VOLUME 9

Material Properties of Bamboo

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Preface

Robert Gretton
Chair

Bamboo is a fast growing plant with many advantages, both in its cultivation and in the physical attributes of its timber. It is used as an alternative to wood in many applications, and has further potential as a substitute for wood and other raw materials.

Modern construction methods, design, and industrial uses require certainty that the physical parameters of materials are well understood. The durability of materials is often a key parameter.

The first paper presented in Session 9 takes a detailed look at the anatomical and chemical properties of *Bambusa vulgaris* from three sites in Ghana, whilst the second looks at developing improved methods of identification of superior genetic material as an aid to exploitation of their superior traits. This work focused on *Bambusa balcooa.*

Papers three to six look at methods of enhancing the durability and characteristics of bamboo timber with a variety of chemicals and treatments. Paper three looks at heat treatments and consequent changes in physical and mechanical properties. Paper four focuses on improving durability with chemical and botanical treatments, and paper five looks at the performance of *Dendrocalamus strictus* treated with a combination of fire retardants and preservatives. Paper six documents experiments using environmentally-friendly chemicals to enhance resistance to moulds.

Identification of Superior Fiber-Trait-Yielding Genetic Resources of *Bambusa balcooa*: Analysis of Physico-Chemical Properties of Fibers

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Abstract

Rapid depletion of forest trees has considerably reduced the availability of wood fibers for paper and pulp industries, while bamboo fibers have replenished the demand partially. Fibers of *Bambusa balcooa* was preferred among non-wood raw materials for paper and pulp making primarily because of its mechanical strength attributable to the high specific gravity. Therefore, an attempt was undertaken to identify superior fiber yielding genetic resources of *B. balcooa*. Extensive analysis of physical (slenderness ratio, Runkel ratio, flexibility coefficient) and chemical (α-cellulose, lignin) characteristics of fibers among 7500 samples from 12 distant locations revealed accessions of two locations exhibiting unambiguous superiority. A SCAR marker ($Balco_{1128}$, DQ9005800) has been identified that discriminates the elite genotype from the others. Furthermore, presence of an open reading frame within the marker sequence was found in these elite genotypes and also in other bamboo species that yield fibers suitable for use in paper and pulp industry. This strategy of molecular differentiation of elite genotypes within a species could advance the efficient commercial utilization of the available superior germplasm resources.

Introduction

The indiscriminate exploitation of forest resources at global scale has considerably reduced the availability of the wood fibers for paper and pulp production (Ganapathy 1997). Consequently, the non-wood fiber resources are gaining increased attention to fulfill the ever-increasing gap between the demand and the supply in an environmentally sustainable manner. Bamboos constitute a major non-wood forest-fiber source for the paper and pulp production worldwide and a number of species have been identified as more useful (Ganapathy 1997). For instance, *Bambusa balcooa* Roxb. is preferred due to its mechanical strength, attributable to the high specific gravity and long fibers (Kabir et al. 1991; Bhatt et al. 2003). However, the physical and chemical fiber characteristics determine the suitability of traits to produce superior quality paper/pulp at low costs.

B. balcooa is native to North-East India and distributed across the states of Nagaland, Meghalaya, Tripura, Assam, West-Bengal, Bihar and Eastern Uttar Pradesh (Seethalakshmi and Kumar 1998). It is also cultivated in many other Asian countries, tropical Africa and Australia (Ohrnberger 2002). The rich local vegetation of *B. balcooa* prompted us to make an attempt towards selection of elite genotype/s across the available natural genetic resources with respect to their fiber characteristics that are directly correlated with their downstream processing to produce better quality paper and pulp at the same time maintaining the economic and environmental benefits protected.

During this investigation two natural genotypic-resources of *B. balcooa* with superior physico-chemical fiber characteristics were identified that led further studies to develop a molecular marker through which such elite genotype could be discriminated for efficient commercial utilization. A sequence characterized amplified region (SCAR) marker has been developed and was amplified from all the accessions of *B. balcooa* collected from two locations (Bb3 and Bb4) that generated superior fibers, but not from rest of the locations surveyed. In woody bamboo, lack of regular sexual events limits the study on QTL and application of breeding based segregation to test the genetic linkage between a marker and the particular trait of interest; therefore, allelic polymorphisms based selection has been adapted.

Development and judicious application of modern molecular technologies in assessment and enhancement of available forest genetic resources for their successful exploitation have often been emphasized (Burley, 2001). In the past, molecular markers have been successfully employed to study population level genetic diversity in bamboos (Suyama et al. 2000; Isagi et al. 2004; Shrestha et al. 2005; Das et al. 2008). However, their further practical application to identify commercially desirable, elite accessions/genotypes are not available in the literature. This could only be achieved when a pool of morphologically and/or chemically well-characterized bamboo accessions would be available for further characterization by genotyping.

The present study was aimed to define a strategy using selected fiber characteristics as the reference to test if molecular markers could be utilized for elite genotype screening in a plant group that predominately lacks a regular sexual event. Attempts were made to search for the presence/absence of the marker in superior/inferior fiber-yielding bamboo species/genotypes, in order to confirm the unambiguous association of the marker with better fiber yielding genotypes.

This strategy of marker-assisted molecular differentiation of elite genotypes within a species could advance the efficient commercial utilization of the available superior germplasm resources.

Materials and Methods

Plant materials

Stands of *Bambusa balcooa* Roxb. growing in wild habitats across 12 distant locations of West-Bengal, India were surveyed (Table 1). Each of these locations was represented by five randomly selected clumps (culms growing together). Five adult culms from each clump (altogether 25 accessions) of all the selected locations were independently sampled (25x12= 300 samples) for fiber isolation. The middle segments of the 5th

internodes of the secondary branches were used as the starting biological samples, since it represents the average length of all the internodes in a branch (Ververis et al. 2004).

Isolation of fibers from the internodes of Bambusa balcooa

Small slivers prepared by macerating internodes with 10 ml of 67% nitric acid, boiled in a water bath for 10 min and subsequently washed in distilled water (Ogbonnaya et al. 1997). Individual fibers were mechanically isolated from the macerated fiber bundles by using a plastic churner. Care was taken to avoid breakage of intact fibers to facilitate measurement of total length. The fibers were then stained with methylene blue solution (0.5%) and glycerine (1:1 v/v) prior to study in light microscope (Han et al. 1999).

Measurement of physical characteristics of fibers

Measurements of fiber length and diameter; cell wall thickness and lumen diameter (fiber

diameter- cell wall thickness) for 25 fibers, independently isolated from each internode were taken. Therefore altogether 625 individual fiber (25 replications X 5 culms X 5 clumps) from each of 12 locations (25 fibers/accession; 25 accessions/location = 25X25X12= 7500 samples) were characterized by 3 physical parameters. Slenderness ratio, flexibility coefficient and Runkel ratio were the three physical characteristics studied to evaluate the quality of the fibers and their suitability to be used as raw material for paper and pulp production. Equations used to derive these physical traits were as follows: slenderness ratio=fiber length/fiber diameter, flexibility coefficient= (fiber lumen diameter/ fiber diameter) X100 and Runkel ratio=2X fiber cell wall thickness /lumen diameter (Ogbonnaya et al. 1997, Saikia et al.1997).

Estimation of α-cellulose and lignin contents from B. balcooa accessions

Cellulose and acid insoluble lignin contents are the two important chemical indicators for fiber strength and durability. The α-cellulose content was estimated following the colorimetric method based on anthrone reagent (Updegraff 1969). One gram of internodal tissues were ground, mixed with 3 ml of nitric acid and acetic acid solution (1:8 v/v) and refluxed in boiling water for 30 min. Lignin, hemicellulose and xylosans were removed through successive washing followed by centrifugation. The resultant pellet was dissolved in 67% sulphuric acid (v/v), mixed with chilled anthrone reagent (Merck, Germany), incubated for 20 min in boiling water followed by a quick chilling on ice and then kept at room temperature for 10 min prior to assay at 620 nm in a spectrophotometer (Beckman-Coulter, DU-520). The α-cellulose content was quantified based on the obtained OD readings and the standard curve prepared with known concentrations of authentic sample and was expressed as gram percentage of the fiber dry weight. Acid insoluble lignin content (Klason) was determined according to the standard ASTM D-1106- 96 protocol (ASTM, 1996).

Statistical analysis of the fiber characteristics

One-way analysis of variance (ANOVA, $P < 0.05$) for each fiber characteristics (three physical and two chemical) were performed separately using SPSS 10.0 statistical software (SPSS Inc., USA) by applying accessions as the source of variations. Duncan's multiple range test (DMRT) was performed to analyze the

accession proximity with respect to each fiber characteristics. SPSS 10.0 software was employed to analyze the linear correlation coefficient (r) between cellulose and lignin contents from fibers of *B. balcooa* samples from different locations.

Sampling of plant materials

Healthy leaves representing 5 accessions of *B. balcooa* from each locations were collected for genomic DNA isolation (Table 1). Five randomly selected accessions from each location were sampled to study genetic variability among these accessions. In addition leaves from 19 other bamboo species: *Bambusa affinis* Munro, *B. atra* Lindl., *B. auriculata* Kurz., *B. bambos* Voss, *B. burmanica* Gamble, *B. multiplex* 'Riviereorum' R. Maire, *B. multiplex* 'Variegata' R. Maire, *B. oliveriana* Gamble, *B. nutans* Wall. ex Munro, *B. polymorpha* Munro, *B. striata* Lodd. ex Wendl., *B. tulda*, Roxb., *B. vulgaris* Schrad. ex Wendl., *B. wamin* Camus, *Dendrocalamus giganteus* Munro, *D. strictus* (Roxb.) Nees, *Gigantochloa atroviolacea* Widjaja, *Oxytenanthera abyssinica* (A. Rich) Munro and *Pseudobambusa kurzii* (Munro) Ohrnberger were also collected from the germplasms stock maintained at the Botanical Survey of India, Howrah, West Bengal, India.

PCR-compatible genomic DNA isolation and authentication of B. balcooa using species- specific marker

Surface sterilized bamboo leaf tissues weighing 0.1 gm, were sliced into small pieces and homogenized in liquid nitrogen using mortar and pestle. DNA was extracted using 2.5 ml warm CTAB extraction buffer following the method of Doyle and Doyle (1987). After removal of RNA by RNaseA (Sigma, USA) treatment, the concentrations of DNA samples were estimated by using an UV spectrometer (Beckman-Coulter, DU-520) and purity of DNA was checked at A260 and A280.

Plant samples were initially identified as *B. balcooa* based on vegetative characters and subsequently authenticated by the presence of species-specific molecular marker, $Balco_{836}$ (Accession no. AY653073, Das et al. 2005).

Cloning and sequencing of the polymorphic DNA fragment

During an investigation on genotypic diversity among 25 different bamboo species, we found a polymorphic, genotype-specific DNA fragment in *B. balcooa*, which is neither present in other bamboo species nor in all bamboo accessions studied during this investigation (Das et al. 2008).

Approximately 1150bp polymorphic DNA fragment was excised from 1.5% agarose gel and purified by using the MinElute Gel Extraction kit (QIAGEN, USA). The eluted fragment was ligated into pGEM-T Easy Vector (Promega, USA), transformed into competent *Escherichia coli* strain DH5 α and the plasmid DNA was purified from the unambiguous white colonies according to Sambrook *et al.* (1989). Colony screening was performed by PCR amplification and the size of the insert was confirmed by *Eco*RI (Roche, Germany) restriction digestion before sequencing the fragment in ABI Prism 3100 automated DNA sequencer.

Marker specificity assay of PCR products by hybridization analysis

The PCR products of the random decamer primer from the genomic DNA of 20 bamboo species and representative accessions of *B. balcooa* from 12 locations were transferred to two positively charged nylon membranes (Roche) and probed with αP^{32} labelled *Eco*RI excised $Balco_{1128}$ marker. Probe labelling was performed using the Prime-a-gene labelling kit (Promega). Approximately 25 ng of probe DNA was labelled with αP32 dCTP (BARC, India). Transfer of DNA to a nylon membrane, UV cross-linking, prehybridization and hybridization at 68°C followed by repeated washing for min each at 25°C, 55°C and 65°C were performed following the methods of Sambrook et al. (1989). Signals were detected by exposing the membranes to X-ray film (Kodak). The marker fragment was used as a positive control. The objective of the first hybridization experiment was to check the species specificity of the $Balco_{1128}$ marker, and that of the second experiment was to check the genotype specificity of the $Balco_{1128}$ marker as well as to validate the homology of the co-migrating marker band amplified from respective samples of *B. balcooa* collected from 2 specific locations, Bb3 and Bb4.

DNA hybridization analysis was carried-out to authenticate the genotype specificity of the polymorphic fragment following the methods adapted by us (Das et al. 2005).

Designing SCAR primers and amplification of target sequence

The 20 bp long primer pairs (Bb_{1128}F 5'-ACCCCCGAAGATCAGAACCA-3' and Bb_{1128}R 5' - ACCCCCGAAGCCTTAGTGTT-3') were designed from the marker sequence. The genomic DNAs extracted from representative accessions of *B. balcooa* from 12 locations and 19 additional bamboo species were screened with this primer pair. PCR amplifications were carried out with Perkin Elmer Cetus 2400 thermal cycler in 50 µl reaction mixture containing 100 ng of genomic DNA; 1.0 µM of primer; 1X PCR buffer comprising of 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$; 250 µM of each dNTPs and 1unit of Taq polymerase (Bangalore Genei, India). Amplification cycle started with 4 min denaturation at 95°C followed by 35 amplification cycles with 45 s denaturation at 94°C, 45 s primer annealing at 62°C, 1 min elongation at 72°C and finally 10 min at 72°C for elongation of PCR products.

Results

Study of physical and chemical characteristics to evaluate fiber quality

In the current investigation, fibers of *B. balcooa* from each location were characterized by 3 physical (slenderness ratio, Runkel ratio, flexibility coefficient) and 2 chemical (α-cellulose, acid insoluble lignin content) parameters. Standardized maceration technique proved useful in isolating intact, individual fibers from the internodal tissues of secondary branches of adult bamboo culms for the measurement of fiber length and diameter; cell wall thickness and lumen diameter. To minimize plant to plant variability due to variable developmental stages, sampling was done from the 5^{th} internodes (Ververis et al. 2004).

The ANOVA revealed that the slenderness ratio was significantly higher ($P<0.05$) for the fibers isolated from locations Bb3 and Bb4 than that of the other locations studied (Table 2). On the contrary, Runkel ratio was

found lowest in Bb3 followed by Bb4, while highest in Bb5. Flexibility coefficient was highest in Bb3 followed by Bb4 and Bb11 (Table 2).The α-cellulose content was highest in the Bb3 and Bb4 (Table 2) and lowest in Bb2. The lignin content was lowest in Bb4 followed by Bb3, while relatively higher in samples of Bb5, Bb6 and Bb7 locations.

Study of genetic variability to identify B. balcooa genotype-specific marker

Species authentication for *B. balcooa* was confirmed by detecting species-specific SCAR marker $Balco_{836}$ (AY653073). A subsequent genotyping revealed presence of an approximately 1150 bp polymorphic DNA fragment in all the accessions of Bb3 and Bb4 locations (Fig. 1a).

Homology assay of PCR products with marker by hybridization analysis

The cloned marker fragment (1128 bp) was probed to the blot containing PCR amplified DNA from the representative samples of *B. balcooa* from 12 locations. Unambiguous hybridization signals were obtained only from the representatives of Bb3 and Bb4 along with the positive control (Fig. 1b). The absence of any signal in the remaining samples from 10 other locations excluded the possibility of presence of the marker, undetected by ethidium bromide staining, or its homologous sequence in other genotypes studied. The marker fragment was successfully cloned into pGEM-T Easy vector and sequenced (Fig. 2).

Genotype-specific SCAR marker development

The SCAR primer pair, designed from the 1128bp marker sequence (Fig. 2), was employed to screen 20 bamboo species (Fig. 3a) and 60 samples of *B. balcooa* from 12 locations. A single, distinct and brightly resolved band of similar size was observed only in samples from Bb3 and Bb4,, but absent in all other bamboo species (Fig. 3b). This genotype-specific SCAR marker was designated as $Balco_{1128}$ and submitted to NCBI GenBank, Accession. DQ900580.

Discussion

Physical and chemical characteristics to evaluate fiber quality

Slenderness ratio, Runkel ratio and flexibility coefficient are the three essential physical parameters to evaluate fiber quality. Slenderness ratio or felting power of the fiber is the physical indicator for the durability of the product (Rydholm 1965); whereas, fibers with low Runkel ratio (<1.0) are preferred for producing high quality papers (Saikia 1997). The magnitude of fiber strength is usually proportional to the flexibility coefficient.

Fibers collected from different samples of Bb3 and Bb4 locations were predicted to be more durable by having higher slenderness ratio while, samples of other locations, except for Bb2 and Bb5, yield fibers that have preferred Runkel ratio for pulp and paper production. Thus, evaluation of these three key physical characteristics indicates that the members of the Bb3 possess best fiber quality followed by Bb4.

Estimation of the chemical characteristics is equally important for fiber quality assessment. It was found that non-wood source could be pulped in one-third time than that of the woody counterparts due to their lower lignin contents (Ververis *et al.* 2004). Additionally, pulping from low lignin plant materials reduces 30% costs for chemicals and power consumption (Young 1997) and thus preferred by the industries. In contrast, higher cellulose content ensures improved fiber strength. Hu *et al*. (1999) have demonstrated that lignin and cellulose contents could be regulated in a compensatory manner. Expression of *Pt4CL1* gene in transgenic *Populus* resulted in 45% reduction in lignin content, and 15% increase in cellulose content. In the present study, a natural compensation with reduced lignin (38% decrease from the average value of all samples assayed) and higher cellulose content (10% increase) in the Bb4 was evident and supported by a high negative correlation (-0.645) between the cellulose and lignin content (data not shown). The correlated physical and chemical characteristics of the fibers derived from accessions of Bb3 and Bb4 unambiguously demonstrated their suitability for superior paper and pulp production and also indicates cost efficacy. The combined use of the aforesaid parameters could reliably be extrapolated to other non-wood timbers to evaluate fiber quality.

Development of a molecular marker for the screening of superior genotype

Bamboos, despite being an economically important plant group, has been neglected over the years to study genetic diversity within a species by developing molecular marker/s associated with important traits like fiber quality, disease resistance etc. SCAR markers have been applied to the woody trees for diverse purposes that include identification of olive varieties (Hernandez *et al.* 2001); sex determination in papaya (Deputy *et al*. 2002) and Salix (Gunter *et al.* 2003); mildew resistance in apple (Evans and James 2003); species identification in bamboo (Das *et al*. 2005) and in *Pinus* (Mehes *et al*, 2007). Previous studies have reported intra-species phenotypic variations for many bamboo species that include *B. tulda, B. pallida* and *D. hamiltonii* (Kondas 1982; Soderstrom and Young 1983; Kochhar *et al.* 1990). However, no further efforts have been made to associate these phenotypes with genomic variations. Here we report the identification of a SCAR marker, $Balco_{1128}$ (Accession No. DQ900580) that has been successfully employed for identifying *B. balcooa* genotypes possessing superior fiber quality. SCAR markers linked to a major QTL for high fiber strength have been developed in upland cotton (Guo *et al*, 2003). However, lack of regular sexual events, unavailability of a mapping population and scarcity of sufficient genomic information in woody bamboos limit the application of breeding based segregation methods. In bamboo, due to long sexual cycle, it is almost impossible to test the genetic linkage between the identified marker with any superior fiber trait that unambiguously associated with the elite genotype/s. However, molecular evidence coupled with physical and chemical fiber traits possibly indicate that the superior fiber quality is not due to chance rather than genotypic difference.

In silico analyses of $Balco_{1128}$ sequence revealed presence of a putative 375bp ORF fragment (Bb.LK, GenBank Accession no. EU258678) and a 552bp promoter like upstream region. This finding further indicates a possible functional role of Bb.LK in yielding better fibers. Very high sequence homologies were obtained for the predicted ORF fragment with a putative *Oryza sativa* cv. *japonica* protein kinase (AAP13008, E-value=$2e^{-40}$) and a putative *Arabidopsis thaliana* protein kinase (NP171917, E-value=$3e^{-20}$). The conserved domain database search (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) revealed leucine-rich repeat (LRR) protein domain (COG4886) within Bb.LK sequence (E-value: 0.00002). The presence of conserved LRR regions at the C-terminal end and predicted localization of a homologous sequence in chromosome 3 of *Oryza sativa* cv.

japonica through GRAMENE search result (http://www.gramene.org/multi/blastview) suggest a possible functional role of the Bb.LK sequence. Receptor like protein kinases (RLKs) with LRR plays important roles in diverse processes of plant development (Dievart and Clark, 2004; Torri, 2004). A RLK gene (*GhRLK1*) from cotton has been reported to perform a crucial role in cotton fiber development (Li *et al.*, 2005).

Conclusion

The application of molecular marker techniques in plants to select superior traits has the advantage over the traditional phenotypic markers since they are not environmentally regulated. Moreover, molecular markers linked to a trait are unaffected by the ecological conditions and are detectable in all stages of plant growth and development (Mohan *et al*, 1997). This strategy could also be extended for marker-aided screening of other plant species for selecting elite genetic resources and their subsequent characterization for judicious commercial utilization.

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Table 1. Principal geographical features of the collection sites

Location code	Collection number*	Place of collection†	Latitude	Longitude	Altitude (m)	Max. Temp. (°C)	Min. Temp. (°C)	Average annual rainfall (mm)	Soil Type
Bb1	SB/SIB/02/016	Sibpur	22° 34' N	88° 19' E	10.0	30.7	19.8	1633.6	New Deltic Alluvial
Bb2	SB/SAR/02/018	Saradapally	22° 56' N	88° 11' E	12.0	35.5	16.6	1555.0	Old Alluvial
Bb3	SB/BHA/03/023	Bhadreswar	22° 49' N	88° 21' E	11.6	36.0	17.5	1523.0	New Deltic Alluvial
Bb4	SB/DRA/03/025	Dighra 1	22° 54' N	88° 26' E	11.0	36.0	17.2	1542.0	New Deltic Alluvial
Bb5	SB/DGA/03/037	Dighra 2	22° 54' N	88° 06' E	11.0	36.5	17.5	1510.0	Old Alluvial
Bb6	SB/SRE/O3/046	Srerampur	22° 53' N	88° 24' E	14.2	35.0	18.0	1580.0	New Deltic Alluvial
Bb7	SB/SIN/03/056	Singur	22° 48' N	88° 13' E	13.0	36.0	16.8	1445.0	Old Alluvial
Bb8	SB/MEM/03/067	Memari	23° 11' N	88° 7' E	24.0	36.0	13.2	1621.0	Old Alluvial
Bb9	SB/MAN/04/082	Mankundu	22° 55' N	88° 24' E	18.0	36.5	17.0	1515.0	New Deltic Alluvial
Bb10	SB/RIS/04/087	Rishrah	22° 47' N	88° 22' E	13.8	35.8	17.8	1480.0	New Deltic Alluvial
Bb11	SB/DHI/04/094	Dhitara	22° 55' N	88° 18' E	12.0	36.0	17.2	1605.0	New Deltic Alluvial
Bb12	SB/CHU/04/104	Chuchura	22° 58' N	88° 25' E	18.0	35.0	16.0	1595.0	New Deltic Alluvial

* Collection number of representative accession

† Locations under different districts of West-Bengal, India

Table 2. A comparative account of physical and chemical characteristics of *Bambusa balcooa* fibers collected from 12 locations

Location code	Slenderness Ratio*	Runkle Ratio*	Flexibility Coefficient*	Cellulose content (%dry wt)*	Lignin content (%dry wt)*
Bb1	184.96 (32.66)^{c}	0.92 (0.33)^{bc}	54.16 (11.48)^{d}	58.98 (0.88)^{bc}	26.43 (0.86)^{b}
Bb2	187.95 (35.15)^{c}	1.02 (0.25)^{a}	50.83 (7.47)^{f}	51.72 (1.19)^{f}	25.13 (0.40)^{c}
Bb3	243.93 (32.08)^{a}	0.69 (0.08)^{f}	60.00 (6.09)^{a}	66.25 (1.15)^{a}	20.23 (0.75)^{e}
Bb4	212.99 (41.54)^{b}	0.80 (0.19)^{e}	57.24 (9.71)^{b}	64.53 (1.15)^{a}	17.97 (0.21)^{f}
Bb5	186.09 (34.60)^{c}	1.05 (0.30)^{a}	50.43 (8.24)^{f}	56.12 (1.19)^{d}	27.93 (0.40)^{a}
Bb6	187.31 (32.06)^{c}	0.85 (0.23)^{d}	55.64 (8.45)^{c}	59.75 (0.33)^{bc}	27.60 (0.89)^{a}
Bb7	187.32 (34.08)^{c}	0.96 (0.23)^{b}	52.35 (7.60)^{e}	56.12 (1.19)^{d}	27.83 (0.32)^{a}
Bb8	187.75 (36.97)^{c}	0.92 (0.32)^{bc}	54.22 (10.18)^{d}	58.03 (0.33)^{c}	25.90 (0.20)^{bc}
Bb9	184.43 (30.56)^{c}	0.89 (0.30)^{c}	54.70 (9.06)^{cd}	54.59 (0.33)^{de}	25.73 (0.25)^{bc}
Bb10	185.27 (30.18)^{c}	0.90 (0.31)^{c}	54.48 (9.24)^{d}	53.63 (0.99)^{e}	25.83 (0.40)^{bc}
Bb11	184.52 (51.55)^{c}	0.83 (0.30)^{de}	57.14 (11.87)^{b}	59.94 (1.15)^{b}	23.07 (0.15)^{d}
Bb12	185.15 (28.72)^{c}	0.89 (0.30)^{c}	55.02 (9.81)^{cd}	53.63 (1.52)^{e}	23.87 (0.35)^{d}

*Values within parenthesis represent ±SE; same superscript letters within a column do not differ significantly ($P < 0.05$) according to Duncan Multiple Range Test.

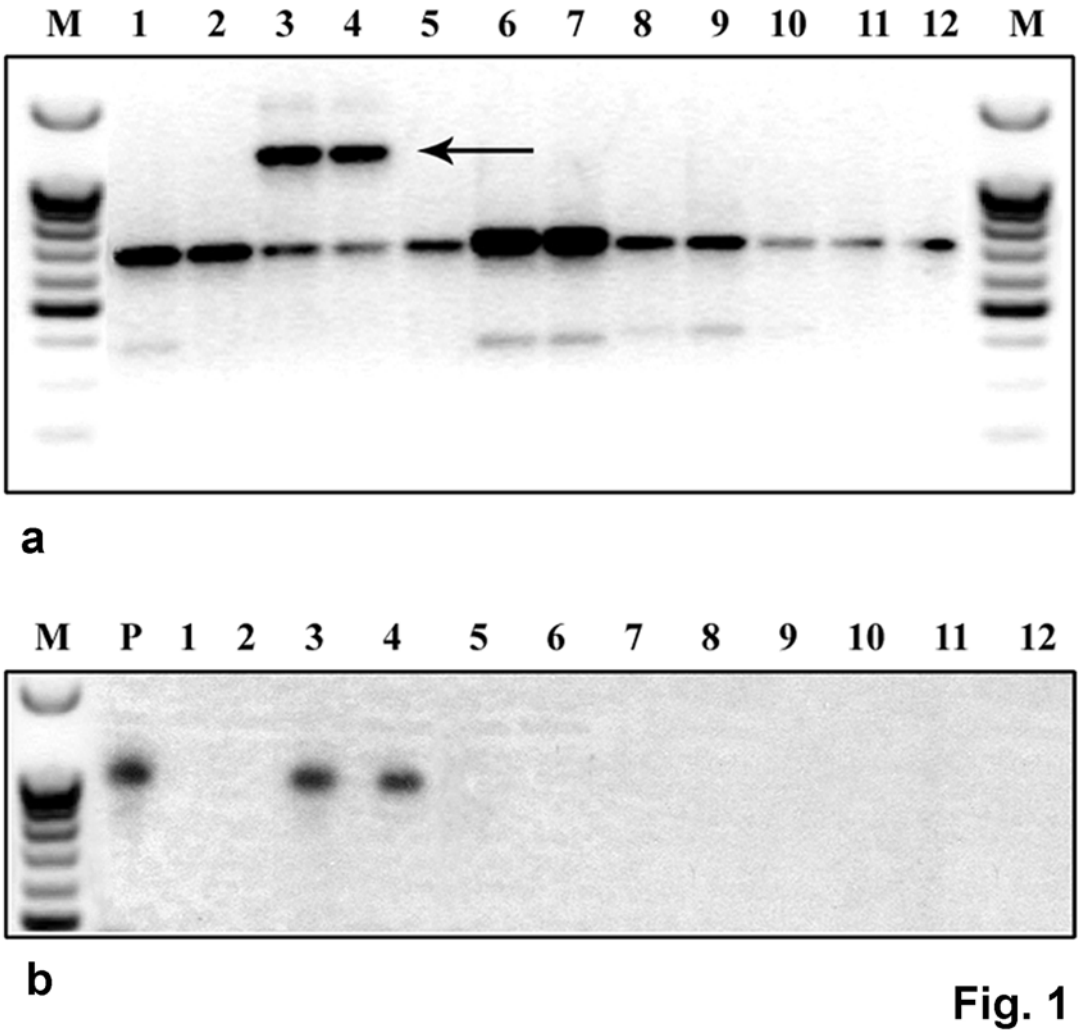

Fig. 1a. Electrophorogram showing PCR amplification products of representative members of *B. balcooa* collected from 12 locations. Lane number represents location in the same order as depicted in Table 1. M= molecular marker (1.5 Kb + 100 bp ladder). The arrow indicates presence of polymorphic DNA fragment (~1150 bp) only in Bb3 and Bb4.

Fig. 1b. Hybridization of ~1150 bp marker fragment with the blot of agarose gel containing PCR amplified products of DNA from representative *B. balcooa* samples collected from12 locations.The hybridization signals of target fragment are present only in Bb3, Bb4 and in the positive control (P).

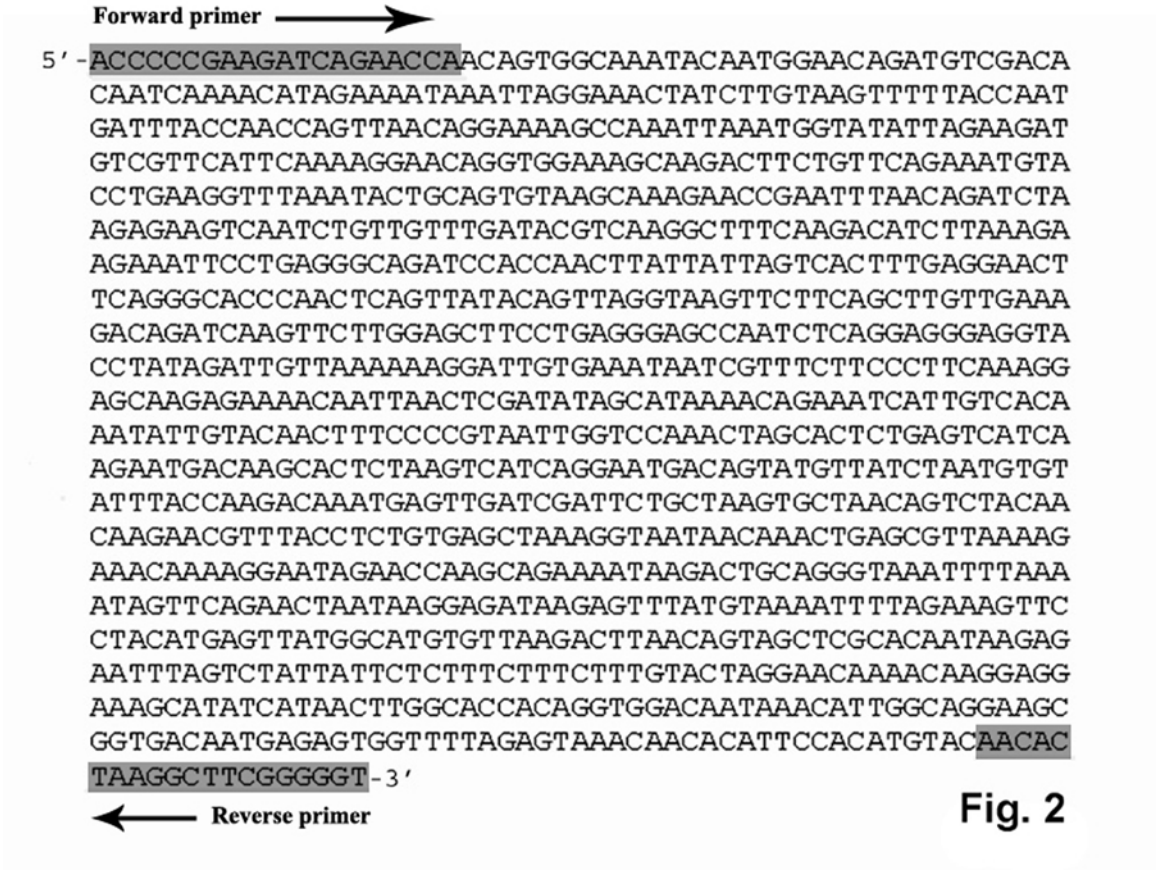

Fig. 2. Nucleotide sequence of the polymorphic DNA fragment showing the positions of SCAR primers, forward primer 5′ to 3′ and reverse primer complementary to 3′-5′ direction as indicated.

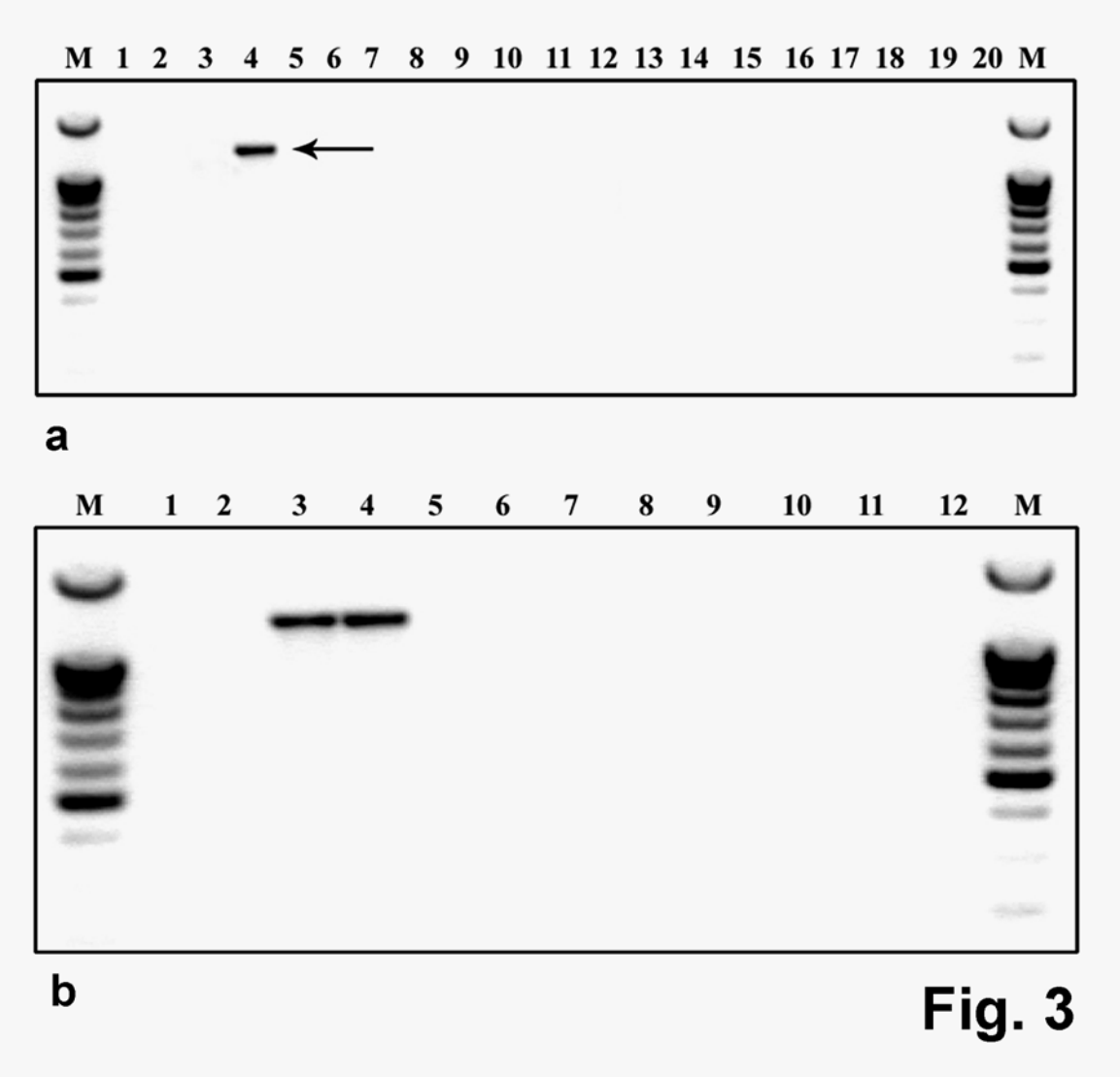

Fig. 3a. Electrophorogram of agarose gel (1.5%) that shows the amplification product only from the genomic DNA of *B. balcooa* using the SCAR primer pair, $Bb_{1128}F$ and $Bb_{1128}R$: Lane 1, *Bambusa bambos;* 2, *B. atra;* 3, *B.auriculata;* 4, *B. balcooa* (Bb4); 5, *B. burmanica;* 6, *B. multiplex* 'Riviereorum'; 7, *B.multiplex* 'Variegata'; 8, *B. nutans;* 9, *B. oliverina;* 10, *B. polymorpha;* 11, *B. striata;* 12, *B. tulda;* 13, *B. vulgaris;* 14, *B. wamin;* 15, *B. affinis;* 16, *Dendrocalamus giganteus;* 17, *D. strictus;* Lane 18, *Gigantochloa atroviolacea;* 19, *Oxytenanthera abyssinica;* 20, *Pseudobambusa kurzii;* the arrow indicates migration of 1128bp marker fragment. M, molecular marker (1.5 Kb + 100 bp ladder).

Fig. 3b. Representative electrophorogram of agarose gel (1.5%) showing amplification products only from single representative of Bb3 and Bb4 of *B. balcooa* using $Bb_{1128}F$ and $Bb_{1128}R$ SCAR-primer-pair, while no amplification products obtained from other 10 accessions. M= molecular marker (1.5 Kb + 100 bp ladder).

The Anatomical and Chemical Properties of *Bambusa Vulgaris* from Three Sites in Ghana

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Abstract

Most of the existing research on the anatomy of bamboo species in Ghana and most West African countries have centered almost entirely on the gross structure of bamboo species. Limited information exists on their microstructure structures and dimensions. This has rendered researchers and industrialists almost handicapped to fully understand and utilize the fascinating native bamboo species. The knowledge of the durability of bamboo culms in natural stand is low. In this present study, the ultramicrostructural characteristics of the predominant bamboo species in southern Ghana- *Bambusa vulgaris var. vulgaris and B. var. vittata* from three major bamboo sites were undertaken. Results revealed range in fibre bundle width from 311-506µm, the diameter of metaxylem vessels of 128-196 µm. The vascular were basically bundle types iii and iv with fibre strands of the heart-blocked and arc-like type. A strange inclusion of parenchyma close to fibre bundle was identified in sample from Assin Fosu. Further, preliminary phytochemical screening also shows the absence of alkaloids-an important decay resistant indicator but the presence of anthraquionone. These findings have added to knowledge the probable reason of the low natural durability of most bamboo species and nature of microstructure. The paper concludes with recommendations for further studies in the structure of other bamboo species including those in trial test in plantations for wider acceptance and enhanced utilization.

Keywords: *Bambusa vulgaris, a*natomy, ultramicrostructure, bamboo internodes,phytochemical properties.

Introduction

Bambusa vulgaris – a native sympodial bamboo species in Africa and Madagascar- has multiple uses (Bystriakova et al. 2004) and is rated as a moderately resistant bamboo species(De Guzman 1978).In Ghana, it is reported to be the predominant bamboo species (Ebanyenle and Oteng Amoako 2007;Tekpetey et al.2007).The detailed knowledge of its technological properties and variation is generally accepted to significantly influences the level of acceptance processing and utilization of bamboo resources. Information on the macro and ultra microstructural features of bamboo, for instance, is necessary for assessing its suitability and quality for specific product. The fibro vascular proportion, vascular bundle types and diameters of vessels influence bamboo behaviour (Liese 1987; 1998, 2000). Liese (1985) reports that anatomical properties of bamboo culm vary according to species, the condition of growth, age of the bamboo and part of the culm. The percentage

distribution orientation of cell such as parenchyma, fibres, and vascular bundles also vary considerably along and across the bamboo culm (Espiloy1985; Liese 1985; Soeprayitno et al. 1985).The bamboo culm comprises about 50% parenchyma, 40% fibres and 10% vessels and sieve tubes (Liese 1987).In an earlier work on *Bambusa vulgaris* Schrad. Ex. Wendl from Ghana the anatomical results revealed similar values proportions of different tissues (Assouan 2002).Little information, however, exist on the ultra microstructure of our native bamboo species and extent of variation in different ecological zones in Ghana to better understand their behaviour during processing and when in service. The relatively low durability of bamboo species have been attributed to low extractive content but qualitative differences in the content of extractive will explain the difference among species around the world. Without these studies, the observed difficulties and the behaviour of our bamboo in service can only be based on speculation rather than empirical evidences. Furthermore, investigating the bamboo chemical composition and phytochemical will offer significant basic data and information on the properties and durability of our bamboo species which have been cited as impeding wider acceptance in Ghana. The use of bamboo in papermaking and architecture in different areas in Ghana will be harnessed. The knowledge of these properties will enhance the mode of preservation treatment and industrial processing of *Bambusa vulgaris*. This is what the paper seeks to elucidate in three bamboo growing areas in southern Ghana.

Material and Methods

Anatomical Studies

Sound internodes of matured bamboo of above four years were harvested from Kumasi (Ashanti Region), Akim Oda (Eastern Region) and Assin Fosu in the Central Region of Ghana between August 20 and 30th, 2006. The samples were placed under shades at the Wood Science Workshop, KNUST and were transported to Open Key Laboratory at International **C**entre for Bamboo and Rattan (ICBR),Beijing, China for the anatomical studies . The 4th internodes from the ground level and the 12th internodes of *Bambusa vulgaris* were chosen for the anatomical studies. Three softened samples were sectioned from the middle part of selected internodes and the Leica Sliding Microtome – M2000R model was used to prepare transverse and longitudinal sections of the softened blocks. Sections were then observed using XL 30 ESEM ^{TM} FEG Environmental Scanning Electron Miscroscope in the ESEM laboratory. Vascular bundle types, the metaxylem vessels, parenchyma and its inclusions; and the arrangement of pitting among the two varieties were observed. The results were presented in Micrographs and Tables accordingly. The thickness of the fibre bundle at protoxylem side of the vascular bundle of Ghanaian Bamboo was also measured over the culm wall of base 4th internodes.

Phytochemical Screening

Some internodes were selected; air dried and was cut into small strips for analysis. The strips were small enough to be placed in a Wiley Mill at Forest Research Institute of Ghana. The material was sieved manually to pass through a No. 40 mesh sieve (425-µm) yet retained on a No. 60 mesh sieve (250µm). The resulting material was placed in glass jars labeled with appropriate code for chemical analysis and acetone and ethanol extraction were undertaken using the soxhlet apparatus. After the ethanol extraction, some portion of bamboo extracts take

Chemistry laboratory, Department of chemistry, Kwame Nkrumah university of Science and Technology for the phytochemical screening.

Results and Discussion

The observation of bamboo samples from the three sites revealed basically two main types of vascular bundles: the classical Types III and IV as reported by Liese and Grosser (2000). These are shown in Fig 1 and 2. This type of bundles is characteristic of sympodial bamboo like *Bambusa vulgaris.* The type iii, however occur occurring mostly on the 12th internodes whilst the type iv were found both in the 4th and 12th internodes in the three sites studied. This supports research work on the classification of vascular bundle type. The identified vascular bundle in the sample are cited to have a good condition for better pulp yield than in other monopodial bamboo species though depending on the age of the samples it might be more difficult to process than the mono types. The implication therefore during processing is that in machining of *Bambusa vulgaris* from natural stands in Ghana difficulty might be encountered in comparison to bamboo species of the monopodial type like *Phyllostachys pubescence* (Moso bamboo) since more polyllamenate fibre walls will be encountered. No expanded topology form of the vascular bundle types was recorded in all the samples observed. The fiber strands were mainly the heart- blocked shaped and arc-like shape described earlier researches (Liese and Grosser 2000.) Fig 1; Fig 2

Parenchyma Cells and Inclusion

A comparison of the shape of the parenchyma cells of moso bamboo seems more rectangular than those observed in the *Bambusa vulgaris* samples as evident in Fig 3 and 4.The implication from its processing cannot be cannot be predicted immediately rather than a significant genetic variation in monopodial and sympodial bamboo types. In figure 5and 6 most of the parenchyma cells were either partially filled or fully filled with starch granules. Many researchers who investigated culms of up to three years found no starch during the first year of growth but many starch granules in older culms. Liese (1997) reported the abundance of starch granules is characteristic of older bamboo culms two years and above. The absence or presence of starch granules in raw bamboo culms influences its susceptibility to termites and insect attack because the starch is a source of food for the insects. (*Fig 4;* Fig 5; Fig 6)

The season of harvesting has also been reported to have a great impact on the abundance of starch in bamboo culms at different height the need to plan for its harvesting is important to sustainable harvest and utilization. Most of the bamboo are in natural stands in Ghana and are mostly found in swampy areas, such bamboo resources may not readily accessible in the rainy season.

Metaxylem of Bambusa vulgaris

Measurement of the vessels diameter from the three sites gave values ranging from 128 to 196µm with minimum value recorded in the outer culm of the 12th internode of *Bambusa vulgaris* from Assin Fosu whilst the highest was from the base (4th internode)of culms from Assin Fosu. On an average, the conducting system, including the phloem, account for about 8% of the total culm and this appears rather small when compared with

the lumen area of softwood tracheids (60-70%), diffuse porous hardwood vessels (20-30%), ring porous hardwoods (15- 30%) and rattan metaxylem (15-20%) (Liese 1994). The variation in the vessel diameter could be responsible for differential conduction of fluid in culm wall.In related work from different zones of the world, varied results for these values were obtained. Espiloy (1987) obtained an average vessel diameter of 165 µm for *Bambusa. blumeana* and 220µm for *Gigantochloa levis* (values at the base were slightly higher). Wu and Hsieh (1991) reported a diameter decrease for *Dendrocalamus latiflorus* from the 6th internode towards the top, but a slight increase in the case of *Phyllostachys edulis*. Kumar and Dobriyal (1992) measured for *Dendrocalamus. strictus* a vessel size of 60 µm at the outer part, 85 µm at the middle and 100 µm at the inner part. Abd. Latif (1995) registered mean values of 147-187 µm for *Bambusa. vulgaris* and 114-137 µm for *G. scortechinii*, both with smaller diameters at the top. Such information is required for application in seasoning and preservative treatment.

The figures 8 is described as the structures of interest which might be a peculiar inclusion of parenchyma cells near the vascular bundles of Ghana bamboo especially *Bambusa vulgaris* . The position of the figure near to thick fibres and the presence of starch granules in other cells near make it difficult to identify. The structure was observed at the 4th internode from Assin Fosu from the Central Region.

Phytochemical Analysis

The phytochemical properties of woody plants including woody bamboo consist of the secondary metabolites-alkaloids, flavonoids, anthraquinones which impart different support to the plant. Results from the work revealed the absence of alkaloids in the sample is a probable reason for the rapid decay of bamboo species in service in Ghana especially when used in the raw state. Alkaloids are generally formed as metabolic by-products; however, their characteristic bitter taste and accompanying toxicity generally help to repel insects and herbivores. The absence of alkaloids coupled with large amount of starch granules in the parenchyma cells as observed in earlier work (Tekpetey 2006) may be the reason for rapid deterioration of bamboo culms when used in service in an untreated state**.** Low level of durability of bamboo culms has reported the abundance of starch and low extractive content as contributing factors (Liese1998). Additionally, the absence of alkaloids in the sample extracts explains better the low durability of Bambusa vulgaris which may be true for many other bamboo species. Relatively, low density species, high moisture content species with low extractive content are susceptible to insect attacks and hence rapid deterioration especially in raining seasons. The need for chemical preservation of bamboo culms may be preferable option to address the low durability rather than a method of reducing the starch content of bamboo culms before their use in service of matured culms. The purgative action of some bamboo species could also be attributed to the presence of anthraquinone in the extracts.

Conclusion and Recommendation

This work has established that there are slight structural variation in fibre bundles and vessel dimensions of *Bambusa vulgaris* from different zones in Ghana .It has also enhanced the knowledge on its treat ability, biological resistance, processing and utilization bamboo in Ghana . The phytochemical screening of the extracts of *Bambusa vulgaris* indicated the absence of anthroquinone glycosides, alkaloids but the presence of flavonoids. It is recommended that further quantitative analysis of phytochemicals of major bamboo species in

different part of the world should be collaboratively undertaken for both indigenous and exotic species. Further collaboration is needed to identify ‘new structures’ and also to understand the nature of bamboo species in most developing countries and it niche for diversified utilization.

Acknowledgement

The financial support from the International Tropical Timber Organization (ITTO) for the study is highly appreciated. The authors are grateful for the technical and financial support of Chinese State Forestry Administration through International Centre for Bamboo and Rattan, Beijing, China to undertake the anatomical and thermal studies under the Key Open Laboratory Research Scholarship. The authors appreciate the suggestions of a renowned anatomist during the draft stage of this article.

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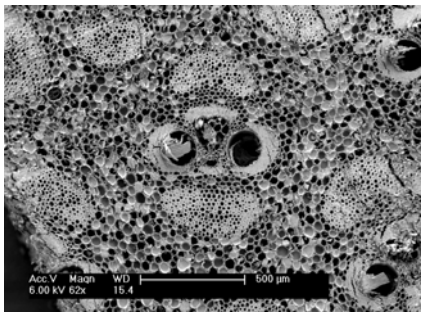

Fig 1

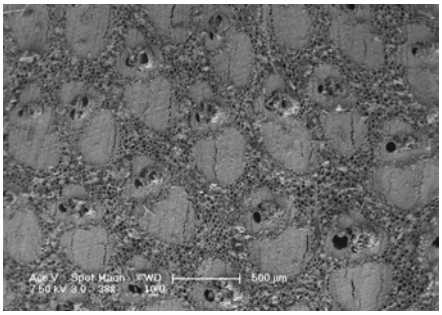

Fig 2

Fig 1- Vascular bundles with two isolated fibre bundles of *Bambusa* v*ulgaris* from Akim Oda, Ghana

Fig 2- Vascular bundle with one isolated fibre- bundle at the Protoxylem Assin Fosu, Ghana

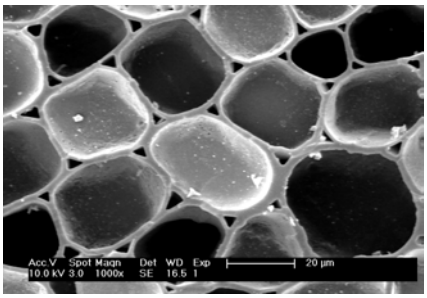

Fig 3- Shape of Parencyma cells of Moso bamboo SOURCE(ICBR,2006)

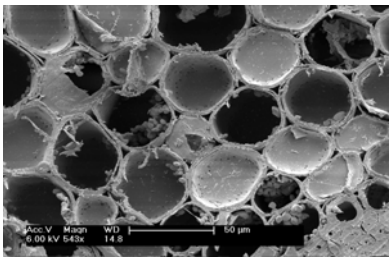

Fig 4-Parenchyma cell structure in Bambusa vulgaris from Ghana

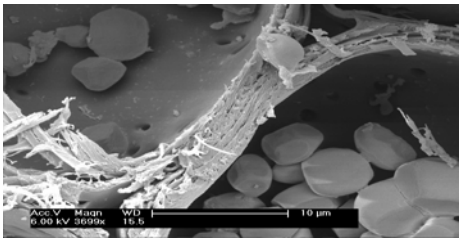

Fig 5- Starch granules in *Bambusa Vulgaris* from Akim Oda, Ghana.

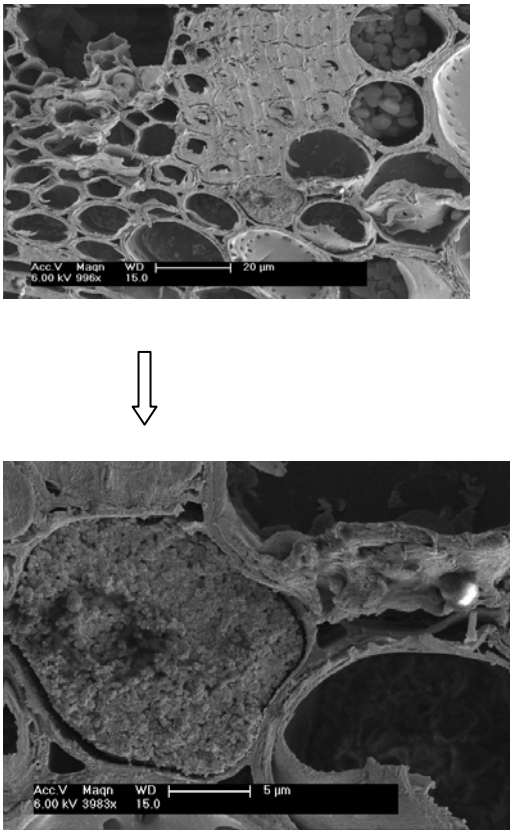

Fig 6 and 7- strange inclusion of parenchyma cells of Bambusa vulgaris from Assin Fosu, Ghana

Performance of *Dendrocalamus strictus* Treated with Combined Fire Retardant and Preservative Systems against Fire, Fungus and Termites

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Abstract

Bamboo is an economic substitute of wood. It is a major building material in several parts of India. Its wider acceptance however is often hindered because of its susceptibility to microbes and insects. Other factor which causes heavy losses of bamboo products is fire. The present work deals with the treatment of *Dendrocalamus strictus* with six fire retardant and preservative compositions. Different combinations of fire retardant and new eco-friendly preservative ZiBOC were tested in the above species for protection against fire, fungus and termites. Bamboo culms treated with six compositions at 15% concentration, were subjected to three tests, viz: flame penetration, flammability and rate of burning as per Indian Standard. Compositions (1) Ammonium sulphate +Ammonium phosphate + ZiBOC,(2) Ammonium sulphate+ ZiBOC , (3) Ammonium phosphate+ ZiBOC ,(4) Magnesium phosulphate+ Magnesium pyrophosphate+ ZiBOC , (5)Magnesium phosphate+ ZiBOC and (6) Magnesium pyrophosphate+ ZiBOC at 15 % were taken for the study. Results revealed that 7.4 kgm^{-3} was the lowest retention achieved by 6 no. composition while 19.90 kgm^{-3} was the highest retention achieved by no.1 composition. Treatment cost for Composition no..1 was comparatively higher as compared to other treatments because of high retention and solubility of chemicals in acid. Whereas, lower cost was observed by Composition no..2 as the chemicals were water soluble. All compositions performed as per standard except Composition no..3 in flame penetration test. Statistical analysis revealed that Composition no.. 4 performed best followed by no.2 and 1.Test against decaying fungus in laboratory and termites in mounds respectively exhibits significant protection by all compositions as compared to control.

Introduction

Bamboo is an important construction material, for simple and modern engineered structures. Bamboo houses of different kinds provide homes for a billion people, not only in rural areas, where it is considered to be moderate priced or even cheap, replaceable material, but also in urban environments. Its wider use as a substitute or alternative for wood is supported by the increasing scarcity and expense of timber in several bamboo-producing countries. Its wider acceptance however is often hindered due to its susceptibility to biological degradation and fire. There are million of bamboo thatched huts spread all over the country which becomes dry in summer, and in which most of the uneducated and poor population lives. In spite of this, although, no actual statistic is available. It has been estimated that less than 0.1 percent of these huts are destroyed by fire before they

disintegrate due to rot or other causes. If construction material like thatch, bamboo, wood etc. is given a preservative cum fire retardant treatment by soaking, it is probable that the loss of bamboo houses would be reduced to a very small fraction of the present number.

A tropical environment with high humidity and temperatures accelerates the natural bio-cycle. These hazards have their impact also in restricting building regulations. Few regions in India are most fire-prone and despite rigorous precaution a considerable loss of life and property occurs every year. Bamboo and wood are extensively used for construction purposes, both are cellulosic material which catches fire easily. A wide range of protective procedures, including chemical preservation methods are known, similar to those for timber under tropical conditions. But they are more seldom than regularly applied due to lack of knowledge about possibilities of bamboo protection, lack of adequate treatment facilities and chemical preservatives, uncertainty about the economics and lack of demand for treated bamboo components etc. Protection of this versatile material, especially in areas where longer service life is desired, can result in immense social and economical benefits. It would increase bamboo availability, facilitate rural employment potential, and save maintenance costs of constructions, which would occur due to replacement of degraded bamboo components.

During the last decade extensive work has been done to develop fire retardant composition of wood and panel products. Various chemicals like ammonium phosphate (mono and di-), ammonium sulphate, boron compounds aluminium sulphate and combinations of these have been recommended (Goldstein 1973; Dev and Kumar 1982). Performance of fire retardant cum preservative composition is also evaluated on plywood (Samani et al. 2007). Some conventional fire retardant, which are mainly water borne inorganic salts, provide a certain degree of decay resistance. These chemicals, if not permanently fixed, are not suitable for exterior purposes. A simple one step process that gives both resistance to fire and microbial decay is rarely known. There is, however, not much data on the performance of such treatments on bamboos is available.

Earlier a comparative study of fire retardant tests for timber and plywood treated with mono-ammonium phosphate at equivalent chemical loading was carried out and the behavior of plywood was found better than the solid wood (Dev et al. 1987). Different workers have worked on different compositions of fire retardants and antiseptic formulations. Most of the studies revealed either higher concentration i.e. 15 % (Purushotham et. al. 1963) of compositions or higher retention in wood i.e. 48 kg/m^3 is required (Dev et. al. 1992). Lower retentions of preservatives cum fire retardant combinations affect the treatment cost, which will be considerably lower at lower retentions.

This study evaluated various fire retardant mixture and preservative ZiBOC that could be used as a combined treatment for wood products. Related investigations on improving the decay resistance alone is already reported (Tripathi et al 2005). In the present study ZiBOC a new safe preservative developed at FRI Dehardun , India is tested along with other chemicals known to impart fire resistance. Because of the presence of borax, zinc and copper salt in ZiBOC in sufficient amounts and the different fire retardant compositions, it is expected that it will provide protection against various degrading agencies.

Material and methods

Green mature bamboo culms (3 nos.) of *Dendrocalamus strictus* of 3-5 years of age were collected from bambusetum of Forest Research Institute Dehradun , India . This study consisted of two parts. The first part consisted of selecting six compatible combinations and testing fire resistance capability in bamboo through various methods (IS: 5509: 2000). The second part of the study involved treatment of bamboo and its evaluation for decay resistance against fungus; brown rot (*Trametes versicolor*) and white rot (*Oligoporus placentus*) in laboratory and termites in mounds (*Odentotermes obesus* Rambur) in field.

Materials:Preparation of Preservative:

Zinc chloride ($ZnCl_2$), Borax ($Na_2B_4O_7$ .10 H_2O) and copper sulphate ($CuSO_4$ .5 H_2O) (AR grade) Merck limited, India taken in a particular proportions made water soluble with the help of co-solvent (Tripathi 2008).

Chemicals: Chemicals used for fire retardants were of commercial grade. The different chemical compositions (treatments) used are shown in table 1.Compositions ; (1) Ammonium sulphate +Ammonium phosphate + ZiBOC,(2) Ammonium sulphate+ ZiBOC , (3) Ammonium phosphate+ ZiBOC ,(4) Magnesium phosphate + Magnesium pyrophosphate+ ZiBOC , (5) Magnesium phosphate+ ZiBOC and(6) Magnesium pyrophosphate+ ZiBOC at 15 % were taken for the study .Composition 2 is water soluble whereas , all other compositions required addition of sulphuric acid for solubility, where sulphuric acid acted as co-solvent and resulted a homogenous transparent solution. The amount of acid mixed is mentioned against each composition (Table-1).

Procedures:

Treatment of bamboo: As bamboo has tapering ends, culm upto the length of uniform diameter was selected for sample preparation. Round Bamboo samples of one feet (30 cm) were cut and further four splits were made from each piece. Twenty four splits were taken for treatment with each composition. Six splits were taken as control. The samples were than treated with six different formulations of fire retardant chemicals and preservatives at 15 % concentrations by diffusion method (Kumar et. al .1994). Specimens of 1 feet length left in S.S. tank for 7 days and removed after that, excess treating solution was blotted from the specimens. After treatment the retentions of the chemicals were calculated.

Fire resistance test:

The samples after treatments were kept in humidity chamber to obtain moisture content 19-22 %. The samples were then tested for performance against fire by flame penetration test, inflammability test and rate of burning test as per IS 5509 (2000) and 1734 part-III (1972), where specifications mentioned are for fire retardant plywood. Treated bamboo splits were tested as per the standard except thickness and width, which was taken as such. Rest of the test procedure was followed as such. Data was subjected to statistical analysis using SPSS. ANOVA for different test was calculated and represented in table 2 and 5 .Critical difference (CD) was calculated through scheffe's test to find out which treatment differs significantly.

Flame penetration test :

125 mm long bamboo splits were taken, thickness and width of the split bamboos were taken as such without any alteration. The test specimens were taken and held horizontally 50 mm above the nozzle of a blowpipe flame. The test specimen was rotated in a horizontal plane at 75 rpm in such a way that the center of the flame forms a circle of 25 mm diameter on the specimen. The time taken for the flame to penetrate the thickness of the bamboo was recorded and calculated for per mm thickness of bamboo and shown in table 3. The time taken for the flame to penetrate the thickness of the bamboo was recorded. The time (T) taken in minutes is calculated as per the following formula:

$$T = \frac{15t}{6}$$ where t is the thickness of bamboo (mm).

Rate of Burning Test :

The test specimens of size 100 X 12.5 mm of full thickness of bamboo were prepared and suspended in a fire tube and adjusted at a height of 30 mm from the flame of the burner. A standard LPG gas flame was used to ignite the test specimens. Time taken to loose weight from 30 to 70 % was recorded.

Flammability test :

The test specimens of size 125 mm in length and of full width and thickness of bamboo were taken and held 15 mm apart. One specimen was held 40 mm higher than the other. An ordinary burner having 3 mm bore is fixed horizontally so that the flame touches the lower end of the inner face of the lower sample. The axis of burner is centrally disposed 22 mm above the lower edge of the lower specimen, the end of the burner being 12mm away from the face of the specimen. LPG gas was fed to the burner at the pressure of 100 mm of water, resulting in a blue flame which, when unobstructed is 100 mm long. The time taken for the higher specimen to be ignited after the ignition of the lower specimen was recorded.

Fungicidal efficacy:

The bamboo blocks 1.9 (L) × 1.9 (W) x and full thickness (T) cm^3 were prepared from *Dendrocalamus strictus* splits conditioned to a constant weight and treated with 15 % concentrations of the fire retardant compositions by dip diffusion process so as to attain maximum penetration and retention. For each composition six replicates were used. Soil block bioassay was conducted as per IS: 4873 (2008) against *Oligoporus placentus* (brown rot) and *Trametes versicolor* (white rot). After a time period of 14 weeks the blocks were removed, conditioned as done previously and the percent weight loss was calculated, as a measure of decay. Untreated samples served as control.

Termicidal efficacy:

Untreated control and treated specimens with dimensions 10 (L) x 2.5 (W) x full thickness (T) cm^3 were taken for the study. Termite resistance test was conducted in termite mound and methodology was followed as reported by Shukla (1977). The test was conducted to evaluate the natural termite resistance of bamboo as well

as efficacy of compositions in bamboo. This method was designed to get accelerated data while, by creating conditions near to nature. 12 replicates were taken for each composition and treatment was done by dip diffusion process for one week. Samples were removed and shade dried. The treated and untreated samples of bamboo were arranged randomly and then neatly woven with the help of wire in the form of a garland. The specimens were exposed to *Odentotermes obesus* Rambur in active termite mounds, in an un-weathered and unleached condition consecutively for two year. Samples were installed in the month of May of 1st year and removed in November of the same year, results were recorded and the specimens were installed again in May in the 2nd year and removed in November as done earlier, so as to have exposure for two successive termite seasons. Specimens were cleaned off mud and debris and evaluated visually for damage (IS: 4873 1993).

Results and Discussions

Table-1 shows 6 composition of fire retardant and preservative in different ratios. Cost of the preservative shows that Composition no.. 2 is the lowest in cost while Composition no.. 1 and 4 are comparatively higher. Minimum retention i.e. 7.4 kg/m^3 was achieved by Composition no.. 6 whereas high retention i.e. 17.90 and 15.90 was achieved Composition no..1 and 4 respectively.

Fire resistance test: Six combinations of fire retardant and preservative were evaluated for fire performance using different tests. The mean weight loss in rate of burning test for each combination, number of replicates evaluated in each group and ANOVA was calculated and shown Table- 2&3. Statistical analysis shows significant ($p<0.05$) difference between treatments and control which lie in different subsets. Table-3 shows that no statistical difference could be found among treatments. Therefore, no particular treatment stood out as better than the rest. Besides that time of glow and maximum temperature attained in treated and control was also noted. The maximum temperature rise in control sets during testing was upto 560 ^{0}C while temp. Observed in sets of treated samples was remarkably reduced.

Taking a similar approach in examining the fire performance of each combination, treated and untreated replicates, statistical analysis for flame penetration test and homogenous subsets are shown in Table- 5 & 6. All treatments were significantly effective ($P<0.05$) as compared to control. Subsets of compositions show that replicates of control failed badly while comp.no.3 performed slightly better than control. While rest of the compositions performed excellently. Table-6 shows that comparison 4 stood out best followed by 2 and 1. Inflammability test revealed that replicates treated by six combinations took more than minimum time prescribed i.e. 2.5 min. /mm thickness (I.S: 5509 ; 2000) for inflammability. Control replicates were ignited in considerably short time. Table 7 shows performance of treated bamboo samples with different fire retardants in inflammability test. Results show that temperature observed in the samples treated by all compositions was remarkably low.

Decay resistance:

Fungicidal efficacy: The blocks of treated and untreated bamboo samples were examined for fungicidal decay. There was no visual evidence of decay in any treated block. 23.7% and 38.9% weight loss was observed in control replicates against *Oligoporus placentus* and *Trametes versicolor* respectively. The results of the decay

test of treated blocks (Table-8) show that in general protection against both the fungus was imparted by all the compositions. The protection ranged between 34-76% against *Oligoporus placentus* whereas, higher protection i.e. 55-82 % was achieved against *Trametes versicolor*. On comparing effectiveness in terms of percent protection composition 3 and 1 were least effective whereas composition 4 had imparted maximum protection against both the fungus. Almost comparable results were observed by Composition no.. 2 and 4 and .5 and 6. In view of the importance of decay tests in assessing the merits of a wood preservative performance of composition tested no. 2 and 4 found most promising. Samples treated by all compositions revealed excellent protection against termite except no. 3 where a slight attack of termite (Visual rating sw) on samples was observed. While control samples were badly destroyed DW (visual rating 5.0) (Shukla 1977).

In this study the fire resistances showed that all the composition were fairly similar except no.3 which resulted marginally status as compared to recommended ones in flame penetration test. Decay test against termite and fungus exhibited best performance by Composition no..2 and 4. Comparing cost of treatment and chemical, Composition no.. 2 was found cheapest. and effective even at very low i.e. 11.50 kg/m^3 retention as compared to other known fire retardant chemicals (Dev and Kumar 1982) Therefore, it can be recommended for testing on pilot scale.

Table 1: Fire retardant compositions used for treatment of *Dendrocalamus strictus*

Composition no..	Compositions (treatments)	Ratio	Acid (H_2SO_4) added (ml) for Solubility in 80 liter of water	Cost/Kg (Rs.)	Avg. Retention (kg/m^3)	Treatment cost/m^3 (Rs.)
1	Ammonium sulphate+ Ammonium phosphate+Ziboc	5:5:5	1150	114	17.90	2028
2	Ammoniumsulphate+ Ziboc	10:5	Water soluble	60	11.50	690
3	Ammoniumphosphate +Ziboc	10:5	800	126	9.80	1242
4	Magnesiumphosphate+ Magnesiumpyrophosphate+Ziboc	5:5:5	500	114	15.90	1801
5	Magnesiumphosphate+ Ziboc	10:5	500	126	14.6	1849
6	Magnesium pyrophosphate+ Ziboc	10:5	500	94	7.4	688

Table 2: ANOVA for rate of burning test

Source	df	Mean Square	F	Sig.
Composition	6	82.776	59.105	.000
Error	35	1.400		

Table 3 : Mean weight loss in rate of burning test arranged in homogeneous subsets

COMPOSITION	Subset		Period of flaming(Sec.)	Maximum temp. at the top of tube (^{0}C)	After glow time (min)
	1	2	>0.2		
6.00	52.0428		>0.2	130	3
4.00	52.3179		>0.2	330	3
2.00	52.5782		>0.2	135	1
3.00	52.6584		>0.2	230	4
1.00	53.7541		>0.2	345	2
5.00	54.5167		>0.2	165	2
Control		62.5317	>0.2	560	4

Table 4: Performance of different compositions in rate of burning test

Composition	Average weight of sample(gm	Average wt. after 30%weight loss	Expected w. after 20 min.) / Average weight after70	Average actual wt. found after 20 minutes	Remarks
1	18.13	10.57	5.44	6.33	Pass
2	10.1	7.07	3.03	3.73	Pass
3	9.9	6.93	2.97	3.64	Pass
4	12.76	8.93	3.83	4.76	Pass
5	12.76	8.93	3.83	4.3	Pass
6	13.73	9.61	4.12	5.2	Pass
Control	15.73	11.01	4.72	3.35	fail

Table 5: ANOVA for flame penetration test

Source	df	Mean Square	F	Sig.
Compositions	6	7.638	44.453	.000
Error	35	.172		

Table 6 : Average time taken in flame penetration test arranged in homogeneous subsets

Compositions .no.	Avg. thickness of sample (cm)	Time required (min.) as per mm. thickness	Average time taken by replicates (min/mm thickness)				
			1	2	3	4	5
Control	1.0	2.5	0.83 (fail)				
6	0.7			2.15 (marginal)			
3.	0.86			2.59 (pass)			
5	0.65			2.50 (pass)	2.50 (pass)		
1	0.7				3.20 (pass)	3.20 (pass)	
2	1.05					3.70 (pass)	3.70 (pass)
4	0.8						4.21 (pass)

Table 7: Inflammability test of *Dendrocalamus strictus*

Composition no.	Average thickness (cm)	Average time taken (min.)	Remarks
1	0.96	30	Pass
2	0.93	30	Pass
3	0.8	30	Pass
4	0.8	30	Pass
5	0.9	30	Pass
6	0.9	30	Pass
Control	0.8	13.4	Fail

Table 8: Efficacy of different compositions against fungus in laboratory and termites in field.

Bioassay		Average weight loss (%) caused by white and brown rot fungus						
		Com.1	Com.2	Com.3	Com.4	Com.5	Com.6	Control
Fungus	*Oligoporus placentus*	14.8 (37%)*	5.6 (76%)*	5.1 (34%)*	15.6 (78%)*	7.3 (69%)*	7.3 (69%)*	23.7
	Trametes versicolor	16.3 (58.09)	7.1 (82.2)	17.3 (55.5)	6.9 (81.2)	13.0 (66.5)	14.1 (63.7)	38.9
	Deterioration rating caused by termites							
Termite Mound	*Odentotermes obesus*	N	N	sw	N	N	N	Dw

*Values in parenthesis are % protection as compared to control.

N = Normal (0 score) ; Dw : Destroyed by termites (5 score)

Sw : slight termite attack (2.5)

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Properties Evaluation of Dendrocalamus giganteus Treated by Heat

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Abstract

Despite a great number of applications for bamboo, two drawbacks are concerned to this raw-material. The first one is a short life span of most of the species, due to its high starch content at the parenchyma cells. The second one is an inadequate behavior when bamboo is exposed to climatic changes. In searching to minimize these drawbacks, bamboo strips from *Dendrocalamus giganteus* Munro culms were submitted to several temperatures: 140, 180, 220, 260 and 300 °C. Physical and mechanical properties of the specimens were evaluated, aiming to detect ideal conditions for apply thermal treatment, and at same time did not reducing the original bamboo characteristics. Results showed that bamboo strips properties were very sensible to temperature effect. Higher temperature provoked damages on bamboo structure, denoted by decreasing of the density and an important reduction on ultrasonic pulse velocity. Modulus of rupture was more sensible to detect temperature effects on thermal treated bamboo (TTB).

Keywords: bamboo, Dendrocalamus giganteus, thermal treatment, NDE

Introduction

Due to bamboo decay by insects and fungi, several treatment process were developed aiming to obtain a more adequate raw-material for several purposes, as building constructions, handicrafts, furniture, particleboards and laminates. In some of this industrial process, heat is applied (mainly for bamboos belonging to *Phyllostachys* genus).

If specific conditions are employed, heat can change lignocellulosic structure of bamboo, provoking then irreversible transformations as was reported by Brito (1992) for wood.

Bamboo natural dry is an example of a type of thermal treatment. However, in these conditions the effects produced on bamboo are much less aggressive when compared to the drastic condition that occurs in the case of a thermal treatment. In simple words, this is similar to a coffee toasted production. Chemical substances changes occur denoted by color, taste and flavor modifications of the coffee seeds, when compared to the original raw-material.

Thermal treatment is also a simple way to improve dimensional stability of the wood (Brito et al. 2006). If optimal conditions (temperature range and time of heating) are employed, thermal treated bamboo (TTB) can performs better than natural ones, eliminating chemical treatments which are normally hazardous to the environment.

Thermal treatment is like a pre-carbonization type in the range of 220 to 300 ºC. In these conditions, hemicellulose is modified, by removing water, acetic acid, phenols and others compounds of small calorific power (Luengo et al. 2008). One intermediate material between vegetal biomass and charcoal is produced at the end of this process.

The main objective of the thermal treatment is to concentrate biomass energy in a product after a short time, employing small heating rate and moderate temperatures. Physical and chemical properties of the vegetal biomass change according to temperature and wood specie (Felix et al. 2003; Brito et al. 2008).

One of the most important steps of the thermal treatment is the range of selected temperature. For *Eucalyptus grandis* wood, temperatures of 30 (control), 120, 140, 160, 180 and 200 °C were tested by Pessoa et al. (2006. After thermal treatments, specimens were submitted to decay. It was observed a favorable effect of the higher temperature on the death of the xylophage organisms.

Because its high content of sugars, carbohydrates, resins and starch, bamboo decayed by insects, bacteria and fungi. Among these organisms, bamboo borer (*Dinoderus minutus* Fabricius) provokes considerable weight loss, mainly for tropical bamboos. The intensity of the attack by borer and the magnitude of the weight loss depend on bamboo specie, age of the culms, season and treatment type applied to the bamboo culms (Hidalgo-Lopez 2003).

Like others natural materials, bamboo properties show great variability, mainly due to the specific condition of its growing. So, to evaluate bamboo properties it is necessary at apply tests to several specimens. Non destructive tests (NDT), mainly by means a ultrasound, is an alternative to the mechanical classic tests, because the ultrasonic pulse velocity (UPV) across the specimen probably can detect changes in its structures (by high temperature effect, for example), in a fast way (Calil Jr et al. 2004).

The effect of the thermal treatment on wood composite was evaluated by stress wave by Del Menezzi et al. (2007), denoting the accuracy of this method to estimate physical and mechanical properties of the OSB (oriented strand board).

Methodology

Strips preparation for thermal treatments

Seasoned culms (5 years old) from bamboo *Dendrocalamus giganteus* Munro were collected at School of Agricultural Engineering at the Campus of Campinas State University. By means a special device, strips of 30 cm x 2 cm x 1 cm were produced. Strips were dried at indoor conditions until moisture content value ranged 10 to 15%.

Except control, to eliminate moisture content effect on thermal treatment, all of the strips were initially dried at 100 °C and placed in plastic bags. Initially, 110 strips were randomically separated and submitted to thermal treatment at selected temperatures (22 samples by each temperature).

Thermal treatments were conducted at Forestry Science Department Laboratories from College of Agriculture Luiz de Queiroz (ESALQ/USP), in an oven with heat system by electric. Strips were submitted to 140, 180, 220, 260 and 300 °C. To avoid combustion, N_2 was employed for the last three temperatures. Initial temperature was 100 ºC, and a heat rate of 0.1388 ºC/min was adopted, according to Pessoa et al. (2006). Specimens remained during 1 hour in an oven, when target temperature was reached, and a time necessary to reach temperature equilibrium. Strips loss weight was evaluated by comparison of the mass before and after the thermal treatments.

Tests applied to Thermal treated bamboo (TTB)

TTB properties evaluation is necessary to select optimal range temperature aiming to obtain a more adequate product to a specific application, as furniture, for example. Specimens were evaluated according to the adaptation of the Brazilian Standard for wood (NBR 7190/97).

TTB color

Samples color parameters (L - lightness, a - absorbance and b - reflectance) was obtained in a Spectrophotometer CM-260d Minolta.

Density

Density (in gcm^{-3}) was obtained before and after thermal treatments directly from the samples.

Swelling and water absorption

Specimens were obtained after flexure test (5 cm x 2 cm x 1 cm) and soaked in water during 24 h. Central region (fractured ones) of the specimens was rejected. Weight gain and swelling (at three anatomical directions – axial, radial and tangential) after 24 h, were compared with control. A sophisticated research about changes of wood-water system by heat treatment was recently presented by Almeida et al. (2009),

Non destructive test (NDT) by ultrasound

Ultrasonic pulse velocity (UPV) across the specimens was evaluated after and before the thermal treatments applied to the strips. A Steinkamp BP-7 device, with transducers of exponential surface, with a 45 kHz resonance frequency, was employed. UPV was obtained by distance (in mm) divided by time of propagation (in μs).

With density and the VPU of the strips after and before thermal treatments, it was evaluated the dynamic modulus (E_d), by

$$E_d = \rho . V^2 . 10^{-9} \ (GPa)$$

ρ - density (kg/m³);

V – Ultrasonic pulse velocity (m/s).

2.2.5 Flexure

For the modulus of rupture (MOR) in bending, it was randomically selected 10 specimens by each thermal treatment. It was adopted a span of 150 mm in an EMIC device model DL/300 kN and a speed of displacement of 1 mm/min.

Results

TTB at 140 °C (second ones) shows almost the same color of the bamboo reference. Parameters obtained for both temperatures were: lightness 75.16 and 76.35; absorbance 6.24 and 5.27; reflectance 25.74 and 24.66, respectively. Similar behavior was observed for 220 when compared to 260 °C. So, in terms of color analysis, TTB can replace several tropical woods. On the other hand, TTB at 300 °C, the last ones in the figure, show an excessive deformation and a darkness color (as a charcoal) denoting an inadequate temperature for possible TTB applications (Figure 1).

Figure 1 – TTB according to the thermal treatment temperature (air dried – control; 140, 180, 220, 260 and 300 °C (upper).

Weight loss decreases more intensively at 300 °C, in order of 50%, corroborating the severity of this temperature on bamboo structure (Figure 2).

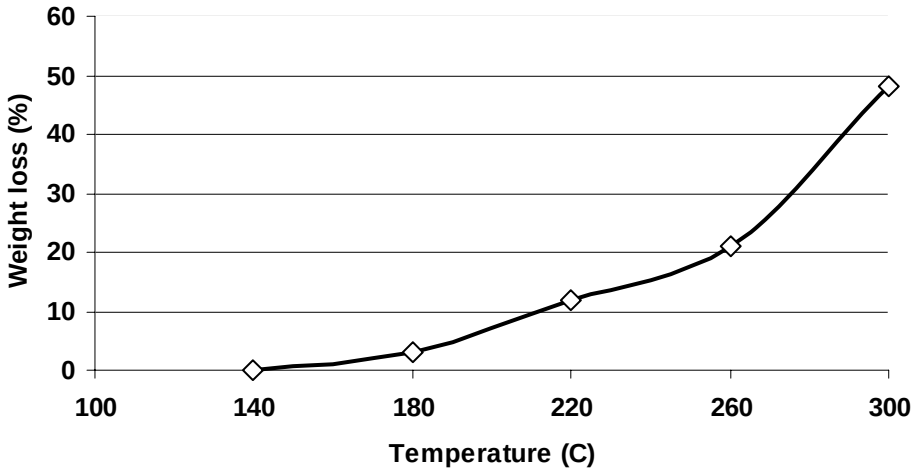

Figure 2 – TTB weight decreasing (%) as a function of the temperature.

For higher temperatures, TTB properties change drastically (Table 1). Density decreases because weight loss is more intensive than volume shrinkage (Figure 3). UPV (Figure 4) and dynamic modulus - E_d (Figure 5) grow-up because moisture content of TTB is reduced. However, for 300 ºC, these parameters are sensitive enough to detect important structural failure on TTB.

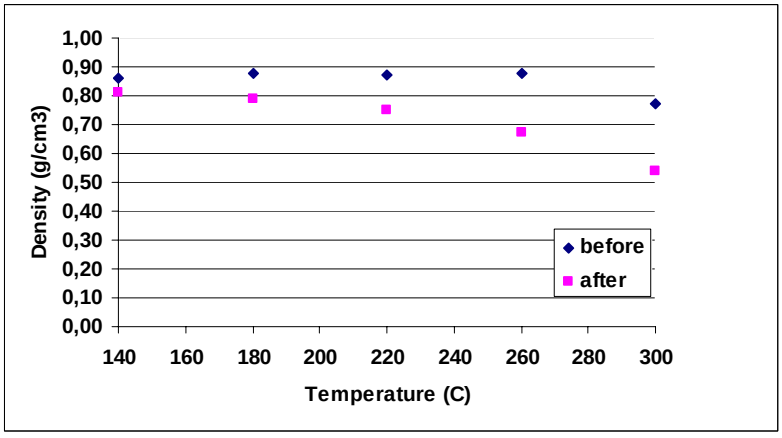

Figure 3 – Density of TTB.

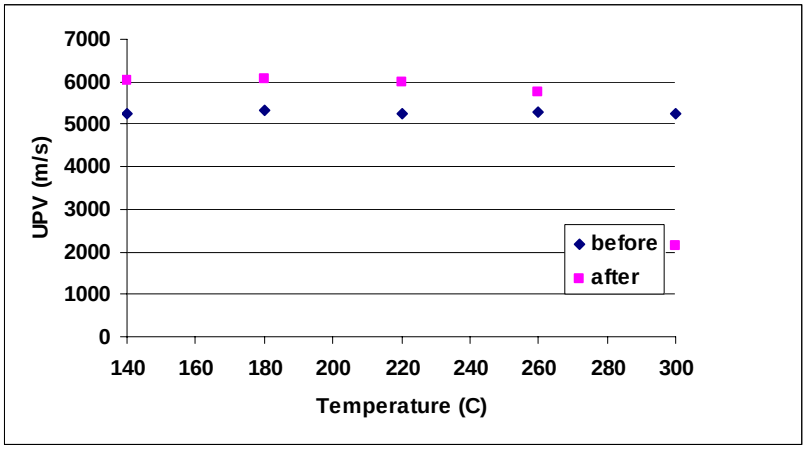

Figure 4 – Ultrasonic pulse velocity (UPV) on TTB.

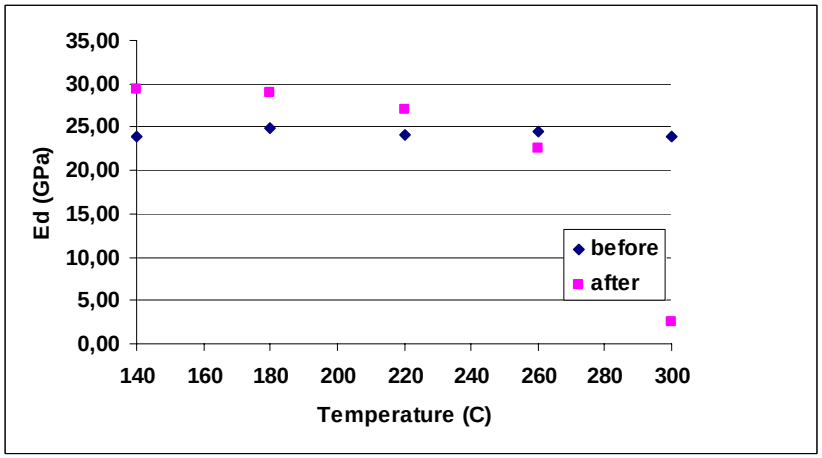

Figure 5 – Dynamic modulus of TTB.

For TTB at 140 and 180 ºC, the comparison of the modulus of rupture (MOR) seems to be more sensible than UPV or E_d to detect micro-cracks in the specimens (Table 1). MOR decreases quickly as temperature of treatment increases (Figure 6).

Table 1 – Properties of TTB.

Temperature	30 ºC	140 ºC	180 ºC	220 ºC	260 ºC	300 ºC
Density ($g.cm^{-3}$)	0.87^{a}	0.81^{ab}	0.79^{bc}	0.75^{c}	0.67^{d}	0.54^{e}
UPV ($m.s^{-1}$)	4354^{d}	6041^{a}	6055^{a}	5973^{b}	5774^{c}	2147^{e}
E_d (GPa)	16.56^{d}	29.42^{a}	28.92^{a}	26.95^{b}	22.47^{c}	2.47^{e}
MOR (MPa)	194.30^{b}	232.21^{a}	157.33^{c}	122.86^{d}	79.07^{e}	11.58^{f}

Different letters at the same line signifies a significant difference at 95% probability level by Tukey's Test.

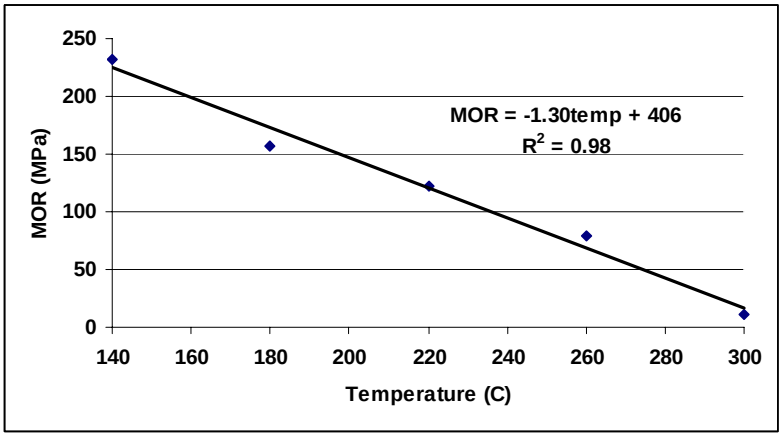

Figure 6 – Modulus of rupture (MOR) of the TTB as a function of temperature.

Swelling after 24 hours was negligible (0.12 to 0.18%) for axial direction, except for treatment at 300 ºC (1.60%), indicating in this case the degradation of the specimens. Swelling at radial direction was higher than tangential direction (this is an opposite behavior when compared to normal wood). For temperature higher than 220 ºC, it can be observed a tendency of the stabilization (2%) of both anatomical direction changes (Figure 7). However, specimens at higher temperature became brittle and it was observed tendency to cracking and torsion (Figure 8). Also, at 180 ºC, it was possible to detect that parenchyma wall and starch were damaged (Figures 9 and 10).

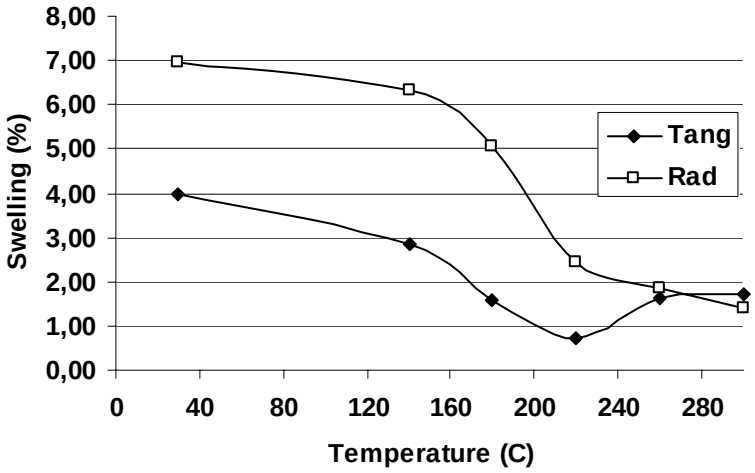

Figure 7 – Swelling according to anatomical direction (radial and tangential).

Figure 8 – Specimens after swelling.

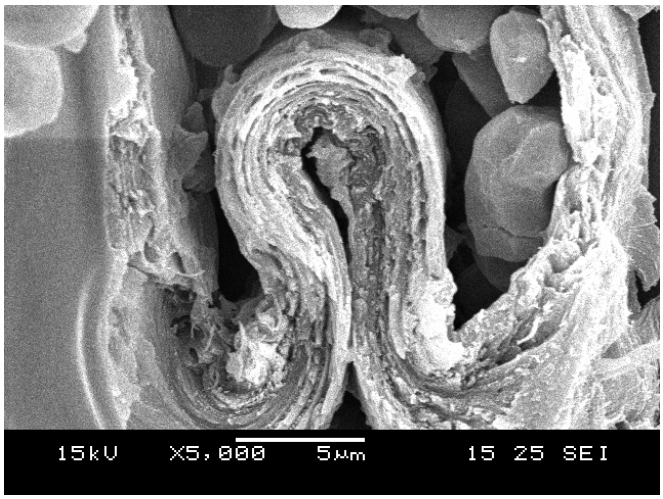

Figure 9 – Parenchyma wall shrinkage.

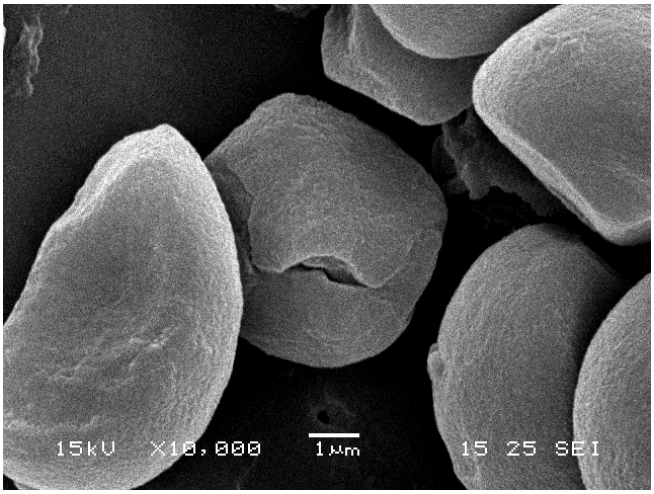

Figure 10 – Cracks on starch surface.

Conclusions

Thermal treated bamboo (TTB) has potential to be applied for several applications depending on temperature range. At 180 ºC, there is an important change on bamboo structure. Temperature greater than 260 ºC provoked considerable damage on bamboo structure and can prevent its special application as furniture. Structural changes at TTB can be performed by non destructive evaluation (NDE), but modulus of rupture was more sensitive to detect internal micro-cracks. Thermal treatments improve the dimensional stability of bamboo when soaked in water.

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Chemical Protection of Bamboos, *Bambusa bambos* and *Dendrocalamus strictus* for their Commercial Utilization

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Abstract

Though highly perishable, utilization of bamboo in various commercial applications is increasing in India. Enhancement of durability of two commercial bamboo species *Bambusa bambos* and *Dendrocalamus strictus* by chemical treatment was studied. To test the efficacy of different new commercial insecticidal chemicals viz Cypermethrin, Bifenthrin , Imidaclorpid, Chlorpyriphos, Fenvalerate, Cypermethrin Carbamate and two neem based biopesticides, dip, spray and pressure treatment were used. The treated bamboo stakes were implanted in the termite testyard as per IS 4833-\1968 and evaluated over a period of 36 months for their efficacy. The studies indicated that Bamboo pressure treated with Bifenthrin was more effective followed by Imidaclorpid, Chlorpyriphos, Fenvalerate and Cypermethrin against termite attack. Carbamates and Neem based Biopesticides were found less effective.

Keywords: Bamboo, durability, chemicals, botanicals, termite, *Bambusa bambos, Dendrocalamus strictus*

Introduction

Bamboo is a very important forest resource that benefits the life of people in a myriad ways including meeting the need for structural uses like posts, pole fencing, scaffoldings, house building etc. Bamboo compares favorably with such timber as 'sal' and 'teak' in strength properties. Bamboo, considered as one of the strongest structural material available, often succumbs to termite attack and biodeterioration during storage. In tropical humid areas, enormous quantities of bamboo culms stored in forest depots, mill yards, etc. deteriorate. The natural durability of bamboo is low and varies from 1 to 36 months, depending on the species and climatic conditions During storage for upto 12 months, about 25 -40% damage of culms has been reported in India (Thapa *et al* 1992) The severity of termite attack and deterioration depends on the duration of storage, bamboo species and environmental and storage conditions. Subterranean termites account for at least 80% damage and drywood termites account for more than 20% (Su and Scheffrahn 1990). Most bamboo used for structural purposes in rural and tribal housing deteriorates within a couple of years and the demand for frequent replacements puts a heavy pressure on the resource.

Chemical treatment using various insecticides and preservatives has been the most widely used method to control post –harvest pests of bamboos. Various pesticides have been recommended and used in different

countries. 5% water soluble copper-chrome-arsenic composition (CCA); 5-6% water solution of copper-potassium dichromate-borax (CCB); 5-6% water solution of boric acid borax –sodium pentachlorophenate in 0.8:1:1 or 1:1:5 ratio (BBP); 2-3% water solution of borax:boric acid in 5:1 ratio and 10% or 20-25% water solution of copper sulphate. These are mostly applied by soaking under normal pressure, cold or heated conditions or under high pressure (Singh and Tewari 1979,1981 a,b;Kumar *et al.* 1985; Thapa *et al.* 1992) Chlorpyriphos and other synthetic pyrethroids have recently become available for use in organic solvent type formulations (Satish kumar 1995) Bamboo stakes pressure treated with Chlorpyriphos, Cypermethrin and Alphacypermethrin were free from damage for more than 28 months. The efficacy of chlorpyriphos against subterranean termites has confirmed the findings in the earlier reports (Remadevi and Raja.Muthukrishnan 1997, 2004). *B.bambos* pressure treated with acephate and permethrin showed no damage upto 12 months of implantation. Thereafter, acephate treated bamboo stakes showed total damage within 48 months after implantation and permethrin treated bamboo stakes showed 60% damage at 54 months after implantation (Remadevi *et al*. 2005) Timber treated with Chlorpyriphos@ 1% and 2% a.i and Fenvalerate @1% and 2% a.i showed 100% protection till 24 months of implementation. (Sundararaj *et al*. 2007)

Enhancement of durability with some economically viable preservatives may help in reducing the frequent replacement of the natural resources. Developing information on enhancement of durability of bamboo helps the end users. Presently, many new bio- pesticides and insecticides are available in the market and an attempt has been made to test the efficiency of some synthetic pesticides and two neem based formulations in protecting two bamboo species *Bambusa bambos* and *Dendrocalamus strictus* against termites.

Materials and Methods

Field evaluation of 8 chemicals and 2 botanicals on two bamboo species viz *Bambusa bambos* and *Dendrocalamus strictus* were done at the termite test yard of the Institute at Nallal Field station as per IS 4833-1968. The field status in the Longitude of 77.38^0E and latitude 12.58^0 N and the soil condition is red, loamy and acidic. The rainfall in this area was 650-750mm and the water retaining capacity was 37.77 at 15 cm depth. The test site was abundant with 4 species of termites. All the experiments were conducted with five replicates. Bamboo culms free of borer and fungus attack were purchased from the market. Bamboo stakes of 30 cm length having a node were taken from the different levels of culms and they were labeled using galvanized sheet labels.

The chemicals evaluated includes two neem based formulations viz 1) Nimbecidine (neem) 0.03%EC and 2) Gromin (neem)EC 1% w/w min. and synthetic insecticides viz 1) Chlorpyriphos (Lethal) 20% TC 2) Chlorpyriphos (Dursban) 20% TC 3) Cypermethrin (Cypercid)10% EC 4) Indoxicarb (Avaunt)14.5% SC 5) Fenvalerate 20%EC (Fencid) 6) Imidachlorpid 17.80%SC (Confidor) 7) Imidaclorpid (Termex) 350EC 8) Bifenthrin 2.5 TC (Biflex).

The treatments were done by spraying, dipping and pressure impregnation. Stakes treated with water served as control and the experiment was done with 5 replicates using the two bamboo species *B. bambos* and *D.strictus* as follows:

Treatment 1(T1): Spray treatment, by spraying and drenching the entire stake with the chemical.

Treatment 2(T2): Dip treatment by submerging the stake in the chemical for 48 hours

Treatment 3(T3): Vacuum for 15 minutes followed for by 50 pounds / sq. inch pressure for 60 minutes.

All the treated and untreated (controls) bamboo test stakes were air dried and thoroughly mixed up and firmly implanted in a randomly block design with half their length buried in the soil 60 cms apart from each other along with a set of untreated bamboo stakes of both the bamboo species and tested as per (IS 4833-1968) at the termite test yard in the Nallal Research field station (Hoskote) of the Institute of Wood Science and Technology Bangalore. Observations of the stakes were made at interval of 6 months. During each observation each stake was pulled out of the soil and visually assessed on the extent of damage and re-implanted for further observations.

Results and Discussion

The termite fauna identified in the test yard were *Odontotermes horni (*Wasmann*), O. obesus* (Rambur), *O. redemanni* (Wasmann) and *Microtermes obesi* (Holmgren). Among these *O. obesus* (Rambur) is considered to be a major wood-destroying termite. The data presented in Table 1&5 shows that the untreated controls are totally damaged over a period of 12 months after implantation.

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Table-1 Percentage of destruction to Bambusa bambos stakes impregnated with different insecticides by subterranean termites and different months after implantation

Chemical	Dosage	Treatment	Mean per cent damage in stakes at different MAI*					
			6M	12M	18M	24M	30M	36M
Nimbecidine (neem) 0.03%EC	1%	T-1	0	10	20	35	40	60
		T-2	0	0	10	10	20	35
		T-3	0	0	0	0	15	25
Gromin EC 1%w/w min.	1%	T-1	0	10	15	25	35	65
		T-2	0	10	25	35	40	50
		T-3	0	0	10	20	30	40
Lethal 20% TC (Chlorpyriphos)	1%	T-1	0	0	0	0	0	10
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Dursban 20%TC(Chlorpyriphos)	1%	T-1	0	0	0	0	10	10
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Cypercid 10% EC (Cypermethrin)	1%	T-1	0	0	0	0	0	10
		T-2	0	0	0	0	0	5
		T-3	0	0	0	0	0	0
Avaunt 14.5% SC (Indoxicarb)	1%	T-1	10	30	40	60	75	80
		T-2	0	0	0	10	10	25
		T-3	0	0	0	0	5	10
Fencid 20%EC (Fenvalerate)	1%	T-1	0	0	0	5	10	15
		T-2	0	0	0	0	5	10
		T-3	0	0	0	0	0	0
Confidor 17.80%SC (Imidachlorpid)	1%	T-1	0	0	0	0	0	0
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Termex 350EC (Imidachlorpid)	1%	T-1	0	0	0	0	0	5
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Biflex 2.5TC (Bifenthrin)	1%	T-1	0	0	0	0	0	0
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Control			40	100	-	-	-	-

MAI – Months after implantation

One way ANOVA showing significant difference between the chemicals used for protection of *Bambusa bambos*.

Table.2: Spray treatment, by spraying and drenching the entire stake with the chemical.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	48909.09	10	4890.909	21.90702**	9.38E-16	2.007792
Within Groups	12279.17	55	223.2576			
Total	61188.26	65				

Table.3: Dip treatment by submerging the stake in the chemical for 48 hours

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43144.7	10	4314.47	37.34492**	6.03E-21	2.007792
Within Groups	6354.167	55	115.5303			
Total	49498.86	65				

Table.4: Vacuum for 15 minutes followed for by 50 pounds / sq. inch pressure for 60 minutes

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43252.27	10	4325.227	47.53789**	1.9E-23	2.007792
Within Groups	5004.167	55	90.98485			
Total	48256.44	65				

** shows significant difference at 0.05 Level of significance.

Table-5 Percentage of destruction to *Dendrocalamus strictus* stakes impregnated with different insecticides by subterranean termites and different months after implantation

Chemical	Dosage	Treatment	Mean per cent damage in stakes at different MAI*					
			6M	12M	18M	24M	30M	36M
Nimbecidine 0.03%EC (Neem)	1%	T-1	0	15	20	35	40	55
		T-2	0	10	10	25	35	45
		T-3	0	0	10	15	25	35
Gromin 1% w/w min. EC	1%	T-1	10	20	30	40	45	50
(Neem)		T-2	0	0	10	25	35	55
		T-3	0	0	0	10	35	45
Lethal 20% TC (Chlorpyriphos)	1%	T-1	0	0	0	0	0	15
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Dursban 20% TC (Chlorpyriphos)	1%	T-1	0	0	0	0	10	15
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Cypercid 10% EC (Cypermethrin)	1%	T-1	0	0	0	0	0	0
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Avaunt 14.5% SC (Indoxicarb)	1%	T-1	0	35	40	45	50	75
		T-2	0	0	0	15	25	35
		T-3	0	0	0	10	10	10
Fencid 20% EC(Fenvalerate)	1%	T-1	0	0	0	25	35	45
		T-2	0	0	10	10	15	25
		T-3	0	0	0	0	0	0
Confidor 17.80%SC (Imidachlorpid)	1%	T-1	0	0	0	0	0	0
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Termex 350EC (Imidachlorpid)	1%	T-1	0	0	0	0	10	15
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Biflex 2.5TC (Bifenthrin)	1%	T-1	0	0	0	0	0	0
		T-2	0	0	0	0	0	0
		T-3	0	0	0	0	0	0
Control			30	100	-	-	-	-

MAI – Months after implantation

One way ANOVA showing significant difference between the chemicals used for protection of *Dendrocalamus strictus*.

Table.6: Spray treatment, by spraying and drenching the entire stake with the chemical.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	43975.76	10	4397.576	18.82841**	2.2E-14	2.007792
Within Groups	12845.83	55	233.5606			
Total	56821.59	65				

Table.7: Dip treatment by submerging the stake in the chemical for 48 hours

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	40877.27	10	4087.727	23.63469**	1.85E-16	2.007792
Within Groups	9512.5	55	172.9545			
Total	50389.77	65				

Table.8: Vacuum for 15 minutes followed for by 50 pounds / sq. inch pressure for 60 minutes

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	41335.61	10	4133.561	31.55755**	2.98E-19	2.007792
Within Groups	7204.167	55	130.9848			
Total	48539.77	65				

** shows significant difference at 0.05 Level of significance.

Anova was conducted to know the effect of different treatments viz., spray, dip and pressure treatments separately for the two bamboo species *Bambusa bambos* and *Dendrocalamus strictus* (Table 1 & 5)

Anova indicates that the effectiveness of the chemicals was significantly different in all the treatments viz., spray, dip and pressure treatments. (Table 2-4 & 6-8)

The data presented in table 1 and table 5 shows that the untreated controls are totally damaged over a period of 12 months after implantation. The two bamboo species *Bambusa bambos* and *Dendrocalamus strictus* pressure

treated (T-3) with the chemicals Bifenthrin, Imidachlorpid, Chlorpyriphos , Fenvalerate and Cypertmethrin showed good results. The bamboo stakes treated with Bifentrhin 2.5 TC showed 100% protection against termites with all the three treatments T-1, T-2 and T-3 during the entire study period of 36 months. Bifentrhin TC is presently the most advanced development in today's growing world of termiticides. Based on a unique pyrethroid called bifentrhin, Biflex TC brings all levels of termiticidal activity. The active ingredient present in this termiticide, prevents the termites from crossing the barrier and kills them in contact. Even when the barrier weakens over a period of time, it still provides protection since it reduces the pressure of attack through repellency and do not attack or penetrate the barrier.

Imidaclorpid also known as a new generation termiticide, is a non-repellent systemic cum contact insecticide, being a latest and fastest growing molecule also gave good protection to bamboo with its lethal action against termites. This chemical is known to act on acetyl chlorine, binding the nerve receptor cells of termites leading to lasting impairment. Consequently, the feeding termites stop feeding and die. However, although the treatments (T-2) and (T-3) proved effective against termites, it was observed that treatment (T-1) i.e bamboo stakes spray treated with Imidaclorpid, slowly lost its efficacy over a period 36 months. Pressure treated bamboo stakes with Chlorpyriphos, Fenvalerate and Cypertmethrin showed more or less the same resistance against termite attack i.e Bamboo pressure treated (T-3)with Chlorpyriphos, Fenvalerate and Cypertmethrin were totally free from termite attack during the study period of 36 months. Earlier studies indicated that bamboo stakes pressure treated with Chlorpyriphos, Cypermethrin and Alphacypermethrin were free from damage for more than 28 months. The efficacy of chlorpyriphos against subterranean termites has confirmed the findings in the earlier reports (Remadevi and Raja.Muthukrishnan 1997, 2004).Bamboo stakes treated with Carbamate were found less resistant to termite attack. Feeding on these treated stakes (T1) for this chemical commenced during 12 months of exposure.

Bamboo stakes treated with two neem based formulations fared badly to termite attack. Even bamboo stakes pressure treated with these neem base formulations were prone to termite damage after 12 months. The above observations were similar for both the species of Bamboo i.e *Bambusa bambos* and *Dendrocalamus strictus* tested in the termite test yard.

Conclusion

Under Indian conditions, the study revealed that the two commercially available bamboo species *Bambusa bambos* and *Dendrocalamus strictus* treated with Bifentrhin 2.5 TC has proved a better termiticide compared to Chlorpyriphos , Imidachlorpid Fenvalerate and Cypermetnrin, which also gave good protection during the study period. Carbamates showed less resistance to termite attack. Two Neem products also proved less effective against termite attack.

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Protection of Bamboo by Environment-friendly Chemicals against Short-term Molding

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Abstract

The protection of the bamboo species *Bambusa stenostachya* and *Thyrostachys siamensis* against molds was tested with environment-friendly chemicals in the laboratory. Bamboo samples were treated with various organic acids and their salts. Mold growth on the specimens was evaluated 1, 2, 4 and 8 weeks after inoculation with a conidia mixture of seven moulds isolated from bamboos in Vietnam. A second experimental set used specimens which were not inoculated but contained the mold flora from the original bamboo samples. Treatments with 10% acetic acid, 7 % propionic acid as well as with a mixture of 3% boric acid and 7% propionic acid inhibited mould growth totally.

Keywords: Bamboo, Molding, Antifungal treatment

Introduction

The bamboos Bambusa stenostachya (Tre gai) and Thyrostachys siamensis (Tam vong) are two of the important species of Vietnam. They are used for production of furniture and for housing mainly to export (Phan 2004). Generally, several fungi from the groups of deuteromycetes (molds), ascomycetes and basidiomycetes colonize the culms and leaves of bamboos (Mohanan 1997). Molds can also occur on the surface and at the cross-ends of culms in a humid atmosphere as they require high relative humidity above 70%. Especially, exposed bamboo material during storage, processing, transport in container and its final use is affected by molds (Liese and Kumar 2003).

Figure 1 shows molded bamboo culms at arrival in Hamburg harbour after container transport from Vietnam.

For protection of wood and bamboo against molds and other fungi, pentachlorophenol had been widely used. However, pentachlorophenol is banned due to its high toxicity in many parts of the world as well as in Vietnam (Tang 2009). Therefore, bamboo manufacturers have pressing problems to protect bamboo for home use and export. Since Vietnam exports large quantities of bamboo culms and utilities in containers to Europe the damage due to mold growth at arrival has become quite serious. Manufacturers greatly need cost-effective but also environment-friendly treatment methods.

Welling and Lambertz (2008) used chemicals of alkaline pH value in order to reduce molding of pine sap wood specimens and obtained protection with potassium and sodium carbonate. Liese and Walter (1978) showed the efficacy of an acid formulation (boric acid) on the protection of sugar cane bagasse against molding. Our emphasis was on the use of free acids. The protective efficacy of organic acids like acetic, boric, citric, formic, propionic and sorbic acid has been applied long time for food and as antiseptica (Wallhäußer and Schmidt 1967). Therefore, we combined the preventing effect of acids with the additional protective effect of their low pH-value against microorganisms (Schmidt 2006) for short-term protection against molding.

Material and Methods

Bamboo Specimens

Each ten mature bamboo culms of 3 - 4 years age of *Bambusa stenostachya* Hackel and *Thyrostachys siamensis* Gamble were collected from a bamboo-plantation in Tay Ninh province, South Vietnam. From the fresh culms, samples of 70 mm length were taken halfway between the internodes and longwise split. Their moisture content was 100 to 120 %.

Chemicals

Acetic, boric , citric , formic , propionic, sorbic acid, and the salts potassium citrate, sodium acetate, sodium borate and sodium propionate from laboratory providers were applied in the formulations listed in Table 1.

Table 1. Acids and salts used for anti-mold test

Formulation	Acid / salt	Concentration (%)	pH –value
1	acetic acid (AA)	7	3.0
2	AA	10	2.8
3	citric acid (CA)	7	2.7
4	CA	10	2.6
5	formic acid (FA)	7	3.8
6	FA	10	3.7
7	propionic acid (PA)	7	2.9
8	PA	10	2.8
9	sorbic acid (SA)	0,6	3.7
10	Na-acetate (NA)	7	8.4
11	NA	10	8.5
12	Na-propionate (NP)	7	8.0
13	NP	10	8.1
14	boric acid (BA)+Na-borate (BS)	2% BA + 3% BS	8.7
15	BA + NP	3% BA + 7% NP	7.0
16	BA + NC	3% BA + 7% NC	7.9

17	BA + K-citrate(KC)	3% BA + 7% KC	8.3
18	BA + AA	3% BA + 7% AA	3.0
19	BA + SA	3% BA + 0.3% SA	3.9
20	BA + CA	3% BA + 7% CA	2.5
21	BA + PA	3% BA + 7% PA	3.0
Control	H_2O	-	-

Fungi

The seven molds used were isolated from bamboo at the Nong Lam University of Ho chi Minh City, Vietnam. Identification by DNA-ITS sequencing as described by Schmidt (2000) revealed *Aspergillus niger, A. flavus, A. oryzae, Aspergillus* sp., *Mucor* sp., *Paecilomyces variotii* and *Penicillium* sp.

Treatment, Inoculation and Incubation

Two specimens of the two bamboo species were dipped 5 min. in the respective treatment solution and placed in a small plastic basin 10 x 10 x 6 cm (Fig. 2). Specimens were not sterilized before incubation. For one test series, artificial infection with a water-based mixture of conidia of the 7 molds was performed with a small brush. The other series contained the molds from the natural flora of the bamboo plants and from the sample processing. The basins were incubated at 30°C and 75 % RH. Evaluation was done after 1, 2, 4 and 8 weeks.

Assessment of Mould Growth

The development of mold growth on the surface of the bamboo specimens was assessed according the ranking shown in Table 2.

Table 2. Rating for determining mold growth on bamboo specimens

Rating	Description	Definition
0	no coverage	no growth
1	1-10 % coverage	slightly overgrown
2	11-25 % coverage	moderately overgrown
3	26-50 % coverage	severely overgrown
4	>50 % coverage	very severely overgrown

Results and Discussion

Figure 3 shows effective and ineffective treatments of the series `artificial infection´.

The treatments with 10% acetic acid (formulation 2), propionic acid (formulations 7 and 8) as well as with the mixture of 3% boric acid and 7% propionic acid (formulation 21) prevented mold growth totally over the whole incubation period of 8 weeks.

The results of both test series are summarized in Table 3.

Table 3. Efficacy of anti-mold treatments (cf. Table 2)

Incubation time (weeks)	1				2				4				8			
Bamboo species	Ts		Bs		Ts		Bs		Ts		Bs		Ts		Bs	
Test series	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Formulation 1	0	0	0	0	1	0	0	0	4	0	3	0	4	3	4	3
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	2	1	4	3	4	4	4	4	4	4	4	4	4	4	4	4
4	2	0	4	4	4	3	4	4	4	4	4	4	4	4	4	4
5	4	1	4	2	4	4	4	4	4	4	4	4	4	4	4	4
6	4	2	3	4	4	4	4	4	4	4	4	4	4	4	4	4
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	2	0	4	3	4	3	4	4	4	4	4	4	4	4
10	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
11	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4
12	0	0	2	0	3	1	4	3	4	4	4	4	4	4	4	4
13	0	0	1	0	4	1	4	4	4	4	4	4	4	4	4	4
14	0	0	3	3	4	2	4	4	4	4	4	4	4	4	4	4
15	0	0	0	0	3	1	4	1	4	4	4	4	4	4	4	4
16	1	1	3	1	4	3	4	4	4	4	4	4	4	4	4	4
17	1	0	3	2	4	3	4	4	4	4	4	4	4	4	4	4
18	0	0	0	0	0	0	0	0	1	0	0	0	3	2	0	0
19	2	0	2	0	4	2	4	3	4	4	4	4	4	4	4	4
20	2	0	2	2	4	4	4	4	4	4	4	4	4	4	4	4
21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Control	3	0	3	0	4	3	4	3	4	4	4	4	4	4	4	4

Ts =Thyrostachys siamensis, Bs = Bambusa stenostachya, A = artificial infection, B = natural mould flora

After 8 weeks, there were no significant differences in final molding between the test series `artificial infection´ and `natural mould flora´´. Some differences occurred within the first two weeks, the inoculated specimens being faster overgrown due to high amount of spores in the inoculum.

Ten percent acetic acid, 7% propionic acid and the boric/propionic acid mixture prevented mold growth in both series completely. All other formulations allowed severe (rating 3) or very severe (rating 4) mold growth. Both bamboo species behaved rather similar regarding mold susceptibility and prevention. The exception was formulation 18, showing moulded *T. siamensis* and clean *B. stenostachya* specimens.

To avoid drying of the bamboo specimens during eight weeks of culture, completely closed plastic basins were used as culture vessels for fungi. As objection to the experimental design, a shortage of oxygen should be considered. However, the air volume in the culture basins of over 500 cm^3 should have been sufficient for the relatively few mold hyphae living on the small bamboo specimens.

For the laboratory experiments, only one infection of bamboo specimens was performed. Under field conditions with larger samples, bamboo is exposed to permanent infection pressure from the surrounding air. Possibly, the applied concentrations do not meet those practical conditions.

The tested chemicals do not fix to the bamboo tissue. Washing out by rain and evaporation of active substances, particularly under direct sunshine, reduce protective efficacy (Willeitner and Liese 1992). For practical use, samples must be protected from rain and sunshine all the time, both being during container transport.

Conclusion and Recommendations

The laboratory experiments have shown that molding of bamboo can be prevented by simple treatment with environment-friendly chemicals. Results revealed that treatment of bamboo samples with acetic acid, propionic acid and a mixture with boric acid prevented mould growth for 8 weeks. The other chemicals were less effective or ineffective.

Experiments with the effective formulations will now be continued under field conditions in Vietnam with larger bamboo dimensions as culms, round and split as well as handicrafts and commodities. Since molding of bamboo is a serious devaluation in trade, corresponding experiments are recommended for other bamboo countries with their respective species.

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**Figure 1. Molded bamboo culms at arrival in Ha
Vietnam**

Figure 2. Specimens in plastic basins for mould protection test

Figure 3. Artificially infected and differently treated bamboo specimens after 8 weeks of incubation