

Chemical Composition and Resistance to Decay of Thermally Modified Wood from *Casuarina glauca* and *Eucalyptus camaldulensis* Grown in Egypt

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ABSTRACT

In Egypt, *Casuarina glauca* and *Eucalyptus camaldulensis* are main common fast growing hardwood species in plantations, which have a high potential for use in this country. In this study, thermal treatment process was used to improve the durability of these locally produced wood species as an effective mean in wood preservation. In this context, wood specimens were exposed to heat under inert atmosphere in laboratory conditions at temperature of 240°C with different durations to reach mass losses of 5, 10 and 15%. Fungal decay tests were conducted using the brown rot fungus *Poria placenta* and the white rot fungus *Coriolus versicolor*. The chemical composition of the wood and the elemental composition as well as the extractive toxicity before and after thermal modification of the wood were determined to understand the reasons for the improvement of the durability of the wood. Heat-modified wood specimens showed a significant increase in their durability against wood decomposition depending on the severity of the treatment. Wood holocellulose was found to be distinctly more sensitive to the heating process than lignin constituent did. In addition, the weight loss was increase by fungal decay and holocellulose was decreased however, the lignin ratio was increased. The results obtained in this work may provide valuable information as a way towards the use of heat treatment technology in wood preservation in Egypt.

Keywords: *Casuarina glauca*; *Eucalyptus camaldulensis*; Chemical composition; Wood protection; Decay; Heat treatment.

INTRODUCTION

Egypt has virtually no natural forests and almost all the substantive forest is plantation implying *Casuarina* spp. and *Eucalyptus* spp. most common fast growing species. *Casuarina glauca*, an agro-forest tree species with low durability, is widely used by the local people for handles, fences, rafters, shingles, stakes, small sea-water piles, for flooring and turnery (Abdel-Aal, 2014 and Nasser 2014). Meanwhile, *Eucalyptus*

camaldulensis is another fast-growing tree species widely used as wind breaks in the country. It may have importance in some usages, such as pulp, fiber, chip industries and packing box production. Despite many advantages, *Eucalyptus* wood has some disadvantages, such as high swelling, low dimensional stability, and several drying problems, which limiting its use. The climatic conditions of Egypt are favorable for many wood degrading organisms. Therefore, it is essential to protect wood and its products to prolong its service life. In respect to the environment, improvement of the durability for this particular species would be a great step in elaboration of usage for these locally growing wood species. Heat treatment is one of the processes used to modify the properties of wood (Militz 2002; Hakkou *et al.*, 2006 and Windeisen, *et al.*, 2007). During the heat treatment process, hemicelluloses are degraded, lignin auto-condenses, and crosslinks with polysaccharides are created (Tjeerdma *et al.* 1998; Hakkou *et al.* 2006 and Mohareb *et al.* 2011). Because of this modification method, several wood quality properties such as dimensional stability and resistance to bio-deterioration are expected to improve. In general, the temperature and duration for heat treatment vary from 180 to 280°C for 15 min to 24h depending on the heat treatment process, wood species, sample size, moisture content of the sample, the desired mechanical properties, resistance to biological attack and dimensional stability of the final product (Hakkou *et al.*, 2005 and Mouras *et al.*, 2002). In this context, the literatures pointed out that temperature has a greater influence than time on many properties including the biological resistance against fungal decay (Brito *et al.*, 2008 and Mohareb *et al.*, 2010). In addition, the change in properties is mainly caused by thermic degradation of hemicelluloses. Theoretically, the available OH groups in hemicellulose have the most significant effect on the physical properties of wood. Heat treatment lowers

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water uptake and wood cell wall absorbs less water because of the decrease of the amount of wood's hydroxyl groups. As a consequence of the reduced number of hydroxyl groups, the swelling and shrinking will be lower (Korkut *et al.*, 2008). The aims of this study were to improve the decay resistance of that rapid growth Egyptian wood species (*C. gluca* and *E. camaldulensis*) using thermal treatment technique and to determine its chemical composition before and after the heat modification process.

MATERIALS AND METHODS

1. Heat treatment

Heat treatment was carried out on the sapwood of *C. gluca* and *E. camaldulensis* obtained from nine-year-old trees grown in the northwest coast of Egypt (Amria city) in the summer of 2014. Three sound discs (about 5 cm in thickness) were taken from each tree species at stem base. In order to prevent dryness, the discs were placed in plastic bags and transported to the laboratory of wood technology, Forestry and Wood Technology department, Faculty of Agriculture, Alexandria University. Wood discs were stored in freezing temperature until using for further processing. Blocks ($10 \times 20 \times 50 \text{ mm}^3$, the latter dimension corresponding to longitudinal direction) were oven-dried at 103°C until mass stabilization point then heated at 240°C under nitrogen for different times to reach mass losses, generated by the thermo-degradation of 5, 10 and 15%. The oven temperature was increased by $20^\circ\text{C min}^{-1}$ from ambient to final temperature. Mass loss due to chemical degradation during heat treatment was calculated according to the formula:

$$\text{ML (\%)} = 100 \times (m_0 - m_1)/m_0$$

Where, m_0 is the initial anhydrous mass of the sample before heat treatment and m_1 is the anhydrous mass of the same sample after heat treatment.

2. Determination of wood chemical composition

Heat treated and control wood samples were ground and the resulting sawdust was passed through a 40-mesh screen. The sawdust was then Soxhlet extracted with toluene/ethanol (2/1, v/v) mixture (8 hours) and ethanol (8 hours) and dried at 103°C for 48 hours. After solvent evaporation, the extract content was directly determined. A 0.5 g (m_1) of sawdust was mixed with 72% H_2SO_4 (10 ml) for 4 hours at room temperature. The mixture was then diluted with 240 ml of distilled water, heated under reflux for 4 hours and filtered. The residue was washed with hot water and dried at 103°C to a constant mass (m_2). The lignin content was calculated as percentage of residues based on oven dry wood meal weight using the following formula:

$$\text{Lignin (\%)} = 100 \times (m_2/m_1)$$

The complement percentage represents the holocellulose content.

3. Microanalysis

Wood was ground to fine sawdust and passed through different sieves to obtain a powder of size ranged between 0.2 and 0.5 mm. Sawdust was oven dried at 103°C for 48 h and stored in closed bottles before analysis. Elemental analyses were performed on a Thermofinnigan Flash EA1112 microanalyzer. The results obtained are the percentages of carbon, hydrogen and oxygen. From these values, the oxygen/carbon ratio was calculated.

4. Fungal decay

Heat treated and untreated *C. gluca* and *E. camaldulensis* sapwood blocks were cut in specimens of $\times 10 \times 25 \text{ mm}^3$ for fungal durability evaluations and dried at 103°C to constant weight before all decay tests. Petri dishes (9 cm diameter) were filled with sterile medium (20 ml) prepared from malt (40 g), agar (20 g) in distilled water (1 L) and inoculated with a piece of mycelium of a freshly grown brown rot fungus *Poria placenta* and white rot fungus *Coriolus versicolor*. Petri dishes were incubated at 22°C and 70% relative humidity (RH) until full colonization of the surface's medium by the fungal mycelium. Two heat-treated specimens and one untreated sample were placed in each Petri dish and subjected to fungal decay for 12 weeks. Each experiment was triplicated. At the end of the test period, the mycelia were removed and the blocks were dried at 103°C to mass stabilization point and eventually weighed (m_2) to determine the mass loss (ML) caused by the fungal decay:

$$\text{ML (\%)} = 100 \times (m_1 - m_2)/m_1$$

Where m_1 is the initial anhydrous mass of untreated and heat treated wood blocks before fungal exposure and m_2 is the anhydrous mass after fungal attack.

5. Biological efficacy of wood extracts

Mycelium grown in 9 cm Petri dishes, filled with 20 ml of malt-agar medium prepared as above mentioned containing 50, 100, 250, 500 or 1000 ppm of toluene/ethanol extracts. Addition of the extracts was carried out after medium sterilization by addition of the necessary quantity of extract solubilized in the minimum amount of 70% ethanol. Plates inoculated in their center with a small portion of *P. placenta* and *C. versicolor* grown on malt agar. The dishes were kept at 22°C and 70% RH. Growth inhibition was determined when the diameter of the control culture reached 9 cm by measuring the diameter of the colony estimated from the mean of three diameters and calculated according to the following formula:

$$\text{Growth inhibition (\%)} = 100 \times (1 - d_1/d_0)$$

Where, d_0 is the diameter of the control culture and d_1 is the diameter of the culture in the presence of extracts. EC_{50} (the concentration caused 50% reduction in a hyphal growth) with corresponding 95% confidence limits were estimated by probit analysis (Finney, 1971).

6. Statistical analysis

Statistical analysis was performed by using SPSS 21.0 software program (Statistical Package for Social Sciences, USA). Mean values and standard deviations were calculated for all tests. Mean separations were performed by Student-Newman-Keuls (SNK) test in one-way analysis of variance (ANOVA) and differences at $P < 0.05$ were considered as significant. The concentration in mg/L inhibited 50% of fungal mycelial growth (EC_{50}) and its corresponding 95% confidence limits were calculated by probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

1. Heat treatment

Figure 1 shows the calibration curves of mass losses (%) for *C. gluca* and *E. camaldulensis* after heat treatment at 240°C for 24 hours. These curves were developed as an indirect method to predict wood mass loss by heat treatment for test wood species. It is obvious that the mass loss in both treated wood species was increased with treatment time. The degradation was noted to be important during the first stage of thermal treatment and becomes less important in the second

stage, in which the mass loss become more stable with the increasing of heat treatment time. It is obvious that the heat-treated *E. camaldulensis* samples were significantly affected and reached to their second stage before the heat-treated of *C. gluca*. This phenomenon could be attributed to the differences in wood density and its chemical composition mainly of the highest extractive contents in *E. camaldulensis* when compared with *C. gluca* (Tables 1 and 2). In addition, the resulting mass loss due to heat treatment is an important parameter in the thermal modification of wood because it has a direct relationship with the properties of the resulting wood after thermal modification (Viitaniemi *et al.*, 1997; Kamdem *et al.*, 2002 and Wang and Cooper 2005). In general, the evolution of the loss of mass with respect to time differs from the wood species to the other according to various factors, such as chemical and physical characters.

2. Wood chemical composition and microanalysis

The results of chemical compositions and microanalysis of untreated and heat-treated *C. glauca* and *E. camaldulensis* wood are given in Tables 1 and 2, respectively. It can be noted that the percentages of lignin content increased gradually with the severity of heat treatment with an observed decrease in holocellulose contents.

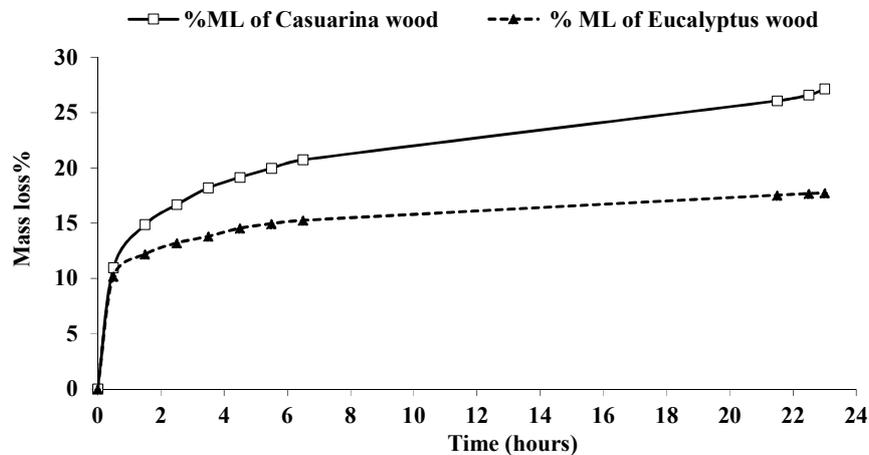


Figure 1. Mass loss (%) of *C. gluca* and *E. camaldulensis* after heat treatment at 240°C for 24 hours

Table 1. Chemical compositions and microanalysis of untreated and heat-treated wood samples of *C. glauca*

Mass loss due to heat treatment (%)	Chemical composition (%)			Microanalysis (%)			
	Lignin	Holocellulose	extracts	C	H	O	O/C
Control	29.1	64.9	6.0	46.74	5.8	47.46	0.76
6.12	29.2	61.9	8.9	49.04	5.76	45.2	0.69
9.52	32.8	58.4	8.8	50.26	5.76	43.98	0.66
14.64	35.4	57.8	6.8	50.4	5.65	43.94	0.65

Table 2. Chemical compositions and microanalysis of untreated and heat-treated wood samples of *Eucalyptus camaldulensis*

Mass loss due to heat treatment (%)	Chemical composition (%)			Microanalysis (%)			
	Lignin	Holocellulose	Extracts	C	H	O	O/C
Control	34.3	55.9	9.8	51.25	5.16	43.6	0.64
4.84	39.4	50.2	10.4	52.44	4.96	42.61	0.61
11.22	41.9	49.3	8.8	52.9	4.7	42.41	0.60
16.58	44.8	48.6	6.6	56.1	5.09	38.82	0.52

The highest influence of the heat treatment on holocellulose could be attributed to the loss of hemicellulose or fragile pentoses and hexoses during the heat treatment (Kamdem *et al.*, 2002). For this reason, lignin content of heat-treated wood was higher than that of the untreated control in both tested wood species. Consequently, the observed increase in lignin ratio does not imply the formation of lignin during the heat modification process but the reduction of other wood constituents. In this regard, the data obtained in this work emphasized that the holocellulose content in the heat-treated wood samples are more susceptible to the thermal degradation when compared with the lignin. These results are in agreement with those obtained by Chaouch *et al.*, (2010) and Mohareb *et al.*, (2011). Furthermore, a slight increase was recorded in the extract contents for both examined wood species. This can be attributed to the generated extractives during wood thermo-degradation (Hakkou *et al.*, 2006). In addition, the microanalysis results showed a gradual increase in carbon content with a remarked decrease in oxygen content in both thermally modified wood species. These results are similar to those obtained by Chaouch *et al.*, (2010), who reported that the oxygen decreased to the dehydration reactions during wood thermal degradation. Consequently, the estimated O/C ratios were decreased by heat treatment intensity. These results are in accordance with Šušteršič *et al.*, (2010), who found a good correlation among heat treatment, mass losses, fungal decay, weight loss and elemental composition allowing utilization of lignin content and O/C ratio as a possible marker for prediction the conferred decay durability after thermal modification.

2. Wood chemical composition and microanalysis

The results of chemical compositions and microanalysis of untreated and heat-treated *C. glauca* and *E. camaldulensis* wood are given in Tables 1 and 2, respectively. It can be noted that the percentages of lignin content increased gradually with the severity of heat treatment with an observed decrease in holocellulose contents. The highest influence of the heat treatment on holocellulose could be attributed to the loss of hemicellulose or fragile pentoses and hexoses during the heat treatment (Kamdem *et al.*, 2002). For this reason, lignin content of heat-treated wood was higher

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3. Fungal decay

Mass losses (%) of *C. glauca* and *E. camaldulensis* blocks with heat treatment after fungal decay using *P. placenta* and *C. versicolor* fungi were given in Table 3. Significant improvements have been achieved in both wood species against brown rot fungus *P. placenta* and white rot fungus *C. versicolor* due to chemical modifications after heat treatment. The mass loss resulted from *P. placenta* and *C. versicolor* decaying of untreated *C. glauca* and *E. camaldulensis* were 37.4%, 22.9%, 32.3% and 19.7%, respectively. Meanwhile, the mass loss of the heat-treated specimens at the highest thermal intensity level in *C. glauca* and *E. camaldulensis* were 4.9% and 3.6% for *P. placenta* decaying, respectively. While the recorded mass loss with *C. versicolor* were 3.9% and 1.5% for *C. glauca* and *E. camaldulensis*, respectively.

Table 3. Mass losses (%) of *C. glauca* and *E. camaldulensis* blocks heat treated after fungal decay using *Poria placenta* and *Coriolus versicolor* fungus

Heat treatment (%)	<i>C. glauca</i>		Heat treatment (%)	<i>E. camaldulensis</i>	
	<i>P. placenta</i>	<i>C. versicolor</i>		<i>P. placenta</i>	<i>C. versicolor</i>
Control	37.4 ^a ±1.92	32.30 ^a ±1.09	Control	22.9 ^a ±0.62	19.70 ^a ±1.22
6.12	21.90 ^b ±1.08	24.50 ^b ±0.94	4.84	12.70 ^b ±1.01	11.60 ^b ±0.85
9.52	12.10 ^c ±0.92	9.70 ^c ±0.78	11.22	5.60 ^b ±0.49	5.90 ^c ±0.94
14.64	4.90 ^d ±0.81	3.90 ^d ±0.43	16.58	3.60 ^c ±0.54	1.50 ^d ±0.36

Values are average of three replicates and values in parentheses are standard deviations. Different letters in the same column indicate significant differences according to the Student-Newman-Keuls (SNK) test ($P \leq 0.05$).

These results indicated an significant enhancement in the durability of the thermally modified *C. glauca* and *E. camaldulensis* wood. It was reported that the improvement of the biological performance for the thermo-modified wood mainly attributed to the degradation of carbohydrates (Welzbacher and Rapp 2007; Willems *et al.*, 2013 and Altgen *et al.*, 2016). This means that of less amorphous cellulose and hemicelluloses are available to adsorb moisture, which is a limiting factor for the growth of fungi and its decomposition. The correlations between weight losses resulting from fungal degradation and lignin percentages, carbon contents and O/C ratio for the heat-treated *C. glauca* and *E. camaldulensis* wood are illustrated in Figures 2 and 3, respectively. Interestingly, a good correlation was observed between carbon content, lignin proportion and O/C ratio in both investigated wood species. The findings emphasis that chemical constituents of wood tested could be used as valuable indicators for the conferred wood durability after heat treatment. These obtained data are similar to those described by Esteves and Pereira (2009), Calonego *et al.*, (2010) and Mohareb *et al.*, (2011).

They stated that the chemical compositions of wood are correlated to the occurred mass loss due to thermal degradation reactions, which in the meantime are directly linked to weight losses resulting from fungal attack.

4. Biological efficacy of wood extracts

To investigate the role of the generated extracts in *C. glauca* and *E. camaldulensis* during heat treatment, an *in vitro* bioassay test was conducted on the brown rot *P. placenta* fungus using mycelia radial growth inhibition technique (Figure 4). The results recorded in Table 4 showed that the extract of *C. glauca* wood after heat treatment could contribute in the gained decay resistance. The calculated EC₅₀ value decreased from 73.05 mg/L in the control samples to 58.47 mg/L with the extract obtained from the thermo-modified wood at 15% mass loss. Based on this finding, the conferred durability in *C. glauca* after heat treatment seems to be an outcome for modification of its hydrocarbons chemical composition and the extracts after heat treatment.

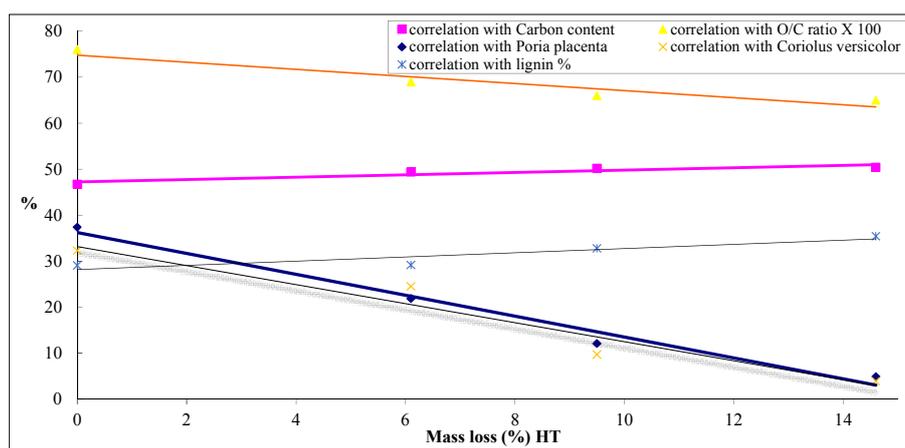


Figure 2. Correlation between mass losses resulting from heat treatment and wood chemical compositions for the heat-treated *C. glauca* wood

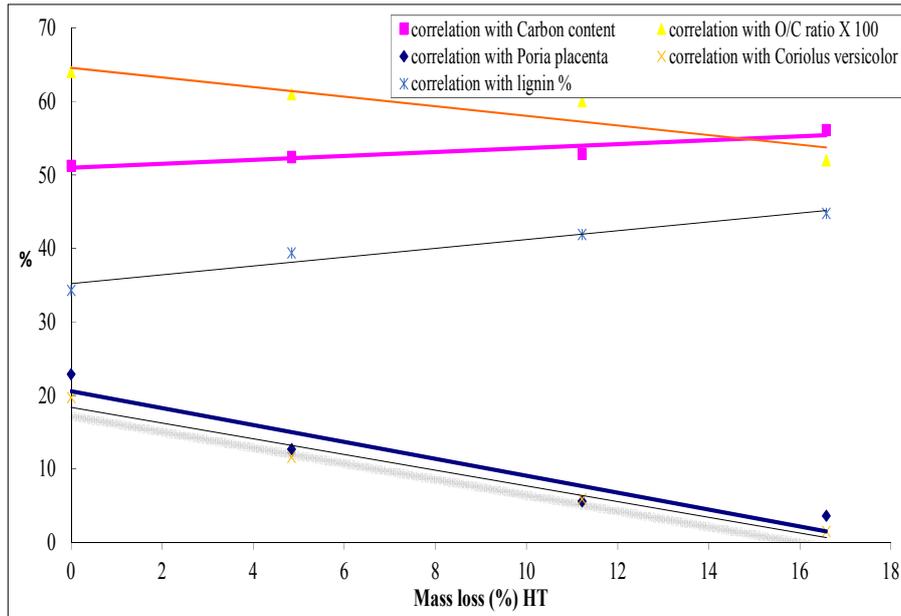


Figure 3. Correlation between weight losses resulting from fungal decay and wood chemical compositions for the heat-treated *E. camaldulensis* wood

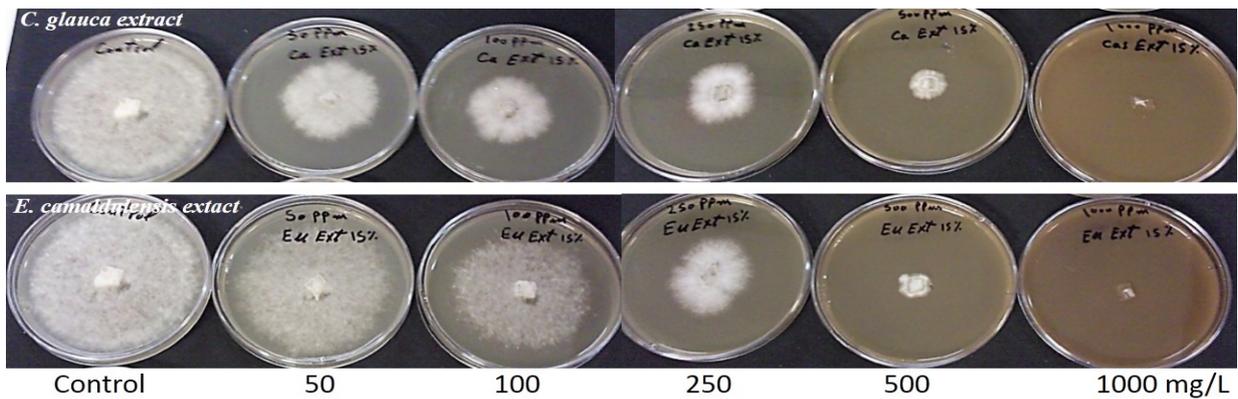


Figure 4. The *in vitro* antifungal activity of *C. glauca* and *E. camaldulensis* extracts from heat treated wood at 15% weight loss against *Poria placenta* using mycelia radial growth technique

Table 4. The *in vitro* antifungal activity of *C. glauca* and *E. camaldulensis* extracts from control and heat-treated wood at 15% Mass loss against *P. placenta* by using mycelia radial growth inhibition technique

Wood extracts	EC ₅₀ ^a (mg/L)	95% confidence limits (mg/L)		Slope ^b ± SE	Intercept ^c ± SE	(χ ²) ^d
		Lower	Upper			
Casuarina extract (control)	73.05	40.10	105.18	2.31±0.22	-4.31±0.35	6.98
Eucalyptus extract (control)	50.41	40.22	59.41	2.72±0.34	-4.63±0.65	1.99
Casuarina extract (15% ML)	58.47	42.79	73.23	1.70±0.21	-3.00±0.44	0.159
Eucalyptus extract (15% ML)	105.74	13.36	253.10	2.19±0.18	-4.43±0.40	28.77

^a The concentration causing 50% mycelial growth inhibition.

^b Slope of the concentration-inhibition regression line ± standard error.

^c Intercept of the regression line ± standard error.

^d Chi square value.

Conversely, the generated extracts of *E. camaldulensis* wood during thermal treatments seem to have no significant effect on wood durability, since the EC₅₀ value increased from 50.41 mg/L in the control samples to 105.74 mg/L with the extract of the thermal modified wood at 15% mass loss. Accordingly, the achieved decay resistance in thermally modified *E. camaldulensis* wood is mainly attributed to the occurred alteration in its cell wall chemical composition. In this context, several published results indicated that the extracts generated during heat treatment may improve wood durability (Peters *et al.*, 2009) while the other stated that the extractives have no effect on wood durability after heat treatment (Hakkou *et al.*, 2006).

CONCLUSION

The influences of heat treatment on the chemical composition and the durability of *C. glauca* and *E. camaldulensis* woods were investigated in the current study. The results showed a significant alteration in chemical compositions of the wood after heat treatment. Important reductions were noted with the holocellulose contents while significant increases were recorded with lignin proportions in the treated wood. The thermally modified local wood *C. glauca* and *E. camaldulensis* were tested against both brown and white rot fungi *P. placenta* and *C. versicolor*, respectively. A great enhancement was observed in wood durability for both examined wood species against fungal decay after heat treatment. Also, the results pointed out a good correlation between lignin content, O/C ratio and the mass loss resulting from fungal attack, which paving the way for using of these parameters as possible markers for wood durability after heat treatment. The results indicate that the extracts from *C. glauca* after heat treatment play an additional role in the resistance of wood decay resistance besides the modification of its cell wall chemical compositions after heat treatment. On the contrary, *E. camaldulensis* extracts have no effect after heat treatment on its durability and the main cause for wood decaying resistance seems to be the modification of its cell wall with chemical constituents. Finally, the data gathered in this work draws attention to the heat treatment technology and the possibility of using this process for wood preservation in Egypt as a valuable alternative to toxic chemical treatments.

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الملخص العربي

التركيب الكيميائي والمقاومة للأعفان الفطرية لأخشاب الكازوارينا البيضاء والكافور البلدي النامية في مصر والمعالجة حرارياً

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وكذلك نسبة الأكسجين/كربون المحسوبة عند تلك المستويات مما يجعل من تلك المكونات الكيميائية مؤشرات معتبرة لمستوى المقاومة المتحقق بالخشب نتيجة المعاملة بالحرارة. ومن ناحية أخرى فقد أوضحت النتائج في هذه الدراسة أن المستخلصات الناتجة في خشب الكازوارينا أثناء عملية التحلل الحراري (نتيجة المعاملة بالحرارة) تلعب دوراً إضافياً في زيادة المقاومة للفطريات بجانب التحوير الذي يحدث في المركبات الكيميائية الرئيسية بالخشب (الهالوسليلوز واللجنين) نتيجة المعالجة الكيميائية. وعلى العكس من ذلك فقد وجدت الدراسة أن المستخلصات الناتجة بالخشب عند المعالجة الحرارية بخشب الكافور ليس لها دور في زيادة المقاومة للفطريات وإنما تكون المقاومة للفطريات بخشب الكافور بعد المعالجة الحرارية ناشئة من التحوير الذي يحدث في المركبات الكيميائية الرئيسية في الخشب (الهالوسليلوز واللجنين) فقط مما يعكس تباين تأثير المعالجة الحرارية على المستخلصات باختلاف نوع الخشب. وبناء على نتائج هذا البحث فيوصى باستخدام تكنولوجيا المعالجات الحرارية للخشب في مصر بحيث تكون بديل هام لمعاملة الأخشاب بغرض حفظها من التحلل الفطري بدلاً من استخدام المواد الكيميائية السامة في هذا الغرض وإيضاً للأثر الإيجابي على البيئة المحلية نتيجة التقليل من استخدام المواد الكيميائية الحافظة للأخشاب واستبدالها بتلك الطرق المعتمدة على الحرارة.

هذا البحث يتناول دراسة تأثيرات المعالجة الحرارية على التركيب الكيميائي والمقاومة للأعفان الفطرية لأخشاب الكازوارينا البيضاء والكافور البلدي المتحصل عليها من أشجار محلية نامية في مصر. وقد أوضحت النتائج حدوث تغيير جوهري في تركيب المكونات الكيميائية الرئيسية بالخشب بعد المعاملة بالحرارة في وسط خامل حيث وجد إنخفاض معنوي في كمية الهالوسليلوز الموجودة بالخشب في حين زادت نسبة اللجنين في الأخشاب المعالجة حرارياً عند مقارنتها بالعينات الكنترول في كلا نوعي الخشب تحت الدراسة وقد تم أيضاً مقارنة مقاومة تلك الأخشاب المعاملة بالحرارة تحت مستويات مختلفة من المعالجة الحرارية ضد نوعين فطريين ينتميان إلى الأعفان الفطرية البنية والبيضاء على التوالي وقد وجد ارتفاع كبير في مقاومة تلك الأخشاب المعاملة بالحرارة ضد الفطريات المحللة للخشب عند مقارنتها بالأخشاب غير المعالجة (الكنترول) وذلك لإنخفاض كمية الكربوهيدرات المتاحة لامتصاص الرطوبة (الهالوسليلوز) نتيجة التحلل الحراري والمعروف أن الرطوبة هي العامل المحدد لنمو الفطريات وإحداث أثرها المتمثل في تحلل الخشب في حين تزداد نسبة المواد الفينولية الذي يمثلها الجنين بالخشب. وكذلك أوضحت النتائج وجود ارتباط ملحوظ بين المقاومة المتحصل عليها في الاخشاب ضد الأعفان الفطرية وبين نسبة اللجنين المقدرة عند كل مستوى من مستويات المعالجة الحرارية