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Section 1 Biology

Laboratory evaluations of woods from Pakistan and their extractives against *Postia placenta* and *Trametes versicolor*

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ABSTRACT

Natural durable wood species are those which exhibit innate tolerance to wood decay organisms such as fungi and termites. The goal of this study was to evaluate 4 wood species (*Dalbergia sissoo, Cedrus deodara, Morus alba* and *Pinus roxburghii*) from Pakistan in order to determine their resistance to both a model brown (*Postia placenta*) and white (*Trametes versicolor*) rot fungus compared to a durable reference species (*Tectonis grandis*). In a 12 week soil bottle test, *C. deodara* and *M. alba* were found to be resistant to decay, *D. sissoo* was moderately resistant and *P. roxbughii* was non-resistant. This resistance was greatly reduced when blocks were leached with a series of solvents. When a non-durable species was treated with extractives from these species, decay resistance did not improve against either brown or white rot test fungi.

Keywords: naturally durable wood, wood decay fungi, E-10 soil bottle bioassays, extractives

1. INTRODUCTION

Naturally durable woods are groups of tree species that possess characteristics that either slow or impede the activity of biological agents of wood deterioration. Natural durability has been attributed to a wide range of factors including density/specific gravity, hardness, and most notably extractive content (Scheffer 1966). Extractives are non-structural components of the living tree that are often produced in response to wounds or adverse physiological conditions that would otherwise diminish the health of the tree. Extractives are highly variable and have been found to consist of a wide range of chemical compounds such as terpenoids, alkaloids, condensed tannins, and many others (Taylor *et al*. 2002).

A lack of understanding of the mode of action for the various chemical components of wood extractives has hindered the standardization and building code acceptance of naturally durable woods for certain applications such as ground contact (Morris *et al.* 2011). Another confounding factor is that durability varies widely both within and between trees of the same species. In addition, certain wood species (especially hardwood species) with desirable durability often have characteristics that decrease their utility such as growth habit, wood properties, corrosiveness or adhesive bonding (Imamura 1989). Effective extractive utilization is one possible way to overcome these limitations by transferring bio-active compounds from a durable wood to a non-durable wood species (Kirker *et al.* 2013).

The wood species used in this study are commonly available woods of Pakistan and are also being evaluated for potential activity against termites including *Heterotermes indicola*

(Wassman) and *Odentotermes obesus* (Rambur), which are major destructors of residential dwellings in this region (Manzoor and Mir 2010). The wood species evaluated in this study are as follows:

- **Teak (*Tectona grandis* L.)** wood was used as a durable reference in this study as it has a proven track record of service (DaCosta *et al.* 1958, (Class 1 durability-Scheffer and Morrell 1998)) and is also available in this area. Past studies indicate that the durability of teak is largely due to the presence of quinones in the heartwood (Lukmandaru and Takahashi 2009). Recent reports on environmental impacts of plantation teak production indicate that availability is not an absolute (Pandey and Brown 2000), and that durability varies due to location of origin (Haupt *et al.* 2003) and stand management practices (Bhat and Florence 2003). Additionally, teak is considerably expensive and the remainder of the wood species represent more affordable potential alternatives to teak.
- **Dalbergia (*Dalbergia sissoo* Roxb)**, is an important tree in low-lying areas of Pakistan. *D. sissoo* grows wild in ditch banks and roadsides and is an important commercial species for production of furniture and other goods (Sheikh 1989, Tewari 1994a). *D. sissoo* is classified as a class 1 (very resistant) wood species based on previous studies (Scheffer and Morrell 1998). Since 2005, *D. sissoo* stands have been experiencing high mortality due to dieback from several suspected pathogens (Khan *et al.* 2000). Current efforts are underway to identify resistant cultivars for this area, but mortality of *D. sissoo* in this region has been reported as high as 80% in forests and 40-60% of roadside stands (Bajwa *et al.* 2003) indicating high volumes of harvested timber of *D. sissoo* may be currently available. Past studies have indicated that heartwood extractives of *D. sissoo* contain high levels of stilbenes, other polyphenols (Seshadri 1972) and neoflavonoids (Mukerjee *et al.* 1971).
- **Deodar Cedar (*Cedrus deodara* Roxb G. Don.)** is also an important tree species in Pakistan. *C. deodara* is extremely common in the higher elevations of Pakistan and has been a valued wood for centuries. A wide range of chemical compounds from *C. deodara* have been previously studied and determined to have numerous medicinal properties (Chaudhary *et al.* 2011). *C. deodara* is listed as moderately resistant (class III) to decay (Scheffer and Morrell 1998).
- **White Mulberry (*Morus alba* L.)** is native to China, but is widely distributed in Pakistan and throughout the Himalayan region due to its connection with silk production. *M. alba* is listed as an invasive plant in Pakistan (Qureshi *et al.* 2014), as well as North America, as it hybridizes with the native Red Mulberry (*Morus rubra*) and decreases its vigor (Burgess and Husband 2006). Heartwood of *M. alba* is listed as non-resistant (class IV) (Scheffer and Morrell 1998) but has been shown to contain simple phenolics, benzophenones, flavonoids, and other chemical compounds (Rowe and Conner 1979) and has been studied extensively for its nutritional and medicinal properties (Omidirin *et al.* 2012).
- **Chir Pine (*Pinus roxburghii* Sarg.)** is a readily available wood species in Pakistan. It has a wide range of uses including building material, heating and has published medicinal value (Tewari 1994b). Although *P. roxburghii* is listed as non-resistant (class IV) to decay (Scheffer and Morrell 1998) extracts of this species have been found to have medicinal and antibacterial properties and heartwood extractives of *P. roxburghii* have been reported to contain abietic acid, longifolene and low levels of several stilbenes (Shuaib *et al.* 2013).

More efficient utilization of local trees of Pakistan would provide for potential value added products. Extractives could be obtained from mill cull and less desirable parts of the tree that are normally left behind, landfilled or burned. Effective wood utilization is also a prime directive of the US Forest Service in order to remove dead and diseased inventory from otherwise productive managed forest stands.

2. EXPERIMENTAL METHODS

2.1 Wood Material and Preparation of Extractives

Raw lumber of Dalbergia (*Dalbergia sissoo)*, Cedar (*Cedrus deodara)*, Mulberry (*Morus alba)* and Chir Pine (*Pinus roxburghii)* were purchased from a timber market located at Jhang road Faisalabad (Pakistan), and shipped to FPL Starkville, Mississippi (USA). Marine grade Teak (*Tectona grandis*) was acquired from a supplier in the United States (McIlvain, Pittsburg, PA) and used as a durable reference material in this study. Large boards from each species were evened with a planer and cut into 19×19×19 mm blocks. Shavings from this process were used for the preparation of extractives. Air dried wood shavings (12 g) were soxhlet extracted using 300 ml of ethanol: toluene (2:1) as solvent according to ASTM D1105-96 ″Standard Test Method for Preparation of Extractive-Free Wood″ with minor modifications (ASTM 2014). Shavings were added to soxhlets with a small amount of cotton placed below and above to contain the shavings and extracted for a total of six hours. An aliquot was evaporated at reduced pressure by using rotary evaporator (BUCHI, Rotavapor R-114) and extraction yield was calculated per gram of wood shavings (Ordonez *et al.* 2006). The extractives were stored in small vials after preparation of stock solution of 10mg/ml in refrigerator at 4°C. Although origin and growth characteristics were not obtained for these woods, mean extractive content was calculated for each wood species and is presented in Table 1 along with other relevant wood characteristics.

Table 1: General characteristics of the wood species tested in this study *(specific gravity of blocks conditioned at 33°C and 62%RH= SG, EC= percent extractive content by weight).

Species	Origin	SG*	EC[%]*	Type of wood
Tectona grandis	Myanmar, India	0.55-0.66	5.51	Hardwood
Dalbergia sissoo	Indian sub-continent	0.62-0.82	9.11	Hardwood
Cedrus deodara	Himalaya mountains	Unknown	9.67	Softwood
Morus alba	China	0.55-0.69	5.4	Hardwood
Pinus roxburghii	Pakistan, India, Nepal	0.43-0.49	7.40	Softwood

2.2 Preparation of Extractive Free Wood

Heartwood blocks were extracted according to ASTM D1105-96 with the following modifications. Conditioned blocks (33°C, 62±3%) were numbered and weighed prior to being placed in the soxhlets and extracted for six hours using ethanol: toluene (2:1). Blocks were then washed with alcohol to remove excess toluene and secondarily extracted for six hours in ethanol (95%) alone. Ethanol extracted blocks were air dried overnight and then boiled for six hours with 3L of distilled water and water was changed after every hour.

2.3 Extractive transfer methods

Weighed and conditioned (33°C, 62 ± 3% R.H.) southern pine (SP, *Pinus taeda L.*) and cottonwood (CW, *Populus deltoides*) sapwood blocks (19×19×19 mm) were pressure treated with different concentrations (2.5, 5 and 10 mg/ml) of each durable wood extractive. For control treatments, blocks were treated with solvent only (ethanol: toluene) or water. Blocks were pressure treated by placing five blocks in a 300 ml beaker containing the treatment solution in a vacuum- pressure chamber. Vacuum was turned on for 30 minutes and after that pressure was applied at 40 Psi for one hour. After pressure treatment, blocks were dried using paper towel, weighed, and conditioned again at 33°C and 62±3% R.H.

2.4 Laboratory decay bioassays

Two soil bottle experiments were conducted according to AWPA E10-12 (AWPA 2014). In the first experiment southern pine feeder strips were used to start soil bottles with either *Postia placenta* (Fr.) MJ Larsen & Lombard (Mad-698-R) or *Trametes versicolor* L. (Loyd) (Mad-697). Cottonwood blocks (20mm cubes) were treated with extractives from the 5 wood types at 3 rates: 2.5, 5 and 10mg/mL and compared to untreated, water treated and solvent treated controls. In the second experiment, southern pine feeder strips were used to start soil bottles with *P. placenta*, while red maple (*Acer rubrum L*) feeder strips were used to start *T. versicolor* bottles. Un-extracted blocks of the 5 wood species were compared to extracted blocks in assays where hardwoods were challenged with the white rot fungus and softwoods were challenged with the brown rot fungus. Both sets of soil bottle experiments were conducted for 12 weeks at 27°C in a 70% relative humidity (RH) walk-in incubator. Blocks were weighed before and after experiments after conditioning at 27°C and 30% RH and weight loss was calculated. Mean weight loss due to decay was modelled in Minitab in order to determine statistical significance and mean comparisons were made using Tukey's HSD. Durability indices were calculated from each test by comparing mean weight loss of the material to that of the non-durable reference as referenced in EN 350-1 (EN 1994) using the following formula:

$$X^{*} = \frac{average\ weight\ loss\ of\ the\ test\ specimens}{mean\ weight\ loss\ of\ the\ reference\ material}$$

**X=0-0.14 (very durable), 0.15-0.29 (durable), 0.30-0.59 (moderately durable), 0.6-0.89 (slightly durable, 0.9 and greater (not durable).*

3. RESULTS AND DISCUSSION

3.1 Extracted vs. sound blocks and non-durable controls

The first set of laboratory bioassays were to evaluate the effect of extractives on the durability of each of the wood species. Coniferous species were compared to SP control and are presented with statistical results in Table 2. SP control lost 58% due to decay by *P. placenta* while un-extracted Chir Pine and Cedar lost 22% and 9%, respectively. Durability indices (DI) for Chir Pine and Cedar were 0.38 (moderately durable) and 0.15 (very durable), respectively. However, when extractives were leached using the ASTM D1105-96 procedure, weight loss of Chir Pine and Cedar was increased to 35% (DI=0.6, slightly durable) and 49% (DI=0.84, slightly durable), respectively.

Table 2: Average weight loss (Mean) and standard error (SE) of extracted and un-extracted softwood species. Mean groupings of extracted and un-extracted softwood species exposed to *P. placenta* in a 12 week soil bottle assay. Groupings with different letters are statistically different.

Wood type	N	Mean	Grouping	S.E	DI*	CLASS
Control SYP Un -extracted	9	58.29	A	1.05	1.00	ND
Cedar extracted	10	48.94	A B	5.12	0.84	SD
Chir Pine extracted	10	35.07	B C	9.04	0.60	SD
Chir Pine Un-extracted	10	22.28	C D	5.44	0.38	MD
Cedar Un-extracted	10	8.62	D	1.7	0.15	HD

**DI=durability index as calculated in EN350-1 (ND=Non-durable, SD=slightly durable, MD=moderately durable, and HD=highly durable).*

The 3 hardwood species (*Dalbergia*, *Mulberry* and *Teak*) were compared to cottonwood as a non-durable control which lost 48% of its original weight when exposed to *T. versicolor*. Teak and Mulberry were both found to be highly durable compared to the reference material, 3 and 6% WL respectively, and Dalbergia was found to be moderately durable at 17%WL. When extracted, teak was reduced to durable (0.20 DI), Mulberry was moderately durable (0.59 DI) while Dalbergia was only slightly durable (0.67 DI) with extractives removed.

Table 3: Average weight loss (Mean) and standard error (SE) of extracted and un-extracted hardwood species. Mean groupings of extracted and un-extracted softwood species exposed to *T. versicolor* in a 12 week soil bottle assay. Groupings with different letters are statistically different.

Wood type	**N**	**Mean**	**Grouping**	**S.E**	**DI***	**CLASS**
Control CW un extracted	10	47.607	A	1.85	1	ND
Dalbergia extracted	10	31.738	B	3.5	0.67	SD
Mulberry extracted	5	28.095	B	1.35	0.59	MD
Dalbergia un extracted	10	16.974	C	2.59	0.36	MD
Teak extracted	10	9.532	C D	0.339	0.20	D
Mulberry un extracted	10	6.534	D	0.604	0.14	HD
Teak un extracted	10	2.908	D	0.164	0.06	HD

**DI=durability index as calculated in EN350-1 (ND=Non-durable, SD=slightly durable, MD=moderately durable, D=durable and HD=highly durable).* CW=cottonwood (*Populus deltoides*).

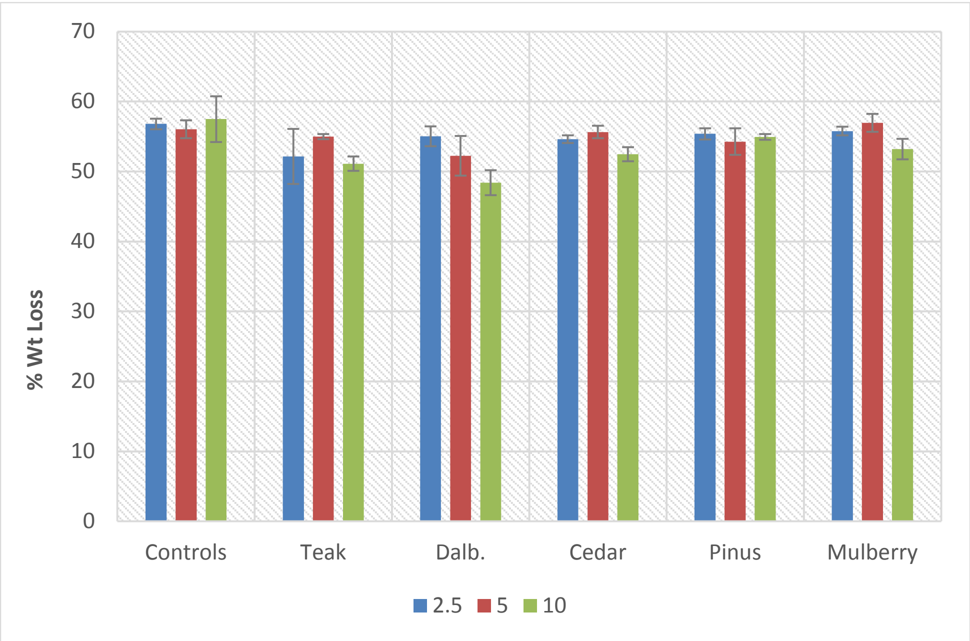

Figure 1: Mean weight loss of softwood blocks treated with extractives of Pakistani wood species at 2.5, 5 and 10 mg/ml exposed to *Postia placenta* for 12 weeks in a soil bottle.

3.2 Treatment of wood with extractives

The results from these experiments indicate that in our standard soil bottle assay at these rates, none of the extractives tested improved the decay resistance of SP against *P. placenta* (Fig. 1). Results of Tukey's HSD (not shown) indicated that means were not different between any of the treatments. The durable reference (teak) did not show any improvement either when used to treat southern pine test blocks, suggesting that the current dosage of these extractives is below the required threshold or not effective against these strains of test wood decay fungi in the standard soil bottle assay.

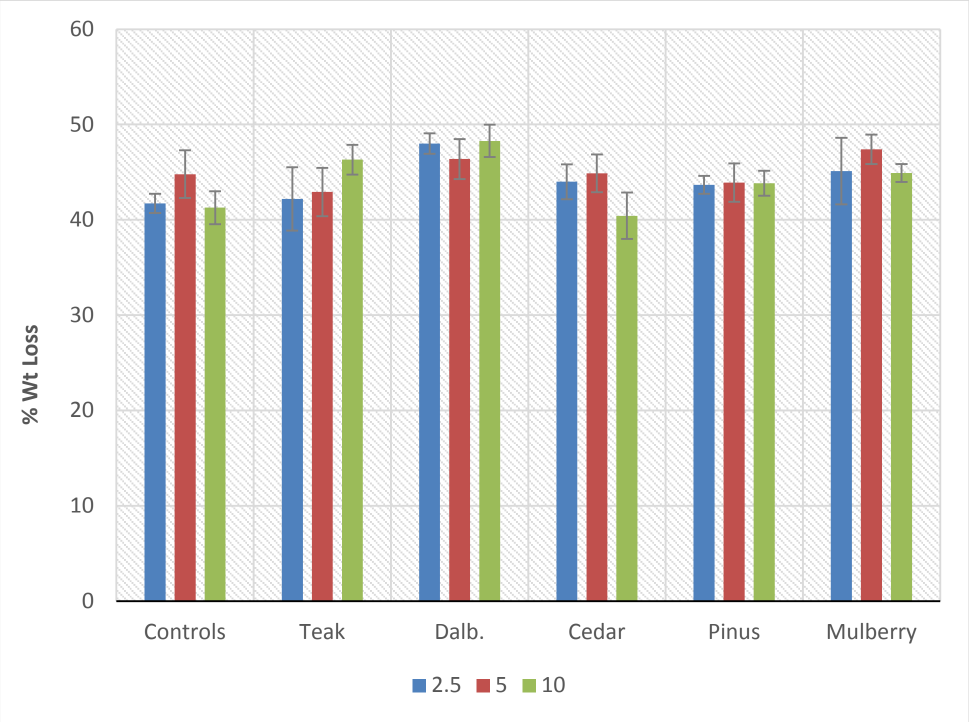

Figure 2: Mean weight loss of hardwood blocks treated with extractives of Pakistani wood species at 2.5, 5 and 10 mg/ml exposed to *Trametes versicolor* for 12 weeks in a soil bottle.

Similarly, none of the extractives tested produced any statistically significant weight losses at the dosages used in this experiment when exposed to *T. versicolor* (Fig 2). Results of Tukey's HSD (not shown) indicated that means were not different between any of the treatments. Again, the durable reference (teak) did not show any improvement either, suggesting that the current dosage of these extractives is below the required threshold or not affective against these strains of test wood decay fungi in the standard soil bottle assay.

3.3 Discussion

The role of extractives is not fully understood for these and many other species. Chemical components of each species are being determined by GC-MS and will be presented elsewhere (See Mankowski *et al*. 2016) in the program. Spatial distribution of extractives may be an important factor in the inherent durability of these woods. They are usually patchy in the native wood (Hillis 1971, Taylor *et al.* 2002) vs. uniform in our "manufactured" durable woods. The E-10 soil bottle is an ideal test for wood preservatives in soil exposure to monocultured wood decay fungi but may represent too harsh an environment for realistic testing of less stringent treatments. Field specimens with the same concentrations have been installed in Saucier, MS and Lahore, Pakistan in order to compare our laboratory results to field performance. At these concentrations, these extractives work well in choice and no-choice feeding bioassays against subterranean termites (*R. flavipes*) (unpublished data) and matched sets against *H. indicola* are underway and well as assays of protozoan mortality and antioxidant activity. Laboratory results from the termite bioassays will be presented elsewhere at this meeting by Babar Hassan.

4. CONCLUSIONS

Results of our laboratory tests were consistent with the available literature for each wood species tested. At the rates evaluated in these tests, extractives of Teak, Dalbergia, Cedar, White mulberry and Chir Pine do not appear to have any protective effects on non-durable wood species, such as southern pine and cottonwood, against these wood decay fungi in an E-10 soil bottle assay. Ongoing field studies will be evaluated in order to correlate laboratory performance with field performance in above and below ground contact.

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