

Resistance of *Cunninghamia lanceolata* Wood Against White-rot Fungi by Using Thermo-Mechanical Treatment

Nguyen Thi Tuyen (Corresponding Author)

Thai Nguyen University of Agriculture and Forestry, Thai Nguyen University, Thai Nguyen 250000, Vietnam

Email: nguyenthituyen@tuaf.edu.vn

Pham Van Chuong

Vietnam National University of Forestry, Hanoi, 100000, Vietnam

Vu Kim Dung

Vietnam National University of Forestry, Hanoi, 100000, Vietnam

Nguyen Viet Hung

Thai Nguyen University of Agriculture and Forestry, Thai Nguyen University, Thai Nguyen 250000, Vietnam

Tran Duc Hanh

Vietnam National University of Forestry, Hanoi, 100000, Vietnam

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Abstract

Heat treatment is an effective method to enhance the biological durability of wood without the use of preservatives. This study aims to analyze the effect of thermo-mechanical treatment on the durability of wood against the attack of some white rot fungi. The central composite design (CCD) method with the help of Design Expert 12.0 software was used to investigate the effects of temperature, compression time, and compression ratio on the white rot fungus resistance of *Cunninghamia lanceolata* wood. The obtained results revealed that the thermo-mechanical treatment of *Cunninghamia lanceolata* samples showed improved antifungal resistance compared to the untreated ones. After 4 months of testing in laboratory conditions, all wood samples with heat-mechanical treatment showed better resistance to fungi. Moreover, the different temperatures, compression ratios, and compression time bring out the different mass loss rates. The obtained results indicate that the wood samples modified at the temperature of 200°C, and 0.6 min/mm thickness combined with the compression ratio ranging from 40÷42% gave the lowest loss rate. Particularly, the resistance test for *Lentinula edodes* gives the best results when the wood compression time is at 0.7 min/mm thickness. Also, this work would provide a scientific and theoretical basis for the relationship between thermo-mechanical treatment and the biological durability of *Cunninghamia lanceolata* wood.

Keywords: Biological durability; Thermo-mechanical treatment; White rot fungus; *Cunninghamia lanceolata*; wood.

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1. Introduction

Cunninghamia lanceolata (Lamb.) Hook belongs to the family of *Taxodiaceae*, found in China, Laos, Vietnam, Malaysia and Cambodia and has a commercially important role in timber production [1]. In Vietnam, *Cunninghamia lanceolata* is mostly found in the northern highland provinces, including Cao Bang, Lao Cai, Ha Giang, and so on. *Cunninghamia lanceolata* is a straight-stemmed, fast-growing tree with beautiful grain. Its advantages are excellent material properties, attractive aroma, moderate hardness, straight texture and ease of processing [2]. Xing and Wakako [3] showed that *Cunninghamia lanceolata* was classified as a wood with moderate resistance when tested in the laboratory and in the field with rot fungi. Heat treatment for wood in general and thermo-mechanical treatment in particular is the new approach to overcome one or more disadvantages of wood by changing its properties. Fu, *et al.* [4], investigated the mechanical properties of heat treatment pine wood based on the changes of chromatic values. The authors established the Artificial Neural Network (ANN) model to study the relations between chromatic values and mechanical properties in heat treatment wood. This proposed model could be suitable to evaluate the mechanical of heat-treated wood in a non-destructive, swift and inexpensive method. Skyba [5], also showed that thermo hydro mechanical (THM) treatment caused to change the chemical composition and the porosity of wood, which makes the wood properties change: Balanced moisture content, dimensional stability, natural/bio-persistence, mechanical and adhesive properties. Also, THM treatment enhances the durability of Norway spruce wood against colonization and degradation by brown rot fungi, but does not improve the wood durability against white rot fungi. Bonigut, *et al.* [6] made the study on the dimensional stability and irreversible thickness swell of thermally treated oriented strandboards (OSB). The positive effects on the dimensional stability and irreversible thickness swell were reported when compared to the untreated ones. Bruno Esteves and Helena Pereira [7] studied heat treatment for pine wood and found that the ability to resist the effects of fungi depends on the temperature and time in treatment process.

Candelier, *et al.* [8], made a review on the effect of heat treatment on the natural strength of wood against brown rot and white rot fungi, and termites as well. The literature review indicated that the different softwood and hardwood species [beech (*Fagus sylvatica* L.), poplar (*Populus nigra* L.), ash (*Fraxinus excelsior* L.), pine (*Pinus sylvestris* L.) and silver fir (*Abies pectinate* Lam.)] treated at different temperatures (180°C, 200°C, 210°C, 220°C and 240°C) show the improvement in decay resistance towards brown rot fungi. However, the resistance to termites is only slightly improved when compared to untreated wood. Wood heat-treated at 215°C and 228°C has a higher natural strength. Taghiyari, *et al.* [9], studied the effects of heat treatment on the physico-mechanical properties of pine wood. The obtained results revealed that the high mass loss was observed at temperature 185°C and the extreme thermal degradation. The mechanical properties of pine wood decreased significantly due to the hornification and formation of irreversible hydrogen bonds. Moreover, they also investigated the influence of nanosilver on heat treatment and found out that significant increase of mass loss for the samples heat-treated at 185°C was reported, and the properties of specimens heat-treated at 145°C were significantly fluctuated. Also, some studies have been focused on the thermo-mechanical treatment of plantation wood. The mechanical strength and physical properties have been evaluated [10, 11].

In recent years, there have been the studies on improving the natural durability of wood without the use of harmful chemicals [12]. Treatment methods that apply a mechanism to limit the water penetration into the wood and make the wood structure after treatment have the ability to limit or resist microbial growth [13, 14]. The hemicelluloses are generally considered as an important nutritive source and a crucial factor in the hygroscopic wood behavior for the development of wood rotting fungi, so the degradation of hemicelluloses due to thermal treatment will play an very important role. On the other hand, the modification of lignin network also contribute to decrease the fungal enzymatic attacks [15]. However, the studies on the relationship between the thermo-mechanical treatment parameters and the biological durability of wood, especially *Cunninghamia lanceolata* are still quite limited. In order to improve the biological durability of wood, it is necessary to study and analyze the nature of the relationship between the parameters of thermo-mechanical treatment and the biological durability. Therefore, the authors are motivated to investigate the effect of heat treatment at different temperatures on the resistance to white rot fungus (*Lentinula edodes*, *Ganoderma lucidum*, *Trametes versicolor*) of *Cunninghamia lanceolata* (Lamb.) Hook.

2. Material and Method

2.1. Materials

2.1.1. Fungal Strains

The test fungi included white-rot fungi (*Lentinula edodes*, *Ganoderma lucidum*, *Trametes versicolor*), which were received from College of Forestry and Biotechnology, Vietnam National Forestry University. These fungi were cultured and grown in Potato Dextrose Agar (PDA) at $25 \pm 2^\circ\text{C}$ until the mycelia mat had covered the inoculated bottles.

2.1.2. Wood Samples

Fifteen-year-old *Cunninghamia lanceolata* (Lamb.) Hook wood samples were grown in Ba Ha district, Lao Cai, Vietnam and were kiln-dried at a maximum temperature of 70°C to a moisture content of 11-14%, and then were treated at different compression ratios, temperatures, and duration. They were denoted from TN1 - TN20 (Table 1). Next, the heat-treated and un heat-treated sample plates with the dimension of 30×20×10mm to test the antifungal capacity of wood.

2.2. Methods

2.2.1. Design of experiment

The central composite design (CCD) method with the help of Design Expert 12.0 software were used with three input variables (temperature, compression time, and compression ratio) and the sample thickness and compression time are depended on time and compression ratio, respectively. The design of experiments is shown in Table 1.

Table-1. Experimental parameters with three input variables affecting thermo-mechanical denaturation

Sample	Temperature (°C)	Time (min/mm thickness)	Compression ratio (%)	Sample thickness (mm)	Compression time (min)
TN1	160	0.5	30	28.6	14.3
TN2	200	0.5	30	28.6	14.3
TN3	160	0.7	30	28.6	20.0
TN4	200	0.7	30	28.6	20.0
TN5	160	0.5	50	40.0	20.0
TN6	200	0.5	50	40.0	20.0
TN7	160	0.7	50	40.0	28.0
TN8	200	0.7	50	40.0	28.0
TN9	146	0.6	40	33.3	20.0
TN10	214	0.6	40	33.3	20.0
TN11	180	0.43	40	33.3	14.3
TN12	180	0.77	40	33.3	25.7
TN13	180	0.6	23.18	26.0	15.6
TN14	180	0.6	56.82	46.3	27.8
TN15	180	0.6	40	33.3	20.0
TN16	180	0.6	40	33.3	20.0
TN17	180	0.6	40	33.3	20.0
TN18	180	0.6	40	33.3	20.0
TN19	180	0.6	40	33.3	20.0
TN20	180	0.6	40	33.3	20.0

2.2.2. Sterilization Method for Wood Samples

The sterilization process of wood samples was carried out according to Vietnam standard TCVN 10753:2015 [16], which is equivalent to European standard EN 113:1997. Prior to the date of placing the specimens in the fungal test vessels, the samples were put in heat proof bags and on each bag contains only the same groups of samples. The bags were sealed and placed in the sterilizer at 121°C for 20 min. The samples were allowed to cool naturally in the bag for 24h and the sterilization process was then repeated again for 10 min. The bags were not opened until the samples were placed in the culture flask.

2.2.3. Cross-Sectional Observation of Wood Samples on Electron Microscopy

The heat-treated *Cunninghamia lanceolata* samples with the best antifungal activity were selected and compared to the non-heat-treated samples with the least resistance. Samples were cut into 5 (R) × 5 (T) × 5 (L) mm small wood samples, fixed on metal bases, and gold-plated with specialized equipment to provide electrical conductivity (plating layer about 10 nm thick). The samples were then observed on a Oxford Instruments INCAx-act 51-ADD0085 SEM electron microscope at Institute of Tropical Technology, Vietnam Academy of Science and Technology.

2.2.4. Methods for Determining Resistance to White Rot Fungus through Mass Loss Ratio under Experimental Conditions

The resistance to white rot fungus of *Cunninghamia lanceolata* wood was carried out according to Vietnam standard TCVN 10753:2015. The wood samples with the same size include 21 samples of wood/mushroom, of which 20 samples were treated, and 1 control sample was not treated. The experiment was conducted with 3 species of fungi (*Lentinula edodes*, *Ganoderma lucidum*, *Trametes versicolor*), and the prepared wood samples were put in the flasks after 7 days of fungal culture. The temperature at 26±2°C and relative humidity of 70 ± 5% were maintained during 4 months for heat-modified *Cunninghamia lanceolata* wood. After tempering, the mycelium on the wood surface was removed with a sharp knife, and the wood samples were dried at 103±2°C to dryness and the loss in mass was determined.

The formula for determining loss mass rate of the sample:

$$H = \frac{m_0 - m_1}{m_0} \times 100$$

Where: H : loss mass rate in percentage of the sample, %;

m_0 : dry mass of the sample before fungi test, g;

m_1 : mass loss after fungi testing, g.

The antifungal ability of modified wood was evaluated according to the levels.

Table-2. Classification of wood's natural resistance to decay

Loss mass rate (%)	Level
$0 < H \leq 5$	Excellent
$5 < H \leq 10$	Good
$10 < H \leq 20$	Average
$H > 20$	Poor

Source: TCVN 10753:2015) [16].

3. Result and Discussion

3.1. Results of Resistance to White Rot Fungus of *Cunninghamia Lanceolata* Wood after Thermo-Mechanical Treatment

The evaluation of the resistance to white rot fungus of thermo-mechanical modified *Cunninghamia lanceolata* wood was carried out under laboratory conditions for 4 months. The obtained results are shown in Table 3, and Figures 1, 2, 3.

3.2. Mass Loss Rate under Laboratory Conditions

Cunninghamia lanceolata wood samples (including 20 thermo-mechanical-modified wood samples and 01 control wood sample) were evaluated for biopersistence on white rot fungi in Potato Dextrose Agar (PDA) medium at 25-28°C, humidity of 70-80%. The results are shown in Table 3 and Figures 1, 2, 3.

Table-3. Average mass loss rate (%) of heat-treated wood with white rot fungus under laboratory conditions

Sample	White Rot Fungus					
	<i>L.edodes</i>	Standard deviation	<i>G. lucidum</i>	Standard deviation	<i>T. versicolor</i>	Standard deviation
TN1	4.12	0.08	6.07	0.07	9.16	0.07
TN2	2.44	0.08	3.39	0.06	4.72	0.13
TN3	3.88	0.06	5.46	0.05	8.22	0.14
TN4	2.30	0.07	3.08	0.04	4.21	0.09
TN5	4.14	0.09	5.74	0.05	8.62	0.12
TN6	2.07	0.06	3.17	0.10	4.42	0.10
TN7	3.82	0.08	5.42	0.07	8.19	0.07
TN8	1.85	0.05	2.89	0.06	3.99	0.11
TN9	4.56	0.04	6.34	0.11	10.05	0.09
TN10	1.52	0.09	2.26	0.13	3.03	0.11
TN11	3.12	0.09	4.14	0.11	6.23	0.09
TN12	2.88	0.08	3.67	0.06	5.72	0.17
TN13	3.44	0.08	4.74	0.04	6.82	0.12
TN14	3.11	0.08	4.43	0.09	6.54	0.13
TN15	3.06	0.07	3.76	0.05	6.06	0.17
TN16	3.01	0.09	3.92	0.04	5.87	0.04
TN17	3.09	0.03	3.89	0.06	5.98	0.17
TN18	3.02	0.04	3.88	0.08	6.03	0.10
TN19	3.00	0.06	3.90	0.06	5.92	0.10
TN20	3.01	0.04	3.85	0.05	6.11	0.04
DC	10.18	0.03	11.21	0.11	15.27	0.07

Figure-1. *Cunninghamia lanceolata* wood samples tested on *Lentinula edodes* (After 2 weeks; a: DC; b: TN9; c: TN10 and 4 months d: DC; e: TN9; f: TN10)

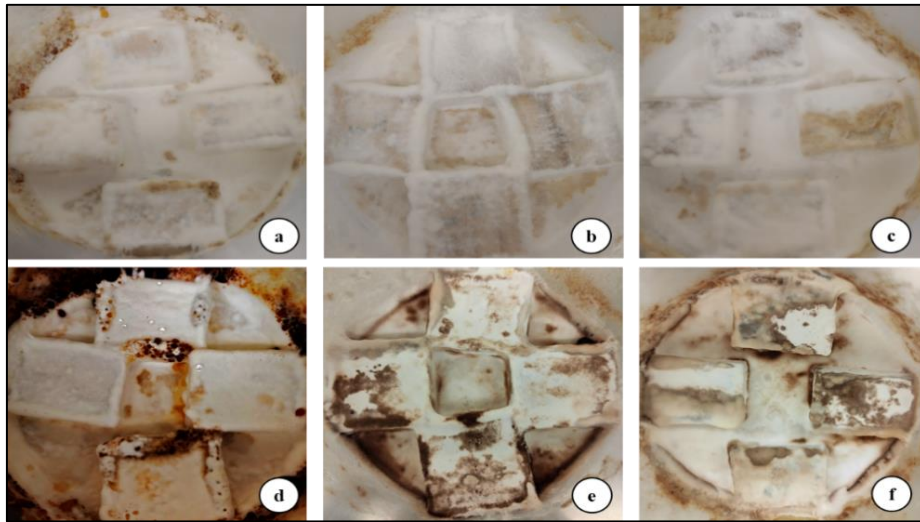


Figure-2. *Cunninghamia lanceolata* wood samples tested on *Ganoderma lucidum* (After 2 weeks; a: DC; b: TN9; c: TN10 and 4 months d: DC; e: TN9; f: TN10)

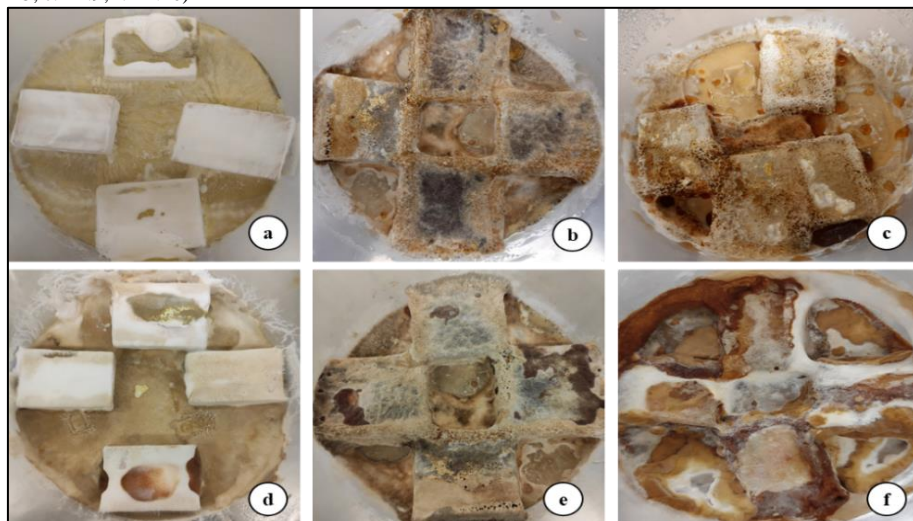
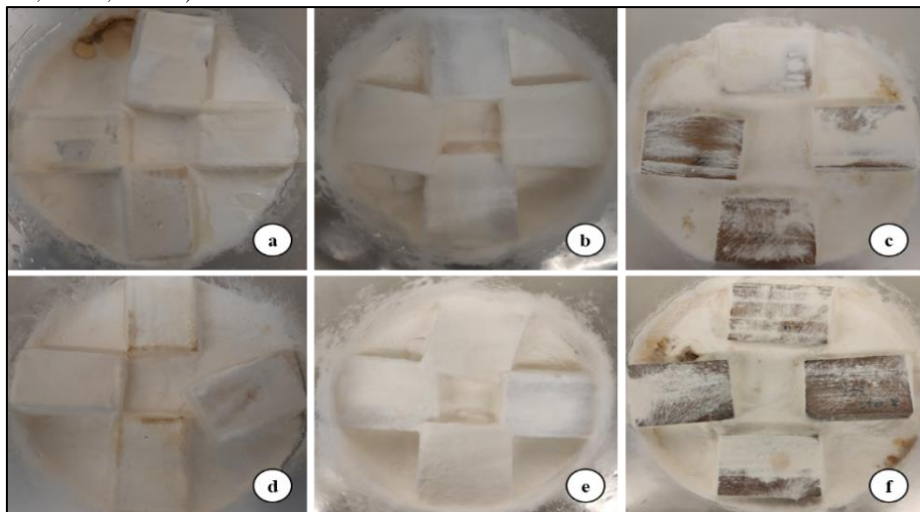


Figure-3. *Cunninghamia lanceolata* wood samples tested on *Trametes versicolor* (After 2 weeks; a: DC; b: TN9; c: TN10 and 4 months d: DC; e: TN9; f: TN10)



The study results on the resistance to white rot fungus of mechanical heat-treated *Cunninghamia lanceolata* wood in experimental condition show that all *Cunninghamia lanceolata* wood samples treated at high temperature improve the resistance to white rot fungus compared with the control samples. Also, the wood samples treated different conditions have the statistical meanings with the control samples after the experimental time of 4 months.

Moreover, the mass loss rates are different in different temperatures, compression ratios, and compression time, and the analysis results reveal that the wood samples modified at 200°C, the compression ratio of 40-42%,

compression time of 0.6 min/mm will bring out the lowest mass loss rate. Especially the test with resistance to *Lentinula edodes* will give the best result when the compression time is at 0.7 min/mm thickness.

Typical results when tested with white rot fungi under experimental conditions indicate that the TN10 wood sample had the lowest percentage of mass loss after four months. For *Lentinula edodes*, this rate is 1.52%, decreasing 6.7 times compared to the control sample, while the TN9 wood sample showed the highest percentage of mass loss (4.56%), about 2.2 times lower than the control sample. For *Ganoderma lucidum*, the TN10 wood sample had the mass loss rate of 2.26% while the TN9 wood sample showed a relatively high mass loss rate of 6.34%. Besides, the wood samples (TN11-TN20) were modified at 180°C and the average mass loss was 4.0 % after 4 months of infection with *Ganoderma lucidum* strain.

It can be explained that the wood cells are bonded together by lignin and hemicellulose, organic compounds that soften when subjected to high temperatures. Therefore, the links in the wood loosen and soften with the increasing temperature to facilitate the process of wood compression. The great forces in compression process reduce the distance between the wood cells, the hollow part, and the amount of oxygen, which is an unfavorable condition for the growth of fungi [12]. Due to high temperature and long treatment time, some wood extracts can be removed or hemicellulose in wood is decomposed, leading to reduce the number of hydroxyl groups (-OH) and improve the resistance to water absorption [17]. The change in the chemical structure of the heat-treated *Cunninghamia lanceolata* wood also shows that the mass ratio of basic substances such as hemicellulose, lignin and cellulose varies when the treatment temperature changes. When the temperature is increased to 180°C, hemicellulose and cellulose change and especially above 200°C. For 200°C over, lignin starts to decompose and increases significantly [18]. Hence, At temperatures of 160°C and 146°C with the compression ratio studied, lignin plasticization in wood was not achieved and the hemicellulose degradation occurred incompletely. The cellulose in the amorphous region has little recombination reaction. Therefore, the elasticity of wood increases significantly [19]. Besides, it shows that the elasticity of wood is inversely proportional to the increase of temperature and pressing time.

Observing the growth of mycelium, it was found that with wood samples treated at high temperature ($\geq 200^\circ\text{C}$), the slow growth of the mycelium did not cover the entire wood sample. In contrast, for the remaining wood samples after periods of fungal infection, the mycelium grew rapidly and covered the surface of the wood sample. Also, the growth rate of the mycelium was relatively fast after only 5 days of placing the wood sample in fungi and the mycelium gradually changed from white to yellow-brown color.

In addition, after 4 months of fungal exposure, when the fungus was removed from the wood surface, color spots appeared in the control and modified wood samples at $\leq 180^\circ\text{C}$. The above phenomenon is consistent with [20].

3.3. Result of Cross-Sectional Observation of Wood Samples on Electron Microscope (SEM)

Among the heat-modified wood samples, the TN10 sample showed the most effective resistance to white rot fungus. Therefore, the TN10 wood sample was selected for observation on electron microscopy to compare the structural change with the DC wood sample after 4 months of bio-persistence testing on white rot fungi.

Cross-sectional observation of wood samples by electron microscopy revealed differences in tissue and cell wall structure between control and TN10 samples (Figures 4, 5, 6), but little difference in mass. For the control samples, the mycelium thrived, especially *Ganoderma lucidum* and *Trametes versicolor*, and the mycelium penetrated deeply into the wood cell wall causing cell separation and structural disruption. The mycelium produced relatively large cavities that were visible in wood samples exposed to *Ganoderma lucidum* and *Trametes versicolor* (Figure 5a, Figure 6a), whereas for the samples caused by *Lentinula edodes*, the mycelium sparsely distributed in the wood structure (Figure 4a); however, it still produces the typical destruction of white rot fungi.

In addition, when the wood decomposition process occurs, the wood structures have been attacked by the fungal enzyme system, causing the wood fiber structure to be damaged and the appearance of fungal spores on the surface of the wood fiber system (Figure 4a, 5a, 6a). A low fiber condition was observed in the TN10 samples (Figures 4b, 5b, 6b).

Treatment with higher temperatures further reduced the number of mycelium in the wood and the degree of cell wall destruction (Figure 4b), and the wood surface is relatively closed. There are few gaps between the wood cells, thereby limiting the mycelium to penetrate the cell wall. As a result, the distribution of fungal enzymes through the walls of solidified wood can be slowed, which may explain why sample TN10 is highly resistant to fungi [21, 22].

However, from observing the invasion of fungi *Ganoderma lucidum* and *Trametes versicolor* on TN10 wood samples, the mycelium system was relatively abundantly distributed in the wood structure, although the wood mass loss rate was not high and the durability was improved when compared to the control sample. The possible reason lying there is that when treating wood with heat, it will change the structure of the secondary wall (S3), creating elasticity for wood cells. Even though there is fungus penetration, the wood structure is less affected [23]. The moisture content of heat-treated wood was found to be much lower than the grain saturation point by the end of the 4-month incubation period. Therefore, no free water is already available in the cell lumina, so that the conditions for mycelial wood growth and degradation are unfavorable.

Figure-4. The control and mechanical heat-treated *Cunninghamia lanceolata* wood samples when exposed to *Lentinula edodes*: (a) Control sample; (b) Heat-treated sample

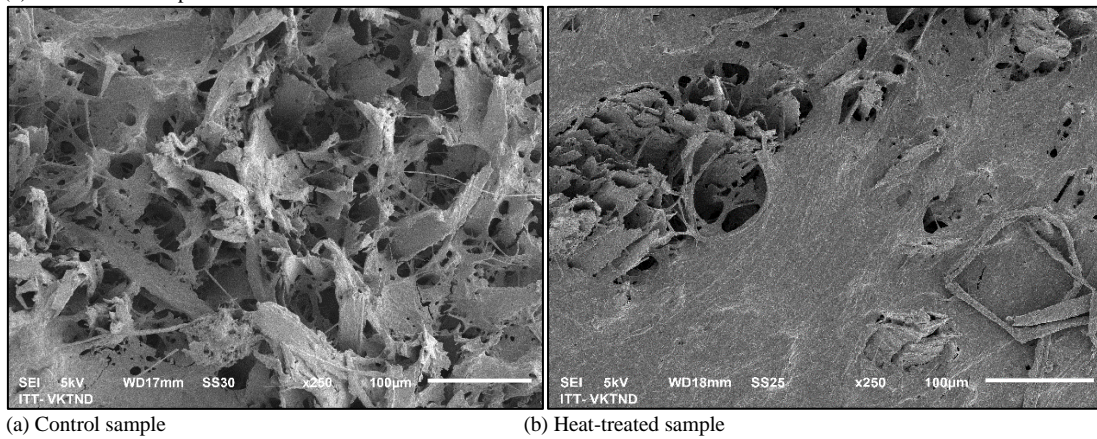


Figure-5. The control and mechanical heat-treated *Cunninghamia lanceolata* wood samples when exposed to *Ganoderma lucidum*: (a) Control sample; (b) Heat-treated sample

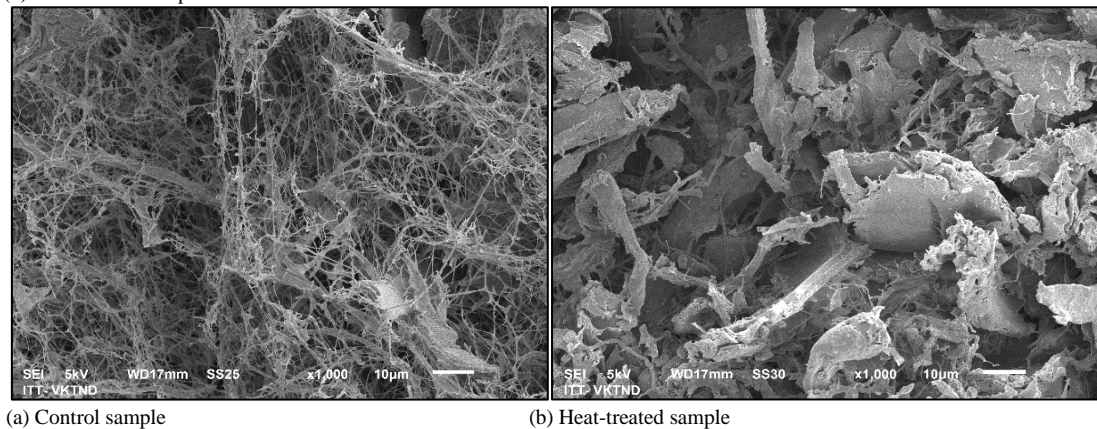
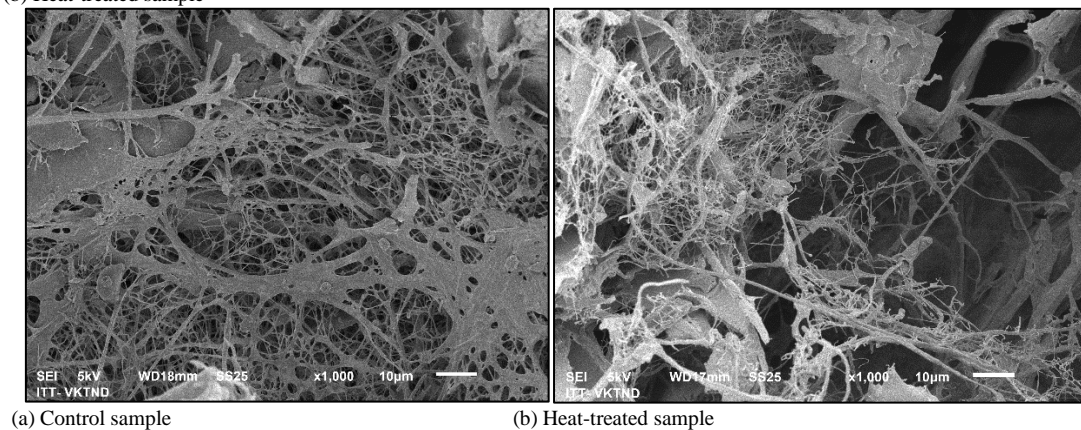


Figure-6. The control and mechanical heat-treated *Cunninghamia lanceolata* wood samples when exposed to *Trametes versicolor*: (a) Control sample; (b) Heat-treated sample



4. Conclusion

The *Cunninghamia lanceolata* wood samples were mechanically heat-treated and showed the improvement of antifungal resistance compared to the control wood samples. This work used central composite design (CCD) method to study the effects of temperature, compression time, and compression ratio on the white rot fungus resistance of *Cunninghamia lanceolata* wood. The obtained results revealed that after 4 months of testing in laboratory conditions, all wood samples with thermo-mechanical treatment revealed better resistance to fungi than the control samples.

The obtained results also show that the different temperatures, compression ratios, and compression time give out the different mass loss rates, and most wood samples modified at 200°C, compression ratio from 40-42%, and compression time of 0.6-0.7 min/mm will achieve the lowest mass loss rate. Specifically, when tested with *Lentinula edodes*, the TN10 wood sample had the lowest percentage of mass loss after 4 months (1.52%), decreasing 6.7 times compared to the control sample, while the TN9 wood sample showed the highest percentage of mass loss (4.56%), about 2.2 times lower than the control sample. For *Ganoderma lucidum*, the TN10 wood sample had a mass loss rate of 2.26% while the TN9 wood sample showed a relatively high mass loss rate of 6.34%. The wood samples (TN11-

TN20) were modified at 180°C and showed the average mass loss of 4.0% after 4 months of active infection with *Ganoderma lucidum*.

In order to have more scientific basis for the changing mechanism of wood characteristics, further studies are needed to focus on the durability and physical characteristics of heat-treated *Cunninghamia lanceolata* wood such as water absorption, brittleness and swelling related to temperature and treatment time.

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