trichoderma* species have been used for biological wound treatment against wood decay fungi on urban trees (Schubert *et al.* 2008). *T. harzianum* shows a white cottony mycelium with green conidiation over the deteriorated surfaces (Shah *et al.* 2012). Furthermore, *T. harzianum* has been found on the surface of wooden substrates and CCA-treated wood (Kim *et al.* 2007; Ljaljević-Grbić *et al.* 2013). During the storage of sapwood logs of Japanese red pine, *Trichoderma* species were the most frequently isolating fungi, which represented more than half of all isolates (Kim *et al.* 2001).

Wood-plastic composites (WPCs), which first appeared in the markets in the 1930s, are hybrid materials of wood and plastic and have been used as a substitute for wood in decking (Wolcott and Englund 1999; Clemons 2002). WPCs have many applications in our daily lives as a replacement of particleboard for indoor and outdoor uses, especially where particleboards have failed. WPCs also have several industrial purposes, such as wall paneling, automotive manufacturing, construction, the production of furniture and consumer products, and the manufacturing of kitchens and bathrooms (Youngquist 1999; Abu-Sharkh and Hamid 2004; Aref *et al.* 2013; Nasser and Aref 2014). Ibach (2010) reported that the same types of mold that affect solid wood can also develop within WPCs. When wetting conditions are present, the molds can be grown over the WPCs' surfaces. In service, decking with WPCs has shown discoloration decay caused by fungi (Morris and Cooper 1998).

Several studies have focused on the biodegradation of WPCs by wood-degrading fungi including *Trametes versicolor*, *Phanerochaete chrysosporium*, *Pycnoporus sanguineus*, *Fuscoporia ferrea*, *Gloeophyllum trabeum*, *Coniophora puteana*, and *Postia placenta* (Morris and Cooper 1998; Naumann *et al.* 2012; Catto *et al.* 2016). WPCs can be protected against fungal growth by the addition of antifungal agents such as wood preservatives or by using natural products like essential oils (Verhey and Laks 2002; Morton *et al.* 2003; Simonsen *et al.* 2004; Xu *et al.* 2015).

The pretreatment of wood particles (cold or hot water extraction) is primarily used to improve the quality of the produced product in terms of mechanical, physical, and thermal stability properties, where they are used to eliminate the wood extractives that cause some problems during the production of WPCs. For example, Nasser and Aref (2014) investigated the effect of aqueous extraction on the performance and properties of wood/polypropylene composites made from *P. dactylifera*, *Juniperus procera*, *Conocarpus erectus*, and *Tamarix aphylla*. They reported that pretreating the wood particles by either cold or hot water and without adding coupling agents resulted in significant improvements in the compatibility of each wood species with polypropylene. However, the effect of these pretreatments on the fungal resistance of the produced WPCs has not yet been studied.

J. procera, locally known as "Arar", is found in Hejaz and southern region of Saudi Arabia (Migahid 1978). The wood of *J. procera* (African pencil cedar, Cupressaceae family) is widely used for construction building, water flumes, draining boards, food containers, roofing shingles, fence posts, and transmission poles.

The mean annual increment (MAI) of *J. procera* was 678 ton/yr, and the extraction was 1840 tons/yr through its main outlets, also, the fuel wood extracted over the MAI was 1161 tons/yr, from Desa'a forest, Northern Ethiopia, Tigray (Teklay and Gebreslassie 2014). *J. procera*, which is composed of approximately 50% carbon, 6% hydrogen, and 44% oxygen, is a durable source of wood for timber and fuel and is resistant to damage by termites, fungi, and light, which makes it useful for furniture, door

and window frames, flooring and floor parquet tiles, firewood, and baking end use purposes (Pohjonen and Pukkala 1992; Kinyanjui *et al.* 2000). The petroleum ether fraction of the dried ground aerial parts of *J. procera* shows the presence of the diterpenoids 4-epi-abietol, ferruginol, hinokiol, sugiol, *Z*-communic acid, hinokiol-1-one 3*β*,12-dihydroxyabieta-8,11,13-triene-1-one, and the sesquiterpene 8*α*-acetoxyelemol (Alqasoumi and Abdel-Kader 2012). Isocupressic acid, (+)-*Z*-communic acid, (+)-totarol, and sugiol isolated from the bark and leaves extracts of *J. procera* were observed to possess good antimicrobial activities (Muhammad *et al.* 1995, 1996). Bark extracts of *J. procera* have shown the presence of two lignans, namely *β*-peltatin A Me ether and deoxypodophyllotoxin (Muhammad *et al.* 1995). Alkanes, monoterpene alcohols or lactones were identified in the essential oil of *J. procera* from Saudi Arabia (Baghlaf *et al.* 1983).

Generally, the tree of *J. procera* has favorable attributes such as drought tolerance and adaptability (Chaffey 1982). The wood has distinctive qualities such as the hardness of wood and resistance to termites and fungal diseases. Because of these aspects, this work was aimed to evaluate the effects of *J. procera* wood-branch particle pretreatments (hot water [70 °C] and cold-water extractions) on the fungal invasion over the surface of WPCs by *T. harzianum* T6776. The fungal infestation and the changes in surface-elemental composition were measured using an environmental scanning electron microscope (ESEM) with dispersive X-ray spectroscopy (EDX) equipment attached to it.

EXPERIMENTAL

Materials

Wood-plastic composite samples

Branches of *Juniperus procera* (as softwood) were debarked, transferred into particles, ground to particle size of -20/+40 mesh (passed through 20 mesh and retained on 40 mesh) and dried (particles were dried in an oven at 100±5 °C for approximately 24 h). The particles were pretreated with two treatments; the first with water extraction (48-h soaking), and the second with hot water extraction (boiling in water for 6 h while changing water every 2 h); the particles in the control treatment did not have any pretreatments. The polymer type used was polypropylene pellets from SABIC Company, Riyadh, Saudi Arabia). The physical properties of PP are melt index (25.0 g/10 min), the density (0.954 g/cm^3), vicat softening point (153°C), tensile strength at yield (36 MPa), and Shore D hardness (104R). The panels were manufactured without any coupling agent with wood/polymer ratio (50/50 g/g) in a melt-blending technique and then compression mold at temperature (180±5 °C), pressure (4.3 MPa), and time (10 min). The produced WPC panels had a target density of 1.0 g/cm^3 with surface area of 300 x 300 mm and thickness of 10 mm. The WPC panels were conditioned at room temperature (65±5 °C) with a relative humidity of 20±2%. Three WPC panels per each treatment were used.

The wood/polypropylene composites (WPCs) were made from untreated *J. procera* branch without bark and polypropylene without maleic acid modified polypropylene "MAPP" (JP-UN-PP). *J. procera* wood-branch was also pretreated by cold-water extraction and polypropylene without MAPP (JP-CWS-PP). In addition, *J. procera* wood-branch was pretreated by hot water extraction and polypropylene without MAPP (JP-HWE-PP). Hot or cold-water extractions for the particles were used to

enhance the performance and compatibility between the *J. procera* wood-branch and the PP (Nasser and Aref 2014; Nasser *et al.* 2017).

The details of the fungal inoculation of WPCs with *Trichoderma harzianum in vitro*, weight loss and SEM-EDX measurements of the samples after inoculation, and visual observation after 2 and 4 months of inoculation of the manufactured WPCs are shown in a previous part of the work (Nasser *et al.* 2017).

Statistical analysis

The significant differences in weight loss and quantitative elemental results between the un-inoculated and inoculated WPC samples made from *J. procera* wood-branch were analyzed using one-way analysis of variance (ANOVA) (SAS Institute software 2001) with the probability set at 0.05 ($P < 0.05$). The difference between means was tested by least significant difference (LSD) tests.

RESULTS AND DISCUSSION

Weight Loss

Table 1 presents the weight loss (WL) results of WPCs made from *Juniperus procera* wood-branch un-inoculated and inoculated with *Trichoderma harzianum*. As expected for *T. harzianum*, which is not wood decay fungi, no change in weight was observed for the WPCs made from JP-HWE-PP and a positive but small and insignificant weight change was found for the WPCs manufactured from JP-CWS-PP and JP-UN-PP, with respect to the weight of the control. WPCs made from *J. procera* wood-branch showed little or no growth of *T. harzianum* when they were untreated or treated with cold or hot water extraction.

Previous studies showed that fungi could cause WL in WPC with respect to the wood fillers only (Albertsson and Karlsson 1988; Iiyoshi *et al.* 1998; Fabiyi *et al.* 2011).

Table 1. Weight Loss (Based on Wood Fraction) of WPCs with Different Pretreatments of *J. procera* Wood-Branch Following Two Months of Incubation with *T. harzianum*

Material Treatment	Weight loss (%)		
	Mean	SD	SE
Control	0.000	0.00	0.00
JP-UN-PP	1.269	1.80	1.04
JP-CWE-PP	0.119	0.20	0.11
JP-HWE-PP	0.000	0.00	0.00

* Each value represents the average of three replicates.

SEM-EDX Analyses

Inoculated WPCs made from J. procera wood-branch

Table 2 shows the EDX analysis of WPCs made from untreated *J. procera* wood-branch particle and polypropylene (JP-UN-PP), inoculated and un-inoculated with *T. harzianum*.

No significant change was found in the C content between the control (51.88%) and the inoculated (51.51%) WPC made from JP-UN-PP. Moreover, the O content increased by an insignificant amount, from 40.70% in the control to 41.09% in the

inoculated WPC. *T. harzianum*-inoculation of WPC made from JP-UN-PP resulted in a significant increase in Si amount from 0.16% to 0.43%, a significant increase in the S amount from 0.33% to 0.78%, and significant decreases in the elemental composition of Cl from 3.10% to 1.98% (Table 2). The small concentrations of P (0.15%) and Al (0.07%) were completely consumed as the WPC made from JP-UN-PP was inoculated with *T. harzianum*.

Nearly no changes were observed for C and O elements between the control and the inoculated WPC samples made from *J. procera* wood-branch particles and polypropylene, where the particles were pretreated by cold-water extraction (JP-CWE-PP) (Table 2).

Significantly ($P<0.0001$), the Si element peak increased from 0.37% to 1.56%, the Cl amount decreased from 4.09% to 3.15%, the K content from 1.78% to 1.53%, and Ca from 3.33% to 2.58%. A similar trend was found in WPCs made from *J. procera* wood-branch particles that were pretreated by hot water extraction with respect to the changes in elemental compositions of C, O, Na, Mg, S, and Ca.

In comparison with the SEM images for the WPCs controls made from JP-UN-PP (Fig. 1), JP-CWE-PP (Fig. 2), and JP-HWE-PP (Fig. 3), nearly no fungal growth was seen over WPC surfaces of inoculated samples.

SEM-EDX results for untreated and pretreated particles of *J. procera* wood-branch with cold or hot water treatment used for the production of WPCs and exposed to the biodeterioration by *T. harzianum* showed that nearly no changes occurred. These results could be related to the extractives present in the wood, which suppressed the fungal growth. Kinyanjui *et al.* (2000) reported that the extracts from *J. procera* had potential anti-termite properties and that the compound cedrol, which is a tertiary tricyclic alcohol, was found to be in the greatest proportion in the oily layer. Phenolic compounds like 3-methylfuran, 2H-pyran-2-one, 3,4,5-trimethyl-2-cyclopenten-1-one, and 2-methylphenol were detected by GC/MS from the extracts of *J. procera* (Kinyanjui *et al.* 2000). Resin of *J. procera* has been reported to be active against wood colonizing fungi (white heart-rot fungus, *Pyrofomes demidoffii*), and the phytochemical screening revealed the presence of different chemical groups like terpenoids, alkaloids, phenolic compounds, fixed oils, saponins, and flavonoids (Bitew 2015).

The changes in the elemental compositions of the inoculated WPCs compared with those of the control could be related to the fact that the fungal remediation processes *via* organic acid production can remove inorganic metals (Kartal *et al.* 2006). In addition, it was reported by researchers that molds like *T. harzianum* consumed C and N for their growth (Mansour *et al.* 2015a,b; Mansour and Salem 2015).

A recent study by Salem (2016) showed that C content was lower in the inoculated woods of *Pinus rigida, Juglans nigra,* and *Fagus sylvatica* with some molds (*Penicillium selerotigenum*, *Paecilomyces variotii*, and *Aspergillus niger*) than in controls.

Fe was observed in the inoculated WPCs but not shown in the uninoculated WPCs manufactured from untreated and pretreated particles from *J. procera* with cold-water extraction. Koenigs (1974) reported that elemental Fe could be involved in the deterioration of wood, which would result in the oxidative degradation of cellulose by fungi; also, elements such as Na, K, Mg, Ca, and Al in decayed wood could be related to the chemical substrates in wood (Schmidt *et al.* 1981; Tyler 1982).

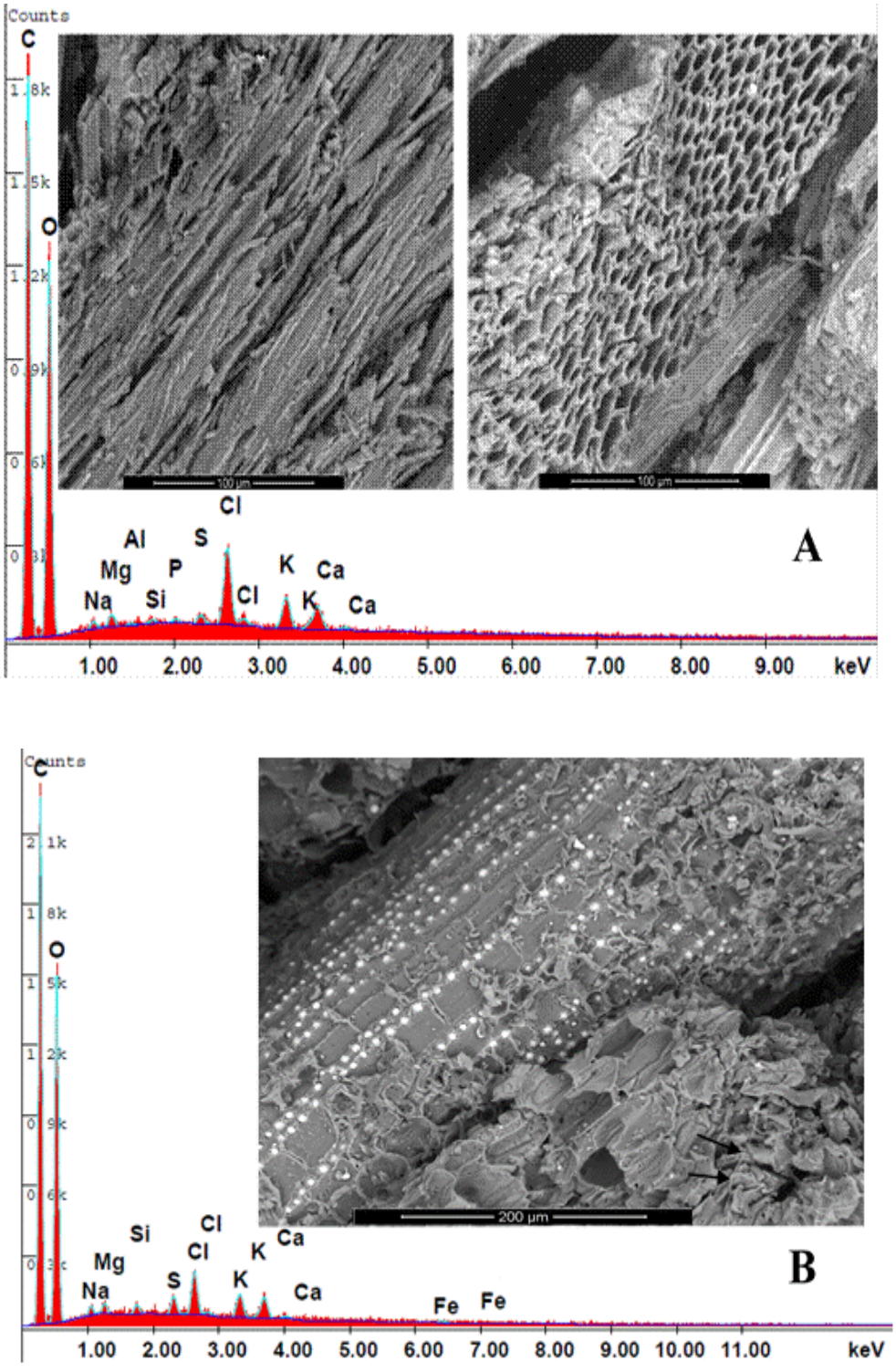


Fig. 1. SEM-EDX graph of WPCs made from untreated *J. procera* wood-branch, (A) Control (uninoculated), (B) (Inoculated (fungal hyphae of *T. harzianum* (arrows) after 2 months)

Table 2. Elemental Analysis of WPCs made from *J. procera* Wood-Branch (JP) and Polypropylene (PP) as Affected by *T. harzianum*

Element (%)	UN-JP/PP		JP-CWE/PP		JP-HWE/PP		$LSD_{0.05}$	R^2	CV %
	Un-inoculated	Incubated	Un-inoculated	Incubated	Un-inoculated	Incubated			
C	51.88±0.09^{bc}	51.51±0.36^{c}	49.65±0.39^{d}	49.68±0.48^{d}	52.18±0.02^{b}	53.02±0.17^{a}	0.542	0.96	0.59
O	40.70±0.26^{ab}	41.09±0.34^{a}	39.46±0.65^{d}	39.90±0.40^{cd}	40.15±0.03^{bc}	39.40±0.12^{d}	0.647	0.81	0.91
Na	0.42±0.02^{c}	0.57±0.03^{b}	0.36±0.06^{c}	0.24±0.12^{d}	0.45±0.01^{c}	0.74±0.04^{a}	0.109	0.90	13.21
Mg	0.45±0.04^{a}	0.51±0.06^{a}	0.30±0.06^{b}	0.45±0.04^{a}	0.35±0.015^{b}	0.48±0.03^{a}	0.083	0.79	10.96
P	0.15±0.01^{c}	0.00^{d}	0.31±0.005^{b}	0.29±0.025^{b}	0.17±0.02^{c}	0.45±0.04^{a}	0.043	0.98	10.45
Si	0.16±0.02^{cd}	0.43±0.07^{b}	0.37±0.13^{bc}	1.56±0.28^{a}	0.00^{d}	0.00^{d}	0.235	0.96	31.37
S	0.33±0.02^{d}	0.78±0.03^{c}	0.31±0.005^{d}	0.24±0.01^{e}	3.13±0.01^{a}	2.49±0.01^{b}	0.031	0.99	1.47
Cl	3.10±0.07^{b}	1.98±0.05^{c}	4.09±0.07^{a}	3.15±0.05^{b}	0.31±0.01^{e}	0.80±0.02^{d}	0.096	0.99	2.43
K	1.42±0.20^{b}	1.42±0.03^{b}	1.78±0.07^{a}	1.53±0.11^{b}	0.00 ^{c}	0.00^{c}	0.178	0.98	9.78
Ca	1.29±0.15^{e}	1.28±0.06^{e}	3.33±0.21^{a}	2.58±0.01^{c}	2.85±0.04^{b}	1.99±0.05^{d}	0.200	0.98	5.06
Fe	0.00^{c}	0.41±0.08^{b}	0.00 ^{c}	0.35±0.01^{b}	0.38±0.03^{b}	0.60±0.01^{a}	0.069	0.97	13.42
Al	0.07±0.02^{a}	0.00^{b}	0.00 ^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.014	0.93	69.98

* According to Fisher's least significant difference test, means with the same letter within the same row are not significantly different at the 0.05 level of probability. Values are the mean of three replicates ± standard deviation.

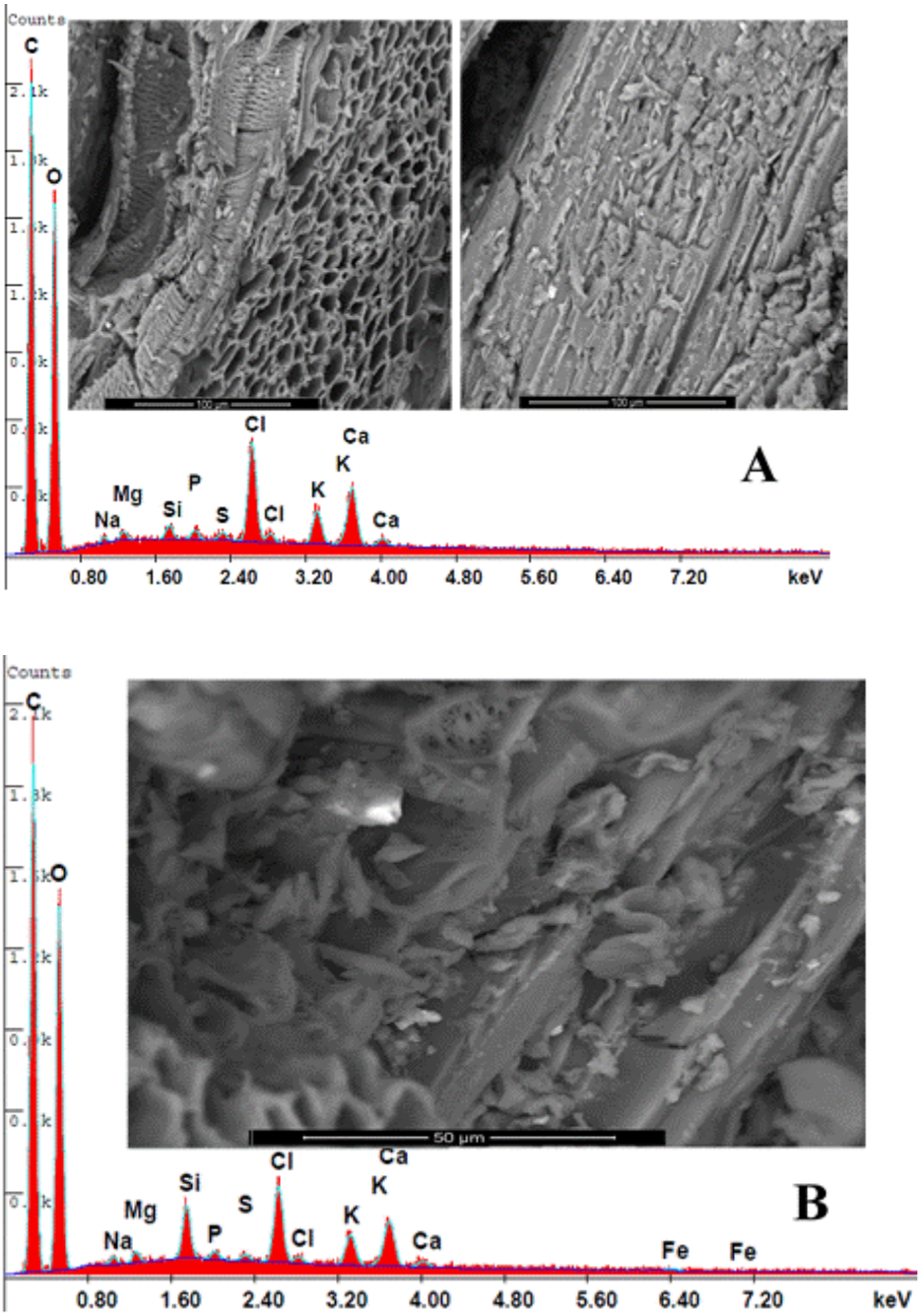


Fig. 2. SEM-EDX graph of WPCs made from pretreated *J. procera* branch with cold-water extraction; (A) Control (uninoculated), (B) (Inoculated with *T. harzianum* after 2 months

Previously, researchers have focused on particle pretreatments that used high temperatures, such as 140, 155, and 170 °C, to show its effects on the mechanical, physical, and thermal properties as well as mold resistance of the manufactured wood-based composites (WPC and particleboards). This high temperature caused degradation of the main chemical components of the wood (hemicellulose) and suggested that water and mold resistance of WPC could be improved (Kim *et al.* 2008, 2009; Hosseinaei *et al.* 2011a,b, 2012). Furthermore, the removal of free sugars, starches, and lipids may explain

the mold resistance of WPC manufactured with hemicellulose extraction at high temperature by hot water extraction (Hosseinaei *et al.* 2012).

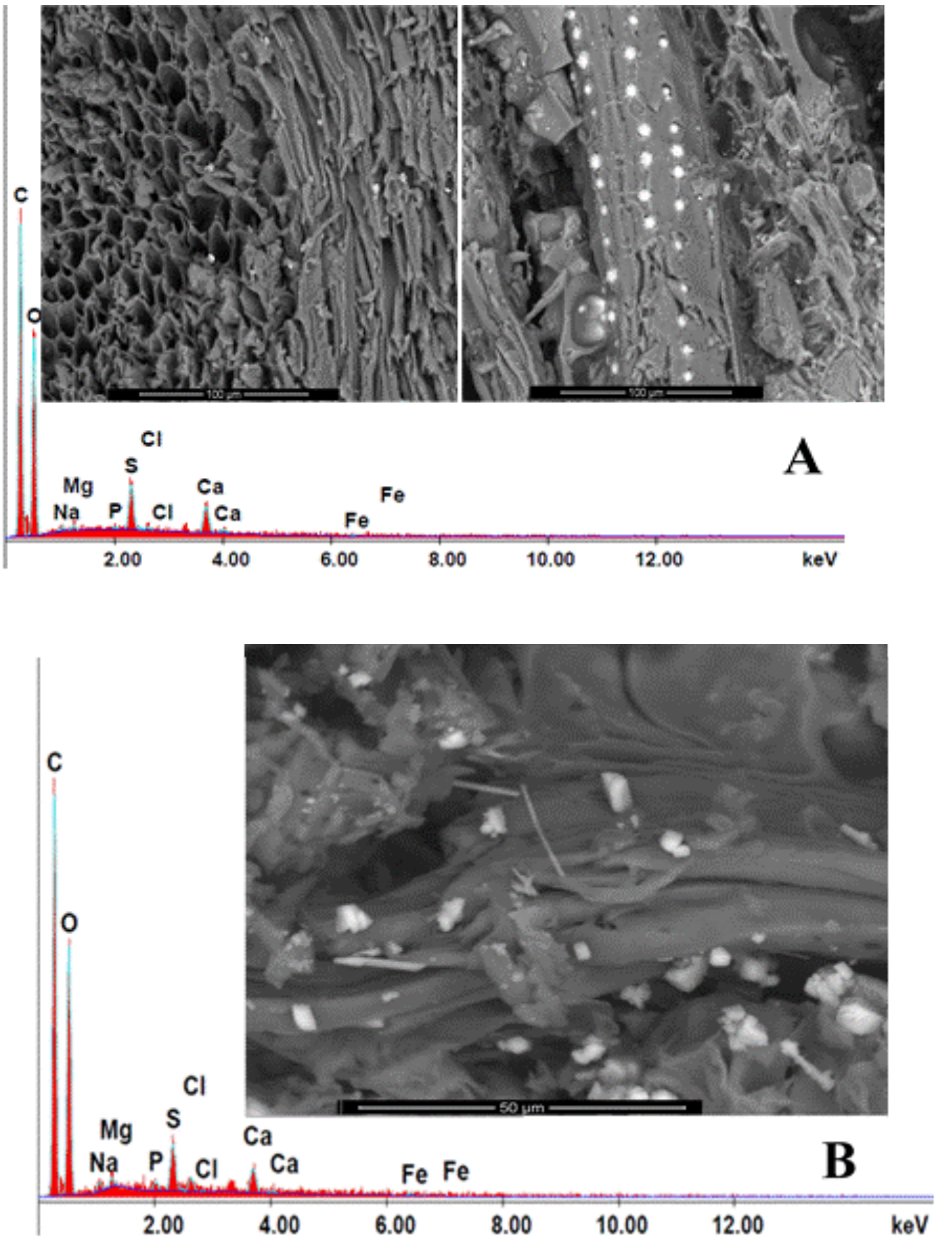


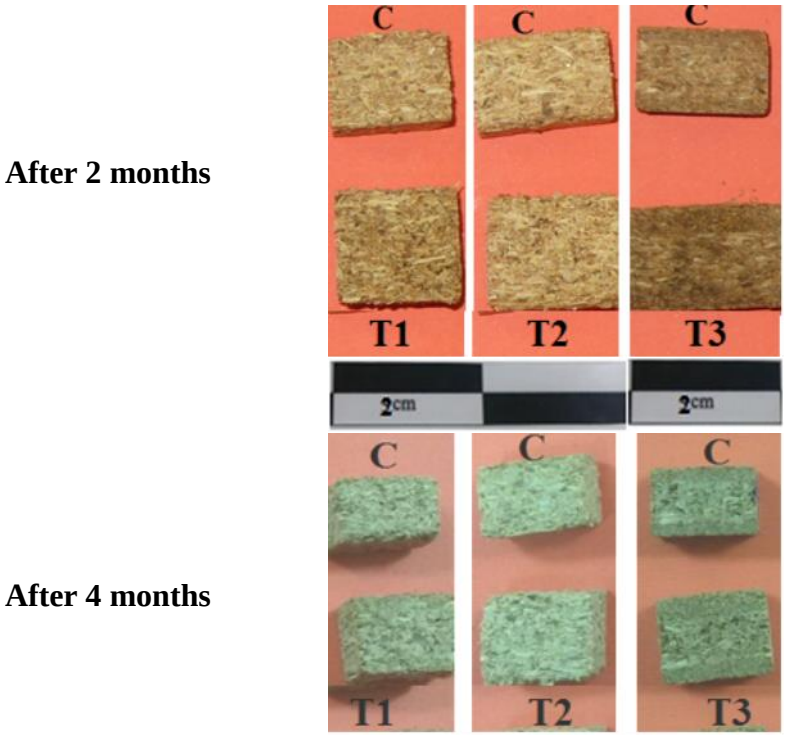
Fig. 3. SEM-EDX graph of WPCs made from pretreated *J. procera* wood-branch with hot water extraction; (A) Control (uninoculated), (B) (Inoculated with *T. harzianum* after 2 months)

Visual Observation of the Incubated WPC Samples

The visual observations of the inoculated WPCs made with *T. harzianum* in comparison with the control were recorded at two months after inoculation and at four months after Petri dish storage at room temperature (Fig. 4).

It can be seen that after two and four months, the WPC samples from *J. procera* wood-branch inoculated by *T. harzianum* did not visually display any growth of *T. harzianum*.

As stated in the literature, the higher plants are a bioresource and produce many different secondary metabolites or natural extracts, which exhibit a broad spectrum of activities against several pathogens (EL-Hefny *et al.* 2017; Hussein *et al.* 2017; Salem *et al.* 2017). These nature extracts are toxic or causes inhibitory to pathogenic fungi, as well as they display the natural resistance of wood (Wagener and Davidson 1954; Cos *et al.* 2006, Mansour *et al.* 2015a,b; Salem 2016; Salem *et al.* 2016 a,b). From these statements, the results of the present work confirmed the natural resistance of *J. procera* wood, where previously, resin had shown activity against wood-colonizing fungi (Martin *et al.* 2002; Bitew 2015). In addition, diterpenoids and sesquiterpene constituents isolated from *J. procera* showed bioactivity against some plant pathogens (Muhammad *et al.* 1995, 1996).



C-Control; T1- JP-UN-PP; T2- JP-CWS-PP; T3- JP-HWE-PP.

Fig. 4. Visual observation of the inoculated WPCs made from *J. procera* wood-branch by *T. harzianum* after two and four months

From the above results, the fungal discoloration must be controlled in the produced WPCs panels, which can be controlled and prevented by rapid kiln drying or by treating of wood surface chemicals as was stated from the literature (Zabel and Morrell 1992; Verhey and Laks 2002; Morton *et al.* 2003; Simonsen *et al.* 2004; Xu *et al.* 2015). Therefore, our results observed that the using of durable wood species like *J. procera* in the production of WPCs had good effects on the prevention of the mold infestation over the surfaces of the produced panels.

CONCLUSIONS

1. SEM-EDX images of WPCs made from the pretreated *J. procera* wood branch particles with either cold or hot water extracted WPCs showed little or no growth of *T. harzianum*.
2. The production of WPCs from a durable wood species (*J. procera*) had good effects on the suppression of fungal growth of *T. harzianum*, where the SEM-EDX measurements showed nearly no changes in elemental composition as well as fungal infestation.
3. From the present results and the data related to the durability of *J. procera* wood, it is suggested to manufacture WPCs from this wood when wet conditions are expected. This way, mold growth could be suppressed.

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