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In vitro Detection and Optimization of Salicylic Acid from the Rhizobacterial Strains *Pseudomonas aeruginosa* UPMP3 and *Burkholderia cepacia* UPMB3 for Plant Defense

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Abstract

Salicylic acid (SA) produced by different plant growth-promoting rhizobacteria (PGPR) is a key phytohormone that regulates plant growth and defenses against pathogens. *Pseudomonas aeruginosa* UPMP3 and *Burkholderia cepacia* UPMB3 are the most important types of plant growth-promoting rhizobacteria isolated from oil palm rhizosphere. The aim of this study was to detect and optimize SA production by the two PGPR *in vitro*. Production of SA was extracted, purified, detected, confirmed and optimized from these two rhizobacterial strains through Thin Layer Chromatography analyses (TLC). Different parameters i.e. casamino acid, pH, temperature, static and shaken condition were considered to optimize the SA production. Salicylic acid production by the two strains was confirmed by TLC analyses, in which the R_f (Retention factor) value was 0.74 respectively that were matched with the authentic SA. Both of these Rhizobacterial strains produced SA, with a maximum yield of 16.29 and 11.13 µg/ml in casamino acids at a concentration of 0.50%, 13.13 and 10.11 µg/ml under pH 7.0, 13.14 and 10.34 µg/ml under 30°C temperature, 12.95 and 9.95 µg/ml at 150 rpm in shaking condition for 3 days incubation period respectively. Therefore, the present study indicates that the rhizobacterial strains *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 have merits to be beneficial bacteria for the plant protection inducing defense mechanism.

সারসংক্ষেপ

উদ্ভিদের বৃদ্ধি উদ্দীপক বিভিন্ন রাইজোব্যাকটেরিয়া (Plant Growth Promoting Rhizobacteria) কর্তৃক উৎপাদিত স্যালিসাইলিক এসিড (SA) একটি প্রধান ফাইটোহরমোন, যা উদ্ভিদের বৃদ্ধি এবং রোগ সৃষ্টিকারী জীবাণু বা পোকামাকড়ের আক্রমণ থেকে রক্ষার উদ্দীপক হিসেবে কাজ করে। *Pseudomonas aeruginosa* UPMP3 and *Burkholderia cepacia* UPMB3 দুইটি গুরুত্বপূর্ণ রাইজোব্যাকটেরিয়াল স্ট্রেন যা অয়েলপাম উদ্ভিদের রাইজোসফেয়ার থেকে শনাক্ত করা হয়েছিল। এই পরীক্ষার লক্ষ্য ছিল উল্লিখিত দুইটি রাইজোব্যাকটেরিয়া থেকে ইনভিট্রো অবস্থায় স্যালিসাইলিক এসিড শনাক্ত ও উৎপাদন নিশ্চিত করা। স্যালিসাইলিক এসিড নিষ্কাশন, বিশুদ্ধকরণ, শনাক্তকরণ এবং নিশ্চিতকরণ Thin Layer Chromatographic বিশ্লেষণের মাধ্যমে করা হয়েছিল। স্যালিসাইলিক এসিড-এর সর্বোচ্চ উৎপাদনে বিভিন্ন প্যারামিটার যেমন- কাসামিনো এসিড, পিএইচ, তাপমাত্রা, স্থির ও ঝাঁকানো অবস্থা বিবেচনা করা হয়েছিল। এই দুইটি রাইজোব্যাকটেরিয়াল স্ট্রেন হতে স্যালিসাইলিক এসিড-এর শনাক্তকরণ নিশ্চিতকরণে Thin Layer Chromatography

কৌশল দ্বারা উভয় ব্যাকটেরিয়ার R_f -এর মান ০.৭৪ পাওয়া গিয়েছিল যা প্রকৃত বা আসল SA-এর সমকক্ষ। উভয় ব্যাকটেরিয়ার ক্ষেত্রে ৩ দিনের ইনকিউবিশন সময়ে ০.৫০% কাসামিনো এসিডের ঘনত্বে SA এর সর্বোচ্চ উৎপাদন হয়েছিল যথাক্রমে– ১৬.২৯ ও ১১.১৩ মাইক্রোগ্রাম/মিলি.; ৭.০ পিএইচ এ ১৩.১৩ ও ১০.১১ মাইক্রোগ্রাম/মিলি.; ৩০° সেন্টিগ্রেড তাপমাত্রায় ১৩.১৪ ও ১০.৩৪ মাইক্রোগ্রাম/মিলি. এবং ঝাঁকানো অবস্থায় ১৫০ আরপিএম এ ১২.৯৫ ও ৯.৯৫ মাইক্রোগ্রাম/মিলি.। সুতরাং, এই পরীক্ষার ফলাফল ইঙ্গিত করে যে, *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 দুটি রাইজোব্যাকটেরিয়াল স্ট্রেন-এর মধ্যে উপকারী বা কল্যাণকর ব্যাকটেরিয়ার গুণাগুণ আছে যা স্বপ্রতিরোধি পদ্ধতির মাধ্যমে উদ্ভিদ রক্ষায় কাজ করে।

Key words: *Burkholderia cepacia* UPMB3, *In vitro*, Plant defense, *Pseudomonas aeruginosa* UPMP3, Salicylic acid, Thin layer chromatography.

Introduction

Plant growth promoting rhizobacteria are the dominant driving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility. Currently, the biological approaches for improving crop production are gaining additional interest among agronomists and environmentalists following integrated plant nutrient management system. In this context, there is an on-going rigorous research worldwide with greater impetus to explore a wide range of rhizobacteria possessing novel traits like biological control of phytopathogens and insects (Hynes *et al.* 2008) along with the normal plant growth promoting properties such as, phytohormone (Ahemad and Khan 2012c), siderophores (Tian *et al.* 2009), antibiotics (Bhattacharyya and Jha 2012), hydrogen cyanaide (HCN) and ammonia production, nitrogenase activity (Khan 2005; Glick 2012) phosphate solubilization, heavy metal detoxifying potentials (Ahemad and Khan 2012c; Ma *et al.* 2011), and pesticide degradation/ tolerance (Ahemad and Khan 2012a; b). Salicylic acid is produced in significant amounts by certain plant growth promoting rhizosphere bacteria, and some of these rhizobacteria have the ability to induce systemic resistance against diseases in plants. The application of SA to plants has long been known to lead to protection against plant pathogens through the elicitation of systemic acquired resistance (Bakker *et al.* 2014). Different *Pseudomonas* and *Burkholderia* species have emerged and potentially promising group of PGPR. Salicylic acid (SA) is an essential signal elicitor for the induction of Induced systemic resistance (ISR) and the orchestration of the events that occur during the hypersensitive response (HR) (Carl *et al.* 2005). Indhiragandhi *et al.* (2008) reported that *Acinetobacter* sp., *Pseudomonas* sp. and *Serratia* sp. shows production of salicylic acid, which is important component in the induction of defense in plants. Earlier, some previous studies reported the influence and role of salicylic acid for protection of plants under various biotic and abiotic stresses including salinity (Horváth *et al.* 2007; Dempsey and Klessig 2017) and in growth and development (Rivas-San Vicente and Plasencia 2011).

The presence of SA in plants is well documented and for some plant species levels in excess of 1 µg per gram fresh weight have been reported (Raskin 1992). SA is required as a signaling molecule in systemic acquired resistance (SAR) that develops in plants after attack by pathogens that cause necrosis

(Durrant and Dong 2004). Systemic Acquired Resistance (SAR) is effective against a broad range of pathogens and the protection can be long-lasting. Manipulation of this induced defense mechanism thus has potential for plant protection. Exogenous application of SA or SA mimics can indeed protect plants against a range of pathogens (Oostendorp *et al.* 2001). Whereas animals and plants both respond to application of SA, effects on fungi have hardly been studied. Production of SA by bacteria has been reported frequently, in many cases related to the production of siderophores under iron limited conditions. Next to playing a role in iron acquisition the effects of SA on plants suggest that the production of this metabolite by bacteria can have a significant impact on plant-microbe interactions in the rhizosphere. Later on, many works had been done in order to substantiate the role of SA during plant-pathogen interactions and to substantiate the fact SA plays an important role in SAR induction in tobacco and cucumber plants (Malamy *et al.* 1990; Métraux *et al.* 1990). Further; many researchers established the prime role of SA in induced systemic resistance (ISR) induction (Yalpani *et al.* 1991; Audenaert *et al.* 2002). SA plays its key role in inducing both local and systemic induced resistance initiated after an immediate plant pathogenic attack (Saikia *et al.* 2003). De-meyer and Hofte (1997) showed *P. aeruginosa* can induce systemic resistance through SA production. They assumed that PGPR can trigger on activation of phenyl alamine ammonia-layase (PAL) activity leading to increased SA biosynthesis and suggested that SA may be indirectly involved in introducing ISR induced by PGPR with special reference to cucumber root disease. So, they considered SA as an important translocated signal that gets accumulated in PGPR treated host plant roots and greatly involved in inducing ISR. Siddiqui and Shaukat (2003) showed *P. aeruginosa* enhanced defense mechanism by inducing systemic resistance in tomato plants through production of SA. Thus in this study it will be worthwhile, to review the efficacy of *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 as the producer of salicylic acid in optimized condition.

Materials and Methods

Sources of the bacterial strains

Two plant growth promoting rhizobacteria *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 were collected from Plant Protection Department, Universiti Putra Malaysia. These bacterial strains were isolated from oil palm rhizosphere. Both strains have been characterized, sequenced and deposited with NCBI Gen Bank (Accession no. GQ183951 - *P. aeruginosa* strain UPMP3 and GQ183952 - *B. cepacia* strain UPMB3). The bacterial strains were also identified based on Biolog® identification system (Zaiton *et al.* 2006; Azadeh *et al.* 2010). In the current study, these bacterial strains were prepared from stock cultures stored at 4°C and subsequently sub-cultured on nutrient agar when required.

Reconfirmation of the bacterial strains using Biolog Reader

P. aeruginosa UPMP3 and *B. cepacia* UPMB3 were grown on nutrient agar (NA) medium for routine use, and maintained in Nutrient Broth (NB) medium with 15% glycerol at - 80°C for long-term storage. The bacterial isolates were identified and reconfirmed with the Biolog® identification system (version 4.2). The procedure for identification utilized 96 wells of microplate containing 95 different dried carbon

source plus control. Single colony of fresh bacteria from 24 hours old culture growing on NA was inoculated on Biolog Universal Growth (BUG) medium. The bacterial inoculation fluid were tested for turbidity and then inoculated in a GN III Biolog 96-well microliter plate with 100µL per well. The microliter plates were incubated at 30-33°C for 24 hours, and the resulting pattern of coloured wells analysed using the Microstation™ system and Biolog MicroLog™ software to give the bacterial identification.

In vitro detection of salicylic acid

Preparation of culture supernatant for SA

The rhizobacterial strains *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 were cultured on NA medium. After 24 hours incubation, the bacteria were grown again in casamino acids broth medium. The pH of the medium was adjusted to 7.0. The experiment was carried out in 250 ml Erlenmeyer flasks containing 50 ml of medium inoculated with 24 hours pre-cultured bacteria. Inoculated flasks were incubated at 34 ± 2°C on an incubator shaker at 200 rpm for 5 days in the dark condition. Thereafter, the bacterial cells were harvested by centrifugation at 8,000 rpm for 10 minutes at 4 °C and the supernatant was used for the screening and extraction of SA.

Screening of SA production

To screen SA production, the supernatant was acidified with 1 N HCl at pH 2.0. Then the supernatant was extracted with chloroform (2 × 2 ml). One ml of extract was added with 2 ml of 2M $FeCl_3$ and 1 ml of distilled water. The salicylic acid reacted with 2 M $FeCl_3$ to form a purple iron SA complex (purple iron colour indicator) with a maximum absorbance at 527 nm. The production of purple iron colour indicated the ability to produce SA of the both bacterial strains. Colour change was recorded in the spectrophotometer.

Extraction and purification of SA

Salicylic acid was extracted and purified following the method described by Shanmugam and Narayanasamy (2009). For extraction and purification of SA production, 50 ml of cell free supernatant was reduced to 20 ml by evaporation under vacuum and acidified at pH 2.0 with 1N HCl. The supernatant was extracted twice with double volume of chloroform. Extracted chloroform fraction was evaporated to dry in a rotatory evaporator at 40°C and dissolved in 1 ml of methanol. After filtering through 0.45 µm membrane filter, the extract was kept at -20°C for confirmation by TLC.

Confirmation of SA production by TLC analysis

Twenty µl of the extracted and purified SA were spotted on pre coated silica gel plates. Then the plates were developed in a solvent system consisting of chloroform: acetic acid: ethanol at the ratio of 95: 2.5: 2.5 (v/v). The plates were viewed under UV light (254 nm and 365 nm) immediately after removal from the developing chamber. The SA was detected by observing a UV reflected band with an R_f value corresponding to that of standard SA.

Optimization and Quantification of SA Production

The efficiency of *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 to produce SA was tested in casamino acids medium with different concentrations of casamino acids (0.10-0.70%) at different pH (3-9), temperature (20-45°C) and in static and shaken conditions (50-250 rpm). The broth cultures were incubated for 5 days and cells were separated by centrifugation at 8000 rpm for 10 minutes. The supernatant was collected and SA was measured spectrophotometrically at 530 nm. All the

harvesting procedures were carried out in dim light with samples maintained in covered ice baths.

Effect of casamino acid concentration on SA production

To check the effect of casamino acid on SA production, casamino acid was prepared with different concentration (0.10-0.70%) and inoculated with the selected strains. 1% inoculum of optical density $(O.D)_{600}$ 1.0 was incubated for 1-5 days respectively. After incubation the broth was centrifuged at 8000 rpm for 10 minutes. The supernatant was collected and measured SA for quantification.

Effect of pH on SA production

To optimize different pH level on SA production by the strains UPMP3 and UPMB3, selected concentration of casamino acid medium was adjusted to pH as 3, 4, 5, 6, 7, 8 and 9. Medium was inoculated with 1% inoculum and incubated for 1-5 days respectively. After incubation, the broth was centrifuged at 8000 rpm for 10 minutes. The supernatant was collected and measured SA for quantification.

Effect of culture conditions on SA production

To optimize different culture conditions on SA production, bacterial strains were grown in selected concentration of casamino acid medium at a range of 1-5 days in static and shaken (50, 100, 150, 200, and 250 rpm) conditions at 28±2°C on incubator shaker respectively. After incubation the broth was centrifuged at 8000 rpm for 10 minutes and collected the supernatant. Then SA was measured for quantification.

Effect of temperature on SA production

To optimize the temperature on SA production by the bacterial strains were grown in the selected concentration of casamino acid medium at a range of 1-5 days in different temperature (25, 30, 35, 40 and 45°C) on incubator shaker. After incubation the broth was centrifuged at 8000 rpm for 10 minutes. The supernatant was collected and SA was measured for quantification.

Statistical analysis

All experiments were performed as Completely Randomized Design (CRD). Data were analysed using statistical analysis system (SAS v9.3) and means were statistically compared using LSD test. The significance level was set up at p <0.05. Three replications were considered for each treatment and repeated twice.

Results

Confirmation of bacterial strains using Biolog reader system

P. aeruginosa UPMP3 and *B. cepacia* UPMB3 were confirmed with the Biolog® identification system and categorized into *P. aeruginosa* and *B. cepacia* taxonomic groups. The bacterial strains were identified as shown in Table 1 and Fig. 1.

Table 1. Identification and reconfirmation of *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 from Biolog® identification system.

Rhizobacterial strain	Probability	Similarity	Distance	Type
P. aeruginosa UPMP3	100	0.57	6.68	GN-NENT
B. cepacia UPMB3	100	0.84	2.83	GN-NENT

Based on Biolog® identification system the bacteria UPMP3 was identified as *P. aeruginosa* and UPMB3 as *B. cepacia* with similarity reading of 0.57 and 0.84 respectively. Probability values were both 100.

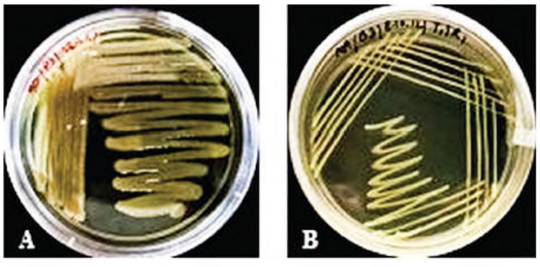


Figure 1. Bacterial strains on culture media. A: *P. aeruginosa* UPMP3 B: *B. cepacia* UPMB3.

Screening and confirmation of SA production by TLC

The bacterial strains were screened for SA production. *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 showed purple iron colour react with $FeCl_3$ which indicated their ability to produce SA (Fig. 2).

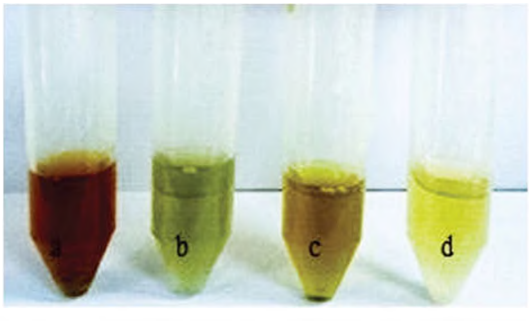


Figure 2. Screening of SA produced by P. *aeruginosa* UPMP3 and *B. cepacia* UPMB3. a: UPMP3 (purple iron colour), b: UPMP3 (control), c: UPMB3 (purple iron colour), d: UPMB3 (control).

Detection and confirmation of SA production by TLC were carried out with the appearance of blue bands matched with that of authentic SA bands on pre-coated silica gel plates under UV illumination at 365 nm. The R_f value was found 0.74 both for *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 that was similar to the R_f value (0.74) of the authentic SA (Fig. 3).

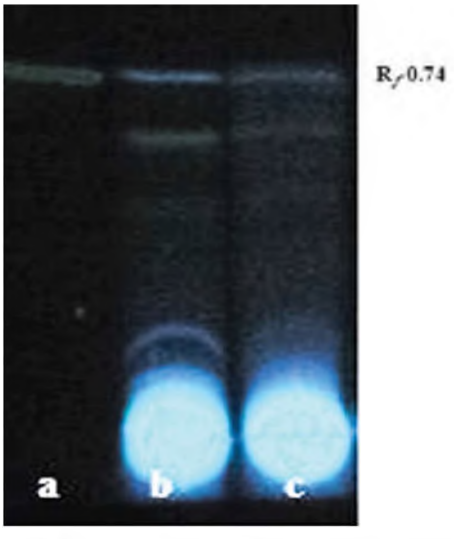


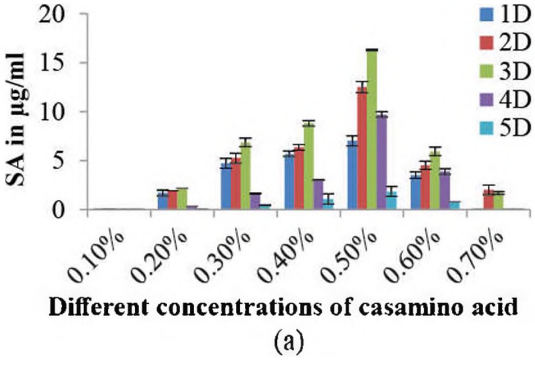
Figure 3. Detection of SA by TLC, a: Standard SA, b: UPMP3, c: UPMB3.

Optimization and Quantification of Salicylic Acid

The optimal conditions for the SA production by UPMP3 and UPMB3 were standardized.

Effect of casamino acid concentration on SA production

Different concentrations of casamino acid as a substrate ranging from 0.10 - 0.70% was tested for the production of SA from UPMP3 and UPMB3. The optimum SA production was found 16.29 µg/ml from UPMP3 and 11.13 µg/ml from UPMB3 in 0.50% casamino acid concentration on the 3rd days of incubation period (Fig. 4a &4b).



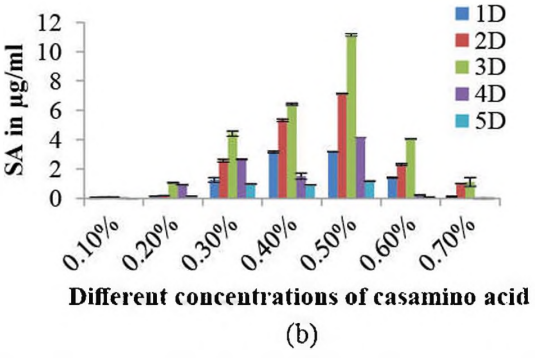
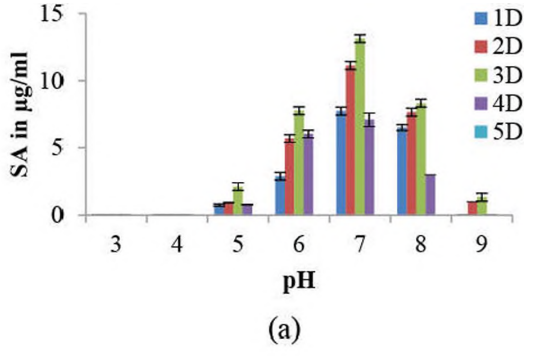


Figure 4. Production of salicylic acid by UPMP3 (a) and UPMB3 (b) at different concentration of casamino acid. Vertical bars represent standard error.



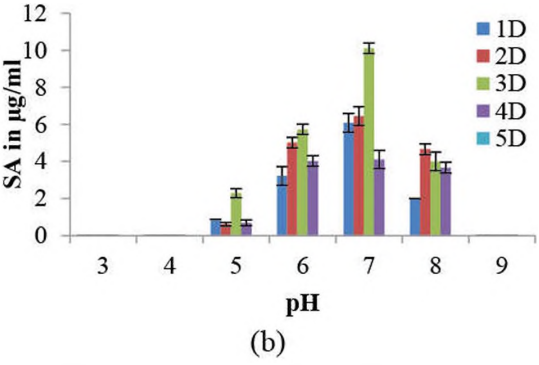


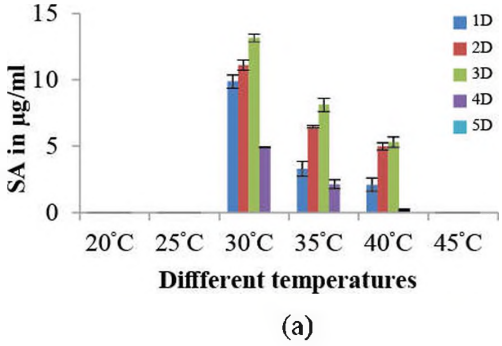
Figure 5. Production of salicylic acid by UPMP3 (a) and UPMB3 (b) at different pH. Vertical bars represent standard error.

Effect of pH on SA production

Among different pH tested, the pH 7.0 favoured the maximum SA production of 13.13 µg/ml from UPMP3 and 10.11 µg/ml from UPMB3 as against 2 µg/ml -8 µg/ml in rests of the pH on the 3rd days of incubation period (Fig. 5a & 5b).

Effect of Temperature on SA production

Among different temperature tested, P. aeruginosa UPMP3 and B. cepacia UPMB3 produced maximum SA of 13.14 µg/ml and 10.34 µg/ml respectively at 30°C on the 3rd day of incubation period. The SA production in rest of the temperature ranged between 2 µg/ml - 11 µg/ml. It has been observed that in both the lower and higher temperature (20, 25, and 45° C), there was no SA production (Fig. 6a & 6b).



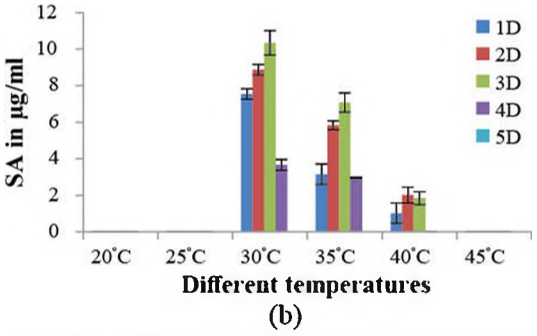


Figure 6. Production of salicylic acid by UPMP3 (a) and UPMB3 (b) at different temperatures. Vertical bars represent standard error.

Effect of culture condition on SA production

Among the static and shaken conditions, culturing *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 at shaken condition with 150 rpm was found to be optimum for the production of SA 12.95 µg/ml and 9.95 µg/ml successively on the 3rd days of incubation period than static condition (Fig. 7a & 7b).

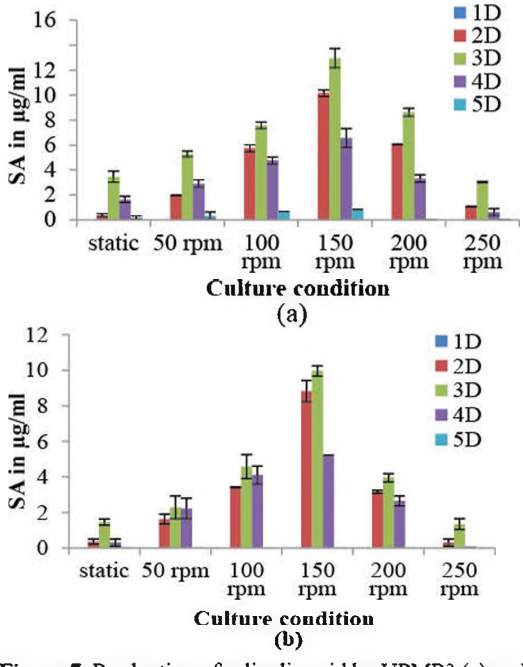


Figure 7. Production of salicylic acid by UPMP3 (a) and UPMB3 (b) at static and shaken conditions. Vertical bars represent standard error.

Discussion

Salicylic acid is known to play major roles in regulating plant defense responses against various pathogens, pests and abiotic stresses such as wounding and exposure to ozone (Balbi and Devoto 2008). It is generally involved in the activation of defense responses against biotrophic and hemi-biotrophic pathogens as well as the establishment of systemic acquired resistance (SAR). Recently, it has been shown that, methyl salicylate, which is induced upon pathogen infection, acts as a mobile inducer of SAR in tobacco (Park *et al.* 2007). SA levels increase in pathogen challenged tissues of plants and exogenous applications result in the induction of pathogenesis related (PR) genes and enhanced resistance to a broad range of pathogens. The PGPR activity and its potential capability of salicylic acid production for sustainable plant protection is well established (Parvin *et al.* 2015). SA in the culture filtrate of the *P. aeruginosa* IE-6S^{+} strain was detected in TLC as a blue spot at an R_f value of 0.91 after exposure to ammonia fumes. *In vitro* SA production by *P. aeruginosa* IE-6S^{+}, determined spectrophotometrically, revealed that the bacterial inoculant cultivated in casamino acid liquid medium synthesized SA at 3.9 ± 1.1µg/ml (Imran and Shaukat 2003). *Bacillus licheniformis* MML2501 produced SA that was confirmed by the blue bands that appeared on pre-coated silica gel viewed under UV illumination and the R_f value was 0.61 which was similar to the authentic SA (Shanmugam and Narayanasamy 2009). In order to determine the ability of the B. licheniformis MML2501 for the production of salicylic acid, experiments were conducted under *in vitro* and *in vivo* conditions. Under optimal pH, temperature, concentration of substrate and shaken conditions, *B. licheniformis* MML2501 showed maximum

production of 18 µg/ml of SA, which is important component in the induction of plant mediated defense enzymes (Shanmugam and Narayanasamy 2009).

The antagonistic potential of the isolated purple non sulfur bacterium (PNSB) *Rubrivax gelatinosus* RASN4 strain was tested in terms of their potentiality of SA production (*in vitro*), which showed a strong positive indication through screening prior to their quantification. During estimation, the amount of *in vitro* SA production by PNSB strain RASN4 was determined up to 27.3 mg/l as maximum highest level (Gupta and Sinha 2020). In TLC, appearance of blue bands both in case of samples and authentic SA standard control, showing same fluorescence postulated strongly the capability of *in vitro* bacterial SA production potentials of isolated rice rhizospheric PNSB RASN4 *Rubrivax gelatinosus* strain (Gupta and Sinha 2020). Production of SA is also influenced by temperature. Ran *et al.* (2005) reported that the relatively high production of SA *in vitro* by *P. fluorescens* strain WCS374 is even enhanced at supra-optimal temperatures (i.e. 31–33°C). This increase was also observed for *P. fluorescens* CHA0r, but not for *P. fluorescens* WCS417r or *P. aeruginosa* 7NSK2. Similarly salicylic acid has been found synthesized in the culture supernatants of *Pseudomonas aeruginosa* and *Pseudomonas cepacia* (Viska *et al.* 1993). Similar effect of temperature (30 °C) on SA production was observed in this study. Indiragandhi *et al.* (2008) also reported that *Serratia* sp. PSGB13, *Acinetobacter* sp. PRGB16 and *Pseudomonas* sp. PRGB06 produces extra cellular salicylic acid with the concentration of 10.0 ± 0.7, 7.2 ± 0.6 and 6.8 ± 0.4 g/ml respectively. De Meyer and Hofte (1997) stated that some plant growth promoting bacteria (PGPB) do trigger a salicylic acid dependent signaling pathway by producing small amount of salicylic acid in rhizosphere. Apart from the production of secondary metabolites, induced systemic resistance in plants by rhizobacteria may also be attributed to the diseases suppression. ISR mediation through salicylic acid is already well established (Van loon *et al.* 1998). Enhancement of induced disease resistance by salicylic acid dependent pathways against bacterial pathogen was carried out in *Arabidopsis thaliana* (Van Wees *et al.* 2000). *Pseudomonas* is the best studied genus for SA production and SA-producing species include *Pseudomonas aeruginosa*, *P. aureofaciens*, *P. corrugata* and *P. fluorescens*. *P. fluorescens* holds the largest number of SA-producers studied, including strains WCS374 and WCS417, CHA0, Pf4–92, Pf12–94, Pf151–94 and Pf179–94 or PICF3, PICF4 and PICF7 (Saikia *et al.* 2003; Mercado-Blanco *et al.* 2004). The latter strains were reported to produce only minor amounts of SA when grown in standard succinate medium (SSM) (Mercado-Blanco *et al.* 2004). Among them, the olive (*Olea europaea* L.) root endophytic strain PICF7 has been shown to be an efficient biological control agent (BCA) against Verticillium wilt of olive (*Verticillium dahliae*) and able to trigger a broad range of defense responses in olive root tissues (Schilirò *et al.* 2012). *Pseudomonas fluorescens* strains WCS374 and WCS417, isolated from potato (*Solanum tuberosum* L.) and wheat (*Triticum aestivum* L.) rhizospheres respectively have been investigated for plant growth promotion and biocontrol activities in several plant species and against diverse pathogens (Bakker *et al.* 2007a; De Vleesschauwer and Höfte 2009). *In vitro* production of SA by strains WCS374 and WCS417 was measured in SSM with low iron availability. Strain WCS374 can

be considered as a SA 'super-producer' (up to 55 µg per ml) *in vitro*. This amount is approximately 10 times higher than that detected for WCS417 as well as for other SA producers under similar culturing conditions (Leeman *et al.* 1996). *P. fluorescens* CHA0, a well-studied PGPR strain, was originally isolated from roots of tobacco (*Nicotiana tabacum* L.) plants grown in soil naturally suppressive to black root rot, a disease caused by *Thielaviopsis basicola* (Stutz *et al.* 1986). For this strain *in vitro* SA production was detected under low iron conditions and effects of carbon sources and minerals on production have been investigated (Duffy and Défago 1999). At elevated temperature, SA production by CHA0 is enhanced (Ran *et al.* 2005a). Besides SA-producing rhizosphere pseudomonads, other bacterial genera have been demonstrated to produce SA. *Serratia marcescens* strain 90–166 was also characterized as an SA-producing rhizobacterium. SA biosynthesis by strain 90–166 is affected by the culture medium with the highest production in Kings medium B that has low iron availability (Zhang *et al.* 2002). Whereas measuring bacterial SA production in vitro is rather straightforward, detection of bacterial SA in the rhizosphere is challenging. On cucumber roots colonized by *P. aureofaciens* 63–28 or *P. corrugata* 13, Chen *et al.* (1999) measured elevated levels of SA as compared to control roots. However, the elevated levels were magnitudes higher than those produced by the bacteria *in vitro*, and the authors concluded that the bacteria stimulated the plant itself to accumulate SA.

Many PGPR have the ability to produce SA in an iron availability dependent way and SA is detected on plant roots (Hayat *et al.* 2013), although likely originating from plant root tissues upon interaction with rhizobacteria. Given the fact that several PGPR can elicit induced systemic resistance (ISR) in plants (Kloepper *et al.* 2004) and that application of SA to plants leads to induced resistance against a range of pathogens (White 1979; An and Mou 2011). Like all other PGPR bacterial strains *Pseudomonas aeruginosa* (Siddiqui and Shaukat 2003), *Bacillus licheniformis* (Shanmugam and Narayanasamy 2009), *B. cereus, B. mycoides, B. pumilus, B. sphaericus* and *B. subtilis* (Abdel-Monaim 2017; Saikia *et al.* 2003) *Pseudomonous fluorescens*, *Serratia marcesceus (*Zhang *et al.* 2002), many PNSB rhizobacterial strains can show the capability of SA production. Parvin *et al.* (2015) confirmed the potentiality of PGPR bacterial strains *Pseudomonas aeruginosa* UPMP3 *Burkholderia cepacia* UPMB3 for synthesizing salicylic acid (SA) *in vitro* and also proved their role in oil palm seedling growth and development. In present study, prime focus was emphasized on plant growth promoting rhizobacterial strains *P. aeruginosa* UPMP3 and *B. cepacia* UPMB3 to assess its potentiality for *in vitro* SA production as it show promising plant growth promoting rhizobacterial activity in order to biofomulate a potent microbial biofertilizer bioinoculant together with some phytopathoremidiatory traits. This study also indicated that the rhizobacterial strain UPMP3 and UPMB3 as a high salicylic acid producing organism has the merits to be explored for its ISR mediated defense induction in plants and can also be used in the xenobiotic environment.

Conclusion

In recent years, salicylic acids (SA) of microbial origin are being used as an extensive strategy in order to protect the plants from plant pathogens to mitigate plant disease for its control. Salicylic acid, exogenously produced

by the bacterial microorganisms, plays a pivotal role in plant growth and development together with the enhancement of their crop productivity through their phytopatho-remediatory effects inducing system resistance against a wide range of fungal and bacterial phytopathogens. Such capability of exogenous Salicylic acid production by the strain UPMP3 of *P. aeruginosa* and UPMB3 of *B. cepacia* might have been exploited for bio formulating an efficient microbial biofertilizer with additional potentiality of phytopathore-mediation in addition to their other PGPR activity traits. This will provide an additional synergistic advantage for bio formulating a potential microbial bioinoculant for promising agricultural crop productivity in Indian subcontinent.

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Chemical Characterization of Tolla Bamboo (*Bambusa longispiculata*) of Different Ages and Heights

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Abstract

Bamboo is an abundant source of biomass that is underutilized despite having a chemical composition and fiber structure similar to wood. However, there is limited information about the chemical characterization of its culms for its utilization and processing. This paper investigated the main chemical compositions of 1, 2, 3, 4 and 5 years old *B. longispiculata* (Talla bamboo) at their three specific positions (top, medium and bottom). All the tests were conducted following the standard TAPPI (Technical Association of the Pulp and Paper Industry) methods. The water-soluble extract of the bamboo is in the range of 3.94-7.09%, 4.56-7.20% and 19.12-27.64% for cold water, hot water and caustic soda (1% NaOH) solubility respectively for 1-5 years old bamboo at their different ages and heights. The top culm position of 5 years old bamboo had the maximum holocellulose content (72.83%). The bottom part of 5 years old bamboo showed the highest (6.39%) benzene-ethanol extract. On an average 3 to 5 years old of bamboo showed the highest lignin content (30.86%) while the minimum lignin content (25.75%) was observed for the top culm position of one-year-old bamboo. Thus, the chemical characterization in the bamboo species will facilitate the alternative use of their processing and utilization-related industry.

সারসংক্ষেপ

কাঠের মতো রাসায়নিক বৈশিষ্ট্য এবং ফাইবার গঠন থাকা সত্ত্বেও বায়োমাসের উৎস হিসেবে বাঁশকে ব্যবহার করা হয় না। বয়স এবং উচ্চতার ভিত্তিতে বাঁশ সাধারণত বিভিন্ন ধরনের রাসায়নিক বৈশিষ্ট্য প্রদর্শন করে। এই গবেষণাপত্রে ১, ২, ৩, ৪, ও ৫ বছর বয়সি তল্লা বাঁশের (*Bambusa longispiculata*) বিভিন্ন উচ্চতায় (শীর্ষে, মাঝে এবং নিচে) আদর্শ TAPPI পদ্ধতিতে রাসায়নিক বৈশিষ্ট্য নির্ণয় করে এর উপযোগিতা নির্ধারণ করা হয়েছে। গবেষণা থেকে দেখা যাচ্ছে যে, ১-৫ বছরের বাঁশগুলোর বয়স ও উচ্চতার ভিন্নতা অনুসারে পানিতে দ্রবণীয় নির্যাস যথা- গরম পানি, ঠান্ডা পানি এবং কস্টিক সোডায় (NaOH) দ্রবণীয়তার হার ছিল যথাক্রমে ৩.৯৪-৭.০৯%, ৪.৫৬-৭.২০% এবং ১৯.১২-২৭.৬৪%। পাঁচ বছর বয়সি বাঁশের শীর্ষ অংশে সর্বোচ্চ হলোসেলুলোজ উপাদান (৭২.৮৩%) পরিলক্ষিত হয়েছে। পাঁচ বছর বয়সি বাঁশের নিচের অংশে সর্বোচ্চ (৬.৩৯%) বেনজিন-ইথানল নির্যাস দেখা গেছে। এছাড়াও তিন থেকে পাঁচ বছর বয়সি বাঁশে সর্বোচ্চ গড় লিগনিন উপাদান ছিল (৩০.৮৬%) যেখানে এক-বছর বয়সি বাঁশের সর্বনিম্ন লিগনিন উপাদান ছিল (২৫.৭৫%)। এই রাসায়নিক বৈশিষ্ট্যগুলো প্রান্তিক ব্যবহারকারীদের জন্য দিক-নির্দেশনা হিসাবে কাজ করতে পারে। পরিশেষে, কাঠের বিকল্প ব্যবহার হিসাবে তল্লা বাঁশের রাসায়নিক বৈশিষ্ট্য নির্ণয় এর ব্যবহার সহজতর করবে।

Key words: Age and height position, *Bambusa longispiculata*, Extractives, Holocellulose, Lignin, Pulp and paper.

Introduction

Over the past few decades, bamboo has received more attention as a renewable, cheap, fast-growing, and easily available material. It is also compatible with existing processing technologies (Tong *et al.* 2005). The overall demand for wood is rising as a result of the growing world economy and population, while the amount of wood supply is expected to decline due to the global biomass demand for green energy (Van der Lugt *et al.* 2008). Consequently, the search for alternative raw materials in place of wood has come into focus (Pannipa 2013). Research on bamboo has progressed rapidly due to its wide availability and material characteristics comparable to wood (Viel *et al.* 2018). Non-timber forest products mitigating the pressure on slow-growing forest resources and the growing demand for qualitative timber (van der Lugt *et al.* 2008). Recently, there are also growing interested in the utilization of bamboo for pulp production (Rasheed *et al.* 2020; Sridach 2010), nanofiber extraction (Visakh *et al.* 2012), composite materials (Amada and Untao 2001; Chiu and Young 2020; Jain *et al.* 1992; Muhammad *et al.* 2019; Tong *et al.* 2005; Viel *et al.* 2018), and biofuel production (Sun *et al.* 2014; Yang *et al.* 2019).

Bamboo is called "Wood of the poor" in India, "Friend of the people" in China, and simply "Brother" in Vietnam, for its many versatile essential uses (Khin *et al.* 2006). Bamboo is a naturally occurring giant grass that grows abundantly in most tropical countries (mainly Asia) except Europe and Antarctica (Lakkad and Patel 1980). *B. longispiculata* is an evergreen clumping bamboo, cultivated in the tropics which is used as an ornamental and construction materials. *B. longispiculata* is native to Bangladesh, India and Myanmar besides it can be normally be found along riverbanks, roadsides, and disturbed sites (Judziewicz *et al.* 2000; Clayton *et al.* 2019). It can grow up to a height of 15 m. Bamboos are usually monocarpic species, living for many years before flowering, then flowering and seeding profusely for 1-3 years before dying (Flora of China Editorial Committee 2019).

Compared to other lignocellulosic biomass, bamboo has unique characteristics in chemical composition. The most abundant organic polymer on the planet is cellulose, which accounts for 40 to 50 % of the mass in bamboo (Li *et al.* 2014; Zhang *et al.* 2018). Hence, the chemical characterization of bamboo is emergent in determining its suitability for various applications and treatments. The accurate compositional analysis enables the evaluation of potential conversion of yields and process economics (Sluiter *et al.* 2010). Since knowledge of bamboo's basic properties is extremely limited, understanding its physio-chemical properties is essential for the effective exploitation of bamboo. However, more research is required to determine its diversified applications.

The plant's characteristics depend on its age and height position (Majumdar *et al.* 2015). Bamboo's chemical composition changes according to species, environment, age, and location, as well as culm height (Liese and Weiner 1996). Such variation changes during the growth and maturation of bamboo (Xiaobo 2004). The understanding of the variation in the chemical composition of bamboo at their different age and height is important for their potential uses. Nevertheless, the chemical properties variation of *B. longispiculata* has not yet been reported yet. Hence, this study was conducted on a detailed analysis of chemical composition at their different ages and heights, to better understand the effect of these factors

on further uses and processing of the wood processing industry.

Materials and Methods

Raw materials

Healthy, straight and defect-free 1 to 5 years-old Bamboo *(B. longispiculata)* was collected from Keucia silviculture research station, Bangladesh Forest Research Institute (BFRI), Satkania, Chattogram (92°24' E and 93°15' E longitude and 24°22' N and 25°8' N latitude), Bangladesh in April, 2021. The area is marked by hot humid summer and dry cool winter. The mean maximum and minimum temperatures of the area are 30.2°C and 12.6°C respectively with a relative humidity of 79% and annual rainfall of 2919.1 mm. Reagent grade (≥95% purity) sodium hydroxide (NaOH), acetic acid (CH_3COOH), Sodium chlorite ($NaClO_2$) and sulfuric acid (H_2SO_4) were collected from Carolina Biological Supply Company, New York City, USA. Analytical grade (≥95% purity) benzene and ethanol were sourced from Merck KGA, Darmstadt, Germany.

Preparation of raw materials

For the present study, mature culms were randomly selected and felled. The average height of the bamboo was 65 feet. The preparation of raw materials was carried out based on the methods described elsewhere (Hossain *et. al.* 2022c). The bamboo culms were cut above 15 cm from ground level and then subdivided equally into the top, middle and basal portions according to their total length. The strips were not small enough to be placed in a Wiley Mill. So, at first, the bamboo was chipped into very small pieces by a Hammer mill and dried in the sun. Then the sawdust of bamboo species was ground to fine particles (size 40-60 mesh) with Wiley mill for chemical analysis. The material was then placed in a shaker with sieves to pass through a No. 40 mesh sieve yet retained on a No. 60 mesh. The fine particles were stored in an air-tight container labeled with appropriate code to permit a complete reaction of samples with the reagents used in the analysis as shown schematically in Fig. 1.

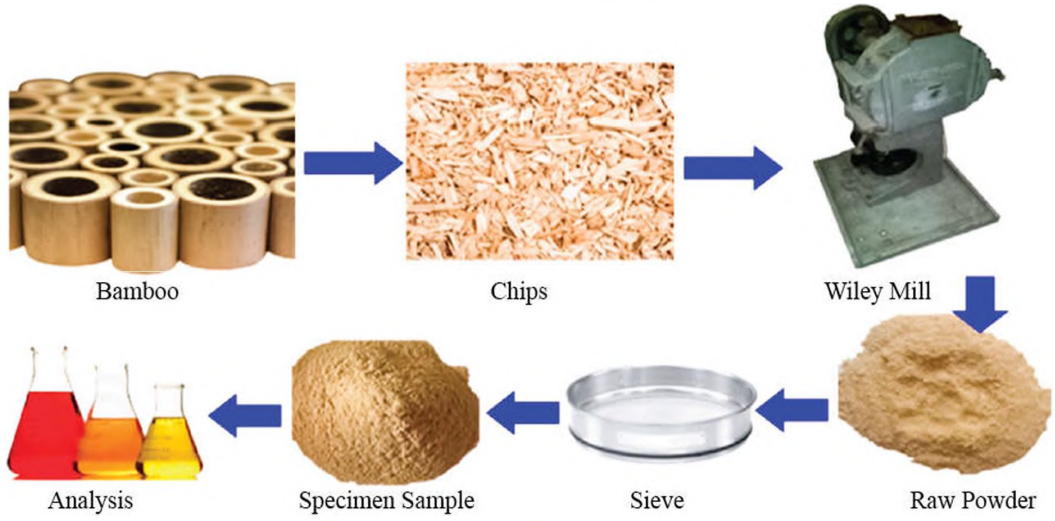

Figure 1. Preparation of bamboo powder sample for chemical analysis.

Experimental methods

Bamboo sample was analyzed at a minimum in triplicate, and the mean values were recorded. All the results were carried out on a percent basis. Chemical analysis was carried out based on the methods described elsewhere (Hossain *et al.* 2022 a, b). Proximate chemical analysis was conducted on air dry milled bamboo samples according to the following standard methods as shown in Table 1.

Table 1. Standard chemical methods followed for different experiments

Sl. No.	Name of the experiments	Name of the chemical methods
1	Moisture content	TAPPI T-264 cm-8
2	Cold water solubility	TAPPI T-207 cm-99
3	Hot water solubility	TAPPI T-207 cm-99
4	1% Sodium Hydroxide (NaOH) solubility	TAPPI T-212 om-02
5	Holocellulose	TAPPI T 249-75
6	Solvent extractives	TAPPI T 249-75
7	Total lignin content	TAPPI T-222 cm-02

Results

Solubility

Water solubility

The chemical composition of the culms is an important factor that influences the utilization and processing of bamboo. Fig. 2A & 2B were illustrated the effect of age and position on the cold and hot water solubility content of *B. longispiculata*. The hot water solubility was higher than the cold water solubility. The solubility of both types decreased with increasing age and height. The highest average cold water solubility was observed in the age graduation of *B. longispiculata viz.*, one (6.74%), two (5.30%), three (5.01%), four (4.80%), and five-year-old (4.23%). The bottom part of one-year-old bamboo showed the highest (7.20%) cold water solubility and the top portion of 5 years old bamboo showed the lowest (3.94%) value.

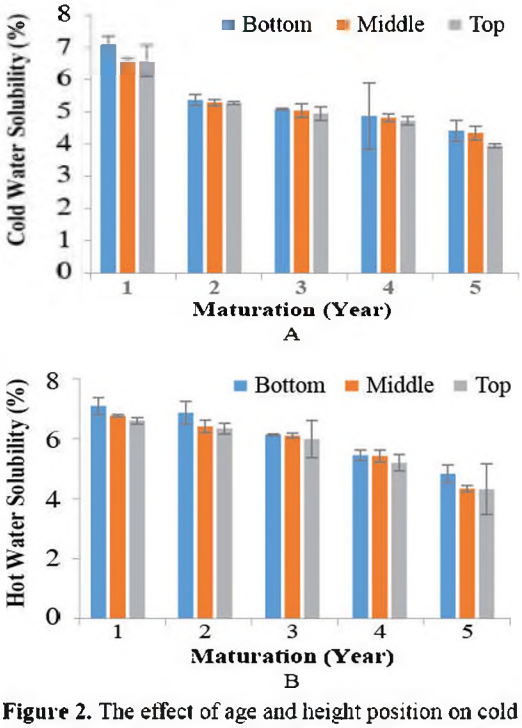

Figure 2. The effect of age and height position on cold (A) and hot (B) water solubility of *B. longispiculata*. The vertical bars represent the standard error.

On the other hand, the highest average hot water solubility values were observed in the age graduation of *B. longispiculata viz.,* 1 year (6.92%), 2 (6.64%), 3 (6.17%), 4 (5.44%), and 5 years old (4.56%). The bottom part of 1 year old bamboo showed the highest (7.20%) hot water solubility and the top portion of five-year-old bamboo showed the lowest (4.38%) values.

Caustic soda solubility

The caustic soda (1% NaOH) solubility of *B. longispiculata*, depending upon age and height is presented in Fig. 3. The caustic soda solubility was decreased with increasing age and height. The highest average values were observed in the age graduation of *B. longispiculata viz.,* 1 year (27.43%), 2 years (25.54%), 3 years (23.39%), 4 years (220.18%), 5 years old (19.31%). The bottom part of one year old bamboo showed the highest (27.64%) cold water solubility and the top portion of 5 years old bamboo showed the lowest (19.12%) value. The total average caustic soda (1% NaOH) solubility of *B. longispiculata* value was observed at 23.17%.

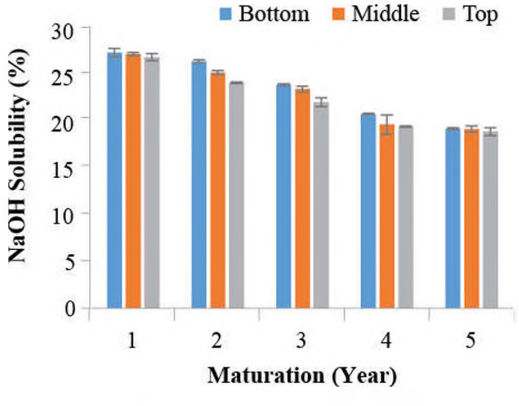

Figure 3. The effect of age and height position on caustic soda (NaOH) solubility of *B. longispiculata*. The vertical bars represent the standard error.

Extractive

From Fig. 4, the variation of extractive content was shown according to age and height position. The lowest extractive content was 0.62% for the top part of a 1 year old while the highest was 6.39% for the bottom part of a 5 years old *B. longispiculata*. The value was observed among the age graduation of *B. longispiculata viz.,* 1 (0.75%), 2 (2.53%), 3 (3.86%), 4 (5.68%), and 5 years old (6.15%). The extractive content was 3.79%. From the result, we observed that the results in different age groups were increased with the increase of age and height. It also observed significantly higher extractive content in 4 and 5 years old bamboo compared to one and 2 years old bamboo.

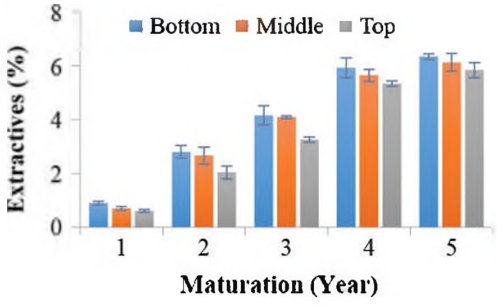

Figure 4. The effect of age and height position on extractives content of *B. longispiculata*. The vertical bars represent the standard error.

Holocellulose

The effect of age and height position on the holocellulose content of *B. longispiculata* was presented in Fig. 5. The bottom part of 1 year old bamboo showed the lowest (66.58%) and the top portion of 5 years old bamboo showed the highest (72.83%) holocellulose content. *B. longispiculata* showed different holocellulose content for 1 (67.04%), 2 (68.56%), 3 (70.12%), 4 (71.95%) and 5 (72.69%) years old bamboos. The average holocellulose content was recorded as 70.07%. The results showed a

general increasing trends for holocellulose contents with an increase in the age and height position of the bamboo.

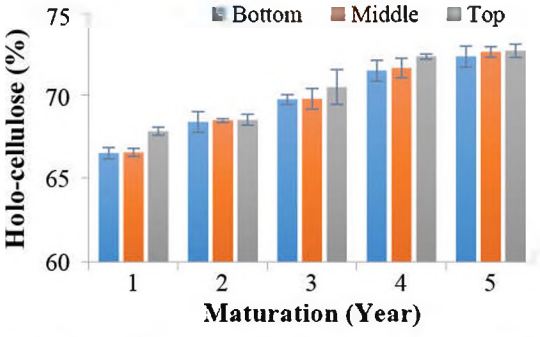

Figure 5. The effect of age and height position on holocellulose content of *B. longispiculata*. The vertical bars represent the standard error.

Lignin

Fig. 6 represented the effect of age and height position on the Klason lignin content of *B. longispiculata*. The bottom portion of 3 years old *B. longispiculata* showed the lowest lignin content (25.57%) and the top portion of 3 years old showed the highest lignin content (31.23%). The lowest average values were observed in the age graduation of *B. longispiculata viz.,* 1 year (26.60%), 2 years (29.51%), 3 years (30.05%), 4 years (31.31%), 5 years old (31. 20%). The total average lignin content was 29.74%.

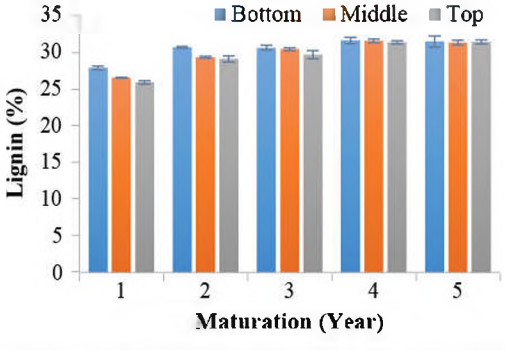

Figure 6. The effect of age and height position on total lignin content of *B. longispiculata*. The vertical bars represent the standard error.

Discussion

For a given bamboo species, the chemical composition cannot be defined precisely, and their composition varies with three parts (top, middle and bottom) and different ages. According to their varying chemical compositions, their utilization also varies.

The extractive contents, particularly cold and hot water soluble are important in the predetermination of water-soluble extractives. The cold water removes a part of extraneous components, such as inorganic compounds, tannins, gums, sugars, and coloring matter present in bamboo. On the other hand, the hot water treatment removed from the bamboo includes tannins, gums, sugars, coloring matter, and starches (TAPPI 2001). The water solubility test indicates the levels of these materials in the wood. The bottom position of all age classes gave the higher value of cold and hot water soluble compared to other portions. The tasted bamboo species did not contain a high concentration of hot water-soluble (10-15%) which may influence the susceptibility to insect and fungal attacks (Khin *et al.* 2006). The obtained result of hot water solubility is more comparable reported by Azeez *et al.* (2016) for *Bambusa vulgaris* These values fall within the acceptable range for paper production.

The alkali extractives (1% NaOH) are low molecular weight carbohydrates that consist of degraded cellulose and hemicellulose in bamboo. The content indicates the intensity of deterioration caused by fungi, heat, light and oxidation that is closely related to decay resistance (Junior and Moreschi 2003; Jiang *et al.* 2015). According to different height levels and ages, the value of alkali-soluble decreased with increasing height and age levels. Balaban and Ucar (2001) said this happens because the

bottom portion has a higher content of organic acid, polysaccharides, polyphenols, and tannin compared to other portions and ages. According to Jiang *et al.* (2015), 1% NaOH soluble extractive content of *B. vulgaris* was determined as 16.50% and according to Selvan *et al.* (2017) it was 33.13% for 5 years old and it was 18.19% for 1 year old which are comparatively similar with our result. Alkali charge must be kept low to preserve the cellulose content and enhance good pulp yield (Sadiku *et al.* 2016). *B. longispiculata* had shown a similar trend in this study. This might prevent the solubility of low molecular weight carbohydrates in a 1% NaOH solution.

It is important to consider that the amount and composition of extractives also depend on the species, part of the bamboo from which they were collected, time of year, and growth conditions, among other factors (Honorato-Salazar *et al.* 2015). In addition, the alcohol-benzene solubles in green bamboo (3.3%-3.9%) are higher than those in wood, such as poplar with a benzene-alcohol extractive content of 2.14% (Gong 2007). Li *et al.* (2007) in their studies reported that alcohol-toluene extractive content increased from the base to the top of the bamboo and showed a continuous increase with age. The presence of new cells may cause a lower amount of extractive content at the top. The higher extractive content is not beneficial to pulp and paper, and bioenergy production. It hinders the delignification and further processing. On the other hand, higher extractive content protects from biodegradation and it is beneficial to some bio-based composites. Therefore, 4 or 5 years old bamboo can be suitable for the biorefinery process. Further study can help to figure out its optimum utilization.

Holocellulose (alpha-cellulose and hemicelluloses) represents the total fraction of polysaccharides in bamboo. Height had a significant effect on holocellulose content. The top portion had a higher holocellulose content and the bottom portion had a lower holocellulose content. Hisham *et al.* (2006) reported that holocellulose content slightly increased beyond 3.5 years and the higher density of old bamboo was probably caused by greater content of lignin, ash, silica, and other extractive materials. The result compares favorably with those reported by Li *et al.* (2007) and Tsoumis (1991) for *Eucalyptus camadulensis* (55.6%), *E. hybrid* (67.80%), and generally for softwood (67%) and hardwood species (71.0 to 89.1%). This bamboo species showed holocellulose content (70.07%) in the range of wood species. 5 years old bamboo can be a potential source of raw material since it contains the highest amount of holocellulose and can be used for pulp and paper, bioenergy, and bio-based composite production.

The lignin present in bamboo is unique. The lignification process changes during the elongation of the culm; the full lignification of the bamboo culm is completed within one growing season, showing no further aging effect (Itoh and Shimaji 1981). Low quantities of lignin in lignocellulosic materials are desirable in the paper industry because they increase the pulp yield (Rowell 1984; MacLeod 2007). The lignin content in green bamboo showed much lower value than that of softwoods and hardwoods (Cai and Tao 2007; Gong 2007). The lignin values of 20-26% place bamboo at the high end of the normal range or 11-27% reported by Bagby *et al.* (1971) for non-woody biomass and closely resemble the ranges reported for softwoods (24-37%) and hardwoods (17-30%) (Fengel 1984; Dence 1992). The average lignin content

of *Yushania alpina, Phyllostachys edulis,* and *Bambusa oldhamii* at the ages of 1–3 years old are 25.27%, 20.35, and 20.9% respectively which are higher compared to the current results (Nahar and Hasan 2013). On the other hand, the high lignin content contributes to the high heating value of bamboo, and its structural rigidity makes it a valuable building material (Scurlock *et al.* 2000).

Conclusion

The study concentrated on a detailed analysis the chemical composition at different ages and heights of *B. longispiculata* to have a better understanding of the effect of these factors on the chemical composition of this bamboo species. The bottom portion of 5 years old bamboo showed the highest benzene-ethanol extractive; it may be advantageous for anti-decay and more suitable for structural application, furniture making and external uses. Based on the different height portions of bamboo, our result indicated a higher value at the bottom portion except for the holocellulose content. The higher lignin content is problematic for the delignification. It is troublesome for the application in pulp and paper, bioenergy, and bio-based composite. Therefore, less than 3 years old bamboo is beneficiary used in the biorefinery process. However, lignin is also a potential source of biorefinery. On the other hand, 5 years old bamboo contains the highest amount of holocellulose. Considering this, four or 5 years old bamboo is a promising source of raw materials in the biorefinery process. The variation of lignin content should be considered for the delignification. The proper ratio height position can solve the problem. Further study with cost-benefit analysis can mitigate all the issues with appropriate applications.

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Improving Strength Properties of Recycled Paper by adding Virgin Jute Pulps

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Abstract

Strength properties are very important for paper grading; usually recycled fibre produce low grade paper. However, it is not easy to produce quality paper from recycled paper without addition of virgin pulp. In this study, the paper was made by mixing new pulps in different proportions with recycled pulp from used paper to explore their quality, and hence their various physical and mechanical properties were tested. A mixture of newsprint books and whiteprint books (1:1) was used to make recycled pulp through hydrapulper (a type of pulp-making machine). The reaction conditions were: temperature 50°C, duration 30 minutes, pulp consistency 10%, sodium hydroxide (NaOH) 0.8% (w/w), sodium silicate (Na_2SiO_3) 0.8% (w/w), detergent 0.15% (w/w) and hydrogen peroxide (H_2O_2) 0.8%(w/w). The resulting pulp was thoroughly washed with tap water and the adhesive and plastic substances were removed with the help of a screening machine. Tossa jute fibre was used to make new pulp because its bastfibre and pulp quality is excellent. The fibres were first to cut into 1.5-2.0 inches and then pulp was made using the neutral sulfite anthraquinone (NS-AQ) method with an alkaline rate of 20% at 175°C. The test papers of 8 cm diameter and 1.25 cm thickness were made by mixing the new pulp with the recycled pulp in seven proportions (90:10, 80: 20, 70: 30, 65:35, 60:40, 55:45 and 50:50).Then the physical and mechanical properties of the test papers such as freeness (rate of water removal from the pulp), tear index, tensile index, burst index, folding endurance were determined. Strength factor (Tear index × Tensile index) of produced paper are 406.29(90:10), 482.05(80:20), 588.15(70:30), 701.55(65:35), 757.26(60:40),745.53(55:45) and 820.83(50:50). The results showed that the quality of paper was increased with the increased ratio of jute fibre. It was also observed that "A" grade paper was obtained from a minimum 35% mixture of Jute pulp.

সারসংক্ষেপ

কাগজের গ্রেডিংয়ের জন্য শক্তি বৈশিষ্ট্যগুলি খুবই গুরুত্বপূর্ণ; সাধারণত রিসাইকেল ফাইবার নিম্নমানের কাগজ তৈরি করে। তবে ভার্জিন পাল্প ছাড়া রিসাইকেল পেপার থেকে মানসম্পন্ন কাগজ তৈরি করা সহজ নয়। এই গবেষণায়, কাগজের গুণগতমান অন্বেষণের জন্য পুনর্ব্যবহৃত পাল্পের সাথে বিভিন্ন অনুপাতে নতুন পাল্প মিশ্রিত করে কাগজ তৈরি করা হয়েছিল, এবং এদের বিভিন্ন ভৌত এবং যান্ত্রিক বৈশিষ্ট্যগুলি পরীক্ষা করা হয়েছিল। নিউজ বই এবং সাদা বইয়ের মিশ্রণ (১:১) হাইড্রোপাল্পার (এক ধরনের পাল্প তৈরির মেশিন)-এর মাধ্যমে পুনর্ব্যবহৃত পাল্প তৈরি করতে ব্যবহৃত হয়েছিল। বিক্রিয়ার শর্ত ছিল; তাপমাত্রা ৫০° সে., সময়কাল ৩০ মিনিট, মণ্ডের ঘনত্ব ১০%, সোডিয়াম হাইড্রোক্সাইড (NaOH) ০.৮% (w/w), সোডিয়াম সিলিকেট (Na_2SiO_3) ০.৮% (w/w), ডিটারজেন্ট ০.১৫% (w/w) এবং হাইড্রোজেন পারক্সাইড (H_2O_2) ০.৮% (w/w)। প্রাপ্ত মণ্ড ট্যাপের পানি দিয়ে পুঙ্খানুপুঙ্খভাবে ধৌত করা হয়েছিল এবং স্ক্রিনিং মেশিনের সাহায্যে আঠালো এবং প্লাস্টিক জাতীয়

পদার্থগুলি অপসারণ করা হয়েছিল। তোষা পাটের ফাইবার নতুন পাল্প তৈরিতে ব্যবহার করা হয়েছিল কারণ এর বাস্ট ফাইবার এবং মণ্ডের গুণগতমান চমৎকার। ফাইবারগুলিকে প্রথমে ১.৫-২.০ ইঞ্চি করে কেটে নেওয়া হয়েছিল এবং তারপরে ১৭৫° সে. তাপমাত্রায় ২০% ক্ষারীয় মাত্রায় নিউট্রাল সালফাইট অ্যানথ্রাকুইনোন (NS-AQ) পদ্ধতি ব্যবহার করে মণ্ড তৈরি করা হয়েছিল। পুনর্ব্যবহৃত মণ্ডের সাথে নতুন মণ্ড সাতটি অনুপাতে (৯০:১০, ৮০:২০, ৭০:৩০, ৬৫:৩৫, ৬০:৪০, ৫৫:৪৫ এবং ৫০:৫০) মিশিয়ে ৮ সে.মি. ব্যাস এবং ১.২৫ সে.মি. পুরুত্বের পরীক্ষণ কাগজ তৈরি করা হয়েছিল। তারপর পরীক্ষণ কাগজের ভৌত ও যান্ত্রিক বৈশিষ্ট্য যেমন ফ্রিনেস (মণ্ড থেকে পানি অপসারণের হার), টিয়ার সূচক, টেনসাইল সূচক, বার্স্ট সূচক, ফোল্ডিং সহনশীলতা নির্ধারণ করা হয়েছিল। উৎপাদিত কাগজের গুণগতমান (টিয়ার ইনডেক্স এবং টেনসাইল ইনডেক্স এর গুণফল) হল ৪০৬.২৯ (৯০:১০), ৪৮২.০৫ (৮০:২০), ৫৮৮.১৫ (৭০:৩০), ৭০১.৫৫ (৬৫:৩৫), ৭৫৭.২৬ (৬০.৪০), ৭৪৫ (৫৫:৪৫) এবং ৮২০.৮৩ (৫০:৫০)। ফলাফলে দেখা গেছে যে পাটের ফাইবার যুক্ত হওয়ার অনুপাতের সাথে কাগজের গুণমান বৃদ্ধি পেয়েছে। আরো দেখা গেছে যে, ন্যূনতম ৩৫% পাটের মণ্ডের মিশ্রণ থেকে চমৎকার মানের ("এ" গ্রেডের) কাগজ পাওয়া গেছে।

Key words: Blending, Jute pulp, NS-AQ pulping, Recycled pulp, Strength properties.

Introduction

The worldwide consumption of writing paper, printing paper, and newsprint is contracting, while packaging, tissue papers and other hygiene products are increasing (Berg and Lingqvist 2017). Paper industry mainly uses 90% wood based virgin fibre for chemical and mechanical pulp (Jahan 2003). Wood based fibre resources are declining at an alarming rate resulting in an acute crisis of raw material supply to the industries. Over the past several years, over 60% of all paper produced in the United States has been recycled and similar amounts are used worldwide (Scott 2011). In Bangladesh bamboos and hardwood species were the main raw materials in Karnaphuli Paper Mills Limited (KPML). However, the paper mill could not reach their targets of daily paper and packaging material production due to scarcity of raw materials and electricity supply. So, it is very urgent to find a new source of pulp making raw materials for attaining continuous supply of papers and boards. Recycled paper could be an important process to meet the deficiency of pulp and preserve trees in the forest. At present, almost all of the paper mills in Bangladesh are using imported pulp and recycled paper as raw materials for paper, paperboard and corrugated board. Paper produced with recycled fibre has great environmental benefits. One metric ton of recycled paper instead of virgin paper saves 4 cubic meters of wood (Shylo and Harmer 2016), 39% of total energy, 58% of greenhouse gasses, 9% of water usage, 56% of ocean acidification, 13% of hazardous air pollutants, 20% of mercury emissions and26% of dioxin emissions (Kinsell 2018). In addition to this, wastepaper can be recycled up to 5-8 times before its fibres become too short and unsuitable for further paper production (Shylo and Harmer 2016; CannyIdeas 2016).

Physical properties of paper made from wastepaper are not suitable due to short fibre (Adu *et al.* 2018). Virgin pulp provides higher grade paper products due to their longer fibre lengths (Sheikhi *et al.* 2013). Quality paper products can also be produced from recycled pulp by mixing with certain percent of virgin pulps; because with recycling fibre lengths

gradually decrease. In the search for a potential long-fibre substitute for softwood pulp, Akhtaruzzaman and Shafi (1995) suggested jute fibre as an appropriate solution. Jute is a natural fibre containing a high value of cellulose and low lignin content (13.7%) (Nahar 1987). The fibre length (2.5 mm) is also good (Shafi *et al.* 1993b). This favors the pulping of jute fibre. It was reported that NS-AQ pulping is a promising process for pulping of jute fibre (Akhtaruzzaman *et al.* 1988; Akhtaruzzaman 1994; Shafi *et al.* 1993a).

Besides, most popular kraft pulping process in the worldwide (Misbahuddin *et al.* 2019) paper industries has some disadvantages including low yield and the paper mill effluent characteristically containing lignin, suspended solid, sulfur and sulfur compound (Ruiz 2011). Bad odor of effluent creates an unhygienic environment. The NS-AQ process of jute pulping reduces this bad odor (Shafi *et al.* 1993a). Therefore, the study is undertaken to improve strength properties of recycled packaging material by blending with virgin jute pulp in various proportions.

Materials and Methods

Raw material collection and processing

Waste paper (newsprint book and whiteprint book) were collected from wastepaper supplier at Aturar Depo in Chattogram. Waste papers were shorted for removing stapler pins, treats and laminating paper. Physical strength of waste paper like tear, tensile, burst and folding endurance were evaluated. Jute fibre was collected from Chattogram local market. The fibres had been cut into 1-1.5″ size and stored into a polythene bag for preparation of pulp.

Hydrapulping of recycled pulp

A mixture of newsprint books and whiteprint books (1:1) was used to make recycled pulp through hydrapulper (Gear ratio: 1:2:1 hydrapulper size: 3.0 ft, Impellor rpm: 800 Impellor, dia: 18.5 inch, Machine serial: P57-1907) a type of pulp making machine.

Table 1. Reaction conditions for hydrapulping of recycled pulp

Parameters	Value	Role
Temperature	50°C	-
Duration	30 minutes	-
Pulp consistency	10%	-
Sodium hydroxide (NaOH)	0.8%	pH maintenance
Sodium silicate (Na_2SiO_3)	0.8%	Ink collector
Detergent	0.15%	Surfactant
Hydrogen peroxide (H_2O_2)	0.8%	Deinking

Thereafter, pulping is done for 10 minutes at 15amp current, adding 150 ml. H_2O_2 for bleaching; again hydrapulping for 15 minutes according to Table 1. The resulting pulp was thoroughly washed with water and the adhesive and plastic substances were removed with the help of a Johnson Vibratory Screening machine. The wet pulp was passed through a screw press to remove excess water and then pulp was refreshed by pulp mixture.

Jute pulping

Six pulps were prepared with neutral sulphiteanthraquinone (NS-AQ) process (Shafi *et al.* 1993a). In this purpose 250 g of oven dry jute fibre were charged in the 2 liter stainless

steel autoclaves placed in an electrically heated air bath. Analytical grades of Na_2SO_3 and Na_2CO_3 were used as cooking chemicals. Cooking time was 3.5 hours at 175°C. The time required to raise this temperature from room temperature was 90 minutes. The liquor to fibre ratio was 7:1 (v/w). Active alkali (AA) dose was 20% and anthraquinone charge was 0.1%. After each cook, the fibres were discharged and the black liquor was collected for residual alkali determination. The cooked fibres were taken in a screen box and washed overnight under running water to wash out the residual liquor. These were stirred slightly with water in a bucket by a slow speed electric mixture. The pulp slurry was then screened through 0.012 inch wide slot ftat (Johnson vibratory) screen to separate any uncooked material from the pulp. The wet pulp was passed through a screw press to remove excess water, and then samples were taken for dry matter content. The pulp yield was determined. The kappa number was determined using TAPPI methods T236 cm-85.

Hand sheet making and physical testing

The test papers of 8 cm diameter and 1.25 cm thickness were made by mixing the new pulp with the recycled pulp in seven proportions (90:10, 80: 20, 70: 30, 65:35, 60:40, 55:45 and 50:50).The pulp samples were beaten in a PFI mill to achieve a Canadian Standard Freeness (CSF) (rate of water removal from the pulp) of 250±3 ml (SCAN-C 21:65) and hand sheets were made. These were then conditioned at 23±1°C temperature and 50±2% relative humidity and tested according to SCAN-C 28:69 for determining the physical strength properties such as tear index, tensile index, burst index and folding endurance (Biswas *et al*. 2017).

Statistical analysis

The physical strength properties were evaluated from five sheets for each beating. Then the mean and standard deviation were calculated. The graphical extrapolated values at 250 CSF were represented by regression.

Results

Active alkali consumption of neutral sulphiteanthraquinone (NS-AQ) pulping of jute was 19.41%. The yield of jute pulping was found 69.71% on average and the average kappa number was 11.60. The lignin content of a pulp sample was (1.74%) calculated by multiplying the kappa number by a factor of 0.15 (Jiang 1992). Physical strength properties such as tear, tensile, burst and folding endurance were evaluated in the newsprint book and whiteprint book (Table 2). Newsprint books and whiteprint books were selected for recycling pulp. The paper sheets made from 100 percent recycled pulp and also recycled pulp blending with different proportions of jute pulps (90:10, 80:20, 70:30, 65:35, 60:40, 55:45 and 50:50) physical strength properties were calculated (Table 3).

Table 2. Physical strength properties of different grade waste paper.

Paper grade	Tear index (Nm/g)	Tensile index (mNm^2/g)	Burst index ($KPam^2/g$)
Newsprint book	7.57±0.82	56.18±2.77	2.18±0.11
Whiteprint book	5.04±0.30	48.22±3.73	2.31±0.35

Table 3. Physical strength properties of paper sheet made from different proportions of mixing of recycled (newsprint book and whiteprint book) and new (jute) pulp.

Recycled Pulp (%)	New Pulp (%)	Beating (rpm)	CSF	Tear Index (mNm^2/g)	Tensile Index (Nm/g)	Burst Index ($KPam^2/g$)	Folding Endurance (log10d)
100	0	0	300	6.38±0.00	32.97±2.27	2.09±0.14	1.13±0.04
		1000	250	6.01±0.28	40.82±1.67	2.40±0.23	1.40±0.10
		1500	220	6.19±0.00	44.07±2.01	2.52±0.19	1.47±0.11
90	10	0	320	8.93±0.29	34.74±2.23	2.49±0.13	1.55±0.09
		1000	290	8.52±0.50	42.60±1.72	2.91±0.30	1.90±0.20
		2000	230	8.40±0.59	51.20±1.17	3.50±0.16	2.01±0.16
80	20	0	400	10.72±0.84	35.52±3.13	2.50±0.12	1.61±0.10
		500	380	11.26±1.05	40.14±2.62	2.79±0.21	1.80±0.11
		1500	320	9.80±0.59	45.81±5.41	3.47±0.18	2.12±0..07
		3500	220	8.97±1.08	54.75±0.90	3.96±0.45	2.35±0.21
70	30	0	440	11.00±0.29	38.93±3.77	2.63±0.26	1.67±0.15
		500	410	12.23±0.58	45.86±1.87	3.25±0.31	1.95±0.10
		2000	310	11.03±0.29	55.57±2.41	4.30±0.23	2.46±0.10
		4000	220	10.69±0.58	58.66±1.72	4.62±0.35	2.64±0.13
65	35	500	450	13.39±0.29	43.71±3.73	3.22±0.71	1.82±0.15
		2000	330	12.80±0.58	55.81±1.97	4.53±0.51	2.41±0.15
		4000	230	11.43±0.29	59.86±2.57	4.78±0.76	2.60±0.08
60	40	500	460	16.81±0.28	43.45±2.77	3.60±0.22	2.12±0.09
		2500	310	13.79±1.55	55.32±2.17	4.52±0.41	2.78±0.11
		4500	200	11.68±1.40	60.52±0.81	5.44±0.21	2.81±0.09
55	45	1000	450	17.32±0.29	49.74±2.26	4.04±0.25	2.16±0.07
		2500	340	13.22±0.77	56.90±1.59	4.94±0.25	2.80±0.08
		4500	230	13.51±1.24	59.94±1.13	5.82±0.36	2.70±0.18
50	50	1000	470	15.82±1.17	51.33±1.73	3.77±0.51	2.12±0.14
		2500	350	15.32±0.49	56.42±3.04	4.84±0.81	2.60±0.11
		4500	240	13.06±0.50	62.35±2.29	5.74±0.80	2.82±0.05

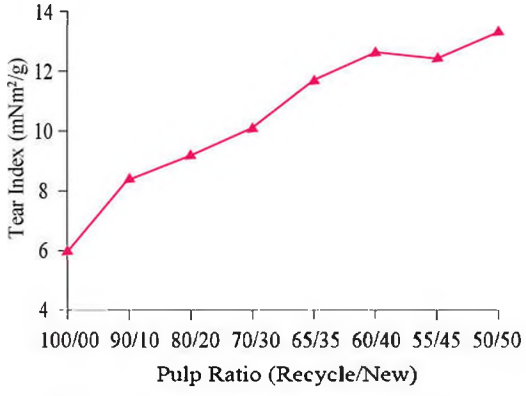

Figure 1. Tear index versus pulp ratio at 250 CSF

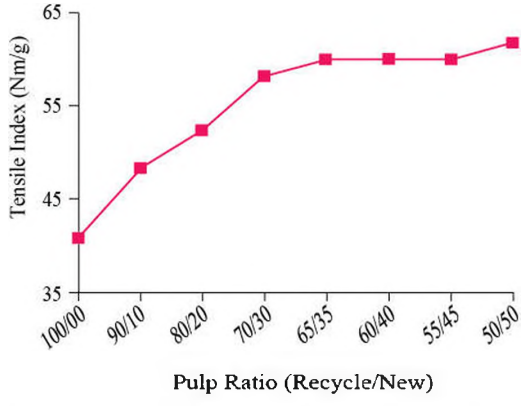

Figure 2. Tensile index versus pulp ratio at 250 CSF

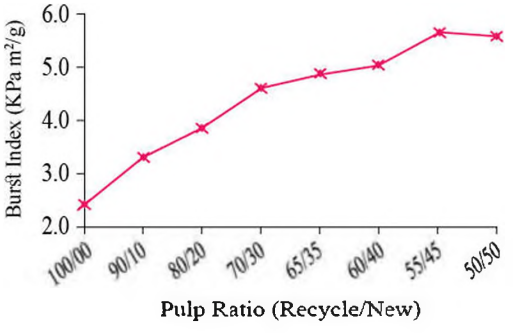

Figure 3. Burst index versus pulp ratio at 250 CSF

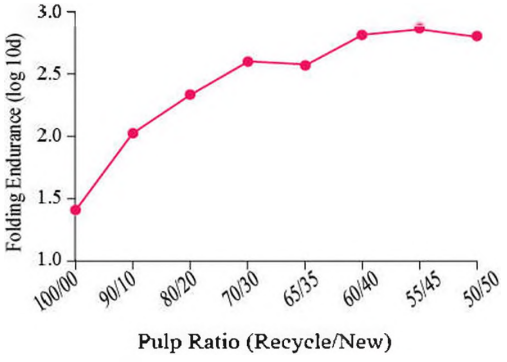

Figure 4. Folding endurance versus pulp ratio at 250 CSF

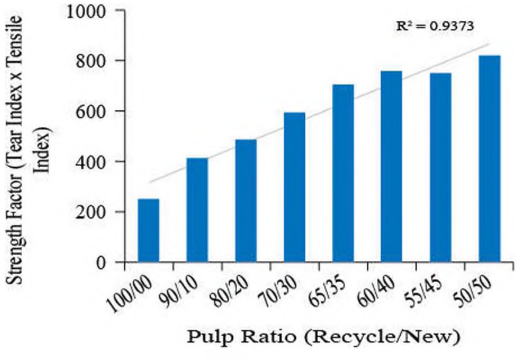

Figure 5. Paper strength at different pulp ratios

The mechanical properties of the paper gradually increased with the increasing portion of virgin pulp which are shown in Fig. 1 to Fig. 4). R^2 value of strength factor of different pulp ratio is shown in Fig. 5.

The following TAPPI test methods (Anon 1992) were used for sheets analysis:tear index (T 414 om-88), tensile index (T 404 om-87), burst index (T 403 om-91) and folding endurance (T 423 om-89). Paper properties are shown in Table 4 where papers made from different ratios are graded following Akhtaruzzaman *et al.* (1997).

Table 4. Properties of paper were made from mixed recycled (newsprint book and whiteprint book) and new (jute) pulp.

Recycled: New Pulp	Excellent (A grade) ≥ 700	Properties (Strength Factor) (Tear Index × Tensile Index)		
		Good (B grade) 700-600	Fair (C grade) 600-500	Poor (D grade) ≤500
100:00	–	–	–	245.33
90:10	–	–	–	406.29
80:20	–	–	–	482.05
70:30	–	–	588.15	–
65:35	701.55	–	–	–
60:40	757.26	–	–	–
55:45	745.53	–	–	–
50:40	820.83	–	–	–

Discussion

Recycled fibres had a poor quality due to the paper forming and drying process and have much lower freeness rates compared to virgin fibres. During refining of the newsprint book and white paper book some features were upgraded but it forms more fines that lead to lower dewatering rate. Drainage of recycled fibres are improved through addition of virgin jute pulp. The important charge groups in fibres are carboxylic acids found in the hemicellulose components (Sjostrom 1989). In this respect, the total charge on virgin pulp is much higher than recycled pulp due to the removal of the hemicelluloses during the recycling process (Rosli *et al.* 2001), contributing to the increase in wetness upon addition of virgin jute fibres. Which is probably due to the increase of pulp's wetness. High yielding long fibre pulp was one of the choices to blend with recycled pulp. The high yield (69.71%) of jute pulp could be the result of high value of cellulose and low lignin content in jute fibre and the NS-AQ method (Akhtaruzzaman *et al.* 1988). A noticeable effect of beating is observed by blending recycled pulp with jute pulp for the drain ability of pulp suspension. It is observed that pulp freeness value of recycled pulp is very low, which signifies a pulp difficult to de-water (Ek *et al.* 2009) at the time of paper making. The tensile index of paper depends on the bonding ability, flexibility, strength, and length of the individual fibres (Nazhad 2005; Fiserova *et al.* 2010). Although beating may decrease both the average length and strength of the fibre, the dominating effect on tensile strength is the increased bonding. (Ek *et al.* 2009).That's why, it is noticeable that the tensile index is increased up to 35% addition of virgin jute pulp. After that, it remained constant or was slightly increased with further addition of jute pulp. The effect of blending virgin jute

pulp with recycled fibres on the tensile index appears that the virgin fibres could restore the tensile strength of the recycled papers after 35% addition, which could be due to the substitution of the passive recycled fibres with the more active fibres of the virgin jute pulp. Virgin jute fibres were able to absorb water and swelled to a higher degree than the recycled fibres. Swelling is an important factor in the development of paper strength by virtue of increasing fibre flexibility; the more flexible the fibres, the more their conformation can be altered that could enhance inter fibre bonding between themselves and the recycled fibres, thus increasing the tensile strength. The inactivity of the recycled fibres is a consequence and drying, which reduces the capabilities of the fibres to swell because of hornification. Szwarcsztajn and Przybysz (1974) reported a similar finding and they attributed it to the presence of more active fibres in virgin jute pulp. Blending with virgin jute fibres had a more pronounced effect where even at a low addition of 10%, the restoration is already completed. This enhancement is ascribed to increase in the bonded area of the sheet resulting from internal and external fibrillation that occurs during beating.

It is clearly noticed from the tear index versus pulp ratio at 250 CSF that the tear index was sharply increased with the addition of new pulp. It has happened for jute fibres of long fibre length, since longer fibres naturally provide more points of bonding and are pulled a longer average distance from within the network (Ek *et al.* 2009). However, it is interesting to note that the effect of addition of virgin jute pulp is negligible. It is possible that the amount of new active sites generated is not sufficient to increase new inter fibre bonding needed to further increase the tear index. Tear strength is found 12 mNm^2/g after adding 35% virgin jute pulp. Burst Index and folding endurance were also linearly increased with the addition of new pulp. Standard strength properties value of prepared paper samples were found at the mixture of virgin pulp 35% and recycled pulp were 65%. The tearing resistance of paper hand sheets, considered in conjunction with the tensile strength, is probably the most commonly used direct measurement of paper strength potential, as others have suggested (Allison 1992). Tearing resistance is a function of both fibre strength and fibre bonding, though limited by fibre strength. It is evident that both tearing resistance and tensile strength increase with pulp blending irrespective of whether the pulp is unbeaten or beaten. This has been explained as a result of replacement of the passive recycled fibres with active virgin jute fibres, which has generated new sites for inter fibre linking. The addition of virgin fibres has, as expected, further enhanced the development of both indices. It is however of significance to note that at high tear strength, the improvement of tensile strength effectively ceases at about 59.91 Nm/g high, suggesting that no further bonding is occurring (tear continue to rise due to the continuing substitution of poor bonding material with virgin fibres). When product of tear and tensile strength value is greater than 700 then that paper is A-grade paper according to Akhtaruzzaman *et al.* (1997). Hence, A-grade paper at the ratio of 35% virgin and recycled pulp was produced where R square value is highly significant.

Conclusion

The study result shows that, it is possible to produce good quality paper from recycled paper upon addition of certain proportion of new pulp. Jute pulp could be commercially used as a new pulp with recycled pulp. NS-AQ

process produced good quality pulp from jute fibre (Tossa) with 69.71% (w/w) yield. At a minimum of 35% addition of jute pulp, high quality A-Grade paper could be produced. However, further research is recommended on blending the recycled pulps with other long fibrous virgin pulp to improve recycled papers strength.

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Treatment of *Albizia lebbeck* Wood by Soaking and Diffusion Method Using Chromated-Copper-Boron (CCB) Preservative

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Abstract

The experiment was undertaken to investigate the retention of 10% Chromated-Copper-Boron (CCB) solution (2:2:1) in Kala-koroi (*Albizia lebbeck*) (L.) Benth. wood applying soaking as well as diffusion method. The assessments were applied for 5, 7, 9 and 11 days for both the method. Retention was recorded 1.96 kg/m^3, 11.78 kg/m^3, 12.92 kg/m^3 and 13.61 kg/m^3 in *A. lebbeck* wood where soaking method applied. Moreover, retention was found 5.22 kg/m^3, 6.43 kg/m^3, 7.32 kg/m^3 and 12.36 kg/m^3 in *A. lebbeck* wood when diffusion method applied. In case of both methods the highest retention was recorded 13.61 kg/m^3 and 12.36 kg/m^3 for 11 days. Considering the Standard of Bangladesh Standards and Testing Institution (BSTI), i.e., 13.61 kg/m^3 and 12.36 kg/m^3 retention can meet the suitability of the study.

সারসংক্ষেপ

কালাকড়ই কাঠের ধারণ মূল্যায়নের জন্য চুবানো এবং ডিফিউশন পদ্ধতিতে ১০% ক্রোমাটেড-কপার-বোরন (সিসিবি) দ্রবণ (২:২:১) দ্বারা পরীক্ষাটি করা হয়েছিল। উভয় পদ্ধতিতে ৫, ৭, ৯ এবং ১১ দিনের জন্য এ পরীক্ষাটি প্রয়োগ করা হয়েছিল। উক্ত সময়ে চুবানো পদ্ধতি প্রয়োগ করে ১.৯৬, ১১.৭৮, ১২.৯২ এবং ১৩.৬১ কেজি/ঘনমিটার ধারণ রেকর্ড করা হয়েছে। অধিকন্তু, ডিফিউশন পদ্ধতি প্রয়োগ করে ৫, ৭, ৯ এবং ১১ দিনের জন্য যথাক্রমে ৫.২২, ৬.৪৩, ৭.৩২ এবং ১২.৩৬ কেজি/ঘনমিটার ধারণ রেকর্ড করা হয়েছে। উভয় পদ্ধতিতে ১১ দিন প্রয়োগ করে কালাকড়ই কাঠের সর্বোচ্চ ধারণ ১৩.৬১ এবং ১২.৩৬ কেজি/ঘনমিটার রেকর্ড করা হয়েছে। কালাকড়ই কাঠের ধারণ ১৩.৬১ এবং ১২.৩৬ কেজি/ঘনমিটার রেকর্ড করা হয়েছে, যা বাংলাদেশ স্ট্যান্ডার্ডস অ্যান্ড টেস্টিং ইনস্টিটিউশন (বিএসটিআই)-এর মান বিবেচনা করে উপযুক্ততা পূরণ করতে পারে।

Key words: *Albizia lebbeck*, Diffusion method, Penetration, Retention, Soaking method.

Introduction

Albizia lebbeck (L.) Benth. is native to tropical Africa, Asia and Northern Australia. It is widely naturalized within sub-humid, semi-arid tropics and subtropical areas where there is a marked dry season and a reliable rainy season. It is found from sea level up to an altitude of 1800 m (Cook *et al.* 2005; Lowry *et al.* 1992; Duke 1983). Native and exotic range of *Albizia lebbeck* of the world distribution is showing in Fig. 1 (Orwa *et al.* 2009). *A. lebbeck* is a common exotic species in Bangladesh. It is planted in roadsides as shade tree, in homestead forests for fuel wood production and in front of school or college premises as ornamental tree. Although, the species grows on all types of soils in

Bangladesh, but frequently planted on the northern and southern parts of the country (Khan and Ungar 1996; Das and Alam) especially on the wet damped soils of the village areas of greater Barishal, Patuakhali and Noakhali district (Uddin *et al.* 2007). The plant is found almost everywhere in Bangladesh. May be that's why the plant has many local names: East Indian walnut, lebbeck, lebbek tree, flea tree, fry wood, koko, woman's tongue tree. It has also vernacular name: Kala-koroi (Chittagong), siris, shirish (Beng.), harish, moroi (Sylhet), bhutkoroi (Dinajpur, Rangpur), (Das and Alam 2001).

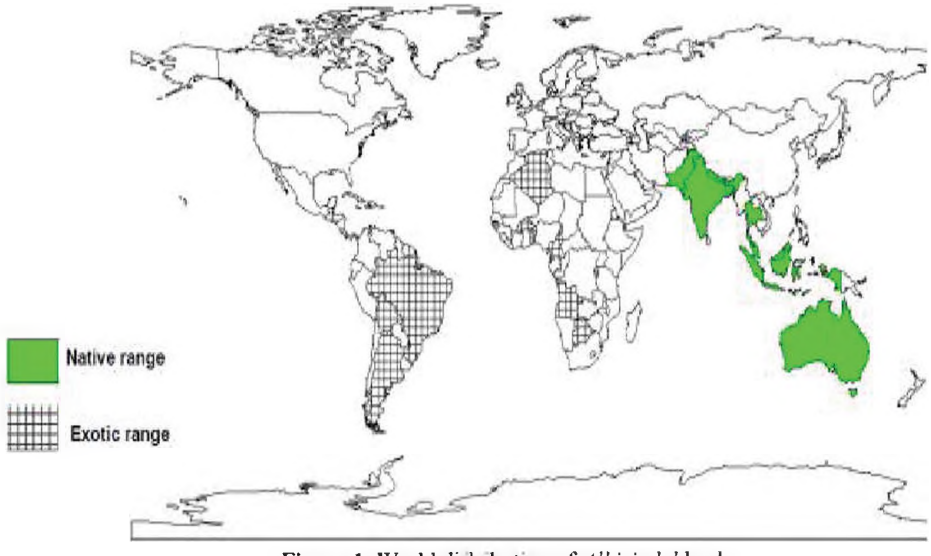

Figure 1. World distribution of *Albizia lebbeck*.

A. lebbeck is a deciduous, perennial medium-sized legume tree. It reaches 3–15 m in plantations and up to 30 m in the open. Its dense shade-producing crown can be as large as 30 m in diameter. Leaves are bi-pinnate with 3–11 pairs of bright green, oblong leaflets, 1.5–6.5 cm long × 0.5–3.5 cm broad. Inflorescences are globular clusters of 15–40 white fragrant flowers. The fruits are 10–30 cm long × 3-6 cm broad, reddish-brown pods that contain 5–15 flat rounded, free moving seeds. They produce an incessant rattle in the wind, reminding women's chatter, hence the name "women's tongue" (FAO 2010; Orwa *et al.* 2009; Lowry *et al.* 1992). *A. lebbek* is a multipurpose tree. As a fodder tree, its foliage, twigs, flowers and immature pods are relished by different classes of livestock (camels, cattle, small ruminants and rabbits) (FAO 2010). It has an extensive, fairly shallow root system thus can be a good soil binder and may be used to prevent soil erosion. It is also a source of firewood and timber. *A. lebbek* is suitable for agroforestry regimes and it is used for shelter belts and as shading tree in coffee and tea plantations (Orwa *et al.* 2009; Duke 1983). Optimal growth conditions are average day temperatures ranging from 19°C to 35°C, annual rainfall between 500 mm and 2500 mm and fertile, well-drained loamy soils. It may,

however, withstand lower and more irregular rainfall conditions. It can also grow on a wide diversity of soils such as acid, alkaline or saline soils, eroded soils and laterites except heavy clays (Orwa *et al.* 2009; Lowry *et al.* 1992). It is tolerant of heavy grazing and fire (Lowry *et al.* 1992). Seedlings are sensitive to frost and heavy browsing but older plants can survive (NAS 1980). Wood-boring is carried out by many insects either to obtain food or as a means of protecting their eggs, larvae and pupae. Many insects and a few other invertebrates are wood-borers. Some of them obtain both sustenance and shelter from the wood, while others use it only as their habitat. Certain species attack only living trees, others are found mainly in freshly felled or dying trees; a few infest only dry woodland, while others attack only old moist wood. Those that attack trees and fresh logs frequently bore and live in the inner bark for a variable period of time, before they penetrate the wood. They also can be considered to be inner back borers. Some insects that attack only freshly killed or felled trees can survive and develop slowly in dried wood. Therefore these species often continue boring into wood that has been dried and processed (Anderson 1960). Wood remains a major raw material for furniture manufacturing in Nigeria despite the relatively recent incursion of plastic, glass and aluminum. Also, wooden furniture manufacturing remains a major source of employment generation in the country (Olorunnisola 2000). It is also the most widely distributed of all wood-based industries in the country. Wood-borers constitute the greatest threat to timber and timber products, even more than other factors combined (Beal 1981). Insects attack the lumbers of *A. lebbeck* which are not seasoned or partly seasoned. The finished timber product is also threatened by termites and other wood borers (Wood and Sands 1978). A worrisome fact about insect destruction of wood is that there is no stage of development that is free of their attacks. There is an array of insect's injuries at one particular time or the other (Ashiru 1996).

A. lebbeck wood is used for fabricating furniture and construction material. Generally Kala-koroi wood is used for making furniture after 25 to 30 years when maximum heart wood formed. Due to scarcity of wood, people are using Kala-koroi wood of 15 to 20 years containing more sapwood content. As a result, the untreated wood deteriorates quickly and therefore need to be replaced frequently within short time. Normally untreated Kala-koroi wood survives 25 to 36 months in outdoor condition. But properly treated wood might be last 4 to 5 times in outdoor condition. Sapwood is rather thick; heart wood dark blackish brown with darker streak and much valued as timber for decorative furniture and veneer. It is also suitable for construction work (Das and Alam 2001).

Materials and Methods

The Wood Preservation Division of Bangladesh Forest Research Institute (BFRI) carried out the treatability and natural durability of Kala-koroi (*Albizia lebbeck*) (L.) Benth. wood species which were collected from Patiya, under Chattogram district. The age of the tree was 20 years. Then the logs were sawn and dried planks at shed of Wood Preservation Laboratory in BFRI to reduce the moisture content. Average moisture content was 72.4% when the wood was collected. Before treatment, all planks were sized into 50.8 cm × 5.08 cm × 2.54 cm. A total number of 48 wood samples were prepared for the experiment (Fig. 2).

Figure 2. Untreated Kala-koroi wood samples.

Figure 3. Treated Kala-koroi wood samples.

Then, all specimens were allowed to dry for reducing moisture content up to fiber saturation point (FSP) at 25–30% moisture content for treatment. Out of 48 samples, 24 samples were taken for soaking method and remaining 24 for diffusion method. 10% CCB aqueous solution was used in both the method. If the percentage is less than 10%, then retention rate become lower than the standard level. If the percentage is higher than 10%, and then retention rate become higher than the standard level but treatment cost rapidly increase, which is not economically viable. Wood will be treated by water-borne preservatives solution for obtaining required retention and reducing experimental period. The physical and mechanical properties of wood increase after treatment using 10% CCB aqueous solution (Shanu *et al.* 2015).

Firstly, for soaking method, every 6 samples were immersed into 10% CCB aqueous solution (2:2:1) for 5 days, 7 days, 9 days and 11 days separately. Tweenty four specimens were staked after treatment by soaking method (Fig. 3).

The average absorption and retention of immersed samples were determined by weighing the samples before and after treatment. Again, the samples were dried. First of all, dry samples were crossed-section for determination of penetration. Then, Chrome-azurals solution was applied in split wood samples which reacted with CCB preservatives and changed color. The blue color indicates the penetration of treated samples. Depth and intensity of blue color indicates penetration range and treatability group of treated samples. Finally, penetration and retention were measured of the specimens.

The maximum and minimum moisture content of wood specimens were 57.21% and 41.76% for diffusion method. Every 6 samples were immersed into 10% CCB aqueous solution (2:2:1) for 5 days, 7 days, 9 days and 11 days separately. The treated samples were removed from 10% CCB aqueous solution and kept 12 hours for drying. The average absorption and retention of immersed samples were determined by weighing the samples before and after treatment. The dried and treated

samples were crossed-section for determination of penetration. Then, Chrome-azurolS solution was applied in split wood samples which reacted with CCB preservatives and changed color. The blue color indicates the penetration of treated samples. Depth and intensity of blue color indicates the penetration range and the group of treated samples. Finally, penetration and retention were calculated of treated wood samples. Afterward, treated and untreated samples were kept in the stake yard for service test (Fig. 4).

Figure 4. BFRI Stake yard

Statistical design of experiment and analysis

The experiments were carried out in a completely randomized design (CRD) with 6 replications. SPSS statistical software was used for the data analysis. Analysis of variance (ANOVA) and least significant difference (LSD) test were carried out to evaluate the significant of differences among the different retentions of treated specimens.

Results

Wood specimens of Kala-koroi (*Albizia lebbeck*) (L.) Benth. were treated by soaking method using 10% CCB aqueous solution for different duration. Penetration and retention of treated samples were measured. Retention of preservatives of wood samples were recorded 1.96 kg/m^3, 11.78 kg/m^3, 12.92 kg/m^3 and 13.61 kg/m^3 when soaked for 5, 7, 9 and 11 days respectively (Table 1).

Table 1. Retention of preservatives in Kala-koroi (*Albizia lebbeck*) (L.) Benth. wood sample using soaking method.

Charge No.	Sample size (cm)	Treatment period (day)	Retention (kg/m^3) ± Standard error
1	2.54×5.08×50.8	5	1.96 ± 0.01
2		7	11.78 ± 0.02
3		9	12.92 ± 0.02
4		11	13.61 ± 0.02
		*F-value	0.68
		p-value	0.42

Note: The data is significant for 5% probability level (*F > p)

Wood specimens of Kala-koroi (*Albizia lebbeck*) (L.) Benth. were treated by diffusion method using 10% CCB aqueous solution for different duration. Penetration and retention of treated samples were measured. Retention of preservatives were recorded 5.22 kg/m^3, 6.43 kg/m^3, 7.32 kg/m^3 and 12.36 kg/m^3 when diffused for 5, 7, 9 and 11 days respectively (Table 2).

Table 2. Retention of preservatives in *Albizia lebbeck* (L.) Benth. wood sample (Size: 2.54 × 5.08 × 50.8 cm) using diffusion method.

Charge No.	Average moisture content (%)	Treatment period (day)	Retention (kg/m^3) ±
1	41.76 ± 0.09	5	5.22 ± 0.01
2	50.29 ± 0.10	7	6.43 ± 0.01
3	54.62 ± 0.07	9	7.32 ± 0.01
4	57.21 ± 0.62	11	12.36 ± 0.01
		*F-value	0.01
		p-value	0.91

Note: The data is insignificant for 5% probability level (*F < p)

Discussion

In this study, the highest retention of Kala-koroi was 13.61 kg/m^3 using soaking method for 11 days which can be supported with BSTI Standard. The retention of wood at 11 days is acceptable with the species of Kala-koroi. Different retention was measured in Kala-koroi applying different method and time period. The lowest retention was found 1.96 kg/m^3 at 5 days for this species when soaking method applied. The rate of retention increased rapidly at soaking period of 5 to 7 days. On the other hand, the rate of retention increased slowly at soaking period of 7 to 11 days. If treatment period was continued for more than 11 days in soaking method, retention would probably motionless.

In 57.21% moisture content, the highest retention was recorded 12.36 kg/m^3 with diffusion method which supports BSTI standard. In this study, the retention at 11 days is acceptable with the species of Kala-koroi. Different retention was found in wood samples due to applying different moister content and time period. The lowest retention was found 5.22 kg/m^3 at 5 days in the species of Kala-koroi when diffusion method applied. The rate of retention increased slowly at treatment period of 5 to 9 days. On the other hand, the rate of retention increased rapidly at treatment period of 9 to 11 days.

According to Bangladesh Standard Testing Institute (BSTI), timbers in direct contact with ground or water, especially in outside locations, such as poles, piles, fence-posts, etc. the required retention for CCA preservative chemical is 8–16 kg/m^3 (Anon 1975). In this study, the retention results of treated samples at 11 days are acceptable for both the methods.

Chandra and Gupta (1972) stated that, 16 kg/m^3 of dry salt was necessary for the effective preservation of the poles in contact with ground. In the experiment, the highest retention was found 13.61 kg/m^3 and 12.36 kg/m^3 for the species which is near up to standard level and matched with Chandra and Gupta (1972). Research report of Commonwealth Scientific and Industrial Research (CSIR) (Du Toit and Conradie 1988) indicated that average sapwood retention levels are required for adequate protection of poles against wood rot and termite attack. Findings of the present study prove that penetration and retention level can be maximized into Kala-koroi wood by applying soaking and diffusion method. Accordingly, this wood can be free from wood rot and termite attack resulting in escalating the durability.

Conclusion

Kala-koroi can be treated with CCB solution. Subsequently the wood can be used commercially. It is mentionable that untreated samples were affected by insects, fungus etc. within 8 to 9 months. Till now, the treated samples are in good condition. Treated and untreated wood samples were kept in BFRI stake yard for service test. Longevity of *Albizia lebbeck* was enhanced in association with soaking and diffusion method. Prescribed to use the wood with the narrated treatment for short time use at outdoor and for life time use at indoor condition.

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Effect of Heat Treatment on Physical and Mechanical Properties of Mahogany (*Swietenia macrophylla* King) and Acacia Hybrid (*Mangium* × *Auriculiformis*) Wood

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Abstract

Heat treatment is often used for improve the dimensional stability of wood. In this study, the effect of heat treatment on physical and mechanical properties of Mahogany (*Swietenia macrophylla* King) and Acacia hybrid (*Mangium* × *Auriculiformis*) wood were examined. Samples were exposed to temperature levels of 110°C, 130°C and 150°C for time spans ranging from 3, 6 and 9 hours. Treated samples had higher mechanical properties compared to control samples. Based on the findings, dimensional stability of all types of wood samples improved with heat treatment. It seems that properties of these wood evaluated were more pronounced with increasing temperature and time durations.

সারসংক্ষেপ

কাঠের মাত্রিক স্থায়িত্ব উন্নতকরণে যথাযথ তাপ প্রয়োগের প্রভাব রয়েছে। এই গবেষণায়, তাপ প্রয়োগের প্রভাবে মেহগনি (*Swietenia macrophylla* King) ও একাশিয়া হাইব্রিড (*Mangium* × *Auriculiformis*) কাঠের ভৌত ও যান্ত্রিক গুণাগুণের কিরূপ পরিবর্তন হয়েছিল তা পর্যবেক্ষণ করা হয়। যথাযথ তাপমাত্রা (১১০°C, ১৩০°C ও ১৫০°C) ও নির্দিষ্ট সময় ব্যবধানে (৩, ৬ ও ৯ ঘণ্টা) পর্যবেক্ষণ করা হয়। পর্যবেক্ষণে দেখা যায় যে, নিয়ন্ত্রিত নমুনার তুলনায় তাপ প্রয়োগকৃত নমুনার যান্ত্রিক গুণাগুণ বেশি ছিল। প্রাপ্ত ফলাফলের উপর ভিত্তি করে দেখা যায়, তাপ প্রয়োগের মাধ্যমে নমুনা কাঠের স্থিতিশীল মাত্রা উন্নত হয়েছে। ক্রমবর্ধমান তাপমাত্রা এবং সময় বৃদ্ধির সাথে সাথে কাঠের গুণাগুণ বৃদ্ধি পেয়েছে।

Key words: Acacia hybrid, Heat treatment, Mahogany, Modulus of rupture, Physical and mechanical properties, Volumetric gravity.

Introduction

Wood is the fifth most important product of the world trade (Christophe *et al.* 2001). Due to its poor dimension stability and low durability under variable atmosphere circumstance, researches on stabilization treatment are carried out to limit the moisture absorption by destroying or, combining the hydroxyl groups of the wood (Yan-jun *et al.* 2002).When wood is heated, its chemical and physical properties undergo permanent changes and its structure is reformed. Solid wood is a versatile and renewable material that is widely used in different applications. However, due to the hygroscopic nature of wood it has some undesirable properties such as poor resistance against biological attack of fungi and insects, and swelling and shrinkage caused by water absorption and desorption. These limit the

outdoor applications of wood (Kocaefe *et al.* 2007). Heat treatment of wood has attracted a lot of attention both in Europe and recently in North America as an environment–friendly wood protection method. The untreated wood is hydrophilic (high affinity for water). During the heat treatment, wood becomes more and more hydrophobic (low affinity for water) with increasing heat treatment temperature. As a result, it becomes dimensionally more stable compared to untreated wood. The high temperature thermal treatment of wood is an environment – friendly method for wood preservation (Korkut and Budakci 2010).

Heat treatment is an effective method to improve the dimensional stability of wood and resistance against bio-degradation. However, there is a noticeable reduction of mechanical properties after heat treatment, mainly due to the high temperatures involved (Esteves and Pereira 2009; Bekhta and Niemz 2003; Korkut 2008). Different methods for thermal modification of wood have been developed in France, Finland, Netherlands and Germany since the middle of the last century. In general, the effects of heat depend on the conditions of the heating process, and temperature is the most important factor to be taken into consideration. This reduction results in increased dimensional stability (Krause *et al.* 2004) smaller moisture-induced movement in service (Militz and Tjeerdsma 2001). It is also held that the environmental credentials of thermally-modified wood (TMW) in terms of ecotoxicity are superior to that of untreated wood and may surpass those of several man-made materials (Van Eetvelde *et al.* 1998). Exposing wood to high temperatures however decreases its strength under various forms of stress (Gonzalez-Pena and Hale 2007). Heat treatment is one of the processes used to modify the properties of wood. It serves to improve the natural quality properties of the wood, such as dimensional stability and resistance to bio-corrosion. It reduces certain mechanical properties, but the dimensional stability and the biological durability of wood increases through heat treatment. Also, heat treatment results in favorable changes in the physical properties of the wood, such as reduced shrinkage low equilibrium moisture content, enhanced weather resistance, a decorative dark color, and better decay resistance. Physical and mechanical properties of about 99 forest and homestead timber species were determined (Sattar *et al.* 1999). But till now no information is available on heat treatment of wood. Basic information on physical and mechanical properties is needed prior to using wood species for making furniture and other construction uses. Since the main goal of modification is to improve dimensional stability and durability, the reduced hygroscopicity of the wood after treatment is an important parameter. Temperature influences on rate of reaction. However, it is temperature is either too high or too low the rate decreases dramatically. So, 150°C will be the ideal temperatures in which maximal rate of reactions are achieved. This is why, Mahogany (*Swietenia macrophylla* King) and Acacia hybrid (*Mangium* × *Auriculiformis*) wood has been examined as one of the best options to make for better research. The aim of this study was to examine the effect of heat treatment on physical and mechanical properties of Mahogany and Acacia hybrid wood.

Materials and Methods

Three representative trees of Mahogany were collected from Satkania in Chattogram and Acacia hybrid from Dhalupara in Bandarban. The age of trees was 30-35 years with 10-14 m

height and 80-100 cm girths. Three samples per bole were selected randomly from each tree consecutively 2.50 m bolts above the stump height. All the bolts were fairly straight and free from natural defects.

Measurement of properties

Small clear specimens were tested, in both green and air-dry states, for the following physical and mechanical properties using the procedure given in ASTM (Anon 1971).

1. Physical properties

Three samples per bole were selected randomly from each of trees consecutive 2.50 m bolts above the stump height of each tree. One disk was taken from each bolt for determination of moisture content (MC), specific gravity and shrinkage. The sample size for MC and Specific gravity was 2.54 cm × 2.54 cm × 5.08 cm and for shrinkage was 5.08 cm × 5.08 cm ×15.24 cm.

$$\textbf{Moisture content (\%)} = \frac{\text{Original weight - Oven-dry weight}}{\text{Oven-dry weight}} \times 100$$

$$\textbf{Specific gravity} = \frac{\text{Oven-dry weight}}{\text{Volume at green or air-dry or oven-dry condition}}$$

$$\textbf{Volumetric shrinkage (\%)} = \frac{\text{Green dimension – Air-dry dimension or Oven-dry dimension}}{\text{Green dimension}} \times 100$$

2. Mechanical properties

For determination of mechanical properties the bolts were marked into 6.35cm^2 according to the standard sawing diagram and were sawn to 6.35 cm ×6.35 cm×2.50 m sticks. The sticks for air-dry test were stacked using suitable stickers inside a drying shed and allowed to attain the equilibrium moisture content. Each treatments group was heated at a given temperature for a given period in a convection oven. The heating period at 110°C, 130°C and 150°C temperature was 3, 6 and 9 hrs conditions. Following heat treatment, all specimens including untreated controls were stacked indoors until the moisture content was about 12-15%. Physical properties like moisture content, specific gravity and volumetric shrinkage. Mechanical properties including modulus of rupture, modulus of elasticity, maximum crushing strength, compressive strength, hardness and shearing strength of heat treated, samples were determined as per specification of American Society for Testing Materials (ASTM) (Fig. 1).

A) Static bending

The size of specimens was 5.08 cm × 5.08 cm × 76 cm is tested on a 71.12 cm span with center loading. It furnishes data on bending strength and stiffness for such uses as beams, joists etc. The parameters of Static bending are as follows:

a) Stress at proportional limit (SPL)

Stress at proportional limit the mechanical value of δPL can be obtained by the equation 1.

$\delta PL = 3Pl/2bh^2$ ..... (1)

b) Modulus of rupture (MoR)

The modulus of rupture R (equation 2) can be found by substituting the maximum load, P1 for the load at the proportional limit.

$MoR = 3P1l/2bh^2$ ..... (2)

c) Modulus of elasticity (MoE)

The modulus of elasticity (equation 3) can be determined and substitution

MoE= $Pl^3 / 4ybh3$..... (3)

Where,

P = Load at the limit of proportionality,

l = Span of the test specimen,

b = Breadth of the test specimen,

h = Depth of the test specimen,

P1= Total load and

Y= Deflection at the limit of proportionality

B) Compression parallel to grain

The size of specimens was 5.08 cm × 5.08 cm × 20 cm. It furnishes data on strength and resistance to deformation when loaded in Compression parallel to grain in a short post.

Maximum crushing strength C,

$$C = P/A$$

Where,

P is the greatest load

A is the area of cross section of the column.

C) Compression perpendicular to grain

The size of specimens was 5.08 cm × 5.08 cm × 15 cm. It furnishes data necessary in computing the bearing area required at the ends of beams, joists or loads applied over limited area.

Compressive strength,

$$\delta PL = P/A$$

Where, P is the proportional limit load

A is the area under the bearing plate.

D) Hardness

The size of specimens was 5.08 cm × 5.08 cm × 15 cm. It furnishes measure of resistance to indentation and wears which is useful in selecting species for flooring, trim, etc.

Figure 1. Different steps of the determination for physical and mechanical properties of Mahogany and Acacia hybrid wood.

Statistical analysis

The co-efficient of variation is the ratio of the standard deviation to the mean. The co-efficient of variation puts the expression of viability on a relative basis.

Results

Physical properties

The average values of physical properties such as moisture content, specific gravity and volumetric shrinkage of Mahogany and Acacia hybrid wood were evaluated and presented in Table 1. The average values of physical properties including standard deviation in both control, during heat treatment at 3 different temperatures (110°C,130°C and 150°C) and durations (3, 6 and 9 hrs) were observed. According to the average values of maximum parameters increased with increasing temperature. In some cases, these values were decreased with increasing temperature in treated samples than in control samples.

Table 1. Specific gravity and volumetric shrinkage of Mahogany and Acacia hybrid (14% M.C).

Species	Temp. (c) **	Time (hr) *	Specific gravity	Volumetric shrinkage (%)
Mahogany	Control	0	0.57*** ± 0.03****	5.8*** ± 0.26****
	110°C	3	0.60 ± 0.06	5.4± 0.23
		6	0.56 ± 0.065	6.9± 0.83
		9	0. 61 ± 0.049	5.63± 0.79
	130°C	3	0.60 ± 0.040	3.19± 0.65
		6	0.57 ± 0.040	3.05± 0.58
		9	0.59± 0.035	3.29± 0.34
	150°C	3	0.56 ± 0.029	3.65± 0.23
		6	0.55 ± 0.048	4.94± 0.52
		9	0.58 ± 0.045	4.19± 0.57
Acacia hybrid	Control	0	0.60± 0.057	11.5± 1.63
	110°C	3	0.60 ± 0.042	2.75± 0.46
		6	0.61 ± 0.048	5.84± 0.93
		9	0. 57± 0.017	2.47± 0.60
	130°C	3	0.59 ± 0.038	2.74± 0.65
		6	0.60± 0.055	2.38± 0.39
		9	0.61± 0.061	4.53± 0.71
	150°C	3	0.59 ± 0.078	2.25± 0.36
		6	0.62 ± 0.044	6.05± 0.97
		9	0.60± 0.068	3.81± 0.62

Note: *=hour (hr), **= Temperature, ***= Average, ****= SD (Standard deviation).
The mean difference is significant at the 0.05 level.

Mechanical properties

The average values of mechanical properties such as Static bending in Modulus of Rupture and Modulus of Elasticity, Compression parallel to grain in Maximum crushing strength, Compression perpendicular to grain in compressive strength, Hardness values of surface Side and End and shearing strength of radial and tangential values of Mahogany and Acacia hybrid wood were evaluated and displayed in Table 2 and Table 3. The average values of strength properties including standard deviation in both Control, during heat treatment at 3 different temperatures (110°C, 130°C and 150°C) and duration (3, 6 and 9 hrs) were supplemented. According to the average values of maximum parameters increased with increasing temperature. In some cases, the results showed that these values were decreased with increasing temperature in treated samples than in control samples.

Table 2. Static bending, Crushing strength and Compressive strength of Mahogany and Acacia hybrid

Species	Temp. (c) **	Time (hr) *	Static bending (kg/cm²)		Max. crushing Strength (kg/cm²)	Compressive strength (kg/cm²)
			MoR	MoE		
	Control	0	***630 ± ****19.30	***100 ± **** 20.20	***381 ± **** 26.50	****132 ± **** 5.56
Mahogany	110°C	3	646 ± 4.94	111 ± 6.88	384 ± 18.60	151 ± 22.18
		6	769 ± 6.30	104 ± 7.71	410 ± 13.03	168 ± 23.11
		9	744 ± 6.30	103 ± 4.23	457 ± 17.59	191 ± 12.18
	130°C	3	747 ± 7.72	91 ± 6.02	380 ± 19.80	177 ± 13.90
		6	845 ± 12.90	89 ± 7.22	441 ± 16.70	177 ± 14.02
		9	846 ± 6.77	94 ± 3.03	424 ± 7.32	195 ± 13.08
	150°C	3	712 ± 12.90	90 ± 5.12	486 ± 10.60	179 ± 6.90
		6	545 ± 13.80	89 ± 15.05	443 ± 8.78	167 ± 16.90
		9	670 ± 15.50	100 ± 12.70	510 ± 11.80	199 ± 17.06
Acacia hybrid	Control	0	742 ± 11.76	90 ± 5.01	320 ± 13.53	97 ± 6.03
	110°C	3	792 ± 10.89	102 ± 6.42	261 ± 13.90	107 ± 6.87
		6	853 ± 14.07	103 ± 4.98	311 ± 14.23	104 ± 9.73
		9	973 ± 9.44	94 ± 15.50	343 ± 4.44	110 ± 5.59

Species	Temp. (c) **	Time (hr) *	Static bending (kg/cm²)		Max. crushing Strength (kg/cm²)	Compressive strength (kg/cm²)
			MoR	MoE		
	130°C	3	827 ± 16.47	101 ± 4.98	387 ± 9.39	110 ± 10.30
		6	905 ± 12.15	98 ± 11.95	348 ± 8.85	135 ± 16.40
		9	1098 ±10.18	95 ± 10.64	373 ± 7.83	249 ± 13.60
	150°C	3	917 ± 6.65	107 ± 7.78	366 ± 11.26	116 ± 12.12
		6	1128 ± 18.74	111 ± 17.8	454 ± 6.29	166 ± 8.54
		9	1120± 6.96	101± 4.98	488± 10.50	127± 8.76

Note: *= Hour (hr), ** = Temperature, ***= Average, ****= SD.
The mean difference is significant at the 0.05 level.

Table 3. Shearing Strength and Hardness of Mahogany and Acacia hybrid

Species	Temp. (c) **	Time * (hr)	Shearing Strength (kg/ cm²)		Hardness (Kg)	
			Radial	Tangential	Side	End
	Control	0	***112 ± ****16.08	***170 ± ****13.49	***497 ± ****3.86	***597 ± ****9.17
Mahogany	110°C	3	124 ± 14.17	137 ± 21.07	495 ± 9.94	584 ± 10.11
		6	115 ± 14.17	166 ± 12.07	528 ± 11.95	657± 5.29
		9	130 ± 10.80	148 ± 13.23	518 ± 15.93	518 ± 15.93
	130°C	3	129 ± 16.04	122 ± 6.69	501 ± 8.18	528 ± 13.23
		6	166 ± 5.23	163 ± 8.35	537 ± 9.23	578 ± 10.28
		9	160 ± 11.08	166 ± 12.05	521 ± 11.49	540 ± 10.21
	150°C	3	133 ± 13.08	138 ± 16.40	559 ± 9.53	638 ± 20.80
		6	144 ± 9.13	129 ± 20.79	654 ± 21.30	704 ± 18.30
		9	11 ±19.52	141 ± 21.42	481 ± 8.28	556 ± 8.39
	Control	0	131 ± 10.13	140 ± 20.80	432 ± 8.52	420 ± 10.40
	110°C	3	124 ± 14.90	128 ± 19.30	373 ± 12.62	420 ± 13.42
		6	117 ± 26.80	135 ± 13.80	380 ± 9.65	390 ± 6.83
		9	117 ± 12.20	143 ± 13.50	398 ± 12.32	392 ± 13.50

Species	Temp. (c) **	Time * (hr)	Shearing Strength (kg/ cm²)		Hardness (Kg)	
			Radial	Tangential	Side	End
Acacia hybrid	130°C	3	97 ± 18.50	105 ± 19.50	456 ± 14.20	472 ± 9.36
		6	90 ± 20.45	129± 12.38	476 ± 16.32	490 ± 18.60
		9	110 ± 14.30	127± 18.50	445 ± 13.70	463 ± 11.30
	150°C	3	92 ± 24.50	92± 23.40	406 ± 17.60	415 ± 10.30
		6	80 ± 23.40	115± 20.50	412 ± 10.30	424 ± 14.50
		9	126 ± 22.60	130± 23.50	428 ± 15.30	476 ± 18.40

Note: *=hour (hr), ** = Temperature, ***= Average,****=SD.
The mean difference is significant at the 0.05 level.

In the case of Mahogany, the results showed that, the specific gravity of mahogany at 110°C temperature was higher at 3 and 9 hrs than 6 hrs. The highest decreases were 0.55 at 150°C for 6 hrs and increases were 0.61 at 110°C for 9 hrs. According to the results obtained, the lowest and highest decreased in volumetric shrinkage occurred for treatments at 130°C and 150°C for 3hrs. The volumetric shrinkage values were decreased with increasing temperature and heat treatment time. Results in Table 2 which displayed the mechanical properties of the specimens exposed to heat treatment. The average values of modulus of rupture at 130°C temperature was higher 6 and 9 hrs than both at 110°C and 150°C temperature. The modulus of rupture at 130°C the bending strength at maximum heat-treatment temperature is in wood treated at 130°C for 6 and 9 hrs are 845 kg/cm²; 846kg/ cm². Modulus of elasticity at 110°C temperature was higher than both 130°C and 150°C temperature. The highest value of modulus of elasticity 111 kg/ cm² at temperature 110°C for 3 hrs whereas the lowest value of 89 kg/ cm² at temperature 150°C for 6hrs. The higher value of compressive strength 199 kg/cm² at temperature 150°C for 9hrs whereas the lower value of compressive strength 132 kg/ cm² in control. The lower value of Maximum crushing strength was 380 kg/cm² at temperature 130°C for 3 hrs whereas the higher value was 510 kg/ cm² at temperature 150°C for 9 hrs. Shearing strength at temperature 130°C for 6 and 9 hrs almost were same. The lower value of shearing strength (radial) was 115 kg/cm² at temperature 110°C for 6 hrs whereas the higher value of shearing strength (radial) was 166 kg/cm² at temperature 130°C for 6 hrs. The higher value of hardness (Side) was 654 kg and End 704 kg at temperature 150°C for 6 hrs whereas the lower value of hardness (Side) was 481 kg and End 518 kg at temperature 150°C for 9 hrs and 110°C for 9 hrs. On the other hand, in the case of Acacia Hybrid (*Mangium* × *Auriculiformis*) the results showed that, the average specific gravity and volumetric shrinkage values of control samples were determined to be 0.60 and 11.5(%). The smallest decrease was observed in the treatments at 150°C for 6 hrs. The highest decreases were 0.57 at 110°C for 9 hrs and increases were 0.62 at 150°C for 6 hrs.

The average values of modulus of rupture at 150°C temperature was higher 6 and 9 hrs than both at 110°C and 130°C temperature. The highest value of modulus of rupture 1128 kg/cm², 1120 kg/cm² at temperature 150°C for 6 and 9 hrs whereas the lowest value of 742 kg/cm² in control. Modulus of elasticity at 150°C temperature was higher than both 130°C and 110°C temperature. The highest value of modulus of elasticity 111 kg/cm² at temperature 150°C for 6 hrs whereas the lowest value of 90 kg/cm² in control. The higher value of maximum crushing strength was 488 kg/cm² at temperature 150°C for 9 hrs whereas the lower value was 261 kg/cm² at temperature 110°C for 3 hrs. The higher value of compressive strength 249 kg/cm² at temperature 130°C for 9hrs whereas the lower value of compressive strength 97 kg/cm² in control. The lower value of shearing strength (radial) 90 kg/cm² at temperature 130°C for 6 hrs whereas the higher value of shearing strength (radial) 131 kg/cm². The higher value of hardness (Side) was 476 kg; and End 490 kg at temperature 130°C for 6 hrs whereas the lower value of hardness (Side) 373kg and End 390 kg at temperature 110°C for 3 and 6 hrs. Maximum strength values were increased with increasing in temperature and heat treatment time except shearing strength.

Discussion

In previous research, Ghalehno and Nazerian (2011) reported and determined that, for the heat treatment of Iranian Hornbeam Wood (*Carpinus betulus*) at 130°C, 160°C and 190°C for 3, 6 and 9 hrs, 130°C for 9 hrs bending strength (MOR) value of 933 kg/ cm² is higher than *Swietenia machrophylla*. After heat modification, the hornbeam wood acquires a color that simulate some tropical species the main targets for industrial heat treatment are improved dimensional stability, increased biological durability, enhanced weather resistance and decreased shrinking. Kaygin *et al.* (2009) reported that, physical properties of *Paulownia elongata* wood improved with increasing treatment temperatures and durations, the specific gravity of lowest values obtained was 0.278 at the treatment of 200°C for 7hrs whereas highest values was also obtained 0.32 for samples treated at 160°C for 3hrs. Gunduz *et al.* (2008) reported that, when wood has been treated at high temperatures and long durations, it is recommended that such wood not be used structurally for load-bearing purposes. Farahani *et al.* (2001) reported that heat treatment is improved resistance to fungal decay for above ground applications. Poncsak *et al.* (2006) reported that heat treated birch and showed a reduction of bending strength (MoR) with increasing heat treatment temperature especially above 200°C. Moreover, modified wood is much cheaper and does not require additional surface finishing and impregnation with protective substances and can be used for floor covering, garden furniture and decor, the arrangement of terraces, playground etc.

Conclusion

Treated wood has its own properties which are not comparable to that one of untreated wood. In this research, strength values of the samples of Mahogany and Acacia hybrid wood increased with increasing time and temperature treatments. The smallest decrease was determined at the thermal treatment of 110°C for 3 hrs. The maximum increased of all properties was observed when samples were treated with a temperature of 150°C for 9 hrs, the increase in strength values when evaluating the effectiveness of using this treatment. In

some cases, it is shown that the strength properties decrease with increasing temperature and time duration. This article focuses on the performance changes of modified wood by heat treatment and its mechanism of action. Finally the research and application of heat treatment technology are summarized and prospected.

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Mammalian Species Diversity in Hazarikhil Wildlife Sanctuary of Bangladesh

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Abstract

A study was conducted from July 2015 to June 2017 in Hazarikhil Wildlife Sanctuary (HWS) of Bangladesh to find out mammalian species diversity, population density and identification of major threats to the mammalian species of this sanctuary. Various methods were used including transect survey to detect diurnal mammals, and camera trap to identify nocturnal mammals. A total of 33 species of mammals were recorded which belongs to 20 families under 9 orders. Among the recorded species, 13 species of carnivores, 4 bats, 2 Primates, and 6 species of rodents constitute the major part of mammalian community. According to IUCN (2015), among the recorded 33 mammalian species, 11 species were threatened (4 critically endangered, 5 endangered, 2 vulnerable), 15 species were least concerned and 7 species were nearly threatened. Hunting and poaching, human-wildlife conflict, use of poison for fishing, road inside the forest, forest fire, encroachment for gardening, non-insulated electric wire, grazing were identified as major threats to the mammals of this sanctuary.

সারসংক্ষেপ

জুলাই ২০১৫ হতে জুন ২০১৭ পর্যন্ত সরাসরি পর্যবেক্ষণ, ক্যামেরা ট্র্যাপ পদ্ধতি ও স্থানীয় জনগোষ্ঠীর সাথে আলোচনা করে হাজারীখিল বন্যপ্রাণী অভয়ারণ্যের স্তন্যপায়ী বন্যপ্রাণী উপর একটি গবেষণা কার্যক্রম পরিচালনা করা হয়। এখানে ৯টি বর্গের অন্তর্ভুক্ত ২০টি গোত্রের ৩৩টি গণের সর্বমোট ৩৩ প্রজাতির স্তন্যপায়ী বন্যপ্রাণী রেকর্ড করা হয়। রেকর্ডকৃত স্তন্যপায়ী প্রজাতির মধ্যে ৪ প্রজাতির বাঁদুর, ২ প্রজাতির প্রাইমেট, ১৩ প্রজাতির মাংসাশী স্তন্যপায়ী এবং ৬ প্রজাতির রডেন্ট অন্যতম। IUCN (২০১৫) অনুযায়ী ৩৩টি রেকর্ডকৃত স্তন্যপায়ী প্রজাতির মধ্যে ১১টি বিপন্ন (৪টি মহাবিপন্ন, ৫টি বিপন্ন, ২টি বিপদাপন্ন), ১৫টি ন্যূনতম বিপদগ্রস্ত এবং ৭টি প্রায় বিপদগ্রস্ত। এই অভয়ারণ্যে স্তন্যপায়ী বন্যপ্রাণীর জন্য বিভিন্ন ধরনের হুমকি হিসেবে অবৈধ শিকার, মানুষ-বন্যপ্রাণীর মধ্যে দ্বন্দ্ব, মাছ শিকারের জন্য বিষের ব্যবহার, অভয়ারণ্যের মধ্য দিয়ে মানুষ চলাচলের রাস্তা, বনের আগুন, বাগান করার জন্য অভয়ারণ্যের জমি দখল, অ-নিরোধক বৈদ্যুতিক তার, গবাদি পশুচারণ ইত্যাদি চিহ্নিত করা হয়েছে।

Key words: Camera trap, Hazarikhil Wildlife Sanctuary, Mammals, Population density, Species diversity.

Introduction

Zoogeographically, Bangladesh is located at the junction of the Indo-Himalayas and Indo-China sub-regions, being one of the few countries where species of two bio-geographic realms are available. Because of its geographic location at the eastern end of the Indian subcontinent, Bangladesh is a transitional zone for the flora and fauna of the subcontinent and that of Southeast Asia (Stanford 1991). The distributional ranges of many wildlife species typical to each of these two biotic sub-regions overlap in Bangladesh, making the country's wildlife very diverse. Bangladesh is the home of 49 species of amphibians, 167 species of reptiles, 690 species of birds and 138 species of mammals (IUCN 2015; Khan 2018). Most of these species are restricted to the forest areas especially the protected areas (Feeroz 2013). At present, a total of 125 species of wildlife belonging to different classes are threatened in Bangladesh. Of these, 38 are mammals, 39 are birds, 36 are reptiles and 10 are amphibians (IUCN 2015). Protected area declaration and proper management is essential for their survival of many species. A total of 56 protected areas consist of 20 National Park and 24 Wildlife Sanctuary, 2 Special Biodiversity Conservation Areas and 10 Eco-Park are in the forest areas of Bangladesh and maintained by forest department (www.bforest.gov.bd).

However, the wildlife diversity of Bangladesh is under tremendous pressure due to different anthropogenic activities. Thirty one species of wild animals have become extinct from the wild over the last century and over the previous one century a total of 11 species of mammalian wildlife have been vanished from Bangladesh (IUCN 2015). Most of the extant species of Bangladesh are facing different categories of threats. Such as habitat fragmentation, degradation, etc. Mammals especially primates, civets and squirrels play a vital role for the expansion of natural forest through seed dispersion (Chapman 1995). Hazarikhil is one of the important Protected Araes of Bangladesh. But the extensive work on mammalian fauna not only in these PAs but also in all parts of Bangladesh is limited. Previously, most work was done on a single species in an area (eg. Caped Langur (Islam 1979), Asian elephant (Zabed 1992) and Western Hoolock gibbon (Ahsan 1994), on a specific group of bats (Ahmed 1975), primates (Ahsan 1984), the herpetofauna (Karim and Ahsan 2014), the wildlife of a specific area e.g. Satchari Forest Reserve now a National Park (Feeroz 2003) and the biodiversity monitoring in a Particular area, e.g. Rema-Kalenga WS (Feeroz *et al.* 2011), Dudpukuria-Dhopachari WS (Feeroz *et al.* 2012) and Teknaf WS (Feeroz 2013) and Chunati WS (Feeroz 2014). However, published/unpublished work is not exclusively available on mammals or other wildlife groups in Hazarikhil Wildlife Sanctuary (HWS). Earlier Bangladesh Forest Research Institute estimated 123 avian species in HWS. Considering the fact the study was planned to estimate the mammalian diversity, population status and threats to the mammals in the HWS.

Materials and methods

Study area

Hazarikhil Wildlife Sanctuary (22°42.196' N91°41.070'E) is located in Fatikchhari upazila of Chattogram district. It lies in the Ramgarh-Sitakunda forests, 173 km southeast of Dhaka and 45 km north of Chattogram in southeastern region of Bangladesh and was declared as Wildlife Sanctuary on 6 April 2010 with total hilly forest areas of 1177.53 ha (www.bforest.gov.bd). It is under Hazarikhil

Range situated near Rangapani tea garden. The sanctuary is bounded by Balukhali beat under Narayanhat Range in the North, in the west Ridge of Ramghor- Sitakunda Reserve Forest, in the south Baromasia block under Hazarikhil Forest Range and in the east by Fatikchari Beat (Fig.1).

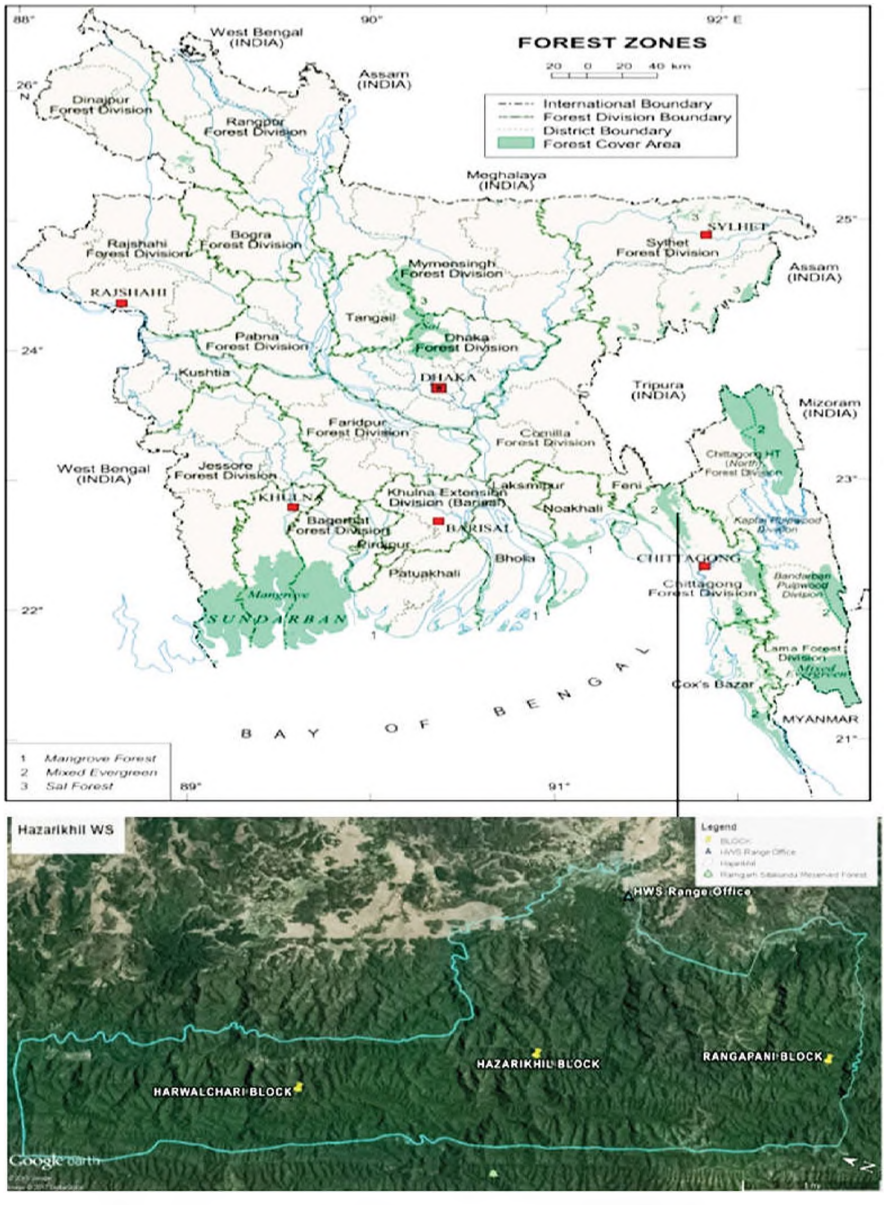

Figure 1. Map showing the location of Hazarikhil Wildlife Sanctuary (Source: www.researchgate.net)

The forest cover of the sanctuary is semi evergreen type. Important tree species include *Dipterocarpus* sp., *Artocarpus chaplasha*, *Tectona grandis*, *Albizia* sp., *Swietenia mahagoni*, *Eugenia* sp., and *Tetrameles* sp. The Sanctuary is surrounded by a low hill range that rises to an average elevation of 350 meters, with the rest of the area lying in the Bengal flood plain. (Hossain *et al.* 2019). Three Mio-Pliocene geological series are represented in the sanctuary area (Surma, Tipam and Dhuptila). On level ground, the soils range from clay to clay loam, whereas on hill land, the soils range from sandy loam to coarse sand. Clay and sandy loams are fertile, and sandy soil is frequently saturated with iron, giving it red or yellowish tinges. Unconsolidated rock soils are moderately to excessively well drained, generally deep, and presumably the oldest soils in this region, whereas consolidated rock soils are created in weathered sandstones, shale, and siltstones, and are probably the oldest soils in this region (Hassan 1994).

A number of sandy bedded stream and charas pass through the sanctuary areas and aquatic habitats associated with forest cover and riparian (stream side) vegetation wildlife species are important part of the entire habitat composition. The sanctuary area enjoys a moist tropical aquatic climate and rainfall is frequent and heavy during the monsoon season (May to October) ranging between 119 mm and 1313 mm (Total 3739 mm). The average maximum annual temperature is 36.8°C and the minimum is 7.85°C, whereas the average maximum and minimum range of relative humidity are 87.50 and 70.75% respectively (BMD, Sitakunda Station, 2015-2018).

Field survey

Field work was carried out from July 2015 to June 2017 following a combination of different methods depending on the species type. Transect survey was conducted both at day and night to cover crepuscular and nocturnal species. A total of 7 transects with 2.5km to 3.5km were set for the survey (Sheng and Xu 1992). Apart from trail survey, camera traps were also used to identify nocturnal terrestrial animals of the sanctuary (Kucera and Barrett 1993). A systematic camera trapping was conducted in pre-established 10 sites of Hazarikhil WS. A total of 54 nights were spent for camera trapping in ten sites. Six Scout Gourd (SG565F-8 M) model cameras were used for camera trapping activities. During days transect observation was done. Total counts of animals have been attempted for species that are relatively small and conspicuously concentrated in an area where the population can be effectively enumerated (Hasan *et al.* 2013). This technique has been used to assess Primate species of HWS. Night survey is a very important method for nocturnal animal. The night survey method has been followed regularly in HWS for observing nocturnal animals. The opportunistic visual survey includes observational evidence of roadside dead animals and the captured animals by the local people have been identified individually. Semi-structured questionnaires along with a photo catalogue of elusive mammalian species were used to survey among the forest dependent local people to assess the threats of the mammals in this sanctuary. The local status of species has been measured on the basis of relative presence as stated by Khan (2018): (1) **Very common-** Species that have a 76-100 percent likelihood of being found in their natural habitats at the time when it is most active (2) **Common-** Species that have a 51-75 percent likelihood of being found in their natural habitats during peak activity (3) **Uncommon-** Species that have a 26-50 percent likelihood of being found

in their natural habitats at the peak of their activity (4) **Rare-** Species that have a 25 percent chance of found in their natural habitats at peak of their activity.

Data Analysis

Species diversity

Simpson's Diversity Index (Simpson 1949) and Shannon- Weaver Diversity Index have been used to know the consequence of species diversity in Hazarikhil wildlife Sanctuary.

Simpson's Diversity Index is

$$D = \frac{\Sigma n\,(n-1)}{N\,(N-1)}$$

Where,

N= Number of individuals of all species

n= number of individual of a selected species

Shannon- Weaver Diversity Index is

H' = - Σpi (ln pi) (Shannon-Weaver 1949)

H' a value called "H prime" that takes into account the relative abundance of the different species (i)

where,

pi = Proportion of individuals found in the *i*th species and

ln = Natural logarithm.

The relative abundance Index (RAI) of the recorded mammalian species in Hazarikhil Wildlife Sanctuary was estimated as

RAI= (Jenks *et al.* 2011).

Where,

A = Total number of detections of a species by all cameras/observations and

N = Total number of camera trap days/survey days by all the cameras/observers throughout the study area following Jenks *et al.* (2011).

Results

A total of 33 mammalian species were recorded in HWS, which is about 26% of total species of wild mammals of Bangladesh. Among the recorded species, 4 species of bats, 2 species of primates, 13 species of carnivores and 6 species of rodents constitute the mammalian community (Table 1). The recorded mammals belong to 20 families of 9 orders. Among them 3 species have been categorized as critically endangered, 6 species as endangered, 2 species as vulnerable, 7 species as near threatened, and 15 species as least concern by the IUCN Red List of threatened species. Of all the mammalian species recorded from HWS, higher numbers were Metatural (30.30%; n = 10), followed by Nocturnal (27.27%; n = 9), Diurnal (21.21%; n = 7), and Crepuscular (21.21%; n = 7) (Table 1).

Table 1. Mammalian species with their local and IUCN-2015 (Bangladesh) status at HWS recorded from July 2015 to June 2017

Sl. No.	Order	Family	English name	Scientific name	Status IUCN 2015 BD	Status in HWS
1.	Eulipotypha	Soridae	Grey musk Shrew	*Suncus murinus*	LC	V, N
2.	Eulipotypha	Tupaiidae	Common tree shrew	*Tupaia glis*	NT	U, D

Sl. No.	Order	Family	English name	Scientific name	Status IUCN 2015 BD	Status in HWS
3.	Chiorptera	Pteropodidae	Flying fox	*Pteropus giganteus*	LC	V, N
4.	Chiorptera	Pteropodidae	Kola Badur	*Cynopterus sphinx*	LC	C, N
5.	Chiorptera	Megadermatidae	False Vampire Bat	*Megaderma lyra*	LC	U, N
6.	Chiorptera	Vespertilionidae	Indian Pipistrel	*Pipipstrellus coromandra*	LC	V, N
7.	Primates	Cercopithecidae	Rhesus Macaque	*Macaca mulatta*	VU	C, D
8.	Primates	Cercopithecidae	Capped Langur	*Trachypithecus pileatus*	EN	R, D
9.	Carnivora	Canidae	Wild Dog*	*Cuon alpinus*	EN	R, M
10.	Carnivora	Canidae	Jackle	*Canis aureus*	LC	R, M
11.	Carnivora	Felidae	Jungle cat	*Felis chaus*	NT	R, Cre
12.	Carnivora	Felidae	Leopard cat	*Felis Bengalensis*	NT	R, Cre
13.	Carnivora	Felidae	Fishing cat	*Prionailurus viverrina*	EN	R, Cre
14.	Carnivora	Herpestidae	Small Indian mongoose	*Herpestes auropunctatus*	LC	C, Cre
15.	Carnivora	Herpestidae	Crab eating Indian	*Herpestes urva*	NT	R, Cre
16.	Carnivora	Mustelidae	Smooth coated Otter	*Lutra perspicillata*	CR	R, M
17.	Carnivora	Mustelidae	Hog Badger	*Arctonyx collaris*	VU	R, N
18.	Carnivora	Ursidae	Asiatic Black Bear*	*Ursus thibetanus*	CR	R,M
19.	Carnivora	Viverridae	Binturong*	*Arctictis binturong*	NT	R, N
20.	Carnivora	Viverridae	Large Indian Civet	*Vivvera zibetha*	NT	R, Cre

Sl. No.	Order	Family	English name	Scientific name	Status IUCN 2015 BD	Status in HWS
21.	Carnivora	Viverridae	Small Indian Civet	*Vivericula indica*	NT	R, Cre
22.	Cetartiodactyla	Sidae	Wild Boar	*Sus scrofa*	LC	R, M
23.	Cetartiodactyla	Cervidae	Barking Deer	*Muntiacus muntjac*	EN	U, D
24.	Cetartiodactyla	Bovidae	Mainland Serow	*Capricornis sumatraensis*	EN	R, D
25.	Pholidota	Manidae	Chinese Pangolin*	*Manis crassicaudata*	CR	R, N
26.	Rodentia	Sciuridae	Hoary -Bellied Himalayan Squirrel	*Callosciurus pygerythrus*	LC	Vc, D
27.	Rodentia	Sciuridae	Orange Bellied Himalayan Squirrel	*Dremomys loriah*	LC	R, D
28.	Rodentia	Muridae	Greater Bandicoot Rat	*Bandicota indica*	LC	R, M
29.	Rodentia	Muridae	Indian Field Mouse	*Mus booduga*	LC	U, N
30.	Rodentia	Muridae	House Mouse	*Mus musculus*	LC	U, M
31.	Rodentia	Muridae	Common house Rat	*Rattus rattus*	LC	V, M
32.	Rodentia	Hystricidae	Indian Porcupine	*Hystrix indica*	LC	R, M
33.	Lagomorpha	Leporidae	Indian hare	*Lepus nigricolilis*	EN	R, M

Note: *According to the local people

Status: V= Very Common, C=Common, R=Rare, Uncommon=U; Active

Period: D=Diurnal, N=Nocturnal, Cre = Crepuscular, M = Metaturnal

Over all relative abundance showed that observed status of 60.61% mammals were rare, 15.15 % uncommon, 9.09% common and 15.15 % very common. Carnivores constituted the largest order (in terms of number of species) in the sanctuary consisting of 13 species (40% of the total species) that belongs to 06 families. The small order is Pholidota

contained only one species. Among the total (33) species in HWS under 20 family, four species belong to the family Muridae, three species are under each family of Felidae and Viverridae followed by the 06 family like Pteropodidae, Canidae, Herpestidae, Cercopithecidae, Mustelidae and Sciuridae consist of 2 species each and 11 family represent only one species each.

Status and relative abundance of mammals

During the survey of the mammalian species in HWS 18 species were direct observed and 8 species were camera trapped and rest of the 7 species confirmed by the people comment and sign of presence. Relative abundance index of Mammalian species in HWS recorded from July 2015 to June 2017 is demonstrated in Table 2.

Table 2. Relative Abundance Index of Mammalian species in HWS recorded from July 2015 to June 2017

Sl. No.	English name	Scientific name	Total CT/O days	Sighting	CT pic	Total Observation	RAI
1.	Grey musk Shrew	*Suncus murinus*	54	47	0	47	87.04
2.	Common tree shrew	*Tupaia glis*		15	0	15	27.78
3.	Flying fox	*Pteropus giganteus*		54	0	54	100.00
4.	Kola Badur	*Cynopterus sphinx*		27	0	27	50.00
5.	False Vampire Bat	*Megaderma lyra*		15	0	15	27.77
6.	Indian Pipistrel	*Pipipstrellus coromandra*		51	0	51	94.44
7.	Rhesus Macaque	*Macaca mulatta*		33	0	33	61.11
8.	Capped Langur	*Trachypithecus pileatus*		21	0	21	38.89
9.	Wild Dog*	*Cuon alpinus*		0	0	0	0
10.	Jackle	*Canis aureus*		17	0	17	31.48
11.	Jungle cat	*Felis chaus*		3	0	3	5.56
12.	Leopard cat	*Felis Bengalensis*		1	8	9	16.67
13.	Fishing cat*	*Prionailurus viverrina*		0	0	0	0
14.	Small indian mongoose	*Herpestes auropunctatus*		31	0	31	57.41
15.	Crab eating Indian Mongoose	*Herpestes urva*		5	0	5	9.26
16.	Smooth coated Otter*	*Lutra perspicillata*		0	0	0	0
17.	Hog Badger	*Arctonyx collaris*		5	0	5	9.26

Sl. No.	English name	Scientific name	Total CT/O days	Sighting	CT pic	Total Observation	RAI
18.	Asiatic Black Bear*	*Ursus thibetanus*		0	0	0	0
19.	Binturong*	*Arctictis binturong*		0	0	0	0
20.	Large Indian Civet	*Viverra zibetha*		0	1	1	1.85
21.	Small Indian Civet	*Viverricula indica*		0	4	4	7.41
22.	Wild Boar	*Sus scrofa*		10	4	14	7.79
23.	Barking Deer	*Muntiacus muntjac*		6	12	18	33.33
24.	Mainland Serow*	*Capricornis sumatraensis*		0	0	0	0
25.	Chinese Pangolin*	*Manis crassicaudata*		0	0	0	0
26.	Hoary-Bellied Himalayan Squirrel	*Callosciurus pygerythrus*		54	0	0	100.00
27.	Orange Bellied Himalayan Squirrel.	*Dremomys loriah*		5	0	5	9.26
28.	Greater Bandicoot Rat	*Bandicota indica*		3	1	4	7.41
29.	Indian Field Mouse	*Mus booduga*		15	0	15	27.78
30	House Mouse	*Mus musculus*		23	0	23	23.00
31.	Common house Rat	*Rattus rattus*		47	0	47	87.04
32.	Indian Porcupine	*Hystrix indica*		5	1	6	11.11
33	Indian hare	*Lepus nigricolilis*		0	1	6	1.85

*According to local people, CT/O= Camera Trap/Observation

Species diversity indices

From the estimation of Simpson's Diversity Index Value of surveyed mammals = 0.549 indicates significant diversity and the calculation Shannon-Weaver Diversity Index was weighed about 2.63 which prophesies that the diversity of the major mammals is rich enough in the study area (Shannon and Weaver 1949). Camera trapping also resulted in sighting Wild Boar four times and Hog Badger five times times, Small Indian civet four times (Fig. 2). Moreover, as an opportunist feeder, they also move form paddy field to homestead gardens surrounding forest village (Feeroz *et al.* 2011).

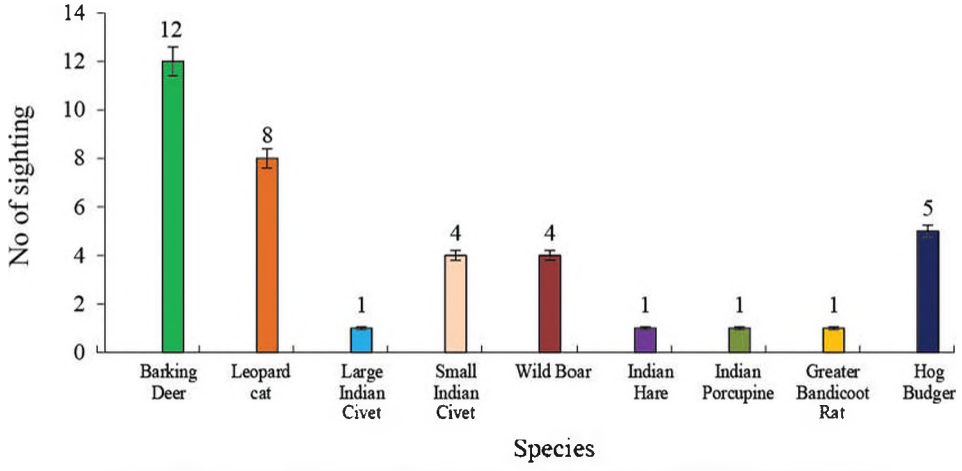

Figure 2. Frequency of photo captured in camera traps during 180 camera nights.

Discussion

Among all the elusive terrestrial mammals, 4 groups of Capped langur were seen in the sanctuary. One group stays outside of the sanctuary all the time. This group has been found habituated to stay in the Harualchari village (near bridge) adjacent to the sanctuary. Sometimes they came to rest house of Tea garden for searching food. The group was also seen several times near streams. Another 3 groups move at last corner of Hazarikhil block which is north portion of the sanctuary where same species was recorded as common in Chunati Wildlife sanctuary and Rema-kalenga wildlife sanctuary (Feeroz 2014; Feeroz *et al.* 2011) and as very common in Teknaf Wildlife sanctuary and Dudhpukuria-Dhopachari wildlife sanctuary (Feeroz 2013; Feeroz *et al.* 2012). Two species of squirrels namely, Hoary-Bellied Himalayan Squirrel (*Callosciurus pygerythrus*) and Orange Bellied Himalayan Squirrel (*Dremomys loriah*) were found in HWS. As Hoary-Bellied Himalayan Squirrel is distributed almost all over the country along with forest ecosystem (Khan 1982; Khan 2015) so this squirrel is also very common in HWS. It is seen everywhere in the forest from deep forest to periphery. Orange Bellied Himalayan Squirrel was rarely observed in HWS during study period. In the spring time it was only seen in low population. On the basis of information of local people another important species Serrow (*Capricornis sumatraensis*) is found in the sanctuary, although could not possible to see the animal but some indirect clue have been found such as dung, foot print, resting place of this animal is identified at several occasions. The species prefers to stay and grazing on the high, vertical hill side and hill top. During the survey a skull with horn of Serrow was collected from the hunter who killed the species during the study period from Baraiyadhala National Park situated adjacent to forest areas of HWS Sanctuary. Among the two species of mongooses found in HWS, Crab-eating Mongoose is larger in size with a metallic gray coarse fur, having a distinctive white stripe on either side of neck. Tail is very bushy at base, but hairless at the tip. They are very shy and occur in small groups. It feeds mainly on frogs, crabs and fish in marshy areas, streams and in

paddy fields. As a burrower they make their den in undisturbed hilly areas of the middle part of the sanctuary. Barking deer was mostly abundant in all the forested areas of HWS. The calling of this animal in the sanctuary is very common. In HWS this species was sighted almost twelve times in camera traps. The reason may be due to diverse food habit of this species from fresh grasses to leaves and twigs of trees. Another mostly common mammal was Leopard cat also has a very good population in HWS. Sometimes people around the sanctuary become confused if it is a Leopard which was sighted eight times by camera traps. The reason may be due to the diverse food habit of this species from rodents to small birds, eggs and also domestic poultry. A major part of HWS surrounded by human settlements, tea garden, wetland and agricultural fields which ensure huge production of rodents and other prey species for Leopard cat. Undisturbed bushy hills and valleys of the sanctuary support large group of Wild Boars and Hog Badger in HWS. Hog-Badger (*Arctonyx collaris)* is one of the most elusive nocturnal mammals of the country and very little is known about this species. Doubtful occurrence of this species was recorded from Teknaf (Khan 1987) and confirmed presence from Teknaf Wildlife Sanctuary in 2010 by camera trapping and also recorded in Dudhpukuria-Dhopachari Wildlife sanctuary (Feeroz *et al.* 2012). Forest resource exploitation in Bangladesh, both legally and illegally, has been accelerated due to increased demand of ever growing population with no exception to forest areas of HWS. Without forest friendly management plan, over collection of trees, fire wood and non-timber forest product as bamboos, cane, sun grass, broom grass made the forest areas of HWS degraded. There is hardly any old tree cover in HWS. Plantation programme has started from the year of 2001-2002 and is ongoing. Alteration of forest habitat to cultivation land and expansion of tea gardens has reduced the forest areas. Hunting, poaching and destruction of habitat was rampant, so many species disappeared from this area. Some of the major threats have been identified are hunting and poaching, human-wildlife conflict, fish poisoning, construction of road inside the sanctuary, forest fire, encroachment for gardening, grazing, encroachment, uncovered electric ware within the sanctuary (From questionnaire survey). It is a serious threat to the arboreal and flying mammals. It may cause death toll of a number of Capped Langur, Rhesus Macaque, Flying Fox and other bat species and small mammals (Source: Local People).

Conclusion

Thirty three wild mammalian species were recorded in HWS. The presence of the species demands wildlife preferred management practice, habitat conservation and habitat enrichment and habitat restoration for their survival and sustainable population.

The study has provided important baseline information for the first time on the mammalian diversity of HWS of Bangladesh. The observed mammalian species diversity indicates a good faunal variety in the study area. It is the time to protect the wildlife diversity by protecting their habitat and enforcing Wildlife (Conservation and Security) Act, 2012. Wildlife are continuously facing severe disturbance to their habitat by destruction. Steps should be taken to strictly prohibit further encroachment of any forest land to build homesteads or convert to crop fields. Raising awareness and consciousness of local people in regard to the importance of

forestry, wildlife, environmental conservation, biodiversity and endangered ecosystem could be mandatory part of the HWS management. Government may provide some incentives in cash and/or kind such as monetary support, small loan, training etc. to help local people managing alternative income generating programme other than collection of forest product. Enrichment plantation with some native, rare and fruit bearing plants i.e. *Artocurpus* sp., *Syzygium* sp., *Baccaurea ramiflora* (lotkon), *Ficus* sp. (Bot), etc. can be planted around beat office and in the degraded sites of HWS to facilitate the foods for mammalian species in the Sanctuary.

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Bamboo Mat Overlaid Particleboard made from Borak (*Bambusa balcooa*) and Mitinga (*Bambusa tulda*) Bamboo

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Abstract

The goal of this research was to determine the physical (water absorption and thickness swelling) and mechanical (modulus of rupture, internal bond strength) properties of the experimental bamboo mat overlaid particleboard (MOPB) from the planer wastage of Borak (*Bambusa balcooa*) bamboo in which Mitinga (*Bambusa tulda*) bamboo was used as mat covering. All the bamboos were 50-60 feet tall and 3-4 years old. Before manufacturing of composite boards, bamboo mats were treated with an aqueous solution of 10% borax-boric acid (w/v) maintaining borax to boric acid ratio of 1:1 to extend their service life. Urea-formaldehyde glue was used for manufacturing the MOPBs as a resin binder. Five single-layer bamboo MOPBs measuring 500 mm × 500 mm × 12 mm were prepared using a laboratory hot press machine with a target density of 750 kg/m^3. The results demonstrate that the MOPBs made from bamboo planer wastage with a density of 750 kg/m^3 have a significant modulus of rupture (MOR) and internal bond (IB) strength value. The values of modulus of rupture of the MOPBs fulfilled both the Indian (IS 3087: 2005) and ANSI (A208.1-1999) standards, whereas internal bond strength values exceeded the Indian (IS 3087: 2005), ANSI (A208.1-1999) and British (BS 5669-2: 1989) standards specifications.

সারসংক্ষেপ

এই গবেষণার লক্ষ্য ছিল বোরাক বাঁশের প্ল্যানার কুঁচি থেকে পরীক্ষামূলকভাবে তৈরিকৃত ব্যাম্বো ম্যাট ওভারলেইড পার্টিকেলবোর্ডের ভৌত (জল শোষণ ক্ষমতা এবং পুরুত্বের স্ফীতি) এবং যান্ত্রিক (মডুলাস অব রাপচার, অভ্যন্তরীণ বন্ধন শক্তি) বৈশিষ্ট্যাবলি নির্ণয় করা যেখানে মিতিঙ্গা বাঁশের চাটাইকে বোর্ডের আচ্ছাদন হিসেবে ব্যবহার করা হয়েছিল। গবেষণায় ব্যবহৃত ৩-৪ বছর বয়সি বাঁশগুলোর উচ্চতা ছিল ৫০-৬০ ফুট। যোজিত বোর্ড তৈরির পূর্বে, বাঁশের চাটাইগুলিকে তাদের আয়ুষ্কাল বৃদ্ধির জন্য ১:১ অনুপাতের বোরাক্স-বোরিক এসিডের ১০% জলীয় দ্রবণ দ্বারা ট্রিটমেন্ট করা হয়েছিল। ব্যাম্বো ম্যাট ওভারলেইড পার্টিকেলবোর্ড তৈরিতে ইউরিয়া-ফরম্যালডিহাইড আঠা বাইন্ডার হিসেবে ব্যবহার করা হয়েছিল। একক-স্তর বিশিষ্ট ৫০০ মি.মি. × ৫০০ মি.মি. × ১২ মি.মি. পরিমাপের ৭৫০ কেজি/মি.^{৩} ঘনমাত্রার পাঁচটি পরীক্ষামূলক ব্যাম্বো ম্যাট ওভারলেইড পার্টিকেলবোর্ড ল্যাবরেটরি হট প্রেস মেশিনের সাহায্যে তৈরি করা হয়েছিল। ফলাফল বিশ্লেষণে দেখা যায় যে, ৭৫০ কেজি/মি.^{৩} ঘনত্বের ব্যাম্বো ম্যাট ওভারলেইড পার্টিকেলবোর্ডের মডুলাস অব রাপচার এর মান ভারতীয় (IS 3087: 2005) এবং এএনএসআই (A208.1-1999) মানদণ্ডের চেয়ে উত্তম। অন্যদিকে উক্ত বোর্ডের অভ্যন্তরীণ বন্ধন শক্তির মান ভারতীয় (IS 3087: 2005), এএনএসআই (A208.1-1999) এবং ব্রিটিশ (BS 5669-2: 1989) মানদণ্ডের চেয়ে বেশি।

Key words: Bamboo mat overlaid particleboard, Internal bond strength, Modulus of rupture, Thickness swelling, Water absorption.

Introduction

Bamboo is a fast growing plant, as some species can reach full growth in just 90 days. It is a type of woody grass, a cylindrical pole or culm in its natural state. There are more than 1200 species of bamboo all over the world. The three forms of root systems are found in different species: sympodial (clumping), monopodial (running), and amphipodial (clumping and running). The bamboo shoots grow nearer to each other to create a clump in the sympodial bamboo, whereas monopodial bamboo appears to be made up of separate culms. The root bases of amphipodial bamboo are a mix of the two (Sharma *et al.* 2014). The output of bamboo plantation is great and the use of bamboo stems is wide. Once successfully planted, bamboo plants keep on rhizoming, shooting and maturing every year. The annual selective cutting and sustainable utilization can be implemented without damaging the ecological environment. The world is facing a rapid decrease in forest resources and suffering serious deterioration of the ecological environment. Therefore, the development and exploitation of bamboo resources are of considerable importance (Zhang *et al.* 2001). Bamboo forests cover around 22 million hectares of the world's surface (Zhang *et al.* 2013). Bamboo has many advantages, including its fast growth cycle of 3-5 years and the ability to be harvested multiple times from a single planting. It has about 2-3 times higher strength and rigidity than wood (Xiao *et al.* 2013; Zakikhani *et al.* 2014). Bamboo boards were first manufactured in China in the 1970s, and output has expanded as the process has become more industrialized (Ganapathy *et al.* 1999). The efficiency of bamboo composite production varies by producer, although it is about 80% for bamboo scrimber and 30% for laminated bamboo (van der Lugt 2008). During the production of the laminated bamboo panel products, more than 30% of the bamboo like branches, nodes, rhizomes and upper and lower portions of the culm, etc. are left unused and treated as wastage (Biswas 2008). The production of particleboard is escalating day by day. Thus the huge demand for particleboards accelerates the utilization of the wastage. The demand for alternative sources of raw materials is increasing rapidly. Unconventional lignocellulosic materials like agricultural residues and non-woody plant fibers may play a major role in minimizing the demand for manufacturing the composite boards (Nemli and Aydin 2007).

Furniture is one of the basic necessities of human life. It is both practical and decorative and in harmony with the indoor environment. Bamboo furniture is imbued with oriental natural color in a simplified and elegant style. Traditional bamboo furniture is made by means of traditional techniques such as crooking, reinforcing, connecting, holing, mortising and board covering. The manufacturing technology of modern furniture of bamboo based panels is similar to that of wooden furniture. In Bangladesh, the manufacturing of bamboo composite products such as laminates and particleboards is not commonly practiced. Developing technology to produce composite items made of bamboo is currently regarded as a very important research field in Bangladesh (Biswas *et al.* 2009). Based on the research, Bangladesh Forest Research Institute (BFRI) has designed various pieces of furniture with bamboo panels, MOPBs and bamboo mat overlaid wood veneer boards. The study was undertaken to find out the gluability of bamboo mat overlaid particleboards for manufacturing composite products as a furniture component.

Materials and Methods

Bamboo chips and planer wastage

Borak (*B. balcooa)* bamboos aged 3-4 years were collected from Banshkhali, Chattogram. The length of all bamboo was 50-60 feet. Only

32 ft of length from each culm was used to prepare strips for the production of the bamboo panels because of their superior wall thickness. After strip preparation, bamboo planer wastage was found. Planer wastage is the flack shape of the particles.

Bamboo mat overlaid particleboard preparation

Bamboo planer wastage was dried at 5% humidity. Bamboo mats were prepared by hand with Mitinga (*B. tulda)* bamboo. Five-single layer MOPB sizes of 500 mm × 500 mm × 12 mm having a target density of 750 kg/m^3 were made using 35.6 kg/cm^2 pressure with the aid of a hydraulic hot press machine. The temperature of the platens of the hot press was maintained at 140°C. Twenty percent liquid urea formaldehyde glue based on oven dried planer wastage of Borak (*B. balcooa*) bamboo was used in the preparation of particleboards. In this experiment, bamboo mats were used on the face and back of particleboards called MOPBs, which were produced in a single operation where mats and particles were pressed at the same time. The boards were then conditioned at 65±5% relative humidity and 20±2°C temperature before they were put to tests.

Test sample preparation

The bamboo MOPBs were cut into various test specimens. The static bending tests (MOR in bending) were carried out according to the specification of IS: 2380 (Anon 1977) with the help of a Riehle screw power type universal testing machine. The IB strength test was also carried out according to the specification of IS: 2380 (Anon 1977) with the exception that wooden blocks of 75 mm × 50 mm × 25 mm were glued in a cold press with the test specimens. Three specimens of sizes 100 mm x 100 mm were taken from each MOPB to determine the Thickness swelling (TS) and Water absorption (WA) properties. The thickness of the specimens was measured with the platform type thickness gauze with an accuracy of 0.01 mm. The test specimens were immersed in 25 mm depth of cold water (25±2°C). At the end of 2 hours and 24 hours, the test specimens were withdrawn from the water, wiped with a damp cloth, reweighed and re-measured the thickness as before. After that, the percentage of water absorption and thickness swelling was calculated. The test results were then compared with the standard specifications given in Table 1.

Table 1. Standards specification for physical and mechanical property of particleboards.

Standards	Board thickness (mm)	Density (kg/m^3)	MOR (kg/cm^2)	IB (kg/cm^2)	TS (%)		WA (%)	
					2hrs	24hrs	2hrs	24hrs
IS 3087 (Anon 2005)			110.00	8.00	10.00	NA	25.00	50.00
ANSI A208.1 (Anon 1999)	6-20	500-900	110.00	4.00	NA	8.00	NA	NA
BS 5669-2 (Anon 1989)			138.00	3.40	8.00 (for 1 hr)	NA	NA	NA

NA: Not specified in test requirements; hr: hour

Statistical design and analysis

The experiment was performed in a completely randomized design (CRD) with five replications. Analysis of variance (ANOVA) test was also carried out for this experiment.

Results

The mean value, standard deviation and coefficient of variance of MOR, IB, TS and WA are given in Table 2 and Table 3. It was observed that the values of MOR and IB were different for the different types of particleboards (Fig. 1 and Fig. 2). From Table 2, it was found that the mean MOR value of the bamboo mat overlaying particleboards (136.70 kg/cm^2) was higher than that of particleboards without any covering (115.64 kg/cm^2). The mean MOR value of MOPB (136.70 kg/cm^2) fulfilled the requirements of the Indian (110.00 kg/cm^2) and ANSI (110.00 kg/cm^2) standard specifications (Table 1). The value was very close to the British standard specification (138.00 kg/cm^2). The IB strength values have been presented in Table 2. It was found that the IB values of the two types of particleboard were very close to each other. The results didn't clearly show any inconsistency. The mean IB value was 10.86 kg/cm^2 for the MOPBs and 10.28 kg/cm^2 for the planer waste particleboards. Both values met the requirements of Indian, ANSI and British standard specifications (Table 1).

Table 2. Mechanical properties of particleboards made from planar wastage of Borak *(B. balcooa)* bamboo.

Types of particleboard	Board thickness (mm)	MOR (kg/cm^2)	IB (kg/cm^2)
Bamboo particleboard	13.27 ± 0.20 (0.04)*	115.64 ± 3.26 (10.66)	10.28 ± 0.60 (0.36)
Bamboo mat overlaid particleboard	14.53 ± 3.71 (0.13)*	136.70 ± 1.85 (3.42)	10.86 ± 0.44 (0.20)

Note: (±) indicates the standard deviation (SD) of means and the values in parenthesis is coefficient of variance (CV)*

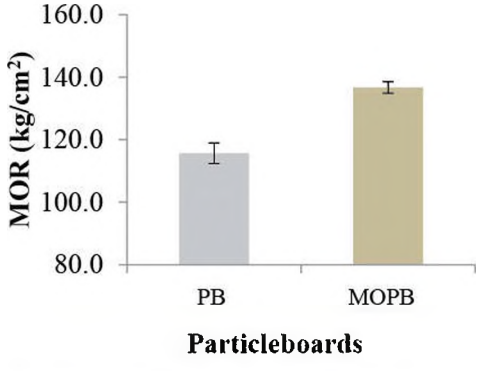

Figure 1. Modulus of rupture of bamboo particleboard and bamboo mat particleboard.

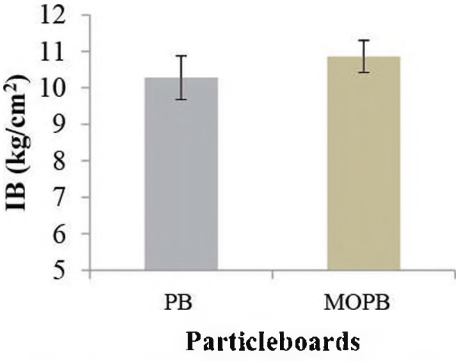

Figure 2. Internal bond strength of bamboo particleboard and bamboo mat particleboard.

The WA and TS properties had also been determined for the two types of particleboards made from bamboo planer waste. The test samples were soaked under water for 2 and 24 hours, weight and thickness differences were measured for the determination of WA and TS (Fig. 3 and Fig. 4). The observed TS values of the two types of particleboards were 14.20 to 14.80% for 2 hours and 18.07 to 16.56% for 24 hours of water soaking (Table 3). After 24 hrs of water soaking, we saw that the average value of WA was 42.44% for planer wastage particleboards and 40.70% for MOPBs. The test results are shown in Table 3.

Table 3. Physical properties of particleboards made from planar wastage of Borak *(B. balcooa)* bamboo.

Types of particleboard	Board thickness (mm)	TS (%)		WA (%)	
		2hrs	24hrs	2hrs	24hrs
Bamboo particleboard	13.27 ± 0.20 (0.04)*	14.20 ± 0.03 (0.01)	18.07 ± 0.60 (0.42)	42.44 ± 1.09 (1.02)	48.91 ± 0.11 (0.01)
Bamboo mat overlaid particleboard	14.53 ± 3.71 (0.13)*	14.80 ± 0.02 (0.01)	16.56 ± 0.57 (0.33)	40.70 ± 0.17 (0.03)	43.52 ± 0.19 (0.04)

Note: (±) indicates the standard deviation (SD) of means and the values in parenthesis is coefficient of variance (CV)*

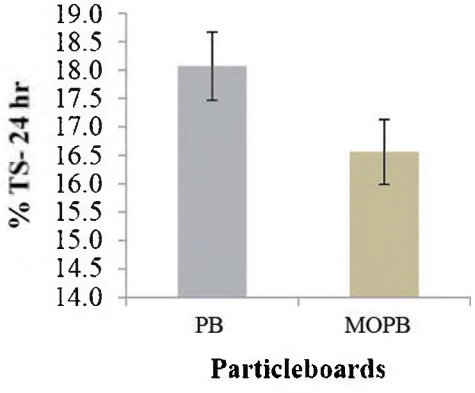

Figure 3. Thickness swelling of bamboo particleboard and bamboo mat particleboardafter 24 hours.

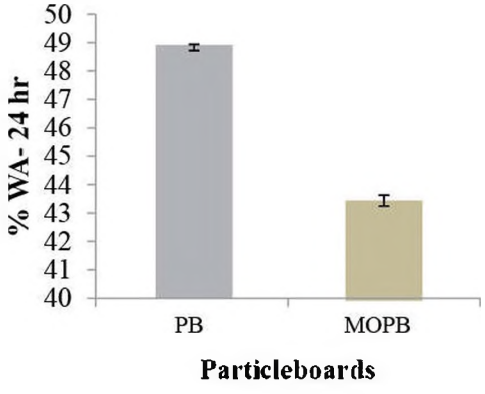

Figure 4. Water absorption of bamboo particleboard and bamboo mat particleboard after 24 hours.

Discussion

After analyzing the data, we can see that the MOR value of particleboards made of bamboo planar wastage is less than that of MOPBs. It is for this reason that both sides of the MOPBs are enclosed with the bamboo mat. According to Kollmann *et al.* (1975), MOR is an essential mechanical property of particleboards in the use of structural components. In the case of IB, the mean value of the MOPBs and the particleboards is almost the same. In practical terms, the MOR strength and quality of any composite board are taken into consideration rather than the internal bonding strength. The IB strength property gives information about the structure of particleboard which ensures a fine adhesive property and good dimensional stability of the particleboard structure.In terms of TS and WA, it has been observed that the WA capacity of the MOPBs is less than that of particleboards made of planer wastage. This is because the insertion of a mat across both sides of the particleboard reduces the water absorption capacity. The sliver of the bamboo mats has a silica coating which has low water absorption capacity. The TS property of the MOPBs was low similar to their WA capacity. Furniture used indoors has less moisture interaction than outdoors. Particleboard is a type of material that is mainly used in the interior of homes. Although accidental water exposure will not decrease the panel's durability or characteristics, it is recommended that household furniture be kept away from water. After 24 hours of water soaking, a value of TS below 12% ensures the dimensional stability of the composite panels when it is used as a material for interior application and furniture production (Popovska *et al.* 2016). Kollmann *et al.* (1975) reported that the highest thickness swelling after two hours of immersion in water should not exceed 6-10% of the original thickness. However, the addition of suitable additives may improve the properties of the particleboards.

Conclusion

The dimensional stability and strength property attribute that the mat overlaying planer wastage particleboard made from Borak (*B. balcooa*) bamboo offers superior dimensional stability and strength properties compared to planer wastage particleboard. The planer waste particleboard with a mat layer can be used for furniture and other household applications. As a result, it is possible to conserve very important forest resources by ensuring maximum utilization of forest products.

Acknowledgement

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Effects of Storage Condition and Period on Seed Germination and Initial Growth Performance of Chapalish *(Artocarpus chama)* Seedlings

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Abstract

Artocarpus chama Bunch, Ham. *ex* Wall. (Chapalish) is a large, deciduous indigenous forest tree species grown in natural forests. The seed of Chapalish is recalcitrant and loses viability within a few days of maturity that makes problem in storage of the seeds. Optimum storage conditions found useful for prolonging the viability of recalcitrant seeds through preventing water loss. The study was taken to identify the suitable storage methods in order to prolong the seed viability of *A. chama*. Nursery trial was conducted at the National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute to evaluate the effects of storage conditions and duration on germination of chapalish seeds. Seeds were stored in five different storage media, *viz.* i) open room (control), ii) sand, iii) chalk powder, iv) ash and v) sawdust with 8 different storage periods (days), *viz.* 10, 20, 30, 40, 50, 60, 70, and 80 days. The effects of storage media and storage periods (days) were assessed through seed germination and seedling growth performance. Viability and seedling growth performance were significantly ($p<0.05$) influenced by storage media, storage periods and their interactions. The results revealed that chalk powder media exhibited significantly higher germination percentage, root length, shoot length and vigor index. It also revealed that it can prolong the viability of Chapalish seeds up to 40 days with 60% germination and was statistically significant at 5% level. The findings may be useful to nursery practioners, foresters, and private plant growers for Chapalish seeds. The storage condition is convenient, low-cost and easily applicable to all nursery owners.

সারসংক্ষেপ

চাপালিশ প্রাকৃতিক বনে জন্মানো একটি বৃহৎ, পর্ণমোচী দেশীয় বনজ বৃক্ষ প্রজাতি। এর বীজ স্বল্পজীবী হওয়ায় পরিপক্ব হওয়ার খুব অল্প সময়ের মধ্যে ভায়াবিলিটি হারায় যা বীজ সংরক্ষণে সমস্যার সৃষ্টি করে। বিভিন্ন ধরনের সংরক্ষণ মাধ্যম বীজের পানি অপচয় রোধ করার মাধ্যমে স্বল্পজীবী বীজের আয়ুষ্কাল দীর্ঘায়িত করার ক্ষেত্রে উপকারী বলে বিবেচিত হয়। তাই চাপালিশ প্রজাতির বীজের গুদামজাতকরণ সমস্যা চিহ্নিতকরণ ও আয়ুষ্কাল বৃদ্ধির সর্বোত্তম পদ্ধতি খুঁজে বের করার জন্য এই স্টাডিটি নেয়া হয়েছে। বাংলাদেশ বন গবেষণা ইনস্টিটিউটের অধীনস্থ বীজ বাগান বিভাগের ন্যাশনাল ফরেস্ট সীড সেন্টারে চাপালিশ প্রজাতির বীজের অঙ্কুরোদগমের উপর বিভিন্ন সংরক্ষণ পদ্ধতি ও উহার সময়কালের প্রভাব মূল্যায়নের জন্য পরীক্ষণটি পরিচালনা করা হয়েছে। ৫টি সংরক্ষণ মাধ্যম যথা : i) খোলা অবস্থায় (কন্ট্রোল), ii) বালি, iii) চক পাউডার, iv) ছাই, v) কাঠের গুড়া এবং ৮টি ভিন্ন ভিন্ন সময়কাল যথা: ১০, ২০, ৩০, ৪০, ৫০, ৬০ ৭০ ও ৮০ দিন সংরক্ষণ করা হয়। বীজের অঙ্কুরোদগম ও চারার বৃদ্ধির নির্ণয়ের মাধ্যমে বিভিন্ন বীজ সংরক্ষণী ও সংরক্ষণ সময়কালের প্রভাব মূল্যায়ন করা হয়েছে। বিভিন্ন মাধ্যমে বিভিন্ন সময়ব্যাপী সংরক্ষণকৃত বীজে জীবনীশক্তি ও চারার বৃদ্ধিশক্তির উপর তাৎপর্যপূর্ণ প্রভাব ($p<0.05$)

পরিলক্ষিত হয়। পরীক্ষণের ফলাফল অনুযায়ী বীজের অঙ্কুরোদগমের হার, চারার বৃদ্ধি এবং শক্তি সূচক (Vigor Index) ক্ষেত্রে চক পাউডারে গুদামজাতকরণ পদ্ধতি তাৎপর্যপূর্ণ সর্বোচ্চ দক্ষতা প্রদর্শন করেছে। পরীক্ষায় আরো দেখা যায় যে, এতে ৪০ দিন পর্যন্ত সংরক্ষণ করা বীজ ৬০% অঙ্কুরোদগম ক্ষমতা প্রদর্শন করেছে যা পরিসংখ্যানগতভাবে ৫% লেভেলে তাৎপর্যপূর্ণ। এই গবেষণার ফলাফল নার্সারি অনুশীলনকারী, বনবিদ ও ব্যক্তিগত উদ্ভিদ চাষিদের জন্য উপযোগী হতে পারে। বীজ সংরক্ষণের এ পদ্ধতিটি সুবিধাজনক, সাশ্রয়ী ও নার্সারি মালিকদের নিকট সহজলভ্য।

Key words: Chapalish, Germination, Recalcitrant, Storage media, Storage period, Vigor index.

Introduction

Artocarpus chama Bunch, Ham. *ex* Wall. (locally known as Chapalish) belongs to the family Moraceae. It is naturally grown in Nepal to Assam, Arunachal Pradesh, Bangladesh, Myanmar and Andaman Islands (Singh 2003). It grows well with a mean maximum temperature of 36°C–40°C, mean minimum temperature of 15°C–30°C and with a rainfall between 203–508 cm. Chapalish naturally found in the foothills, along the bank of a streams, possessing clayey soil with good drainage, moist lateritic and rich deep loam soil (Troup 1921, 1986; Zabala 1990; Das and Alam 2001; Hossain 2015). It is a large deciduous tree with a tall straight bole having a height of 30–40 m and diameter of 1.0-1.5 m (Hossain 2015). Flower head of Chapalish appears in March–April and fleshy fruits ripen in July–August. Fruits are 8–10 cm in diameter and contain few seeds (Hossain 2015). Fruits are eaten by birds, monkeys and other animals, as well as human beings and the seeds are dispersed by wildlife. (Troup 1921, 1986). Seeds of *A. chama* are recalcitrant and losses viability within 10–15 days (Hossain 2015). So, it is imperative to find out a suitable storage method which can prolong its viability to raise seedlings in large scale at nursery.

Seed storage is necessary to ensure a continuous and cost-effective supply of seedlings whenever seedlings are necessary for plantation programs. Recalcitrant (desiccation-sensitive) seeds are metabolically active when shed from the mother plant and possess relatively high moisture content. Even under ambient temperature and low relative humidity, their post-harvest life is very short. Chapalish seeds lose viability when their moisture content falls below 20 to 30% (Farrant *et al.* 1988; Pritchard 2004). Seeds do not withstand drying or are unable to survive low temperatures during storage, which are difficult to store for longer periods of time (Ellis 1984; Hanson 1984). The exact causes of recalcitrant seeds death and its relationship with moisture contents are not fully understood (Fu *et al.* 1993). It is stated that loss of viability could be either due to the moisture content falling below a certain critical value or simply a general physiological deterioration with time (Chin *et al.* 1984). It is also noteworthy that several pre-harvest factors determine the longevity, like the cumulative effect of the environment during seed maturation, harvesting, drying and the pre-storage environment, time of seed harvesting, duration of drying and the subsequent period before seed is placed in storage (Hanson 1984). The principle of successful seed storage for moist recalcitrant seeds is to maintain seeds at a moisture content levels close to that at which they are shed with continuous access to oxygen, because under these circumstances seed deterioration is

minimized since repair mechanisms operate (Villiers 1975). It is also essential that the conditions should prevent or at least delay germination. The use of germination inhibitors (Goldbach 1979; Edwards and Mumford 1983) or cool temperatures (provided the seeds are not damaged by chilling) can be effective.

The standard pattern for storing and testing seeds for germination to ensure uniformity and reproducibility needs tests. It is important to know how long the vitality exists with a considerable level of germination percentage. The storage medium should maintain seed moisture constant at high levels and allow diffusion of sufficient oxygen to the moist seeds. The storage of moist recalcitrant seeds in damp charcoal, sawdust, or moist sand is generally reported to be more efficient than storage in polyethylene bags (Schaefer 1990). However, several seed storage methods are used in agricultural crops. But a few storage methods have been found to be used for forest tree seeds. Khan and Shankar (2004) studied effect of seed mass and different irradiance level on germination and seedling growth and Rahman *et al.* (2012) observed the establishment and initial growth performance at elevated temperature and saline stress of *A. chama*. Begum *et al.* (2020) studied germination and growth of *A. chama* seedlings in three different growing media at nursery. But ample literature is unavailable on the storage media and period of *A. chama* seeds in Bangladesh. *A. chama* is generally propagated by seed origin seedlings in nurseries. However, the seeds have a short storage life and rapid loss in seed viability is a major problem in propagating the species later on. Normal viability of *A. chama* seed remains 10–15 days (Hossain 2015). So, it is necessary to find out a suitable storage medium which can prolong the viability of Chapalish seeds.

That is why the present study was undertaken to investigate seed viability and methods for prolonging the life of Chapalish seeds under different storage conditions (media) and durations (days).

Materials and Methods

The study area

The experiment was conducted at the National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute, Chattogram from June to August 2018. The climate of the study area is tropical in nature and characterized by hot, humid summers and cool, dry winters. The maximum and minimum temperatures in the area vary from 28.31 to 31.9°C and from 15.2 to 25.2°C respectively (Hossain and Arefin 2012). The mean annual rainfall is around 3,000 mm mainly occurring from June to September.

Collection of Chapalish fruit and extraction of seeds

Fruits of Chapalish were collected in June 2018 from selected plus trees in Ukhya, Cox's Bazar. Ripen fruits were split open and seeds were extracted from the bulbs after removing flesh. After washing and drying at room temperature (25±2°C) for 2–3 days; shrieked, discolored and damaged seeds were discarded and only healthy seeds were used for the experiment. The germination trial was carried out by sowing seeds in a bed filled with pure coarse sand only (Fig.1).

Storage of seeds and layout experimental design for different treatments and media

The experiment was conducted in a Randomized Complete Block Design (RCBD) with four replications. Five treatments (media) with eight storage durations were applied to

determine the effects of storage methods and storage duration on seed germination and initial seedling growth attributes. The storage conditions were, i) T_1 = open-room temperature (control), ii) T_2 = sand, iii) T_3 = chalk powder, iv) T_4 = ash, and v) T_5 = sawdust. Fresh seeds (D_1) were sown on the first day of seed extraction from the fruit. Then The remaining seeds were stored for seven storage durations, viz., 10 days (D_2), 20 days (D_3), 30 days (D_4), 40 days (D_5), 50 days (D_6), 60 days (D_7) and 70 days (D_8). After completion of the storage period, seeds from each treatment were sown. 50 seeds per treatment per replication were sown in all durations for germination trial in the seedbed of the nursery. Routine watering and weeding activities were carried out manually when necessary.

Assessment of seed germination and seedlings growth performance

To determine the effects of storage conditions and storage periods on seed germination percent and seedling growth was explored periodically by counting the germinated seeds and measuring the initial growth performance of seedlings. Germination data was collected every alternate day from the first germination until no further seeds germinated. To assess the growth performance, ten seedlings from each replication (40 from each treatment of every period of storage) were randomly uprooted and measured their shoot length and root length separately when the seedlings were at 30 days old. The vigor index (VI) was calculated according to Baki and Anderson (1973).

Data Analysis

The two-way between groups Analysis of variance (ANOVA) was conducted to compare the mean of germination percentage, shoot length, root length and vigor index of Chapalish under five different storage media and eight different storage periods. All data were statistically analyzed using IBM SPSS 28.0 computer software. ANOVA was done with post hoc comparison using Least Significant Difference (LSD) to determine the significant ($p \leq 0.05$) variations among the storage conditions and storage durations.

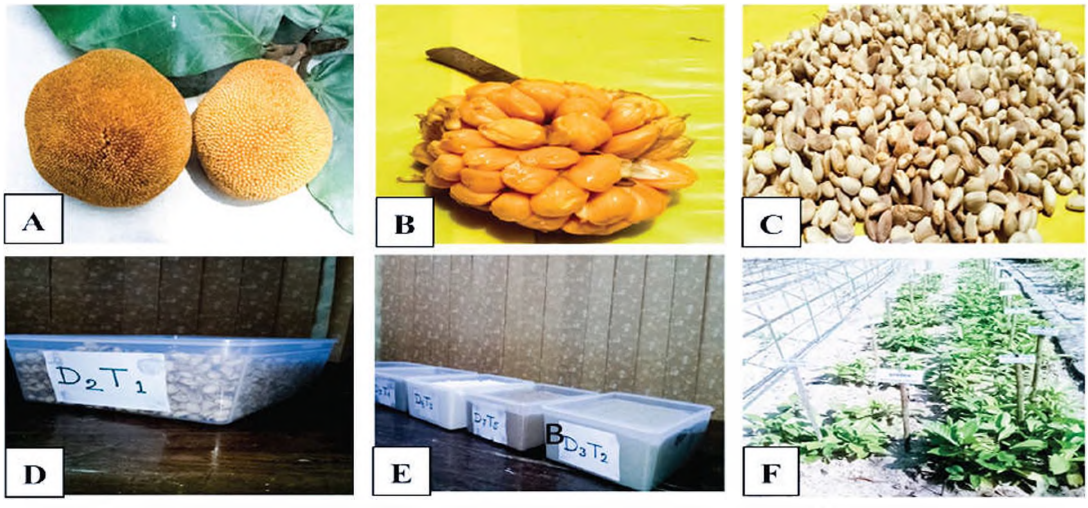

Figure 1. Seed germination of Chapalish. A) Fruit, B) Flesh, C) Seeds, D & E) Different storage media and F) Seedlings of Chapalish in the seed bed

Results

Effect of storage media and period (days) on seed germination percentage

Germination of all the storage conditions and storage durations started 8–10 days after sowing and was completed within 23 days with little variation among the treatments. Different storage conditions and storage periods were found to influence the germination percentage significantly. Fresh seed germination was found to be 85%. Among the storage conditions, the highest germination was observed in the D_2T_3 (83%) which was significantly superior. Treatment D_2T_4 (12%) recorded the lowest germination (Fig. 2). Keeping the seeds at room temperature condition (T_1) is a common practice for many crops. By keeping seeds under room temperature condition maximum germination (45%) was noticed after 10 days of storage and it declined to 21% at 20 days followed by no germination after that. Preserving seeds in sand (T_2) technique was found better in comparison with ash and control treatments. Chapalish seeds could be stored with a germination of 62% up to 30 days which reach to 25% after 70 days of storage. Seeds stored in chalk powder (T_3) showed the highest germination (83%) among the all storage media after 10 days of storage. It was also observed that the seeds of chalk powder medium showed 77%, 71%, 60%, 48%, 41% and 36% of germination for 20, 30, 40, 50, 60 and 70 days of storage respectively which were higher among all the storage media throughout the course of storage period. Keeping the seeds in ash (T_4) is also a conventional practice of seed preservation, but it was only 12% germination which was the lowest after 10 days of storage period and followed by no germination after that. Preserving the seeds in sawdust (T_5) can prolong the seed viability. This treatment showed 82% germination and was better in comparison to ash, control and sand storage treatments after 10 days of storage. Seeds may be stored up to 30 days with a germination of 64% and remained 28% after 70 days of storage.

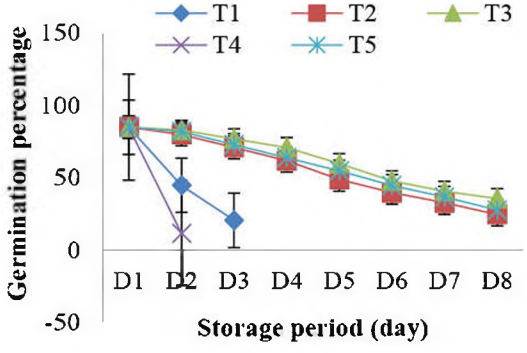

Figure 2. Effect of storage conditions and durations on germination percentage of Chapalish seeds.

A two-way ANOVA was conducted to compare the effect of seed storage conditions (treatments) and period (days) interventions on the germination percentage and viability of Chapalish seeds. The ANOVA showed that there was a significant effect of storage conditions and media on germination percentage, $F(4,120) = 4175.28$, $p < .001$. Another main effect of storage period was found statistically significant on germination percentage, $F(7, 120) = 2277.11$, $p < .001$. The ANOVA yielded a significant effect of the interaction of seed storage period and media on germination percentage, $F(28, 120) = 145.35$, $p < .001$ indicating that the effect of either intervention is correlated with each other (Table 1).

Table 1. Two-way ANOVA for seed germination percentage at different storage media in relation with storage periods

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Storage period	7	70666.300	10095.186	2277.110*	0.000
Storage media	4	74041.600	18510.400	4175.278*	0.000
Storage period x media	28	18043.200	644.400	145.353*	0.000
Error	120	532.000	4.433		
Total	159	163283.1	29254.42		

*Values are significant at $p<.05$ level.

The least Significant Difference (LSD) test was made for grading the calculated values 2.95 at 5% level of those combinations more precisely. Emphasizing the prolonging period of viability in the desired level, D_4T_2 and D_5T_3 were observed same. In the consideration of storage period, it can be said that Chapalish seed stored chalk powder (T_3) for 40 days was optimum and significant at 5% level.

Effect of storage media and period on seedlings growth performance

The shoot length of *A. chama* was significantly influenced by different storage conditions. At ambient (25 ± 2°C) condition (T_1) mean shoot length observed 7.7 cm which was higher than sawdust (7.6 cm), ash (5.4 cm) and sand (7.3 cm) for 10 days of storage. It was significantly lowest to 6.3 cm at 20 days of storage. In sand, it showed 7.3 cm of shoot length which was higher than ash (5.4 cm) at 10 days followed by 6.6, 6.4, 6.3, 6.2, 6.1 and 5.8 cm for 20, 30, 40, 50, 60 and 70 days of storage respectively. Seedlings of chalk powder (T_3) storage showed the highest shoot length (8 cm) at 10 days. It showed 7.5, 7.3, 7.0, 6.9, 6.7, and 6.2 cm for 20, 30, 40, 50, 60 and 70 days of storage respectively followed by sawdust (T_5), sand (T_2), control (T_1) and ash (T_4) respectively. Keeping the seeds in ash (T_4) exhibited the lowest shoot length 5.4 cm at 10 days of storage. For other storage conditions, no seedlings were seen at all. Sawdust (T_5) showed better performance for shoot length of the seedlings. It showed second highest shoot length 6.8, 6.5, 6.3, 6.3, 6.2 and 6 cm for 20, 30, 40, 50, 60, 70 and 80 days of storage respectively in comparison with sand, ash and ambient (25±2°C) storage conditions (Fig. 3).

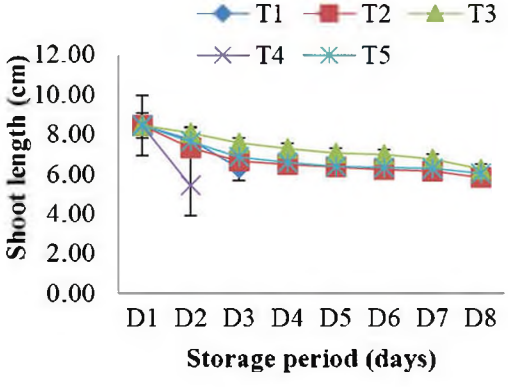

Figure 3. Effect of storage media and duration on shoot length.

A two-way ANOVA was conducted to compare the effects of storage media and storage period interventions on the initial shoot growth performance of the Chapalish seedlings. The ANOVA showed that there was a significant effect of storage media on growth of shoot length, F (4,120) = 19081.87, $p < .001$. The other main effect of storage period was found statistically significant on shoot length, F (7, 120) = 5936.36, $p < .001$. The ANOVA yielded a significant effect of the interaction between storage media and durations, F (28, 120) = 1018.87, $p < .001$ (Table 2).

Table 2. Two-way ANOVA for shoot length at different storage media in relation with storage period

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Storage period	7	463.610	66.230	5936.359*	0.000
Storage media	4	851.560	212.890	19081.875*	0.000
Storage period x media	28	318.280	11.367	1018.867*	0.000
Error	120	1.339	0.011		
Total	159	1634.79	290.4984		

*Values are significant at p<.05 level.

The LSD test was made for grading the calculated values 0.15 at 5% level of those combinations more exactly. Mean shoot length in earlier interval period of all storage media was found almost same and maximum. But emphasizing the prolonging period of viability in the desired level, D_5T_3 and D_5T_2 were observed statistically same. Both combinations are in chalk powder where first one (D_5T_3) was for 40 days with 60% germination and last one was for 50 days of storage with 40% germination. In the consideration of germination percentage, D_5T_3 was found optimum and significant at 5% level. The root length of *A. chama* seedlings was influenced by the different storage conditions and periods at initial stage. Significantly higher root length was observed 9.6, 9.6, 9.1, 8.4, 8.1, 8.0 and 7.5 cm in chalk powder condition (T_3) for 10, 20, 30, 40, 50, 60 and 70 days of storage respectively followed by sawdust, sand, control and ash (Fig. 4). The lowest root length was recorded in ash (7.2 cm) for 10 days of storage.

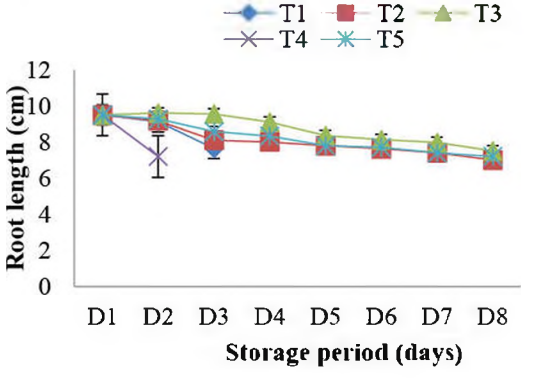

Figure 4. Effect of storage media and period on root length.

To compare the effect of seed storage conditions (treatments) and period (days) interventions on the root length of chapalish seedlings, a two-way ANOVA was conducted. The ANOVA showed that there was a significant effect of the storage conditions and media on the growth of root length, F (4,120) = 24311.67, $p<.001$. The other main effect of storage period was found statistically significant on root length, F (7, 120) = 6620. 63, $p<.001$. The ANOVA yielded a significant effect of the interaction between storage media and duration, F (28, 120) = 1307.84, $p<.001$ (Table 3).

Table 3. Two-way ANOVA for root length at different storage media in relation to storage period

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Storage period	7	598.616	85.517	6620.635*	0.000
Storage media	4	1256.103	314.026	24311.669*	0.000
Storage period x media	28	473.001	16.893	1307.837*	0.000
Error	120	1.550	0.013		
Total	159	2329.27	416.4481		

*Values are significant at p<.05 level.

The LSD test was made for grading the calculated values 0.16 at 5% level of those combinations more exactly. Mean root length in earlier interval period of all storage media was found almost same and maximum. But emphasizing the prolonging period of viability in the desired level, D_3T_5 and D_5T_3 were observed statistically same. Between the combinations first one (D_3T_5) was in sawdust for 20 days and last one (D_5T_3) was in chalk powder for 40 days of storage. So, in the consideration of storage period, D_5T_3 was found optimum and significant at 5% level. The vigor index of Chapalish was calculated to observe the influence of different storage conditions and periods for seedling growth in the seed bed. The average vigor index was 1521.4 for fresh seeds. Among the storage conditions, significantly higher vigor index was observed 1462.9, 1315.1, 1160.9, 922.4, 723.3, 602.7, and 494.3 for 10, 20, 30, 40, 50, 60 and 70 days in chalk powder followed by sawdust, sand, control and ash respectively (Fig. 5). The lowest vigor index was observed in ash 151.1 for 10 days of storage only.

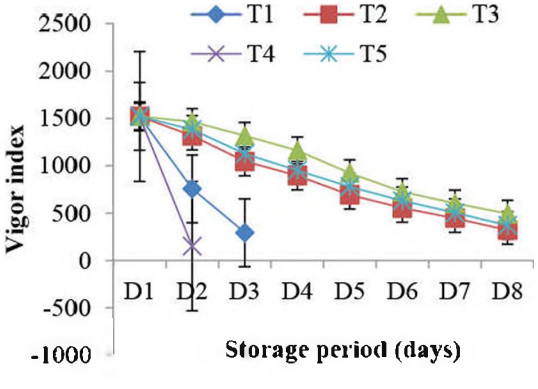

Figure 5. Effect of storage media and period on vigor index.

The effect of treatments and storage period interventions on the vigor index of Chapalish seedlings was comparing in a two-way ANOVA. The ANOVA showed that there was a significant effect of storage conditions on vigor index, F (4,120) = 3723.96, p<.001. Another main effect of storage period was observed s significant on vigor index, F (7, 120) = 3130.63, p <.001. The results also showed an interaction effect between storage conditions and duration, F (28, 120) = 150.60, p <.001, (Table 4).

Table 4. Two-way ANOVA for vigor index at different storage media in relation with storage period.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Storage period	7	25830864	3690123	3130.629*	0.000
Storage media	4	17557981	4389495	3723.962*	0.000
Storage period x media	28	4970339	177512.1	150.5978*	0.000
Error	120	141446	1178.716		
Total	159	48500630	8258309		

*Values are significant at p<.05 level.

The LSD test was made for grading the calculated values 48.07 at 5% level of those combinations more exactly. Emphasizing the prolonging period of viability in the desired level, D_4T_5 and D_5T_3 were observed statistically same. Between the combinations first one (D_4T_5) was in sawdust for 30 days and last one (D_5T_3) was in chalk powder for 40 days of storage. Therefore, the consideration of storage period, D_5T_3 was found optimum and significant at 5% level.

Discussion

Germination of Chapalish seeds for all the storage media and storage duration (days) started 8 – 10 days after sowing. Hoque *et al.* (2019) reported 6–12 days required for *Aquilaria malaccensis* and Alwis *et al.* (2016) observed 7 to 14 days for *Gyrinops walla*. Germination was completed within 23 days and was similar to *Hopea odorata* (Hoque *et al.* 2020a), *Aquilaria agallocha* (Beniwal 1989), *A. malaccensis* (Adelina *et al.* 2004) and *Tamarindus indica* (Haider *et al.* 2020). The highest germination was recorded 83% for Chapalish in chalk powder (10 days) where Begum *et al.* (2020) reported 82% in cocomoss growing media and Hossain (2015) reported 70–80% in the nursery. Gawankar *et al.* (2020) reported 93% for *A. heterophyllus*. The germination percentage of *A. chama* was found inversely correlated with the increase of storage duration (days). The results revealed that the viability of Chapalish seed is highly dependent on storage conditions and storage durations which were in accordance with Manjkhola *et al.* (2005) and Panwar and Srivastava (2015). Keeping the seeds at room temperature conditions is an obvious practice in any crop. The findings revealed the

deterioration in germination by keeping seeds under ambient (25 ± 2°C) conditions about 85% germination was noticed in fresh seeds and it declined to 21 percent after 21 days. Thereafter no germination was noticed at all. This result is similar to *Artocarpus heterophyllus* (Gawankar *et al.* 2020) and *Aquilaria malaccensis* (Hoque *et al.* 2019). Warrier (2009) explained the storage problem of *A. heterophyllus* seeds and suggested that ambient (25 ± 2°C) conditions were not conducive for the storage of this species for more than three weeks. Similarly, Panggabean (1979) observed 80 to 86% seed germination of *A. heterophyllus* seeds after 22 days of storage and none of the seeds germinated after 38 days of storage. Emphasizing the prolonging period of viability in the desired level, the germination of Chapalish seed stored in chalk powder for 40 days was found optimum and significant at $p<0.05$ level. Hoque *et al.* (2020b) found 60% germination for 24 days of storage of *Anisoptera scaphula* stored in sand media. The purpose of seed storage is to maintain the seed in good physical and physiological condition from harvesting through to planting by the farmer. For most crops, time passes between harvesting and planting; the seed has to be kept somewhere during this period, and storage is therefore necessary. Seed storage is the process of preserving viable seeds from the time harvested or collected to the time required for planting or sowing. Seed storage is important as it secures the supply of good quality seeds for sowing whenever needed as well as to maintain the seed in good physiological and morphological condition (Ellis *et al.* 1990; Hong and Ellis 1992). That is why; the prime objective of this investigation was to find out a method of storage for keeping the seeds viable for a longer time. In this context storing in chalk powder appear to be appropriate. Chapalish seeds could be stored in this media with a germination of 60% up to 40 days which is considered at desired level.

The highest mean shoot length (seedling height) of *Chapalish* seedlings after 30 days was 8.4 cm where Begum *et al.* (2020) reported 32.5 cm for the same species and 28.5 cm for Teli garjan (*Dipterocarpus turbinatus*) at six months. The highest mean root length of *A. chama* seedlings was recorded 9.6 cm for 10 days of storage in chalk powder media at the age of 30 days where Begum *et al.* (2020) reported 29.7cm. The height of root length was showed longer than shoot length over the experiment which was in accordance with Hossain (2015) and Troup (1921). The highest mean vigor index was observed 1462.85 for 10 days of storage in chalk powder media at the age of one month where Haider *et al.* (2020) reported 1629 in the treatment of seeds soaked in hot water to 3988 in the treatment of seeds soaked in cow urine for 24 hours in the case of *Tamarindus indica* L.

From above results, it is clear that preserving seeds in chalk powder is the reliable techniques for obtaining a successful germination over a period of even two months. It may be prevented the water loss to a great extent and could maintain 36% germination even at 70 days. It is also revealed that, the viability and seedling growth of *A. chama* is significantly correlated with storage media and period.

Conclusion

The findings of the study indicate that Chapalish seeds could be effectively stored in chalk powder which prolongs seed viability up to 40 days with 60% germination. It is not only considered at desired level but also promising. It may be concluded that the chalk powder is better as a storage media by which vigorous seedlings may be raised after few weeks which

may be useful to nursery owners. The storage condition is convenient, inexpensive and easily accessible to all types of nursery owners, foresters and private plant growers.

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