Turk J Agric For
32 (2008) 331-338

Beneficial Effects of Fungal Treatment before Pulping and Bleaching of *Acacia mangium* and *Eucalyptus camaldulensis*

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Received: 03.07.2007

Abstract: Three white-rot fungi (*Ceriporiopsis subvermispora* (Pilát) Gilb. & Ryvarden, *Phanerochate chrysosporium* Burds, and *Trametes* (*Coriolus*) *versicolor* (L.) Lloyd) were tested for their ability to modify/degrade lignin in cooking and bleaching. Both chips and pulps of *Acacia mangium* Willd. and *Eucalyptus camaldulensis* Dehn. were pretreated with these white-rot fungi for different time periods (8, 12, and 16 days for chips; and 4, 8, and 12 days for pulps). For chip pretreatment, Kappa number decreased with the increase of fungal treatment time, and the maximum reduction in Kappa number was 28% and 25% for acacia and eucalyptus, respectively, after 16 days of fungi inoculation. The variation in Kappa reduction was also found for different fungi species when inoculated in the same wood species. Fungi treated pulps were also easy to delignify in oxygen delignification stage compared to the control pulp without serious viscosity loss. In bleaching, the final brightness was higher in the case of FD_0ED_1 bleached pulps (as much as 8 ISO units) than OD_0ED_1 bleached pulps for both species. By FD_0ED_1 bleaching sequence, it was also possible to reach the full brightness of pulp. Hand sheet properties were also improved significantly by fungi treatment prior to bleaching. Strength properties of hand sheets increased up to 20% by fungi treatment. Considering all the pulping and papermaking properties, the performance of *C. subvermispora* was better compared to other fungi tested, and the response of fungi to eucalyptus was better compared to acacia.

Key Words: Fungi, biopulping, biobleaching, acacia, eucalyptus, inoculation

Introduction

Environmental concern is increasing day by day and awareness regarding proper utilization of existing resources is also increasing. Hence, pulp and paper industries, committed to provide quality paper product to world, are seeking new environmentally friendly technologies ensuring energy saving and proper resource utilization for pulping and bleaching. Therefore, many researchers are concentrated on the potential aspects of pulping and bleaching by biological methods (Nishida et al., 1988; Akhtar et al., 1992, 1993; Fujita et al., 1993; Messner and Srebotnik, 1994; Messner et al., 1998).

Biopulping is an experimental process whereby chips are pretreated with white-rot fungi or microorganisms or lignin degrading enzymes, which appears to be modestly effective as a treatment before mechanical pulping and is also applicable to chemical pulping. White-rot fungi are the most attractive agents for the biological removal of residual lignin from Kraft pulp (Fujita et al., 1993), and are unique among most microorganisms in their capacity to depolymerize and metabolize lignin. They do not only produce a whole set of enzymes necessary for the lignin degradation, but can also act as a transporter for these enzymes by bringing them into the depth of wood chips and create the physiological conditions necessary for the enzymatic reaction. Biological pretreatment would reduce the amount of cooking chemicals, increase the cooking capacity, or enable extended cooking, resulting in lower consumption of chemicals in bleaching. Biopulping also improves various paper strength properties (Akhtar et al., 1992, 1993). Decreased Kappa number and increased brightness by fungal pretreatment were also observed for sulfite pulping and Kraft pulping. Fungal treatment helps to decrease the negative environmental impact of pulp and paper production (Fujita et al., 1993).

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Study for the proper selection of lignin degrading microorganisms in pulping and bleaching and process optimization can help to implement this technology in mill scale.

This study was carried out with 2 major hardwood species, i.e., *E. camaldulensis* and *A. mangium* with 3 most effective white-rot fungi, i.e., *C. subvermispora, P. chrysosporium* and *T. (Coriolus) versicolor* to find out the effects of these fungi on pulping and bleaching of acacia and eucalyptus, and its effects on the papermaking properties.

Methodology

Inoculums preparation

A 4% Malt Extract-Agar (MA) media was prepared for fungi culture. The petri-plates were inoculated with *C. subvermispora* and *T. versicolor* and incubated at 30 °C but *P. chrysosporium* was incubated at 39 °C for 10 days without agitation. The mycelia were separated by blending aseptically in a blender using distilled water. By adding sterile water, 200 ml suspension was prepared with 10 mg ml^{-1} concentration on dry weight basis. The suspension was used to treat wood chips for cooking.

To measure their growth rate, 6 petri plates were inoculated with 5 mm discs of mycelium mat of each fungus and were incubated for 6 days under the conditions given above. The growth rate was calculated every day by measuring the diameter of the mycelium circle.

Chip preparation and fungi inoculation

Industrial chips of 3 to 5 years old *Eucalyptus camaldulensis* and *Acacia mangium* were collected from a pulp mill in Thailand and Indonesia, respectively. The collected chips were screened according to standard SCAN CM 40:94. One part of acacia and eucalyptus chips was treated with the above-mentioned fungi prior to cooking and the rest of the chips were cooked directly (without fungal treatment). For different types of fungus and chips, different flasks, each containing 300 g o.d. chips, were autoclaved for 90 min at 121 °C with 103 kPa. Each flask was inoculated with mycelium suspension of *C. subvermispora*, *P. chrysosporium,* and *T. versicolor* without any addition of supplementary nutrients. The rate of mycelium application was 0.1% on dry weight basis of chips. Sterilized water was added to each flask to raise the moisture content of chips to approximately 50%-60% on a dry weight basis for optimum growth of the fungi. After receiving inocula, the flasks were shaken vigorously for uniform mixing. Each flask with sufficient aeration was sealed and placed in an incubator maintaining the temperature at 29 ± 1 °C for different time periods (8, 12, and 16 days). For *P. chrysosporium*, the temperature was 39 °C. After the specified period of incubation (8, 12, and 16 days), each flask was kept in a cold room for 3 days to stop the further growth of fungi.

Cooking

Dry matter content of both treated and untreated chips was determined according to standard SCAN-CM 39:94. CRS autoclave digester was used for cooking. Eucalyptus chips were cooked by 21% effective alkali, 33% sulphidity and 4:1 liquor to wood ratio at 165 °C temperature for 60 min. Acacia chips were cooked by 19% effective alkali and other conditions remained the same. Dry matter content of pulp was measured according to the standard SCAN-C 3:78 and yield was calculated before and after screening. Kappa number, viscosity, and brightness of pulps were measured according to standard SCAN-C 1:77, SCAN-CM 15:88, and SCAN-C 11:75, respectively.

Bleaching

All the pulps coming from the fungi treated chips were mixed together (separately for acacia and eucalyptus) and homogenized. Kappa number, viscosity, and brightness were measured accordingly and repeated with the control pulp (untreated). Three types of bleaching sequence were followed to bleach the pulp (Table 1). For fungi treatment, pulp was taken in a 1000 ml flask and autoclaved at 121 °C and 15 psi for 60 min for proper sterilization. It was then cooled at room temperature and inoculated with mycelium suspension at a rate of 0.05% o.d. weight of pulp and incubated for 4, 8, and 12 days after vigorous mixing. After the specified time period, the flask was stored in a cold room for 3 days to stop the further growth of fungi. The pulp was then bleached following the sequence.

Oxygen delignification was done at 95 °C for 60 min with 10% consistency and 8 bar oxygen pressure where the $MgSO_4$ and NaOH charge was 0.4% and 0.3*Δ Kappa number (target Kappa reduction, 40%) (Sah, 2003), respectively.

Table 1. The bleaching sequence for different types of pulps.

Name of species	Fungi treated/untreated sequence	Bleaching unbleached pulp	Total number of samples
Acacia	treated	$F^{a}D_0ED_1$	9
Acacia	treated	D_0ED_1	1
Acacia	treated	OD_0ED_1	1
Acacia	untreated	FD_0ED_1	9
Acacia	untreated	D_0ED_1	1
Acacia	untreated	OD_0ED_1	1
Eucalyptus	treated	FD_0ED_1	9
Eucalyptus	treated	D_0ED_1	1
Eucalyptus	treated	OD_0ED_1	1
Eucalyptus	untreated	FD_0ED_1	9
Eucalyptus	untreated	D_0ED_1	1
Eucalyptus	untreated	OD_0ED_1	1

[a] F indicates the bleaching stage by fungi

The bleaching conditions are shown in Table 2. Kappa number, viscosity, and brightness were measured according to the standard after fungi treatment/oxygen delignification. After D_0E stage, Kappa number and brightness were measured for all pulps. After the final stage of bleaching, brightness and viscosity were measured following the standard mentioned before. Bleached pulps from all sequences were used to make the hand sheets.

Table 2. The bleaching condition of D_0ED_1.

Stage	D_o	E	D_1
Time (min)	60	60	180
Temp (°C)	60	70	70
Consistency (%)	10	10	10
ClO2 charge (% act Cl)[b]	0.25xK	-	2
NaOH Charge (% on o.d pulp)	-	2	0.4
Final pH (target)	1.5-2.5	10.0-11.5	3.5-4.5

[b]ClO_2 charge: 0.3*K, where K refers the Kappa number of incoming pulp to the D_o stage after oxygen delignification. For all other samples (fungi treated pulps), ClO_2 charge was the same as the respective oxygen-delignified pulp.

Hand sheet making and paper testing

The bleached pulp was beaten in PFI mill at 2000 rpm. After beating, 70 g m^{-2} hand sheets were made and paper properties were examined along with the refined pulps. Drainability (SR) test was performed following the SCAN-C 19:65 and hand sheet was made following the standard SCAN-C 26:76. Brightness, tear, and tensile index were measured following the standard SCAN-C 11:75, SCAN-P 11:73, and SCAN-P 67:93, respectively. Light scattering coefficient was calculated.

Results and Discussions

Growth rate of fungal hyphae

After 6 days, vegetative hyphae of *C. subvermispora* (CS), *P. chrysosporium* (PC) and *T. versicolor* (TV) formed a strong web-like network all around the discs. *C. subvermispora* showed the best growth performance over the specified time period. The diameter of the fungal growth area was more than twice the initial diameter only after 6 days. Among these 3 fungi, the growth performance was poor for *P. chrysosporium* but it forms a very small cluster all around the petri-plate after the specified time period. If the growth rate is higher, then fungal hyphae can invade new wood cells to degrade lignin. Therefore, *C. subvermispora* should provide the best results, which was achieved in this study.

Kappa number, yield, and viscosity

Yield decreased with the increase of inoculation time. The highest yield was observed with 8 days of inculation for all the fungi and wood species. The decrease of acacia yield was gradual but there was a sharp change after 12 days of inoculation for eucalyptus, which was irrespective to fungi (Table 3). Scott et al. (1995) reported that fungi treatment can reduce the yield slightly. This decrease may be due to both the dissolution of lignin and the concurrent attack on the carbohydrates (Messner et al., 1998). For both species, change of Kappa number was significant, and was about 6.5 and 5.3 for acacia and eucalyptus, respectively, for the longest treatment time. It was also observed that Kappa number decreased at a higher rate up to 12 days without affecting the yield much. However, after 12 days, change of Kappa affected the yield substantially. It is more evident in eucaplytus compared to acacia. Considering both yield and Kappa number, 12 days of inoculation showed the best performance for both wood species, and *C. subvermispora* and *P. chrysosporium* showed the best results for acacia and eucalyptus, respectively (Table 3).

Table 3. Kappa number, yield, and viscosity of pulp after cooking.

Cooking condition		Species	Kappa number	Cooking yield (%)	Viscosity (ml g^{-1})
Without inoculation		Acacia	23.6 ± 0.50	56.6	729 ± 11
		Eucalyptus	21.5 ± 0.03	47.0	672 ± 8
8 days	CS	Acacia	20.1 ± 0.05	56.6	815 ± 5
		Eucalyptus	19.3 ± 0.03	47.9	701 ± 12
	PC	Acacia	21.3 ± 0.10	56.1	806 ± 5
		Eucalyptus	18.9 ± 0.05	47.5	684 ± 5
	TV	Acacia	19.7 ± 0.05	55.7	815 ± 4
		Eucalyptus	18.0 ± 0.05	47.1	689 ± 7
12 days	CS	Acacia	17.5 ± 0.03	55.9	783 ± 8
		Eucalyptus	17.9 ± 0.03	47.6	689 ± 7
	PC	Acacia	18.2 ± 0.04	55.3	781 ± 4
		Eucalyptus	16.9 ± 0.02	48.0	666 ± 7
	TV	Acacia	18.7 ± 0.03	55.6	790 ± 5
		Eucalyptus	16.6 ± 0.02	48.0	679 ± 10
16 days	PC	Acacia	17.1 ± 0.03	55.0	708 ± 3
		Eucalyptus	16.3 ± 0.03	45.1	651 ± 5
	PC	Acacia	17.9 ± 0.07	55.0	701 ± 5
		Eucalyptus	16.6 ± 0.03	45.6	628 ± 2
	TV	Acacia	18.0 ± 0.04	55.1	715 ± 5
		Eucalyptus	16.2 ± 0.02	44.6	576 ± 6

Fungal pretreatment causes swelling and loosens cell wall structures, which increases the porosity of the wood chips. This cell porosity occurs early in the colonization process by lignin-degrading fungi (Nishida et al., 1988; Akhtar et al., 1992; Fujita et al., 1993). Also, these fungi remove and/or modify lignin in wood cell walls that might be removed easily during Kraft pulping (Reddy, 1984; Messner and Srebotnik, 1994; Messner et al., 1998). This higher porosity leads to the production of lower amount of rejects. In this study, it was observed that without fungi treatment, rejects was around 1% but it decreased to less than 0.1% with the fungal treatment. Fungal treatment leading to physico-chemical changes in cell walls improves chemical penetration and, subsequently, aids the kraft pulping processes (Messner et al., 1998). Higher residual alkali of fungus treated chips in this works supports the above statement.

Pulp viscosity of fungi treated chips was higher for 8 days of treatment in all cases compared to the control pulps. It was similar or higher in the control pulps for 12 days of treatment, and after that there was a sudden change. Viscosity followed the trend of yield where the change was sharp after 12 days of inoculation (Table 3). Acacia had always higher viscosity compared to eucalyptus. It was observed that after 12 days of inoculation, there was no significant change of Kappa number, but viscosity was affected drastically. White-rot fungi are very selective to lignin for a certain period of time but it also attacks carbohydrates for a prolonged period (Messner et al., 1998). As viscosity decreased drastically after 12 days of inoculation, it can be concluded that carbohydrates was affected after 12 days of inoculation.

Oxygen delignification

The Kappa reduction at oxygen delignification stage changed between 28.8% and 42.6% depending on wood species, fungi treatment of chips, and incoming Kappa number (Table 4). Fungi treated chips showed higher rate of oxygen delignification compared to control. Fungi use extracellular Reactive Oxygen Species (ROS) (Halliwell, 1965; Koenings, 1974) and peroxidases (Wariishi et al., 1991; Hatakka, 1994) to degrade lignocelluloses. These ROS and peroxidases degrade and/or chemically modify a portion of lignin by breaking a side chain of lignin, which was easily removed in oxygen delignification stage or in the subsequent bleaching stages. It was also observed that acacia had a higher rate of oxygen delignification compared to eucalyptus. The formation of HexA during cooking is higher in eucalyptus compared to acacia (Sah, 2003) and it contributes to the Kappa number up to 40%. However, HexA remains unaffected after oxygen delignification (Malinen, 2003), which indicates less reduction of Kappa in the case of eucalyptus. Viscosity loss in the oxygen delignification stage was also lower in the case of fungi treated chips. The loss of viscosity depends on the degradation of carbohydrates. Partially degraded or modified lignin can easily be oxidized at this stage, which reduces the chance of carbohydrate attack resulting less loss of viscosity.

Brightness and light scattering

Samples having 8 days of inoculation period were selected for hand sheet making depending on optimum brightness and viscosity. It was observed that the final brightness was similar/higher in fungi treated pulps (80%-87% ISO) compared to oxygen delignified and OD_0ED pulps (80%-86% ISO); however, D_0ED_1 stages of bleaching raised the brightness from 70% to 75% ISO. White-rot fungi secrete extra-cellular enzymes and

Table 4. Results of oxygen delignification.

Samples (fungi treated/ untreated)	Initial Kappa	Final Kappa	Delign. Rate (%)	Initial viscosity (mg l^{-1})	Final viscosity (mg l^{-1})
Acacia treated	19.6	11.2	42.6	810	795
Acacia untreated	19.1	13.6	28.8	729	697
Euca treated	15.9	9.7	39.0	701	681
Euca untreated	18.8	12.7	32.4	672	651

peroxidase for the oxidation of lignin and increase brightness similar to most of the bleaching chemicals (Kirk and Shimada, 1985). Among the three fungi, *C. subvermispora* was the best in performance. *C. subvermispora* produces isoenzymes of MnP and lacasse, but no isoenzymes of LiP were found. However, its ligninolytic activity is as high as in the organisms containing LiP. These MnPs are not strongly oxidizing like LiPs but very selective to lignin, which makes *C. subvermispora* superior than others (Kirk et al., 1986; Tuor et al., 1992; Bao et al., 1994). After the final stage of bleaching, brightness of eucalyptus was higher than that of acacia. The same amount of chlorine dioxide was applied to all pulps but the initial Kappa number was quite low in the case of eucalyptus compared to acacia, which could lead eucalyptus to gain higher brightness.

There was no significant effect of fungi treatment on the light scattering properties of hand sheet. It was observed that brightness and light scattering properties decreased with the increase of refining. Higher beating reduces the free spaces in paper so the light scattering coefficient decreases (Leskela, 1998). It might be the cause of the lowest brightness with the highest refining. Eucalyptus had a higher light scattering coefficient compared to acacia with the same refining intensity (Figure 1). Eucalyptus pulp is coarser than the acacia pulp (Malinen, 2003), which needs more refining energy to break it down during papermaking. Therefore, in the same refining, acacia had higher collapsibility than eucalyptus, and thus, lower light scattering coefficient resulted. It was observed that, in hand sheets produced from fungi pretreated chips brightness increased around 3 units without affecting light scattering properties significantly (Figure 2). This increment rate was higher when the initial brightness was lower. Fungi treatment makes a chemical change in lignin, which helps remove the lignin easily in the subsequent bleaching stages (Dube and Kothari, 1983; Kirk and Shimada, 1985; Akhtar et al., 1993). The highest light scattering properties was observed in eucalyptus with the lowest refining intensity.

Tensile index and tear index

There was a positive relationship between tensile index and tear index. Tensile and tear index were higher in acacia than in eucalyptus (Figures 3 and 4). The lower fiber coarseness of acacia gives higher specific surface area and higher flexibility of fiber. Higher refining improves the bonding potentiality of the fiber by increasing the flexibility (Yuniar, 2003) and makes it stronger. Acacia has the higher chance to break down during refining, which increases the bonding area yielding higher tensile and tear strength.

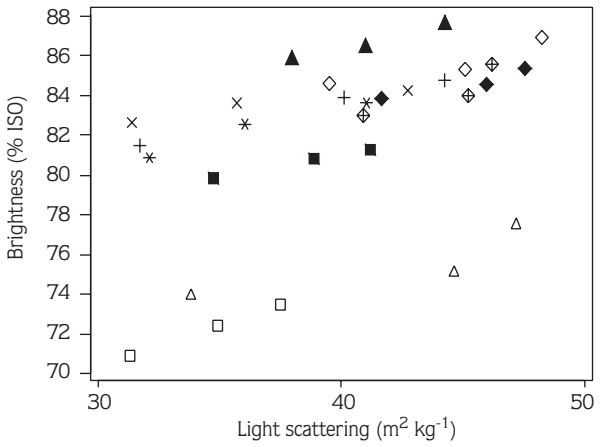

Figure 1. Effects of different fungi and refining intensity on brightness and light scattering of acacia and eucalyptus without fungi treated chips for 8 days of inoculation. {For acacia D_0ED_1 (□), O D_0ED_1 (■), $F(CS)D_0ED_1$ (+), $F(PC)D_0ED_1$ (X), $F(TV)D_0ED_1$ (*) and for eucalyptus D_0ED_1 (△), OD_0ED_1 (▲), $F(CS)D_0ED_1$ (◇), $F(PC)D_0ED_1$ (◆), $F(TV)D_0ED_1$ (⊕)}

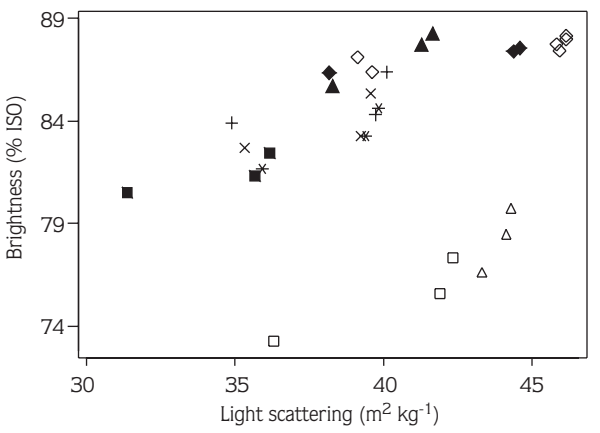

Figure 2. Effects of different fungi and refining intensity on brightness and light scattering of acacia and eucalyptus with fungi treated chips for 8 days of inoculation.{For acacia D_0ED_1 (□), O D_0ED_1 (■), $F(CS)D_0ED_1$ (+), $F(PC)D_0ED_1$ (X), $F(TV)D_0ED_1$ (*) and for eucalyptus D_0ED_1 (△), OD_0ED_1 (▲), $F(CS)D_0ED_1$ (◇), $F(PC)D_0ED_1$ (◆), $F(TV)D_0ED_1$ (⊕)}

Fungi treated pulps had higher tensile and tear index than untreated pulps (Figure 3). The greater delignification in fungi-treated acacia and eucalyptus commences greater cellulose-to-cellulose bonding that may have enhanced the hand sheet strength properties. Fungal exudates that cause the fibers to stick together might be another reason for the enhanced strength properties (Setliff et al., 1990). Tensile and tear strength was higher in fungal treated pulps than in oxygen delignified pulps. During the fungi treatment, wood carbohydrates that were surrounded by lignin became exposed or susceptible to the enzymatic attack as a result of the preferential removal of the surrounding lignin. This was more common in fungi other than *C. subvermispora*, which makes this fungus more attractive. Acacia had higher strength compared to eucalyptus (Figure 4). Higher refining intensity showed better performance than lower intensity refining. The strength properties increased up to 8 days of inoculation and decreased after that.

Conclusion

The results presented and discussed in this study demonstrate the great potential of fungal pretreatment of wood chips/pulps prior to chemical pulping and bleaching. The most prominent benefit of fungal pretreatment of chips was the improved effects on cooking with higher yield and lower Kappa number after only 12 days of fungal treatment. Significant increase of brightness was remarkable in bleaching. The conclusions that can be drawn from this work are as follows:

High Kappa reduction (around 6.5 and 5.3 units for acacia and eucalyptus, respectively) obtained by fungal treatment coincides with the strategy of extended cooking. Fungal treatment also modifies the lignin, which can be removed easily in the subsequent stages like oxygen delignification where there was around 2 units of extra Kappa reduction.

Prolonged (around 8 days) fungi treatment prior to bleaching can eliminate the oxygen delignification and lead to produce higher quality paper. It will save the energy in oxygen delignification and in evaporation and the chemicals used in bleaching.

Compared to prolonged time periods, 12 and 8 days of inoculation of chips and pulps, respectively, was optimum regarding the quality and economy. Among the 3 fungi, *C. subvermispora* was more effective than the others in both cooking and bleaching.

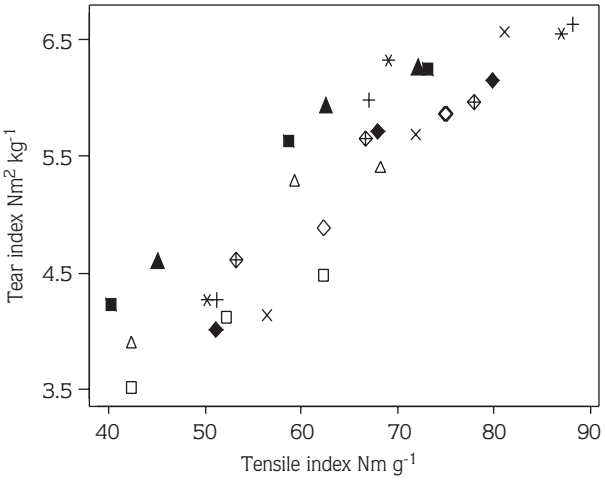

Figure 3. Effects of different fungi and refining intensity on brightness and light scattering of acacia and eucalyptus without fungi treated chips for 8 days of inoculation. {For acacia D_0ED_1 (□), O D_0ED_1 (■), $F(CS)D_0ED_1$ (+), $F(PC)D_0ED_1$ (X), $F(TV)D_0ED_1$ (*) and for eucalyptus D_0ED_1 (△), OD_0ED_1 (▲), $F(CS)D_0ED_1$ (◇), $F(PC)D_0ED_1$ (◆), $F(TV)D_0ED_1$ (⊕)}

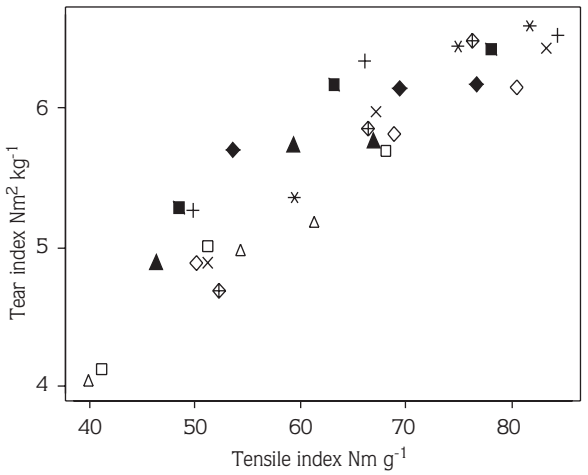

Figure 4. Effects of different fungi and refining intensity on brightness and light scattering of acacia and eucalyptus with fungi treated chips for 8 days of inoculation. {For acacia D_0ED_1 (□), O D_0ED_1 (■), $F(CS)D_0ED_1$ (+), $F(PC)D_0ED_1$ (X), $F(TV)D_0ED_1$ (*) and for eucalyptus D_0ED_1 (△), OD_0ED_1 (▲), $F(CS)D_0ED_1$ (◇), $F(PC)D_0ED_1$ (◆), $F(TV)D_0ED_1$ (⊕)}

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