DEVELOPING ECO-FRIENDLY PRESERVATIVES AND TREATMENT TECHNIQUES FOR *Melocanna baccifera* (ROXB.) KURZ.

By

KANCHAN RAWAT

Department of Forestry

Submitted
in partial fulfillment of the requirement of the degree of Doctor of Philosophy in Department of Forestry of Mizoram University, Aizawl

DECLARATION

I, **Kanchan Rawat**, hereby declare that the subject matter of this thesis entitled "**Developing Eco-Friendly Preservatives and Treatment Techniques for *Melocanna baccifera* (Roxb.) Kurz.**" is the record of the work done by me, that the contents of this thesis did not form basis of the award of any previous degree or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University / Institute.

This thesis is being submitted to the Mizoram University for the degree of **Doctor of Philosophy** in the Department of Forestry.

(KANCHAN RAWAT)

HEAD

Prof. U.K.SAHOO
Supervisor

CERTIFICATE

This is to certify that that thesis entitled **"Developing Eco-Friendly Preservatives and Treatment Techniques for *Melocanna baccifera* (Roxb.) Kurz."** submitted by **Mrs. Kanchan Rawat (**Ph.D. Regn. No. **MZU/Ph.D./775 of 19.05.2015)**, in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Forestry of Mizoram University, Aizawl embodies the record of her original investigation under my supervision. She has duly registered and the thesis presented is worthy of being considered for the award of the Doctor of Philosophy (Ph. D) Degree. The work has not been submitted previously for any degree to this or any other university.

(Dr. Kalidas Upadhyaya)
Joint Supervisor

(Dr. U.K. Sahoo)
Supervisor

ACKNOWLEDGEMENTS

At the very outset, I would like to express my deep sense of gratitude to my Supervisor Prof. U.K.Sahoo, Department of Forestry, Mizoram University for his constant encouragement, valuable advice and contribution of precious time during due course of the study. My sincere thanks are also due to my joint supervisor Dr. Kalidas Upadhyaya, for his continuous guidance.

I am thankful to Prof. B. Gopichand, Head, Department of Forestry, Mizoram University for all the cooperation. I offer my sincere thanks to Prof. S.K. Tripathi, Prof. Lalnundanga, Dr. Kewat Sanjay Kumar, Dr. Nagaraj Hegde, Dr. K.K.Upadhyay and Dr. Suresh Kumar, Department of Forestry who rendered all possible help and support during my work. I extend my deep sense of gratitude to the faculties of FRCBR, Aizawl for helping me during sample collection.

I wish to accord my thanks to Dr. Awadhesh Kumar, Department of Horticulture, Aromatic and Medicinal Plants, Prof. Senthil Kumar, Department of Biotechnology, Smt. Sagarika Das, CIL, MZU for their guidance and support.

My sincere thanks are due to all the research scholars of Department of Forestry, Mizoram University, for their time to time cooperation.

I sincerely thank all the non-teaching staff of Department of Forestry for their help and support.

At last my special thanks go to my family members and my little son Shivansh for endless support during the entire course of this journey.

Place: Aizawl.

Date: (**Kanchan Rawat**)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

NBM	*National Bamboo Mission*
CCA	*Copper Chrome Arsenate*
IPBC	*3-ido-2-propanyl butyl carbonate*
RFRI	*Rain Forest Research Institute*
CNSL	*Cashew Nut Shell Liquid*
ELO	*Epoxidized Linseed Oil*
RSO	*Rape Seed Oil*
EMC	*Equilibrium Moisture Content*
CIP	*Combined Impregnation Process*
CCB	*Copper Chrome Boron*
ARCBR	*Advanced Research Centre for Bamboo and Rattan*
MTCC	*Microbial Type Culture Collection*
PDA	*Potato Dextrose Agar*
ASTM	*American Society for Testing and Materials*
DMSO	*Dimethyl Sulfoxide*
BOD	*Biological Oxygen Demand*
MC	*Moisture Content*
BIS	*Bureau of Indian Standards*
IS	*Indian Standard*
ANOVA	*Analysis of Variance*
CRD	*Completely Randomized Design*

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 Bamboo: The poor man's timber

Bamboo is a well-known fast renewing raw material suitable for many structural and product purposes (Kumar *et al*. 1994). Bamboo shows vigorous growth compared to all other forms of terrestrial vegetation. Use of bamboo for construction and house hold materials like basket, mat, pipes, furniture, musical instruments, pulp and paper, textile, tools, handles etc. is well documented as well as traditionally known (Misdarti, 2006; Krisdianto 2008). It has considerable strength properties even if it has many structural variations like hollowness, nodes, septa, tapering wall from base upwards etc. (Gnanaharan, 2000).

North eastern states, Mizoram in particular are rich in bamboo resources both in abundance as well as in diversity. According to the Department of Environment, Forest and Climate change, Government of Mizoram, bamboo covers 57 % of the geographical area of the state and 27 species of bamboo have been so far reported of the 130 reported in the country (Anon., 2017). Moreover, Mizoram has been declared a timber deficit state because of scarcity of quality trees with considerable growth (The Telegraph, 2019). In Mizoram, National Bamboo Mission (NBM) is run as state bamboo mission through various nodal agencies. The objective is to utilize bamboo for locally useful products, promote local industries, employment generation and so on. Although, plantation and marketing related works are undertaken under the mission, there are many things to be done in product manufacture, industry and over all utilization. Utilization of bamboo at the right time is very important because it faces the risks such as forest fires, insect and pest attack, extreme weather strokes, gregarious flowering and so on. Therefore, as a

preliminary step, properties of bamboo need to be studied to ensure which species is suitable for what specific use.

Bamboo is believed to be useful for each and every purpose from cradle to coffin. It is also relatively cheaper than its counterparts and has been often referred to as poor man's timber across the civilizations. Many people believe bamboos to be trees on the basis of their physical appearance. In fact Bamboos are the giant perennial woody grasses, which belong to the family Poaceae and sub-family Bambusoideae. The bamboo constitutes of a cylinder shaped shell fractioned by transversal diaphragms situated at the nodal position. Bamboo is a type of grass which is commonly grown in Asia having a hard, woody and hollow stem. It is a fast growing grass even in dense conditions and it matures early (Zhang *et al*., 2007). Bamboo culms can be used in their natural or whole form or cut into sections or in strips, slivers and slats. Bamboo culms have been used by people and communities for thousands of years to build houses, fences and bridges. They can be used to make a vast range of utility items, including storage baskets, containers, furniture, agricultural implements and household baskets. There is also a growing demand to meet the needs of new and value added products and application, such as wood substitutes, panels, flooring, roofing and screens.

1.2 Bamboo Resources of north east India

There are about 70 genera and 1200 species (Gyansah and Kwofie, 2011) of bamboo worldwide. India is second only to China in bamboo production. The North-Eastern region harbors more than one third of the Indian bamboo genetic resources having dense bamboo forests. Out of 136 bamboo plant species available in India, 89 taxa are in this region.

The forest area over which bamboos occurs in India is 9.57 million hectares, which constitutes about 12.8% of the total area under forests (Bahadur and Verma, 1980). Naithani (1993) reported 136 indigenous and exotic species under 23 genera, to be found naturally and/ or under cultivation in India, the distribution however is not uniform. The rich areas are confined to the North–Eastern part of the country, Siwalik Hills of Uttar Pradesh, Bastar, region of Madhya Pradesh, Western Ghats in south India and the Andaman Islands. The annual production of bamboo in India is about 4.6 million. Bamboo forests cover a large extent of area in Mizoram. In Mizoram the total bamboo cover is about 7091.66 sq km, which is about 33% of the total geographical land of the state. Bamboo is distributed thoroughly between 400 m and 1520 m above mean sea level. Bamboo is the best renewable natural resource with more than 1500 uses. Its strength is comparable to timber, diversity and abundance in tropical and subtropical parts of world has made it a potential replacement for steel and timber (Scurlock *et al*. 2000). But the major drawback in using bamboo is its low natural durability.

1.4 Chemical constitution of bamboo

The chemical composition of bamboo is similar to that of wood. The main constituents of bamboo culms are cellulose, hemi-cellulose and lignin, which amount to over 90% of the total mass (Kumar *et al*. 1994; Wei *et al*. 2013). The minor constituents of bamboo are resins, tannins, waxes and inorganic salts. Compared to wood, however, bamboo has higher alkaline extractives, ash and silica contents (Tomalang *et al*. 1980; Chen *et al*. 1985). The composition varies according to species, the conditions of growth, the age of the bamboo and the part of the culm. Because the bamboo culm tissue matures within a year when the soft and fragile

sprout becomes hard and strong, the proportion of lignin and carbohydrates is changed during this period. However, after the full maturation of the culm, the chemical composition tends to remain rather constant (Leise, 1985).

The nodes contain less water-soluble extractives, pentosans, ash, and lignin but contain more cellulose than the internodes. The season influences the amount of water-soluble materials, which are higher in the dry season than in the rainy season. The starch content reaches its maximum in the driest months before the rainy season and sprouting. The ash content (1 - 5%) is higher in the inner part than in the outer one. The silica content varies on an average from 0.5 to 4% increasing from bottom to top. Most of the silica is deposited in the epidermis, whereas the nodes contain little silica and the tissues of the internodes almost none. Silica content affects the pulping properties of bamboo (Tomalang *et al*. 1980).

1.5 Bamboo degrading fungi

Fungi are the most prominent bamboo decaying agents. Fungal attack can be seen at three stages of bamboo life cycle i.e. during planting, storing and service. A large proportion of culms can be attributed mainly to fungi which include soft rot, white rot and brown rot. Fungi attack only that bamboo tissue with sufficient moisture content, at least above fibre saturation point (20-22%), air dried bamboo is protected against fungal degradation. Among the different parts, the inner part of the culm is attacked faster than the outer one, which is attributed to the higher content of the nutritious parenchyma in the inner part. The starch content of the parenchyma cells influence to a larger extent for the susceptibility to attack by fungi, especially the blue stain fungi. Among different fungi experimental evidences show that white

rot and soft rot cause more deteriorations in bamboo than by brown rot (Liese 1998). As many as 1100 species of fungi have been recorded from bamboo, comprising 630 Ascomycetes, 150 Basidiomycetes and 330 mitosporic taxa (Hyde *et al* 2002), most of which are reported from asia, with relatively fewer known from India and South Africa. *Fusarium moniliformae* is the fungal pathogen associated with the rot of emerging culms. F. equiseta and *F. moniliforme* are the fungi associated with the rot of growing culms (Mohanan, 1997). As many as 42 fungi belonging to 23 genera have been recorded on seeds of bamboo from India (Mohanan, 1997).

These fungi originate from very fine; air-borne spores present in fruiting bodies, and occur everywhere. They nourish on the substrate in the bamboo culm. Damage becomes noticeable at an advanced stage, when substantial fungal growth is already underway. Fruit bodies appear subsequently on the outer surface and their removal does not stop the decay process inside the culm wall. Depending upon the moisture conditions, three types of fungi occur: surface moulds, stain fungi and decay fungi. Surface moulds grow on the surface and at the cross-ends of green culms, in storage stacks of green and split bamboo where the inner part is exposed and on finished products (Liese and Kumar, 2003). They require a humid and stagnant atmosphere for growth. Stain fungi can penetrate round bamboos from cross cut ends as well as from cuts in the nodes after removal of the branches. They nourish on the starch and carbohydrates. Stain fungi affect mostly split bamboo and slivers. They require high moisture for survival and growth Attack is indicated by shades of blue/grayish-black discoloration on the surface in the form of spots and streaks. It reduces the aesthetic appearance but does not affect the strength properties of bamboo except in severe cases of attack. Fruit bodies are produced on the culm

surface, whereby the hyphae may rupture its hard skin by forming blisters beneath the epidermis to release spore masses (Wahab *et al*., 2005). Decay fungi cause the most serious kind of damage and grow within the lumen of the cells. The enzymes either decompose only the cellulose and hemicellulose leaving behind the lignin leading to brown rot, or they decompose lignin leading to white rot. White rot is more common in bamboo than brown rot. Early decay is difficult to detect. Even before slight colour change or weight loss becomes apparent, the strength properties are much reduced, in particular the impact-bending strength. Early damage can be characterized by dampness in bamboo. At later stages of deterioration the culm is soft to the touch and may be only a fibrous or powdery mass. Advanced brown rot leaves a brownish mass, while white rot leaves white streaks on the culm's surface.

The presence of starch makes bamboo attractive to blue stain fungi as well as borers. Decay fungi need oxygen for respiration and hence limiting the supply of oxygen retards fungal growth. Some fungi, however, can exist even at low oxygen levels associated with high moisture levels (Wahab *et al*., 2005).

Fungi thrive in humid environments. A moisture level of 40 to 80% is ideal for rapid growth. Dry bamboo with moisture below 20% does not promote fungal growth. Temperature ranging from 25°C to 35°C is ideal for fungal growth. Factors like cracks/splits; weathering and fire are abiotic agents which cause degradation in bamboo (Liese and Kumar, 2003). The cracks and splits occur due to stresses caused by sudden drying and direct exposure to sun. Cracks do not really weaken the culm but create points of entry for decay organisms. Splitting of bamboo arises due to nailing without pre-boring, especially in thin-walled bamboo. End splitting can be prevented by coating ends with wax emulsion or coal tar. Weathering of exposed

bamboo occurs due to the interaction of different atmospheric conditions, such as fluctuations of temperature and relative humidity (Kumar *et al.*, 1994). Repeated drying and wetting of exposed bamboo results in widening of minute cracks produced on the surface. Solar radiations cause degradation of cellulose. The wind and dust particles have sand blasting effect on the culm surface giving a weathered look (Derbyshire and Miller, 1981).

1.6 Natural durability of bamboo and need for preservation

Without any protective treatment, most bamboo species have an average natural durability of less than 2 years. Stored under cover, untreated bamboo may last for 4-7 years. These variations in bamboo durability strongly depend on the species, the length of the culm, the thickness of the wall and on the time of harvesting.

The lower portion of the bamboo culm is considered more durable, while the soft inner part of the wall deteriorates faster than the outer harder portion. This is related to the anatomical and chemical nature of the woody cells. Although some of the characteristics of bamboo resemble those of wood, its growth characteristics and microstructure is different (Kumar *et al*., 1994). Unlike timber species like teak, the structure of bamboo is void of toxic deposits. The large amounts of starch present in bamboo make it highly attractive to mould and fungi, termites and powder-post beetles. They cause much damage during drying, storage, and subsequent use. Tests have also shown that bamboo is more prone to soft rot and white rot attack than to brown rot. Bamboo consists of 50-70% hemicellulose, 30% pentosans, and 20-25% lignin (Chen *et al*. 1985). The lignin present in bamboos is unique, and undergoes changes during the growth of the culm. Bamboo is also known to be rich in

silica (0.5 to 4%), but the entire silica is located in the outer layer (1 mm), with hardly any silica in the rest of the wall. Bamboos also have minor amounts of waxes, resins and tannins, but none of these have enough toxicity to improve its natural durability.

Many developing counties (where most bamboos grow) suffer a lack of awareness and professional treatment facilities for bamboo preservation. Furthermore, not all curing methods ensure satisfying results which leads to uncertainties about the advantages of using bamboo all together (Satish and Dobriyal, 1990). A lot of bamboos used for structural purposes in rural housing are untreated (or the wrong species) and deteriorate in just a couple of years, hence the reason bamboo is still considered as a poor man's timber. Not only does the incorrect use affect the reputation of bamboo, it also puts heavy pressure on the resource, since frequent replacement is necessary. Chemical preservatives should be used to protect bamboo products from such degradation (Wahab *et al*., 2005). These are well established methods providing good protection even in adverse conditions. The selection of the appropriate treatment method depends on various factors like state of the bamboo (green or dry), form of the bamboo, round bamboo or splits, end applications, in ground contact, exposure to atmosphere, undercover, structural/non-structural, potential causes of decay such as biotic (fungus/insects) and abiotic (cracks/weathering) (Satish and Dobriyal, 1990). It is important to promote the correct use of bamboo in order to increase the durability, utilization, and popularity of this versatile and environment friendly material. Increasing the shelf-life of bamboo to 50 years or more is certainly possible by applying the appropriate treatments which is also more economical and sustainable in the long run.

The conventional wood preservatives although are found to be very effective against wood destroying organisms, are said to cause environmental pollution and a few of them are hazardous to animals and human beings (Fisher, 1968; Thompson, 1971; Onuorah, 2000). The most common chemicals used by the industries and local artisans are copper chrome arsenate (CCA), Sodium penta chloro phenol, boric acid – borax, IPBC (3-ido-2-propanyl butyl carbonate) and synthetic pyrethroides, most of which have harmful effect on the environment. Over the past few decades, there has been substantial global awareness to develop eco-friendly wood preservatives and those, which do not cause any ill effect on the health of mammals (Onuorah, 2000). There is a continuous search for different methods of bio-control methods for wood preservation and to develop an ideal preservative. An ideal preservative, according to Liese and Kumar (2003) has characteristics such as (i) toxicity to the target organism (wood and bamboo destroying) and minimum toxicity to the non target organism, (ii) permanent fixation inside the bamboo culm, (iii) high penetration inside the bamboo tissues, (iv) easy disposal of treated product, and (v) strength of the treated culm not affected by preservative impregnation. Various plant extracts and oil based formulations such as organic acids, essential oils, eco friendly chemical based presertvatives are continuously being developed, and whose bio efficacy are measured in terms of the improvement in resistance to fungi and based on field performance (Kaur *et al*. 2016). Green plants act as a reservoir for inexhaustible source of innocuous fungicides/pesticides, which are mammalian non-toxic and easily biodegradable than synthetic chemicals. To develop eco-friendly wood preservatives, many studies have been conducted. Most of the reported work is on extractives from heartwood (Onuorah, 2000; Soni, 1975; Gupta and Indra Dev,

1999). However, very few reports are available in which other components of tree such as leaves of *Ipomea carnea* (Adsul *et al*., 2012) and Neem (Swathi *et al*., 2004) possess a number of toxic constituents exhibiting high toxicity against wood-destroying microbes. Efforts have been made by many workers to use these plant products with the amendment of toxic metals and tested for durability against termites or fungi (Jain and Virendra, 1991; Purushotham and Tewari, 1961; Indra Dev and Nautiyal, 2004). With this background, the current study was undertaken involving the following objectives:

1. To standardize the minimum inhibitory concentration of the Neem oil and copper sulphate-boric acid (1:1) formulation against white rot, brown rot and sap stain fungus.

2. To determine the extent of retention and penetration of Neem oil and copper sulphate-boric acid formulation in *Melocanna baccifera* (Roxb.) Kurz treated by two-step process.

3. To estimate the leachability of preservative in treated samples.

4. To evaluate the resistance of treated culms of *M. baccifera* bamboo species against white rot, brown rot and sap stain fungus.

CHAPTER 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Bamboo is very popular in the tropical world for its utility and versatility. It is an interesting raw material with attractive features, suitability for various uses but with limited durability. Therefore, most of the researches are targeting the preservation of bamboo material to enhance its durability especially in Asian countries where bamboo is available in plenty and is part and parcel of rural life. The importance of bamboo in rural economy has been reported by various scholars, from various places. (Liese, 1985; Yudodibroto, 1987; Jha and Lalnunmawia, 2004; Nath *et al.,* 2008). A bird's eye review of literature reveals that although a large amount of work has been carried out on use of different preservatives in enhancing the durability of some woods in different countries around the globe, however, the studies pertaining to the same are few and far between in Indian sub-continent. Moreover, very limited work has been done to enhance the durability of bamboo, the 'green gold' and most versatile plant use. This study therefore is expected to contribute our knowledge to the degree to which the eco-friendly preservatives could enhance durability in bamboo and its parts most influenced by the preservatives and therefore may have some management implications. Various works reported across the globe are classified under the following heads:

2.1 Fungi on Bamboo

2.2 Fungal degradation of bamboo

2.3 Treatability, Preservation techniques and chemicals for bamboo and wood

2.4 Eco friendly preservation techniques

2.5 Northeast India and research gaps

2.1 Fungi on bamboo

Bamboo like any other lingo-cellulosic material is liable to be attacked by fungi. Several instances of fungi associated with bamboo are reported by various workers across the world. Schmidt *et al.* (2013) in an attempt to review the incidences of fungi on bamboo from various parts of the world, reported about 67 species of fungi found on bamboo belonging to Deuteromycetes /Ascomycetes from China, Germany, Philippines, Thailand and Vietnam. Kumar *et al*. (2013) collected litter from bambusetum forest of Rain Forest Research Institute (RFRI), Jorhat for isolation of bamboo associated fungal strains. Moist chamber incubation of the litter revealed 45 fungal taxa belonging to 22 genera were found on grade I litter and 39 fungal taxa found on grade II litter. They found that 24 fungal taxa were common to both grades, Differences were observed in percentage occurrence of fugal species between the two grades of litter. Gogoi *et al*. (2013) found the incidences of blight and rot diseases posing a potential threat to the plantations. They studied the symptomatology, isolation of the causal organism and per cent disease incidence of blight and rot in *B. tulda* plantations in Dimapur district of Nagaland state. Bamboo groves under fifteen villages of Dimapur districts of Nagaland were selected for study and occurrence and symptoms

of the disease were recorded during the year 2009-2010. Four fungal pathogens had been isolated from the diseased samples where same *Fusarium semitectum* Roxb. Was found to be responsible for both blight and rot diseases. The pathogenecity test also confirmed *Fusarium semitectum* is responsible for blight and rot diseases on *Bambusa tulda* in study area.

Mohanan, C. (1994) observed the rot of emerging culms of bamboo caused by *Fusarium moniliforme* var. *intermedium* and rots of growing bamboo culms caused by *F. equiseti* occurred both in plantations and natural stands of Kerala. Both the diseases affected the culm production in plantations and natural stands. Die-back of branches caused by *Fusarium pallidoroseum,* thread blight caused by *Botryobasidium salmonicolor* affecting foliage, culms and branches, foliage blight caused by *Bipolaris maydis* and *Bipolaris* sp. and *Dasturella divina,* causing leaf rust, were recorded in bamboo plantations as well as natural stands. They also reported basal culm decay and withering caused by *Ganoderma lucidum* and *Amylosporus campbelli,* and culm staining and die-back caused by *Apiospra* sp. occurred in old clumps in natural stands and plantations.

2.2 Fungal degradation on bamboo

Schmidt *et al.* (2013) carried out experiment to determine extent of degradation of *Bambusa maculata* and *Gigantochloa atroviolacea* and *Phyllostachys pubescens* caused by white rot *Schizophyllum commune* and brown rot *Coneophora puteana*. Results revealed that all the bamboo species in the test were considerably degraded by the both

the test fungi. They also found out that the samples from bottom portion of tested bamboo culms were more resistant to decay than top portion. Experiments by Leithoff and Peek (2001) on *P. pubescens* and *P. virideglaucescens* in Petri dishes also gave comparable results with moderate decay by *S. commune* and *C. puteana* and stronger decay by soft-rot according to the standard ENV 807. Zhang *et al*. (2007) investigated 34 white-rot fungi and observed up to 15% in *P. pubescens; Pleurotus ostreatus* caused 5.2% and *Trametes versicolor* 13.6%. The white-rot fungus *Lentinula edodes* produced a 13% ML in *P. pubescens* (Kim *et al*. 2011). Suprapti (2010) observed intense decay by the white-rot fungus *Pycnoporus sanguineus* and the brown-rot species *Tyromyces palustris*. Kim *et al*. (2011) observed up to 22% by six white rot fungi in samples of three *Phyllostachys* species and a maximum of 18% by 12 soft-rot fungi. Arantes *et al*., (2012) and Schmidt (2006) stated that the wood-destroying fungi, such as brown-rot (*Serpula lacrymans, Coniophora puteana, Antrodia vaillantii, Gloeophyllum trabeum, Lentinus lepideus*, etc.) and white-rot (*Trametes versicolor,Trametes hirsuta, Schizophyllum commune*, etc.) basidiomycetes, or soft-rot (*Chaetomium globosum, Monodictys putredinis*, etc.ascomycetes, destroy polysaccharides (cellulose and hemicelluloses) and lignin which are present in the cell walls. They also stated that staining fungi like *Ceratocystis pilifera, Aureobasidium pullulans, Alternaria alternata*, etc. cause color changes in inner parts of wood by releasing pigments. Some of these strains are also known to be able to cause soft rot in hardwoods under optimal conditions (e.g. *Phialophora* sp.). Some fungi (white rot) may completely degrade the wood, producing weight loss approaching 96-97% (Haque, 1997). Tanaka *et al.*, (1999)

stated that *Trametes versicolor* is a basidiomycetes white-rot fungus that produces three ligninolytic enzymes and it has an efficient degradation capacity of lignin, polycyclic aromatic hydrocarbons, a polychlorinated biphenyl mixture and a number of synthetic dyes. According to findings reported by Green *et al.,* (2003) brown-rot fungi represent one of the most economically important groups of wood decay microorganisms. Some accounts estimate that as high as 80% of all in-service wood decay is caused by brown-rot fungi shortly after colonizing wood. Gilbertson (1981) verified that the brown-rot fungi made up about 6 percent of the total species of wood rotting basidiomycetes in North America. Chee *et al.,*(1998) in their study, done on the decay potential of thirty eight basidiomycetes fungi samples collected from *Pinus radiata* in New Zealand, reported that among all the samples of fungi twenty isolates caused significant wood weight losses relative to uninoculated controls and thirteen brown rot fungi produced the greatest weight losses (7.7-27.1%). Their study demonstrated that the range of weight loss caused in wood samples by the test fungi was from 0.1-27.1%, relative to uninoculated controls. According to results obtained by them twenty isolates caused significant weight losses of 6.2% or greater ($P<0.05$) and the highest weight losses (7.7-27.1%) was caused by the thirteen isolates of brown-rot fungi including cultures of *Gloeophyllum sepiarium*, *Antrodia serialis*, *Oligoporus leucomallellus*, *Poria sp*., *Coniophora olivacea* and other unidentified isolates. They depicted that the mean wood weight loss by the brown rot fungi (13%) was significantly higher than the white rot fungi (3.8%) but the variation was much greater for the brown rot fungi. Lekounougou *et al*., (2009) tested the initial stages of wood colonization and degradation by growing

the white-rot *Trametes versicolor* on *Fagus sylvatica* wood chips under solid-state fermentation in the presence of malt agar. They suggested that the initial stages of wood colonization on malt agar by *Trametes versicolor* correlated with wood extractives degradation requiring laccase activity, whereas the other wood-degrading systems (peroxidases and polysaccharides hydrolases) were still repressed. Anagnost in 1997 demonstrated the difference between the decay resistance of heartwood and sapwood of red maple against the brown-rot fungus *Oligoporus placentus*, white-rot fungus *Trametes versicolor* and soft-rot fungus *Chaetomium globosum*. They set forth that the heartwood of maple tree showed a slight resistance (lower weight losses) relative to its sapwood and expounded that the action of the white-rot fungus was enhanced by high moisture content, while that of the brown-rot fungus was enhanced by lower moisture content. According to them the test blocks exposed to the white-rot fungus decayed to higher weight losses when supported by sapwood feeder strips than when supported by plastic mesh while in test blocks exposed to the brown-rot fungus, blocks supported on plastic mesh decayed to higher weight losses than those on feeder strips, most likely because of the lower moisture content of the blocks on plastic mesh. Hassan and Abdulkhader (2009) conducted experiment to test the staining ability of *Alternaria sp.*, *Fusarium sp*. and *Paecilomyces sp*. They recorded high degree of rotting and staining for most of the wood species particularly on plywood and maple wood caused by *Alternaria sp.* and *Fusarium sp*. respectively, *Aspergillus sp., Rhizopus sp.* and *stemphylium sp.* were also associated with above fungi, and exhibited a typical wood staining by their spore pigments and showed significant soft rot in vitro. Daniel and

Nilsson (1997) listed *Alternaria, Biporomyces (chloridium), Diplodia and paecilomyces* among soft rot pathogens that degrade the cell wall components of cellulose, hemi cellulose and lignins. Olfat *et al.* (2007) quantified the progressive stages of decay caused by basidiomycetes fungi (*Corolus versicolor*) in five commercial woods viz. *Abies alba, Populus alba, Fagus orientalis, Platanus orientalis* and *Ulmus glabra* on the basis of mass losses to be 22.0%, 39.26%, 42.20%, 37.67% and 16.80% respectively as obtained after the 16 weeks of culture. Hassan and Abdulkhader (2008) conducted experiment to evaluate wood damaging capacity of soft rot and wood staining fungi and efficiency of their preservatives. They proved that the *Alternaria sp.* was the most effective fungus on plywood among *Stemphylium sp*. and *Pencillium sp*. while *Fusarium sp.* exhibited similar pathogenicity on the sycamore wood.

2.3 Treatability, Preservation techniques and chemicals for bamboo and wood

2.3.1 Treatability of bamboo

Vascular bundles play a vital role in treatment of chemicals in bamboo. Since the alignment of vessels is end to end, axial flow is easy and rapid in bamboo culms (Kumar *et al.* 1994). Distribution of vascular bundles is not uniform throughout the cross section of bamboo culms. Smaller, numerous bundles are found on the outer side where as few larger vessels are found on the inner culm portion (Kumar and Dobriyal, 2007). Parenchyma ground tissue in the bamboo culm accounts for half of the tissue by volume which stores the nutrients (Liese, 1987). Vessels occupy only 10 % of the total tissues, which can be easily treated with the chemicals where as parenchyma tissues

need to be penetrated with the preservative chemical which takes longer duration. If parenchyma tissues containing nutrients are not penetrated, these are attacked readily by the fungi (Liese, 1959).

Moisture contained in the bamboo culms influence to a great extent, the treatability of bamboo. For diffusion method of treatment and Boucherie treatment, moisture should be present in the bamboo, and hence ideally 3-4 years matured bamboo show better treatability to water soluble preservative chemicals.

2.3.2 Traditional methods of bamboo protection

Soluble sugars present in the bamboo are major substrate for the degrading agents like fungi and insects. Therefore, one of the techniques was to reduce the content of starch to make bamboo fairly resistant to attack (Kumar *et al*., 1994). Joseph (1958) discussed that in Indian conditions, starch content in bamboo during spring season is higher than winter season, and hence, it was advisable to harvest the bamboo during winter season. He also proposed harvesting of bamboo at right maturity (3-4 years) and post harvest transpiration of bamboo culm by keeping the culms upright or leaning to some duration. Another popular traditional method followed in South East Asian countries is soaking of bamboo in water for 4-12 weeks for leaching of sugars (Sulthoni, 1995). Baking over fire after smearing oil over the culm, lime washing and coatings are other traditional methods of preservation of bamboo (Kumar *et al*. 1994).

2.3.3 Chemical preservation of bamboo

Rose (1969) propagated the fact that copper possesses antimicrobial properties that can inhibit water-borne microorganisms, such as bacteria, viruses, algae and infectious parasites in the drinking water supply. Copper possess an anti-inflammatory and anti-ulcer activity. Copper is a cost effective, broad spectrum and suitable for direct aquatic applications with no toxicity concerns to humans. Therefore the use of coppers on agricultural crops as a fungicide and bactericide became significant to growers. Copper products are used extensively for the management of nuisance algae, aquatic weeds, mollusks, leeches. Copper compounds are used on a variety of agricultural, commercial, and residential use sites as fungicides, bactericides, algaecides, herbicides, wood preservatives, and anti-fouling agents. Copper is also among the few pesticides that are permitted for use on crops with organic certifications. Currently, 16 copper active ingredients (ai) have active food use registrations subject to tolerance reassessment and re-registration review (Sorenson, 1976). Environmental Protection Agency gave numerous benefits to support the significance and continued agricultural uses of copper pesticides. Zeelie (1998) carried out an experiment to investigate the effects of copper and zinc ions on the rate of killing of Gram-negative bacterium *Pseudomonas aeruginosa*, Gram-positive bacterium *Staphylococcus aureus* and fungal yeast *Candida albicans* by antiseptic agents' cetylpyridinium chloride and povidone-iodine (Betadine). They reported that in the 48 test cases copper and zinc ions clearly potentiated the antiseptic agents in 28 (58.3%) cases and exhibited an improved activity in 15 (31.3%) cases. In five (10.4%) cases there was no change in the antiseptics'

antimicrobial activity. They observed an excellent improvement in the killing activity of the antimicrobial agents for copper ions.

In wood preservation copper based preservatives have been successfully used for more than two centuries. Arsenic was mixed with copper to improve its efficacy but arsenic has been banned due to its carcinogenic nature. Chromium was combined with copper and arsenic in order to improve the fixation of copper and arsenic on wood but due to the introduction of biocidal products directive the chromium is likely to be banned. So there seems a major requirement of an alternative to chromium for maintaining the efficacy of copper based wood preservatives and fixation on wood (Humar *et al*., 2006; Green and Clausen, 2005).

Nair (2006) studied the leachability of copper-boron formulations from Alaskan spruce and Hawaiian *Albizia*. Desalegn and Abegaz (2012) conducted a comparative study on various preservation treatments done on indigenous bamboo species of Ethiopia and treated with five preservatives *viz*., borax/boric acid, AC 450, used motor oil, kerosene and crude table salt using four application techniques: pressure and non pressure methods soaking, sap displacement, hot and cold method and dipping. The treated samples were tested against fungi *Trametes versicolor* and *Wolfiporia cocos* and subterranean termites. All preservatives gave protection to different extents.

2.4 Eco friendly preservation techniques

Kaur *et al*. (2016) performed an environment friendly treatment of less durable *D. Strictus*, bamboo species. Various locally available plant extracts and oil cakes

(neem oil, cedar oil, extracts of Jatropha leaves, lantana leaves and Jatropha cake) were investigated using dip as well as pressure treatment methods. Retention of preservatives and antifungal durability of treated product were compared statistically. Retention level of Jatropha cake was found to be the maximum. Leaching test indicated that bamboo treated with neem oil has higher retention levels than other solutions. All the extracts gave protection to bamboo species better than control. Jatropha cake, Jatropha leaves and lantana leaves are found to be effective in protecting bamboo against test fungi. Cedar oil and neem oil are also able to enhance the durability of treated product. Pressure treatment enhanced the effectiveness of these plant extracts by 3-5 times as compared to dip method. Neem oil and kerosene (1:3) in combination with copper naphathanate (0.3%) on pressure impregnation in bamboo provided the best protection (Weight loss 3.5%), as compared to control blocks (WL 65%). Venmalar and Nagaveni (2005) highlighted the evaluation of copper cashew nut shell liquid and neem oil as rubber wood preservatives. Copper was incorporated into cashew nut shell liquid (CNSL) and neem seed oil. The combination of copper& CNSL and copper & neem in pressure treatment have resulted in discernibly high protection against wood rots and termites. Siddiqui *et al*. (2016) evaluate the antifungal ability of Nerium oleander leaves, roots and stem extracts against *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Fusarium oxysporum*. Singh and Majumdar (1996) investigated on the evaluation of anti inflammatory activity of fatty acids of *Ocimum sanctum* fixed oil .This study suggested that linolenic acid present in *O. sanctum* fixed oil can block both cyclooxygenase and lipoxygenase pathways of arachidonate metabolism and could be

responsible for the inflammatory activity of the oil. Mohana and Raveesha (2007) took the aqueous extract of eight plants for antifungal activity against *Fusarium solani* and *Aspergillus flavus* at 10% concentration by three methods namely dry mycelial weight, spore germination and poisoned food techniques. The results revealed that the extracts of *Decalepis hamiltonii* Wight and Arn. (Asclepiadaceae) showed significant antifungal activity. The antifungal activity of aqueous extract of *D. hamiltonii* an edible plant, was further evaluated at different concentrations by poisoned food technique against eight species of *Fusarium*, ten species of *Aspergillus*, three species of *Penicillium*, two species of *Drechslera* and *Alternaria alternata*. It was observed that aqueous extract showed significant antifungal activity against all the test pathogens. Species of *P. chrysogenum* was completely inhibited at 10% concentration. *D. halodes* and *A. fumigatus* were inhibited at 20% concentration, whereas *F. lateritium* and *F. moniliforme*, were inhibited at a higher concentration of 50%. *D. hamiltonii* was further subjected to different solvent extraction using petroleum ether, benzene, chloroform, methanol and ethanol to identify the solvent extract having high activity. It was observed that petroleum ether extract showed highly significant antifungal activity followed by benzene and chloroform extracts, whereas no activity was observed in methanol and ethanol extracts at 2000 µg/ml.

Kumar and Kaushik (2013) in their study explored *Jatropha curcas* for its activity against endophytic fungi. They identified four isolates as *Colletotrichum truncatum*, and other isolates were identified as *Nigrospora oryzae*, *Fusarium proliferatum*, *Guignardia cammillae*, *Alternaria destruens*, and *Chaetomium* sp. In the

study, dual plate culture bioassays and bioactivity assays of solvent extracts of fungal mycelia showed that isolates of *Colletotrichum truncatum* were effective against plant pathogenic fungi *Fusarium oxysporum* and *Sclerotinia sclerotiorum*. Extracts of active endophytic fungi were prepared and tested against *S. sclerotiorum*. Ethyl acetate and methanol extract of *C. truncatum* EF10 showed 71.7% and 70% growth inhibition, respectively.

Krishnayyaand and Grewal (2002) evaluated the effects of different formulations of neem and selected fungicides on *Steinernema feltiae*. The neem formulation, Nimbecidine and neem oil when mixed with a bactericidal soap (commonly used as a surfactant with neem oil) caused 13- 25% mortality of *S. feltiae*. This toxic effect was entirely due to the soap that alone caused about 24% mortality. Neither neem oil, Nimbecidine or soap had any effect on nematode virulence. The fungicide cinnamaldehyde (Cinnamate) was found to be highly toxic, resulting in 100% nematode mortality after 4 h of incubation, followed by hydrogen dioxide/peroxyacetic acid mixture (ZeroTol) that caused 100% mortality after 120 h of incubation.

Singh *et al*. (1980) evaluated the effect of aqueous extracts and oil of *Azadirachta indica* on few soil-borne pathogens, *Fusarium oxysporum, Rhizoctonia solani, Sclerotium rolfsii*, and *Sclerotinia sclerotiorum*, which incite wilt and rot in *Cicer arietinum*. Growth of the four pathogens in liquid medium was inhibited by extracts of leaf, trunk bark, fruit pulp, and oil. Of these four extracts, neem oil showed maximum inhibitory effect. The germination of gram seeds was inhibited at higher concentrations of oil. They observed oil-treated seeds sown in soil infested with the

pathogens singly and intermixed produced disease-free seedlings whereas all the seedlings from untreated seeds exhibited disease symptoms.

Rani *et al*. (2009) screened as many as ninety formulations of neem oil (*Azadirachta indica*) and *Ferula asafoetida*, at different concentrations with α, β-unsaturated carbonyl compounds (1a–1i), were screened *in vitro* against *Sclerotium rolfsii* and *Macrophomina phaseolina* by the Food Poisoning Method. They found that formulation 3 : 1,1a-N2(−) was found to be the most effective against *S. rolfsii*, enhancing the activity of 1a 2.7 times at 66 ppm, whereas 3 : 1,1f-N3 was found to be 5 times more active against *M. phaseolina* than 1f alone. A number of other formulations also showed significant synergistic effect in increasing fungicidal activity. Dhyani (2008) treated poplar and chir wood with neem leaves extracts and neem seed oil and obtained good results on testing against wood decaying fungi and termites. Crude extracts of water and solvent extractable tannin fractions from pine needles were found to exhibit antibacterial and antifungal properties (Selvakumar *et al*., 2007). A study has been carried out on evaluating the efficacy of *Lantana camara* extracts and neem seed oil against the fungal degradation in stored bamboo and bamboo products (Chandra *et al*., 2010). Izran *et al*. (2012) tested the impact of crude palm oil treatment on physical properties and durability of *Bambusa vulgaris var striata.* Kaur *et al*. (2016) tested resistance of *Dendrocalamus strictus* treated with neem oil, cedar oil, extracts of *Jatropha* leaves, *Lantana* leaves and *Jatropha* cake against the white rot fungus *Polyporus versicolor*. Leaching test proved that neem oil had highest retention level.

While results proved that efficacy of all the extracts was better than control, neem oil and kerosene in combination with copper naphathanate provided the best protection

Wahab and Samsi (2004) stated that palm oil treatment gave effective protection to *Gigantochloa scortechnii* against fungi and insects in 6 month ground contact test. Salim *et al*. (2010) carried out research on effect of crude palm oil treatment on physical properties of 3 years old *Gigantochloa scortechinii* Gamble bamboo. Oil heat treatment successfully imparted dimensional stability to the bamboo as volumetric shrinkage of bamboo was also reduced by the treatment conditions (17-53%). The shrinkage properties of bamboo were inversely proportional to the treatment conditions. Wahab *et al*. (2012) subjected hot palm oil treated *Acacia* hybrid wood samples to accelerated laboratory durability test against fungi. Wahab *et al*. (2005) reported improvement in basic density of Semantan bamboo after heat-treatment with palm oil. Manalo *et al*. (2009) tested physical and mechanical properties of three bamboo species *viz*., *Bambusa blumeana*, *B. vulgaris* and *Dendrocalamus asper* after treatment with virgin coconut oil.

Temiz *et al*. (2013) determined the chemical composition of bio-oil from giant cane as well as its efficacy as wood preservative. Scots pine sapwood samples were first treated with bio oil by full cell treatment and then with epoxidized linseed oil (ELO). Additional treatment of ELO decreased bio-oil leaching from the treated wood. Study stated that improved resistance of treated wood samples against white and brown rot was attributed due to the presence of phenolic compounds. Tjeerdsma *et al*. (2005) developed an improved thermal treatment with reactive vegetable oils for wood

treatment at pilot scale. In this study researchers used rape seed oil (RSO), linseed oil (LSO) and modified linseed oil to treat Spruce and Scots pine. Results demonstrated that color of the treated samples was affected to greater extent by than by RSO and LSO. The treatment effected durability better as compared with plain heat treatment. All the treatments caused reduction in MOR, RSO causing the maximum reduction. Optimum temperature suggested for treatment is 180˚C.

Effect of oil heat treatment on physical properties of 3 years old *Gigantochloa scortechinii* Gamble bamboo was investigated by Salim *et al*. (2008). The bamboo splits within epidermis were heat-treated using crude palm oil at temperature 140°C, 180°C and 220°C for duration 30 and 60 min. The results indicated equilibrium moisture content (EMC), density and volumetric shrinkage of heat-treated bamboo decreased as the treatment temperature and time increased. The EMC and density reduction were 4-27% and 11-18% approximately. Volumetric shrinkage of bamboo was also reduced by the treatment conditions (17-53%). Hegde *et al.* (2017) found effective results of heated palm oil treatment in *Dendrocalamus longispathus* against brown rot fungi.

The effectiveness of hot oil treatment process on 15 years old cultivated Acacia hybrid was studied by Khalid *et al*. (2010). The logs were separated into bottom, middle and top portion and treated with palm oil at temp of 180, 200 and 220°C at time period of 30, 60 and 90 minutes. The durability increased with increase in time and temperature. The attack from *G. trabeum* and *C. versicolors* were reduced.

The effects of hot oil treatment on physical and mechanical properties of three species of Philippine bamboo, *viz. Bambusa blumeana, B. vulgaris* and *Dendrocalamus asper* were investigated after exposure to virgin coconut oil at 160 to 200 °C for 30 to 120 min. The results showed improvement in water absorption and thickness swelling properties for all species tested. However, there was a reduction in strength properties as indicated by modulus of elasticity, modulus of rupture and toughness. The improvement in dimensional stability and reduction in strength properties were correlated with temperature but duration seemed to have little or no effect on physical or mechanical properties. (Manalo and Acda, 2009**)**

The improvements of a combined impregnation process (CIP, also known as the Royal process). This treatment combines the protective properties of a wood protection agent and the hydrophobic properties of a subsequent oil treatment in a wood product, Copper-based wood preservatives, which are traditionally used in CIP, especially the toxicity against water-living organisms. The aim of this research is to describe the fixation effectiveness of the following compounds: Chitosan, Propiconazole, Wolmanit CX-8, Tannin, fire protection agent, Alginate. The scots pine sapwood samples (50×25×15) mm were impregnated and oil treated. The treated products were analysed for their preservative-and oil-retention. Preservative fixation time influence on oil treatment was tested. The treated samples were leached according to EN84. Water samples were analyzed for the amount of active ingredient (Larnoy, 2006). Razak (1998) investigated the durability performance of oil cured *G. scortechinii* in a 6 month ground contact test. Results revealed that oil curing had greatly enhanced the durability

against biodegradation especially fungi. There was a decrease in weight loss in oil cured samples before and after 6 month test. Untreated samples showed a weight loss of about 48 %. Weight loss in terms of percentage after 6 month tests varied from 4 % - 34 % with oil cured samples at higher temperature and longer duration losing less weight. Jin *et al*. (2012) investigated the inhibitory effects of bamboo vinegar against bacteria and fungi by a laboratory-based assay. The fungus tested were *Aspergillus niger*, *Mucor racemosus* and *Rhizopus* sp. In their experiment they evaluated the inhibitory effects of bamboo vinegar on the growth of the microorganisms by the determination of inhibition zone diameters and the minimum inhibitory concentrations. The results showed that bamboo vinegar had inhibitory effect on the growth of bacteria and fungi and the degree of growth inhibition showed a dose-dependent response. The inhibitory effect of bamboo vinegar on the growth of the bacteria is found better than that of the fungi, based on the extent of inhibition zone at the same concentration of bamboo vinegar and the minimum inhibitory concentrations experiment. Akter *et al*. (2012) tried the leaf extracts of *Leucas aspera* (Lamiaceae) for determining the in vitro antibacterial, antifungal, and cytotoxic properties. All extracts showed remarkable antibacterial activity against all of the studied organisms except *Escherichia coli*. Methanol extract showed stronger activity compared to ethyl acetate and petroleum ether extracts. It showed highest activity against *Pseudomonas aeruginosa* with zone of inhibition of 15 mm. The standard chloramphenicol did not show any activity against *Shigella sonnei*. But all the extracts showed moderate activity against this pathogen with zone of inhibition ranging from 10 to 13 mm. None of the extracts has shown any significant

antifungal activity against the fungi. Moline and Locke (1993) investigated the antifungal properties of a hydrophobic neem seed extract (clarified neem oil) against three postharvest apple pathogen namely, *Botrytis cinerea* (gray mold), *Penicillium expansum* (blue mold rot), and *Glomerella cingulata* (bitter rot). The antifungal activity of neem seed oil also was compared to that of $CaCl_2$. 2% aqueous emulsion of the clarified neem seed oil was moderately fungicidal to *B. cinerea* and *G. cingulata* in inoculated fruit, but bad little activity against *P. expansum.* Ethylene production was reduced 80% in fruit dipped in 2% neem seed oil compared to wounded, inoculated controls. Neem seed oil was as effective an antifungal agent as $CaCl_2$, but the effects of the two combined were not additive.

2.5 Northeast India and research gaps

As perceived by Tomar *et al*. (2009) bamboo resources are vulnerable in the forests of Northeast India. They suggested that Monopodial bamboo species which are usually stretched in large area like *M. bacciferain* and *D. hamiltonii* can be conserved by declaring these areas as gene sanctuary for these species. The sympodial bamboo species with sparse distribution can be conserved through community based approach and by developing 'Bambusetum'. Also they pointed out that along with creating marketing channel for value added bamboo products, protection of bamboo from degrading agents and improving its shelf life was needed. There are several incidences in northeast India to show the will of tribal people to traditionally conserve the bamboo resources in their respective areas. For instance, Apatani tribe of Arunachal Pradesh

traditionally conserves bamboo (Bije bamboo) with a community knowledge and commitment (Sundriyal *et al*., 2002).

Bamboo forest biomass stores a large quantity of carbon ranging from 40%-45% and nearly half of the total dry biomass is carbon. As mentioned earlier, abundant moisture and starch in bamboo attracts a host of biodegrading agents like rot fungi and insect borers which destroy it completely within a period of 6-18 months during its use/storage after harvest (Mohanan, 1997; Singh, 1998). Bamboo preservative treatment is reported to increase the durability of bamboo significantly thus preserving the carbon sequestered in bamboo. In a study conducted by Gurung and Singha (2013) Copper Chrome Boron (CCB) treated *Bambusa pallida* lost significantly lower weight after 4 years compared to untreated bamboo which lost about 70 % of its weight within a year. Since bamboo species are quite susceptible to insect and fungi attack, traditionally rural people are using simple and cost effective preservation methods. Toxic effects of chromium and arsenic, released from various commercially available preservatives, necessitated the development of environmental friendly treatment techniques for preservation of bamboo species. Indigenous water leaching process is used traditionally to preserve bamboo (Kardam *et al*., 2013). They observed that decay resistance of water leached samples was found to be better than untreated and comparable to chemically treated bamboo species. But, Water leaching alone cannot be considered as long term preservation option for outdoor application of bamboo species. However its integration with other technologies can provide viable resistance to bamboo species. Singha and Borah (2017) evaluated the traditional methods of preservation of *Bambusa tulda*

through water soaking, curing and smoking. The efficacy of water soaking method was tested in the laboratory conditions against *Schizophyllum cummuni* and also in the field. The average biomass loss of bamboo culms during the tests ranged from10.81% (1 month treated) to 19.26 % (control) in case of water soaking method and 25.99% (1 month treated) and 67.66% (control) in curing and smoking method. They also observed increased durability with increase in soaking periods.

Several eco friendly formulations using plant extracts have been tried in India as well as other parts of the world, mostly in Asia (Nakayama *et al.* 2000; Islam *et al.* 2009; Himmi *et al*. 2013; Kaur *et al* 2016 a; Kaur *et al* 2016 b, Kadir *et al* 2014; Boateng and Kirabena, 2019; Xu *et al* 2013). Heat treatment with oil has been tried by Wahab (2012) on wood. However, no work has been reported on bamboo using two step treatment with chemical and heated neem oil. The current study aims at giving heat treatment using eco-friendly oil (neem oil) after the treatment with copper boron solution with a hope of improving fixation of copper – boron in the treated bamboo for long term protection. The author expects the research out come to be useful in holistic and sustainable utilization of bamboo resources.

CHAPTER 3

MATERIAL AND METHODS

3.0 MATERIAL AND METHODS

3.1 Material

All reagents and chemicals were of analytical grade of Sd fine ltd. India and used without further purification. The chemicals were procured from various pharmaceutical companies and used as such without further purification. Neem seed oil/Neem oil was procured from the forest products retailer with brand name Vyas Neem tail.

3.1.1 *Melocanna baccifera* (Muli bamboo)

The genus *Melocanna* has two species namely *Melocanna baccifera* (Roxb.) Kurz and *Melocanna arundina* C.E.Parkinson. *M. baccifera* grows in clumps in which culms are widely spaced. Young culms are green in colour which turns to slight straw colour when matures. The culms are very straight with short numerous branches. The length of the internode varies from 25 cm to 50 cm. The diameter varies from 2 cm to 15 cm. the nodes are very prominent and the wall of the culms is considerably thin. The sheath of the culms turns brown from green when the culms mature. Underside of the sheath is not hairy where as the upper surface sometimes have hairs. In *Melocanna*, only the sheath blade falls off but not the entire sheath. *Melocanna* covers majority of the area of bamboo in Mizoram along with *Bambusa tulda*.

3.1.2 Sample collection

M. baccifera is widely distributed throughout Mizoram. It was selected for this research work on the basis of its susceptibility to the deteriorating and staining fungi. It is important to collect the matured culms for the preservation treatment. Therefore,

matured and fresh culms of *M. baccifera* (Muli bamboo) were randomly harvested from their natural stands growing at Advanced Research Centre for Bamboo and Rattan **(ARCBR**), Aizawl, Mizoram (Figure 3.1). As per the departmental records they were 5-6 years old. ARCBR is located at 23˚43'7" N latitude and 92˚40'23" E longitude with an elevation of 790 m above mean sea level. The mean annual temperature of the site ranges from 25˚C to 36˚C and mean annual rainfall is 1970 mm. The average height of Mizoram's hills to the west of the state is about 3300 ft. The culms were immediately end sealed with paraffin wax from the bottom portion to avoid any moisture loss. The culms were of dark green colour and average diameter of the culms was recorded as 4.50 cm while average thickness was 0.38cm.

Figure 3.1 Procurement of *M. baccifera* culms from ARCBR, Aizawl

3.1.3 Material for Antifungal Test

Fungal strains of bown rot, *Coniophora puteana* (Acc. No. MTCC 1068), white rot, *Schizophyllum commune* (Acc. No. MTCC 1096) and sap stain, *Alternaria alternata* (Acc. No. 2060) were obtained from The Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH Chandigarh and was maintained on Potato Dextrose Agar (PDA) medium at 25 ± 2 ℃. All the laboratory tests were conducted in Science laboratory of Department of Forestry, Mizoram University, Aizawl, Mizoram.

3.2 Methods

3.2.1 Preliminary screening of Neem oil and Copper sulphate-Boric acid formulation

The effective concentration of Neem oil to inhibit the growth of brown rot, white rot and sap stain fungi (Minimum Inhibitory Concentration) was determined in Petri plates by Poisoned Food Technique using Potato Dextrose Agar (PDA) medium (Hunt and Garratt, 1967) and was compared with the performance of Copper sulphate-Boric acid (1:1) formulation exhibited in the same test.

3.2.1.1 Cleaning and Sterilization of Glass Wares and Tools

The glass wares (Borosil make) were cleaned with a solution of sulphuric acid, potassium dichromate in water and finally washed thoroughly with detergent powder and water. Further they were dried for 4 hrs in oven maintained at 110˚C. The glass wares and Petri plates were autoclaved at 15 pounds pressure and 120˚C for 20 min. (ASTM; 1980). All the tools (scalpels, forceps, needle etc.) used were autoclaved at same conditions mentioned above and were cleaned with rectified spirit flamed (till red hot) and cooled every time before conducting experiment.

3.2.1.2 Preparation of Malt Agar Medium

Malt agar medium (3.9 %) was prepared by dissolving 39 g Potato Dextrose Agar medium in 1000 mililiters of distilled water and was heated till boiling. The medium was sterilized in an autoclave at 15 pound pressure and 120˚C temperature for 20 minutes. (Datar, 1995). After autoclaving 30 ml of the medium was poured in each sterilized Petri plate (9 cm diameter) kept in the laminar flow in the culture room. These Petri plates were allowed to cool for 2 hours till the medium has solidified. For each concentration of neem oil and copper sulphate-boric acid formulation along with control (without oil and chemical formulation) 6 replicates were taken.

3.2.1.3 Chemicals and Their Concentration Used in Test

It is expected that antifungal activity of any chemical will depend on toxic nature of its individual components as well as concentrations. Keeping this in view nine different concentrations were taken for each chemical for malt agar bioassay. Neem oil was dissolved in Dimethyl sulfoxide (DMSO) and this solution was further dissolved in malt agar to prepare required concentrations. Same amount of DMSO was also subjected to Petri Plate test to determine its antifungal activity. Since copper sulphate and boric acid both are soluble in water, no other solvent was required to dissolve it. The test was done at following different concentrations:

- **Neem Oil Concentrations:** 0.25, 0.50, 0.75, 1.00, 2.00, 4.00, 6.00, 8.00 & 10.0 % (V/V)
- **Copper Sulphate-Boric acid concentrations:** 0.005, 0.01, 0.02, 0.04, 0.06, 0.08, 0.10, 0.20 & 0.40 %

  Replication: 6

  Total Plates: 360

3.2.1.4 Antifungal Activity Test using Poisoned Food Technique

The Petri plates containing the medium with or without oil and copper sulphate-boric acid formulation were inoculated with an inoculums disc of actively growing 14-16 days old culture of the test fungi. The plates were incubated in the B.O.D. (Biological Oxygen Demand) incubator maintained at 25±2˚C temperature and 70±4% relative humidity. The results were recorded after 15 days in terms of surface coverage (%) by the test fungi over malt agar medium and shown as total inhibition (%). (Kapse, 1996; Wedge *et al.,* 2000). Illustration is given in Figure 3.2. Surface coverage (%) is given by the following formula:

$$Surface\ coverage\ (\%) = \frac{\text{Surface covered by test fungi}}{\text{Total surface area of the petri plate}} \text{x } 100$$

$$Inhibition\ in\ Growth\ of\ fungus\ (\%) = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \text{x } 100$$

Where, Dc = Avg. Diameter of the surface area covered by the fungus in control.
Dt = Avg. Diameter of the surface area covered by fungus in test plates.

The total growth of fungus was rated as per Goyal and Dev (1982). Inhibition in growth of test fungi was statistically analyzed by using OPSTAT software.

Measuring Growth diameter of fungus

Measuring PDA to make required concentration

Pippeting out Neem oil

Pouring PDA into Petri plates

Figure 3.2 Poisoned food techniques for preliminary screening

When there is 0-5 % surface coverage of medium by the mycelium the growth type is sporadic. At 5-25% surface coverage the growth is considered little but at 25-50 % it turns into moderate growth. It is called considerate growth when surface coverage of medium extends from 50 – 75%. Above 75% area coverage by mycelium is termed as complete growth.

3.2.1.5 Determination of Nature of Antifungal Activity

To determine the nature of antifungal activity, the inoculums of the test fungi in which the growth was completely suppressed by the complex were transferred to fresh complex free malt agar medium. The plates were incubated for 15 days and results were recorded as described earlier in section – 3.2.1.4. If the growth is resumed immediately, it is categorized as fungi static and if growth does not resume again then the activity is termed as fungicidal (Iqbal *et al.*, 2004).

3.2.2 Physical properties of *M. baccifera*

To avoid any moisture loss and error in experiment the green culms of *M. baccifera* were as soon put to the tests of physical properties as soon they were brought to laboratory after procurement from stands. The physical properties determined were moisture content, basic density, volumetric shrinkage and volumetric swelling. Samples were taken from all three portions of the culms viz. apical, middle and basal.

3.2.2.1 Determination of Moisture Content of *M. baccifera*

Moisture Content (MC) of green culms of *M. baccifera* was determined by Oven Dry Method following the Bureau of Indian Standards (BIS: 401, 2001). Samples were taken from Apical, Middle and Basal portions of the culms. Green weight of the samples was recorded as Wg and then samples were placed in oven at 100±3°C temperature for 24 hours, after which samples were again weighed. This process was repeated till change in weight became constant and it was recorded as Wo.

Sample Size: 25 mm X 25 mm X culm thickness

Replication = **10**

Total Samples: **60**

Formula used:

$$Moisture\ Content\ (\%) = \frac{Wg - Wo}{Wo} \times 100$$

where, Wg = Green weight, Wo = Oven dry weight

3.2.2.2 Determination of Basic Density of *M. baccifera*

Basic Density of *M. baccifera* was determined by following the Indian Standard 6874: 2008 using formula:

$$Basic\ Density\ (Kg/m^3) = \frac{Mo}{Vg} \times 1000$$

Where, Mo = oven dry mass (g); and

Vg = green volume (cm^3).

The green volume of the samples was determined by water displacement method. A beaker filled with water up to $3/4^{th}$ of its capacity was placed on digital weighing balance. The initial weight was tare to zero. The bamboo sample was carefully merged in water using a thin pin. The change in the value of weighing balance reading was recorded as green volume. The samples were taken from the apical, middle and basal portions.

Sample Size: 25 mm X 25 mm X culm thickness

Replication = **10**

Total Samples: **60**

3.2.2.3 Dimensional Stability of *M. baccifera*

The dimensional stability of *M. baccifera* was established by carrying out volumetric shrinkage and volumetric swelling tests (IS 6874: 2008; Korkut and Hiziroglu, 2014)

3.2.2.3.1 Preparation of specimen for Volumetric Shrinkage and Volumetric Swelling Test

The culms were cut into three separate portions viz. basal, middle and apical portion using mechanical saw. For conducting swelling and shrinkage tests the specimen were cut into 10cm long cylindrical rings from each portion i.e., basal , middle and apical portions. All the samples were cut mechanically using saw. For each test set 10 replicates were taken. There were a total of 60 samples. All the samples were initially weighed (W_1) and marked using permanent marker for measuring various dimensions, to be assured of taking measurements at same points before and after completion of swelling and shrinkage test. All the dimensions were measured at two different points. After marking, the initial length (L_i), outer diameter ($Di_{1)}$ and inner diameter (Di2) of the samples were measured at the marked points (Figure 3.3)

Figure 3.3 Samples for shrinkage and swelling test

3.2.2.3.2 Volumetric Shrinkage Test

For determining anti-shrinkage property of *M. baccifera* the specimens, after recording initial dimensions, were placed in a hot-air oven at 103±2°C till it reached a constant weight (oven-dry condition). The final mass (W_2) and final dimensions (L_f,

D_{f1}, D_{f2}) of the specimens were taken at the oven-dry condition. Since the test was performed to assess the percentage of the samples' shrinkage after being oven-dried. The formula used is as follows:

$$\mathbf{Sh\ (\%) = \ ((V_i - V_f)/V_i\ )\ X\ 100)\ \%}$$

Where, V_i is the volume of samples before oven drying which was calculated using the formula:

$$\mathbf{V_i = \pi L_i\ ((Di_1/2)^2 - (D_{i2}/2)^2)}$$

and V_f is the volume of samples after oven drying which was calculated using the formula:

$$\mathbf{V_f = \pi L_f\ ((D_{f1}/2)^2 - (D_{f2}/2)^2)}$$

3.2.2.3.3 Volumetric Swelling Test

For determining anti-swelling property of *M. baccifera*, the specimens, after recording initial dimensions, were immersed in water completely for 48 hours (Figure 3.4). Then final dimensions were measured at the same points and recorded as L_f, D_{f1} and D_{f2}. As the test was done in order to assess the percentage of swelling in the samples after soaking in water, the formula used is as follows:

$$\mathbf{Sw\ (\%) = \ ((V_f - V_i)/V_i\ )\ X\ 100)\ \%}$$

Where, V_i is the volume of samples before immersing in water which was calculated using the formula:

$$\mathbf{V_i = \pi L_i\ ((D_{i1}/2)^2 - (D_{i2}/2)^2)}$$

and V_f is the volume of samples after immersing in the water which was calculated using the formula:

$$\mathbf{V_f = \pi L_f\ ((D_{f1}/2)^2 - (D_{f2}/2)^2)}$$

Figure 3.4 Immersion of samples in water for volumetric swelling

3.2.3 Two Step Treatment of *M. baccifera*

The green culms of *M. baccifera* were given preservative treatment in two steps. In first step the culms were treated with copper sulphate-boric acid formulation while in second step the previously treated culms were given Oil Heat Treatment. The specimens after one-step treatment and after two-step treatment were taken from apical, middle and basal portions of the treated culms and were subjected to various tests for examining change in physical properties and affect on anti-fungal property of *M. baccifera*.

3.2.3.1 Step One of Preservative Treatment

Fresh culms of *M. baccifera* were treated with different concentrations of copper sulphate-boric acid formulation, which was prepared in 1:1 ratio, using Boucherie method (IS 401: 2001). The treatment was given at three different concentrations viz., 1.0%, 4.0% & 7.0%.

Since both the salts are soluble in water the solution for treatment was prepared by dissolving required amount of salt formulation in water. The preservative solution was the filled in preservative tank of boucherie treatment plant. The treatment was done at 23 psi pressure and was carried for four hours. The blue colour of preservative solution started to drip from the open end of culm after half an hour of starting the treatment indicating reaching of preservative till the end of culm. The treatment was done at Bamboo Development Agency, Chaltlang, Aizawl (Figure 3.5). The culms were left for a week for fixation of preservative. The culms were left to rest for two weeks so that the preservative absorbed by the culms gets fixed properly.

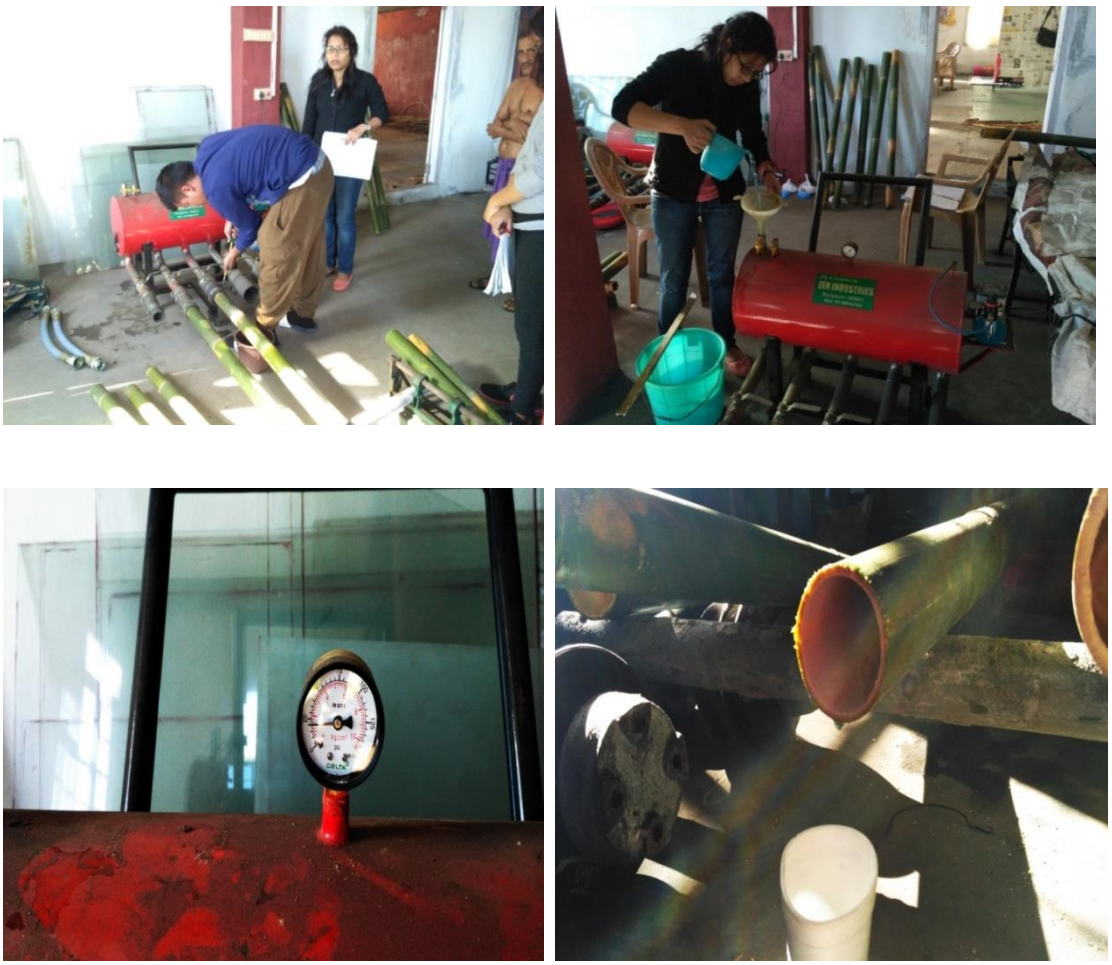

Figure 3.5 Boucherie treatment of the bamboo

3.2.3.1.1 End Penetration of preservative solution in treated culms

After completion of boucherie treatment the culms were checked if the treatment was done thoroughly and uniformly till the other end of the culm as well as across the cross section. End penetration of copper sulphate-boric acid formulation from one end to other end was tested by spot test method under the Indian Standard (BIS IS 1902:2006).

- For Copper: Presence of copper is tested by Chrome Azurole solution which was prepared by dissolving 0.5g Chrome Azurole-S & 5g sodium acetate in 80 ml water and diluted to 100 ml. This solution was spread on the end of treated culm. Appearance of blue colour confirmed presence of copper.

- For Boron: For testing presence of boron two solutions were prepared using the following method:

  Solution 1. 10 g turmeric powder dissolved in 90 ml ethyl alcohol. The solution was decanted and filtered to obtain clear solution.

  Solution 2. 20 ml of concentrated hydrochloric acid diluted to 100 mI with ethyl alcohol and then saturated with salicylic acid (about 13 g per 100 ml).

  Solution 1 was applied on the end of treated culm. After drying of solution 1, Solution 2 was applied over it. Solution 1 changed surface colour to yellow. Solution 2 turned yellow to red colour confirming presence of boron.

3.2.3.1.2 Retention of preservative solution in treated culms (BIS IS 1902:2006)

For detecting the amount of preservative absorbed by the treated culms of *M. baccifera* samples were taken from apical, middle and basal portions. The samples were converted into small chips using knife and then finely ground in an electric grinder. 5g of each sample was analyzed using MPAES spectroscopy instrument in Central Instrumentation Laboratory, Mizoram University, Mizoram.

3.2.3.1.3 Determination of Leaching

Leaching test was done on the samples taken from treated culms in order to estimate the amount of preservative that would leach out when the treated culms would be put to outdoor use under the influence of water/rain. The samples treated with the highest concentration were taken from apical, middle and basal portions. The test was started after two weeks of boucherie test. Three sets of treated blocks were subjected to leaching. All the three sets had six blocks each treated with 7.0 % concentration of copper sulphate:boric acid formulation same time. Leaching was carried out at room temperature. The blocks were kept in a beaker and pressed with a weight to prevent floating (Figure 3.6). Distilled water approximately 9 times more than the volume of the samples was poured into the beaker. After 6, 24, 48, 72 and thereafter at 168 hrs intervals, the leachates were changed with equal amount of fresh distilled water (Anon., 2008). All the sets were subjected to leaching at different time gap after treatment following a time schedule.

Further the leachates were collected and replaced with same amount of fresh deionized water in each set after interval of 24 hrs, 48 hrs, 72 hrs, 96 hrs and 168 hrs. The evaluation of amount of copper and boron present in the leachates was done using

MPAES spectroscopy in Central Instrumentation Laboratory, Mizoram University, Aizawl, Mizoram. .

Sample size: 19mm X 19mm X culm thickness

Replicates for each leachate: 6

Total number of leachate samples: 90

Figure 3.6 Leaching Process

3.2.3.1.4 Volumetric Shrikage of Step-one treated *M. baccifera*

The samples from apical, middle and basal portions of the culms treated with copper sulphate - boric acid formulation were tested for anti-shrinkage property following the same procedure as done in section 3.2.2.3.2.

3.2.3.1.5 Volumetric Swelling of 2-Step treated *M. baccifera*

The samples from apical, middle and basal portions of the culms treated with copper sulphate:boric acid formulation were tested for anti-swelling property following the same procedure as done in section 3.2.2.3.3.

3.2.3.2 Step Two of Preservative Treatment

In the second step the samples from step one treatment were given oil heat treatment. An oil bath chamber was filled with neem oil. The oil bath was a stainless steel cylindrical vessel heated by electric plates connected to a thermocouple and digital temperature controller. The samples were heated in neem oil at 200°C for 30 minutes (Figure 3.7 and 3.8). The samples were immersed in the hot oil when the temperature of the oil reached 80°C to prevent oil from excessively penetrating into the strips and affecting the evaluations of the physical properties (Razak *et al*. 2005). After oil heat treatment the excess oil was cleaned from the samples with tissues paper. The samples changed their color from green to yellow or dark brown after oil heat treatment (Figure 3.9 and 3.10). The samples were weighed before as well as after completion of heat treatment to check the affect of heat treatment on weight of samples. During oil heat treatment some samples were cracked, especially the apical portions of the samples suffered the most. Samples' size and number of samples for neem oil heat treatment varied according to the test they were to be subjected further.

Figure 3.7 Neem Oil Heat Treatment

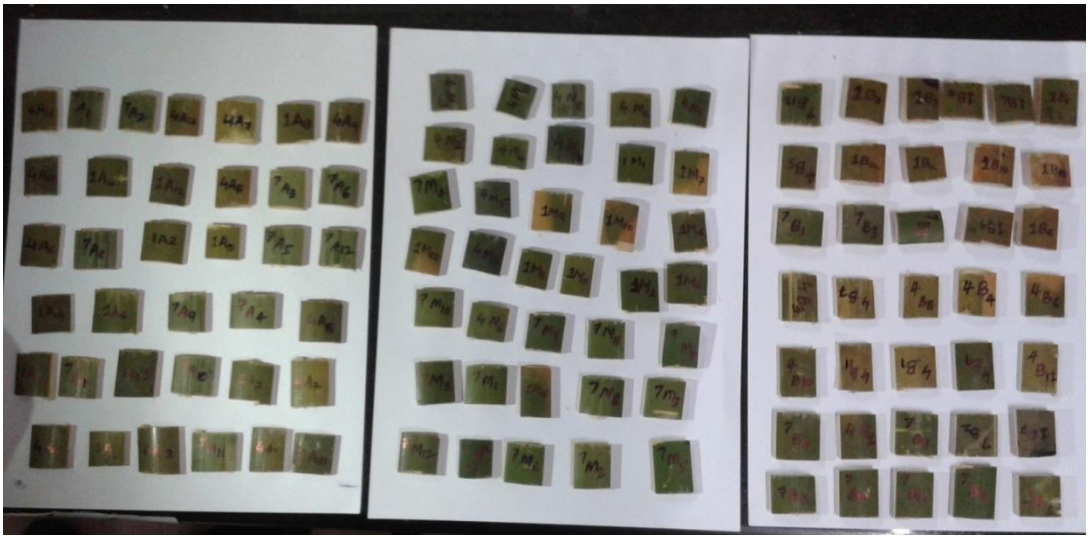

Figure 3.8 *M. baccifera* samples before Oil Heat Treatment

Figure 3.9 *M. baccifera* samples after Oil Heat Treatment

Figure 3.10 Samples of *M. baccifera* after Oil Heat Treatment

3.2.3.2.1 Moisture Content of *M. baccifera* after 2-Step Treatment

The moisture content of the apical, middle and basal portion samples of two-step treated *M. baccifera* was determined following the same method as done in the section 3.2.2.1.

3.2.3.2.2 Basic Density of *M. baccifera* after 2-Step Treatment

The basic density of the apical, middle and basal portion samples of two-step treated *M. baccifera* was determined following the same method as done in the section 3.2.2.2.

3.2.3.2.3 Volumetric Shrinkage of *M. baccifera* after 2-Step Treatment

The samples from apical, middle and basal portions of the culms treated with Two-Step process were tested for anti-shrinkage property following the same method as done in section 3.2.2.3.2.

3.2.3.1.4 Volumetric Swelling of 2-Step treated *M. baccifera*

The samples from apical, middle and basal portions of the culms treated with Two-Step process were tested for anti-swelling property following the same method as done in section 3.2.2.3.3.

3.2.4 Antifungal Property of One-Step and Two-Step treated *M. baccifera*

The effect of Boucherie treatment and combination of Boucherie treatment with Neem oil Heat treatment on the fugal resistance of *M. baccifera* was tested against white rot (*Schizophyllum commune*) and brown rot (*Coniophora puteana*) using soil block bioassay test (Anon., 2008). The selection of test fungi was based on the destructive nature of fungi due to which it causes weight loss in bamboo.

3.2.4.1 Soil Block Bioassay Test

The following steps were done to carry out the test.

3.2.4.1.1 Preparation of Test specimen

The test specimen of size 1.9 cm X 1.9 cm X culm thickness were randomly selected from the apical, middle and basal portion of one-step and two-step treated samples of all the three concentrations viz. 1.0%, 4.0% and 7.0%. Samples from the

untreated culms were used as control. All the samples were seasoned by placing in an oven at 102±3°C for 24 hours. After that they were placed in a desiccator for next 24 hours. The initial weight of the samples was recorded as W_i. The samples were sterilized before inserting into soil bottles. They were steamed at 100˚ C for about 20 minutes at atmospheric pressure in an autoclave in tightly fitted bottles. Six replicates were taken for each treatment. Total 252 samples were taken.

3.2.4.1.2 Preparation of soil culture bottles

Sieved, air-dried garden soil amounting to 125 g with pH between 5.0 and 7.0 was filled (compacted by tapping) in screw capped bottles. 44ml of distilled water was added to the bottles so as to obtain 130% of water holding capacity of soil in the test bottles. Two feeder blocks of size 0.4 X 1.9X 3.5 cm^3 were placed directly on the surface of the soil. The prepared bottles with caps loosened were sterilized in an autoclave at a pressure of 1 kg/cm^2 for 30 min.

3.2.4.1.3 Preparation of test culture

Sterilized culture bottles were thoroughly cooled, the fungus inoculums from freshly grown culture approximately 8-10 mm in diameter was placed on the edge of the feeder blocks. The inoculated bottles were incubated in B.O.D. (biochemical oxygen demand) with slightly loosened lids at 25±4˚C and 70±4% relative humidity for approximately 3 weeks till the feeder blocks were completely covered by the test fungi.

3.2.4.1.4 Introduction and incubation of the test blocks in culture bottles:

Two samples with the inner wall face down were placed on feeder blocks in contact with mycelium in each culture bottle. The bottles containing the test samples

were incubated for a period of 14 weeks in the incubator maintained at 25±4˚C and a relative humidity of about 70±4% (Anon., 2008).

At the end of the incubation period the samples were removed from the culture bottles cleaned off from the adhering mycelium by brush, taking care not to remove the splinters of the bamboo. The samples were dried at room temperature for 3-4 days and after that in the hot air oven and weighed till the constant weight (W_2) was obtained (Figure 3.11).

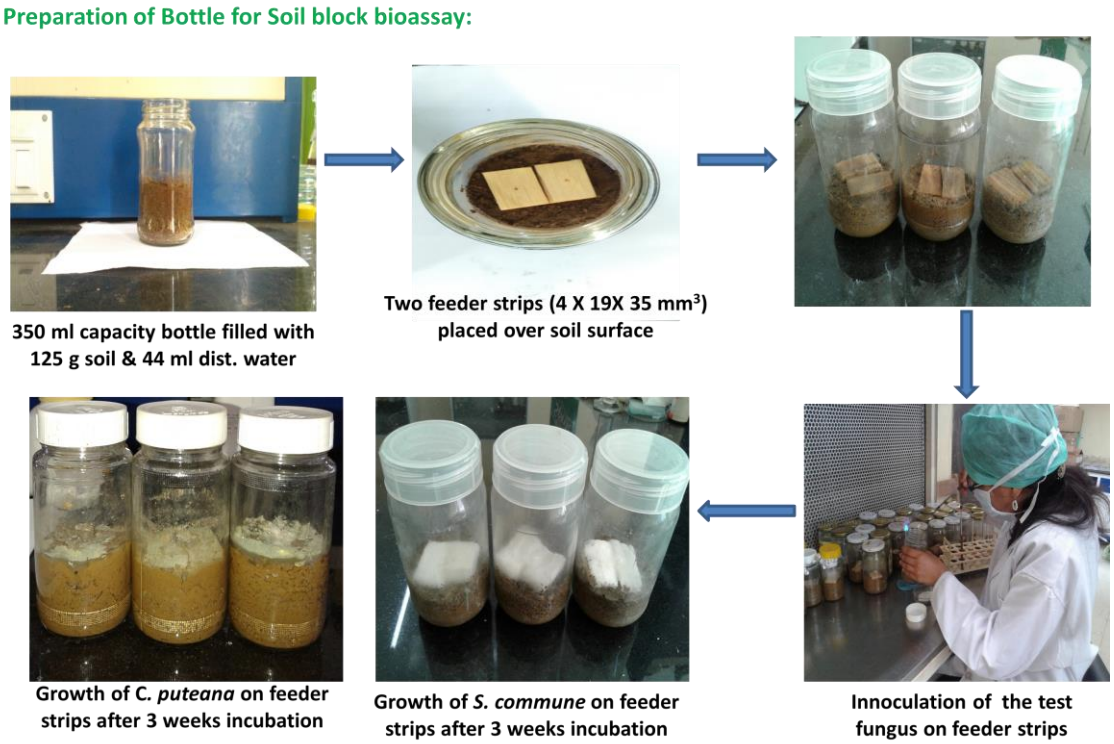

Figure 3.11 Soil block bioassay

3.2.4.1.5 Calculation of Weight Loss

Weight loss (%) was calculated from the conditioned weight of the test samples before and after incubation period.

$$Weight\ loss\ (\%) = \frac{W1 - W2}{W1} \times 100$$

Where W1 = Conditioned weight of the blocks after treatment (before inserting in culture bottles)

W2 = Conditioned weight of the blocks after test

Based on the average weight loss of samples by fungal attack, the decay resistance of bamboo was determined based on class (Martawijaya, 1975, Djarwanto and Suprapti 2004) and expectancy of service life (Seng, 1990) (Table 3.1)

Table 3.1 Classification of bamboo resistance based on the weight loss by fungal attack.

Average weight loss (%)	**Decay resistance**	**Resistance class**	**The expectancy of service life (years)**
None or negligible	Very resistant	I	≥8
Less than 6	Resistant	II	6–8
6 to 10	Moderately resistant	III	3-6
11 to 30	Non-resistant	IV	2–3
More than 30	Perishable	V	< 2

3.2.4.2 Efficacy of one-step and two-step treated *M. baccifera* against sap stain

The laboratory test method used in the experiment for determining the minimum concentration of fungicide or formulation of fungicide, that is effective in preventing bio-deterioration by fungi in selected species of bamboo under optimum laboratory condition gave result in short duration of time which can help in further research.

3.2.4.2.1 Sample Preparation

The samples of size 7.0 cm x 2 cm x culm thickness were randomly cut using sharp saw blade and chisel from the apical, middle and basal portions of one-step and two-step treated *M. baccifera* of all three concentrations viz. 2.0%, 4.0% and 7.0%. Samples from untreated culms were used as control. The specimens were kept in air tight bags to prevent drying.

3.2.4.2.2 Preparation of Petri plates for storing test samples

Three to four layers of absorbent paper were placed on the bottom of each Petri plate of 90 mm diameter and to maintain high humidity during the test period, papers were wet with distilled water until free water appeared. Air bubbles trapped under and between the paper disks were pressed out. Two straight glass rods (2 mm in diameter by 70mm long) were placed on the top of the saturated papers in each plate.

3.2.4.3 Inoculation of Fungus on Test Block Samples

Inoculums of *Alternaria alternata* were taken from actively growing pure culture and mixed uniformly with 100 ml of distilled water using a homogenizer

machine. The spore suspension was stir frequently during inoculation. Inoculation was performed using a spraying method. After inoculation of freshly prepared spores of *Alternaria alternata* on bamboo samples, Petri plates were kept in plastic box which were kept in incubator maintained at temperature around 25 ± 4˚C and relative humidity of 74 ± 4 %. Incubation period was of 4 weeks. Paper pads were rewet with distilled water after every 3 days interval during the incubation period to maintain a "damp condition". The blocks were re-sprayed with the inoculums cocktail at every 3 day interval until the surfaces of the untreated control blocks were overgrown with sporulating fungi (Figure 3.12).

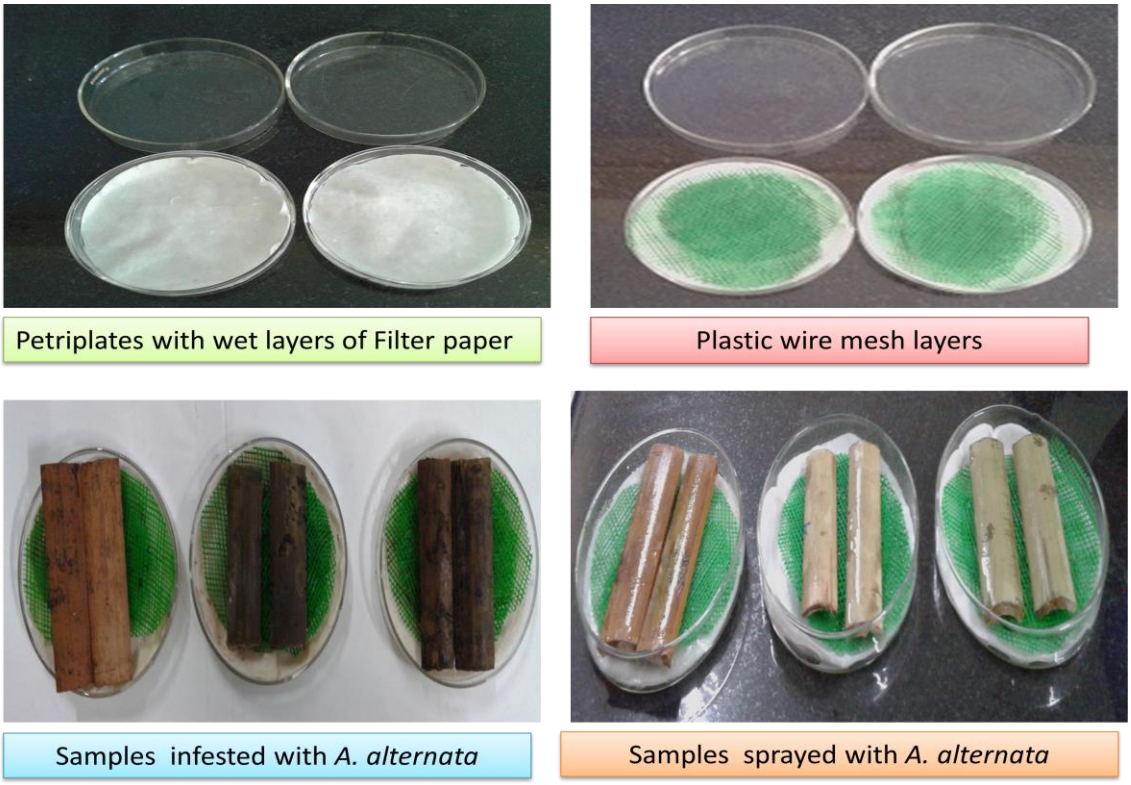

Figure 3.12 Sap stain testing

3.2.4.4 Evaluation of the Test

After 4 weeks, the growth of fungi was estimated visually and scored using a scale of 0 to 5, the 5 being maximum intensity. In the condition of clean bamboo test sample where no stain or fungal growth occurred was given 0 score. When the surface area of bamboo test sample had minor attack with Stain or fungal growth covering less than 20% of upper surface, it was allotted score 1. With light attack of stain or fungal growth on the test sample surface covering 20-40 % area was given score of 2. The moderate attack of fungus covering 40-60 % surface area was denominated with 3 score. While heavy attack covering 60-80 % surface area was attributed score 4, the fungal attack covering more than 80 % surface area of the test sample was applied score 5 (Table 2).

Table 3.2 Evaluation of sap stain attack

Sl No.	**% Surface area covered**	**Score**	**Attack category**
1	0	0	Nil
2	Area >20	1	Minor attack
3	20< Area <40	2	Light attack
4	40 < Area <60	3	Moderate attack
5	60 < Area <80	4	Heavy
6	Area>80	5	Very heavy

3.2.5 Statistical analysis

The data recorded was subjected to statistical analysis to find out the variation between the treatment/factors and the relationship between the observed parameters. The data was analyzed using OPSTAT (Sheoran, 2006).

Analysis of variance (ANOVA): The data was analyzed as per the design used. For the experiments conducted in laboratory that is poisoned food test, soil block bioassay and sap stain test method completely randomized design (CRD) was used. Different parameters taken into account during the course of study were subjected to ANOVA.

CHAPTER 4

RESULTS

4 RESULTS

4.1 Preliminary screening of Neem Oil against Test fungi

The inhibitory effect of Neem oil was determined against the wood and bamboo decaying white rot, brown rot and sap stain fungi at various concentrations using the food poison technique and the results are shown in Table 4.1. The Neem oil was found to be effective against all the tested fungi in the present investigation.

Table 4.1 Efficacy of Neem oil against *S. commune, C. puteana* & *A. alternata*

Treatments (A) (%)	**Fungal strains (B)**			**Mean A**
	***C. puteana* (B1)**	***S. commune*(B2)**	***A. alternata*(B3)**	
A0 (+ve control DMSO)	2.2	5	2.5	3.233
A1(0.25)	50.64	17.698	49.88	39.406
A2 (0.50)	60.53	30.764	60.22	50.505
A3 (0.75)	65.256	41.284	65.73	57.423
A4 (1.00)	67.384	44.666	67.378	59.809
A5 (2.00)	75.478	53.096	75.69	68.088
A6 (4.00)	80.646	66.158	79.456	75.42
A7 (6.00)	86.916	72.546	88.068	82.51
A8 (8.00)	91.436	100	92.868	94.768
A9 (10.00)	100	-	100	100
Mean B	**68.049**	**53.121**	**68.179**	
Factors	**C.D. $^{0.05}$**	**SE(d)**	**SE(m)**	
Factor(A)	1.315	0.664	0.469	
Factor(B)	0.721	0.363	0.257	
Factor(A X B)	2.278	1.149	0.813	

DMSO- Dimethyl Sulfoxide. The values in parantheses under treatment show the concentrations of neem oil

In positive control (DMSO) there was 2.2%, 5.00 % and 2.50% growth inhibition in *C. puteana*, *S. commune* and *A. alternata* respectively. At the lowest tested concentration of Neem oil i.e., 0.25%, *C. puteana* and *A. alternata* showed about 50 % inhibition whereas *S. commune* showed only 17 % inhibition.

With gradual increase in concentration of Neem oil the growth inhibition rate of each fungus also increased. The 50% of growth inhibition was achieved in *S. commune* at 2.0% concentration of neem oil. Complete inhibition for *A. alternata* and *C. puteana* occurred at the highest concentration taken in the experiment i.e., 10.0% while for *S. commune* it was achieved at 8.0% (Table 4.1). Figure 4.1 shows the growth inhibition for *S. commune, C. puteana* and *A. alternata* at various concentrations.

The fungistatic/fungitoxic test revealed that the neem oil was efficient in completely killing the *Alternaria alternata* as the innoclum from preliminary screening test did not revive on fresh malt agar plate therefore it can be categorised as fungitoxic for *A. alternata*. But neem oil proved to be fungistatic for the other fugi i.e., brown rot *Coniophora puteana* as well as white rot *Schizophyllum commue*. Innoculum from the highest concentrations' Petri plates of preliminary screening regrew on fresh malt agar plates (Figure 4.2).

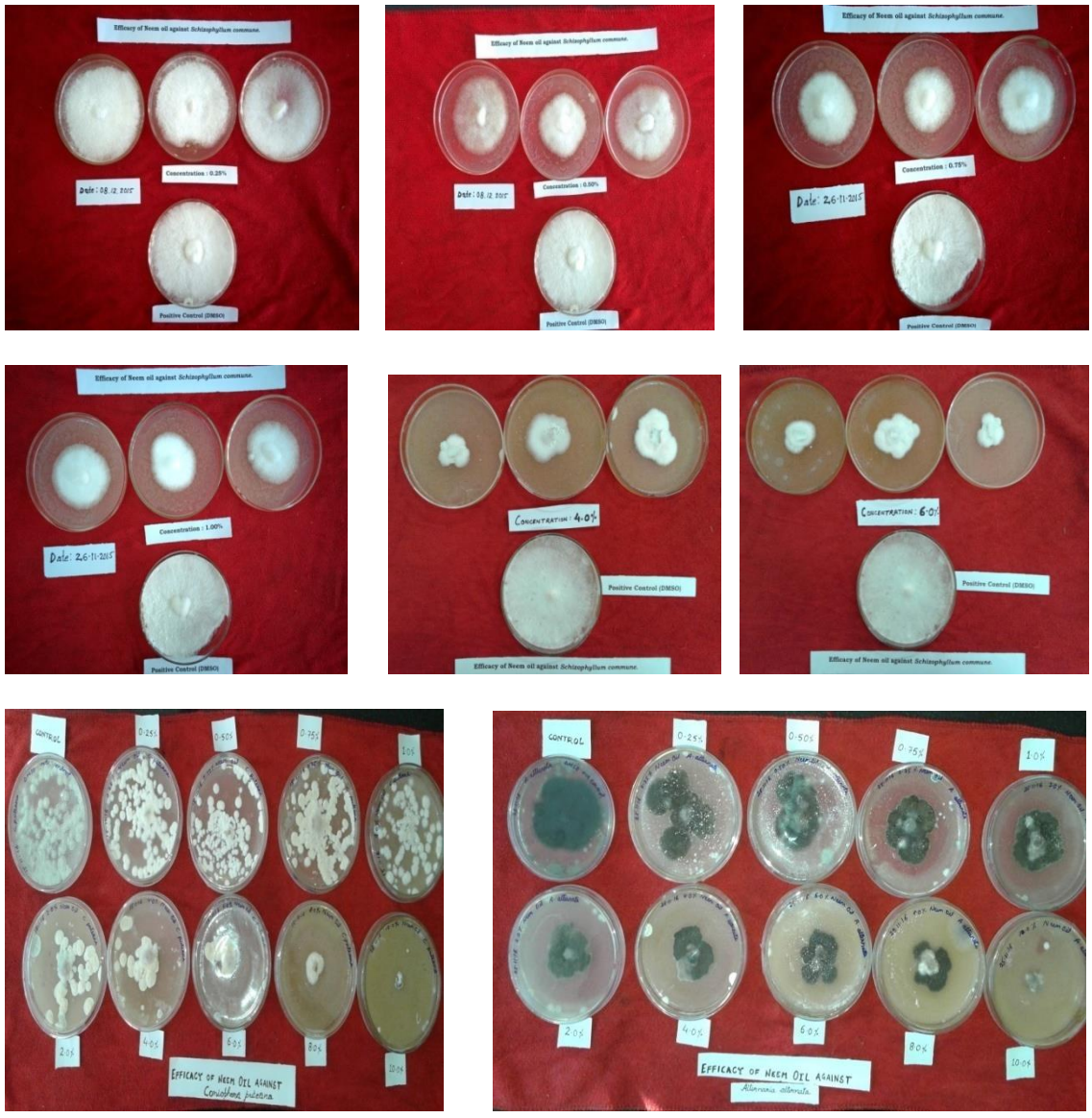

Figure 4.1 Growth inhibition of *S. commune*, *C. puteana* and *A. altrnata* under the effect of Neem oil

Figure 4.2 Fungitoxic/Fungistatic test of *S. commune* and *C. puteana*

4.2 Efficacy of Copper sulphate-Boric acid formulation (1:1) against fungi

From the scrutiny of Table 2, efficacy of Copper sulphate – boric acid (1:1) can be comprehended against the white rot, brown rot; soft rot and sap stain fungi at various concentrations ranging from 0% (control) to 0.4 % (A9) for knowing the minimum inhibitory concentration (MIC). The results revealed that up to 0.02% concentration the growth inhibition was not observed for all the strains of fungi. 100 % growth inhibition was observed in 0.4 % concentration of Copper sulphate – boric acid (1:1) for *C. puteana* and *S. commune* where as for *A. alternata* it was observed at the concentration of 0.2 %. 50 % growth inhibition was observed for *C. puteana* and *S. commune* at 0.06 % concentration and that for *A. alternata* was observed at 0.08 %. Copper sulphate:boric acid formulation proved to be fugitoxic against all the three test fungi.

Table 4.2 Efficacy of Copper sulphate-Boric acid (1:1) formulation against S. *commune*, *C. puteana* & *A. alternata*

Treatment (A) (%)	**Fungal strains (B)**			
	***C. puteana* (B1)**	***S. commune* (B2)**	***A. alternata* (B3)**	**Mean A**
A0 (control)	0	0	0	0
A1 (0.005)	0	0	0	0
A2 (0.01)	0	0	0	0
A3 (0.02)	12.737	0	12.993	8.577
A4 (0.04)	34.237	8.147	23.843	22.076
A5 (0.06)	54.227	33.52	56.17	47.972
A6 (0.08)	66.62	49.813	77.1	64.511
A7 (0.10)	82.357	60.37	85.787	76.171
A8 (0.20)	94.773	71.67	100	88.814
A9 (0.40)	100	100	-	100
Mean B	**49.439**	**35.947**	**50.655**	
Factors	**C.D. $^{0.05}$**	**SE(d)**	**SE(m)**	
Factor(A)	1.949	0.97	0.686	
Factor(B)	1.125	0.56	0.396	
Factor(A X B)	3.376	1.679	1.188	

4.3 Penetration and Retention of Copper sulphate-Boric acid formulation in *M. baccifera*

Penetration test results for the copper and boron have been presented in the Figure 4.3 and Figure 4.4 respectively. Dark blue color as an indicator for presence of copper can be observed in 4% and 7% concentration in apical, middle and basal

portions of Muli bamboo, where as culms treated with 1% copper boron had shown light blue color.

Figure 4.3 End penetration test for copper in three portions of bamboo

Similarly, for boron penetration test the indicative color dark red was observed in 7% concentration for apical portion, 4 % and 7% for middle portion and moderate red color for all the concentrations in basal portion.

Figure 4.4 End penetration test for boron in three portions of bamboo

After the step I treatment (Copper sulphate-Boric acid formulation) from Boucherie method, percent retention of copper-boron was calculated and shown in the Figure 4.5 for apical, middle and basal portion of the bamboo. Among the portions of bamboo, apical portion showed the least retention of chemical where as middle and basal portions were at par with each other. Among the treatment concentrations, it was very clear that higher concentration of chemical has yielded significantly higher

chemical retention. Highest average retention (6 %) was in the basal portion of bamboo treated at 7% concentration of copper boron formulation. At the same concentration middle and apical portions showed 5.9 % and 5.5 % average retention respectively.

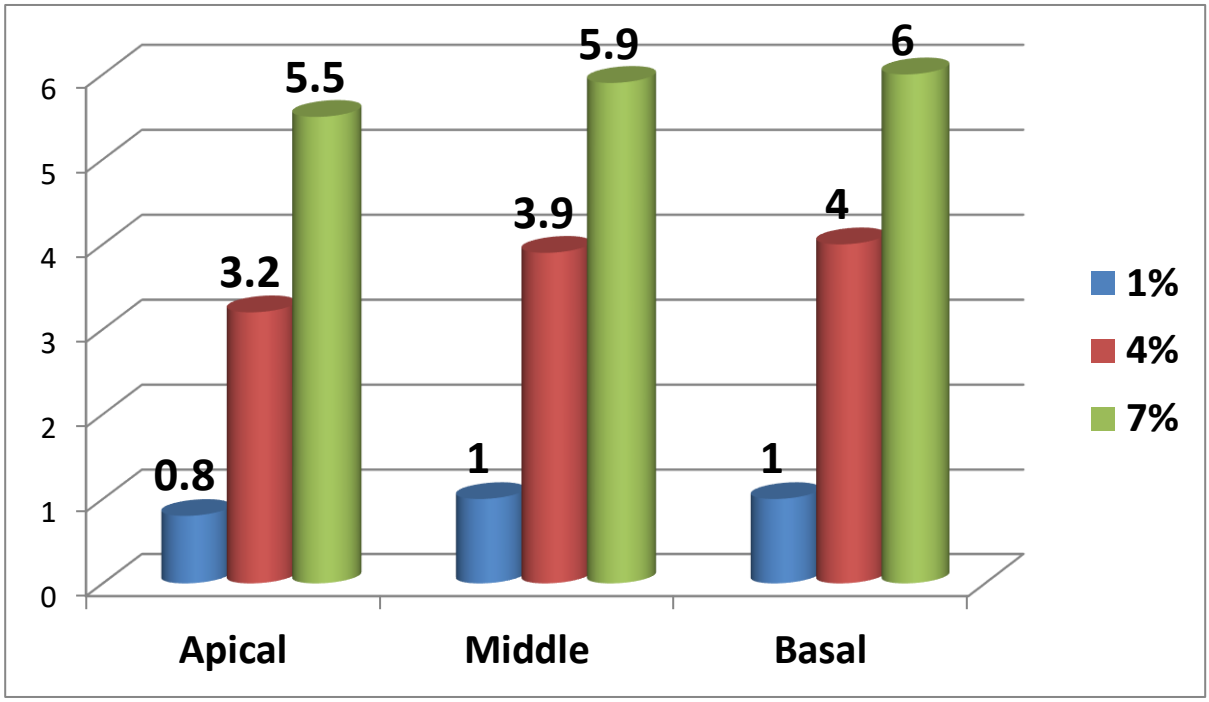

Figure 4.5 Retention of Copper sulphate-Boric acid formulation in *M. baccifera*

4.4 Effect of two step treatment on Moisture content and basic density in *M. baccifera*

Moisture content (%) and density (g/cm^3) were recorded for three different portions of muli bamboo after two step treatment and are summarized in the table 4.3. A significant reduction in the moisture content ($p<0.05$) was noticed after the treatment where as the density was also reduced albeit very slightly. Untreated samples showed moisture content ranged from 39.02 % to 50.23 % from apical to basal portions. After treatment, the moisture was less than 1 % in all the portions of bamboo. However,

before treatment density was in the range of 0.746 (g/cm^3) to 0.817 (g/cm^3) in apical and basal portions which was reduced to 0.69 (g/cm^3) to 0.74 (g/cm^3) after the treatment in apical and basal portions respectively. Bamboo samples before (Figure 4.6) and after (Figure 4.7) step II treatment is shown below. It can be observed that green bamboo lost about one third to half the moisture after the treatment whereas density was notably reduced to an extent of 0.1 (g/cm^3) after the treatment which can be considered a slight decrease.

Table 4.3 Effect of two step treatment on Moisture content and basic density in *M. baccifera*

	Moisture Content %			**Basic Density (g/cm^3)**		
Treatments	**Apical**	**Middle**	**Basal**	**Apical**	**Middle**	**Basal**
Control	39.02±1.65	40.75±3.53	50.23±1.84	0.75±0.076	0.79±0.069	0.82±0.041
Step I at 1% + Step II Neem Oil	0.55±0.10	0.49±0.18	0.33±0.06	0.66±0.005	0.69±0.026	0.74±0.012
Step I at 4% + Step II Neem Oil	0.38±0.07	0.40±0.29	0.53±0.27	0.65±0.009	0.69±0.018	0.74±0.016
Step I at 7% + Step II Neem Oil	0.42 ± 0.18	0.53±0.30	0.41±0.10	0.66±0.010	0.69±0.023	0.74±0.069

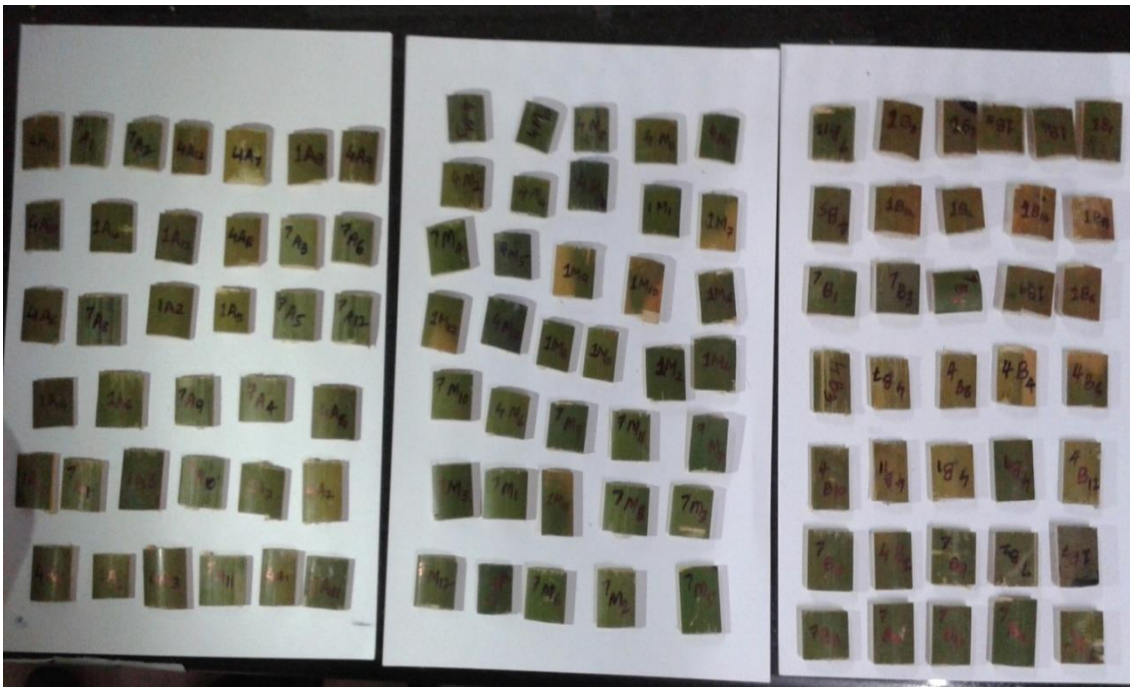

Figure 4.6 *M. Baccifera* test samples before Neem Oil Heat Treatment

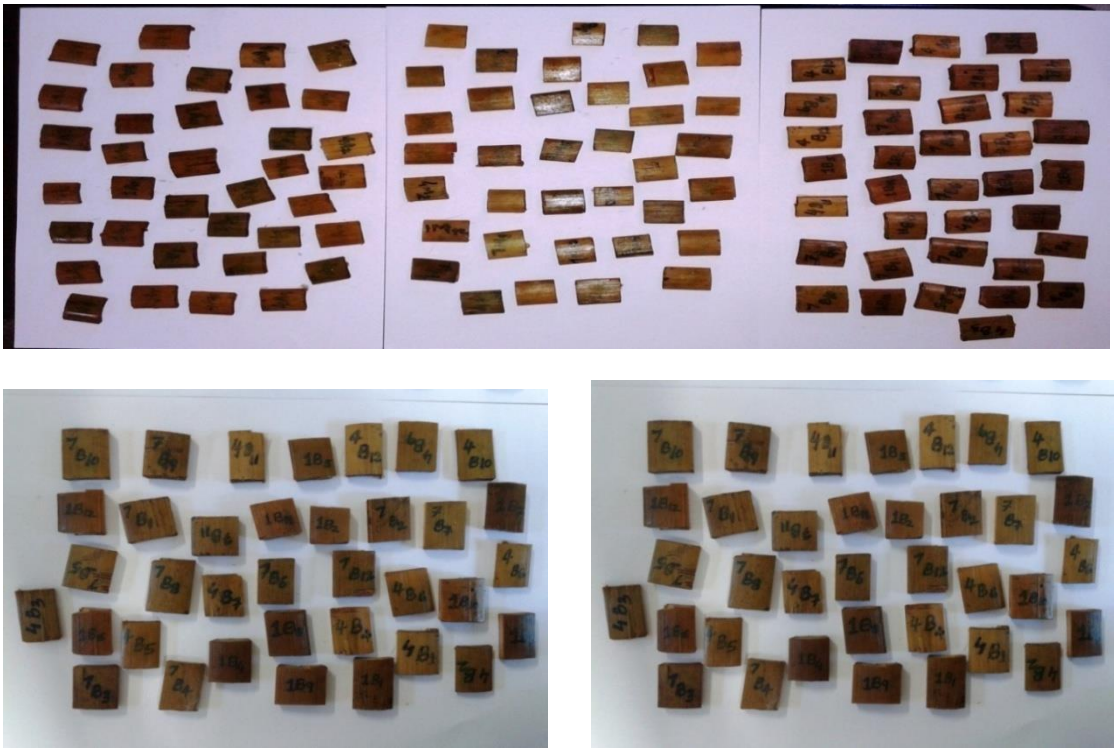

Figure 4.7 Bamboo samples after step II treatment

4.5 Weight loss in *M. baccifera* samples after Two- Step Treatment

Percent weight losses in the treated samples with respect to the initial weight were recorded to know the effect of treatment on weight of different portions of muli bamboo. Figure 4.8 shows an indifferent trend in terms of weight loss (%) among the apical, middle and basal portions as well as for three different concentrations of treatment. Weight loss percent was in the range of 25 % to 34 % due to the effect of treatment. Weight loss was caused by the hot oil treatment. In the basal portion of the bamboo the hot oil treatment caused a weight loss of 30 to 34 % irrespective of the step I treatment, whereas that in middle portion of the bamboo was in the range of 26 to 30 % and in apical portion it ranged from 25 to 30 %.

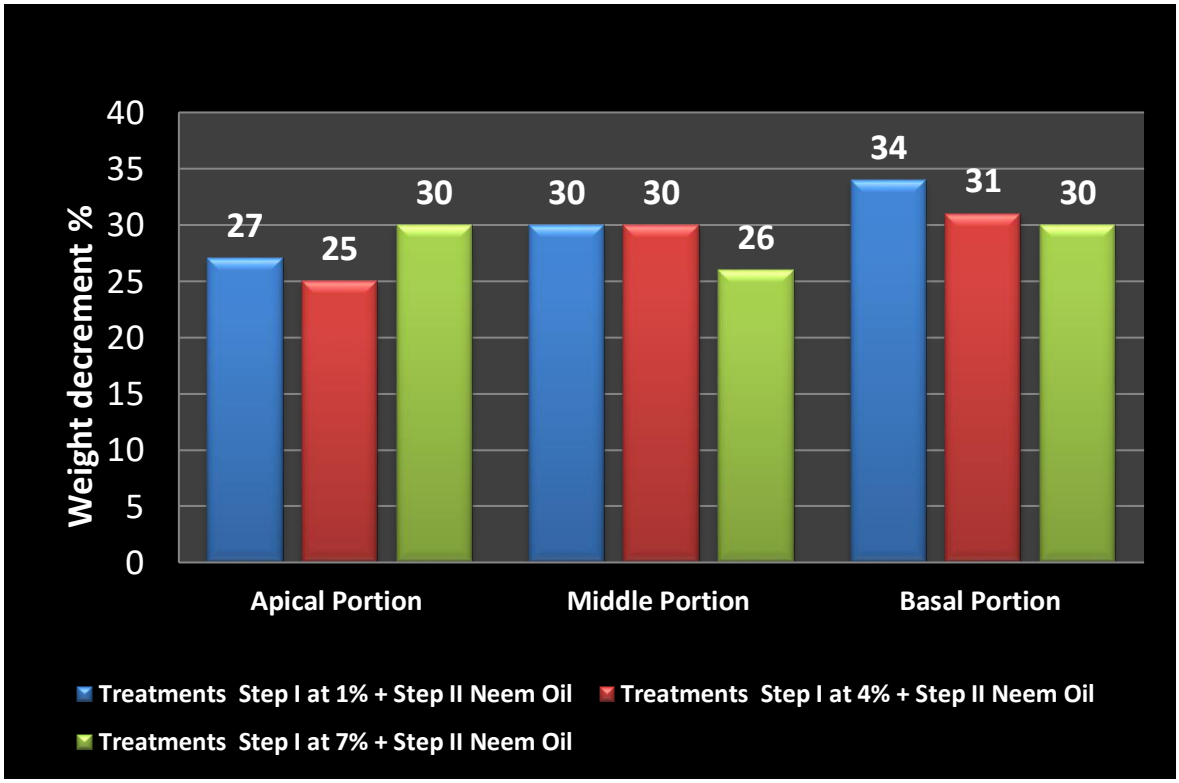

Figure 4.8 Percent Weight loss in *M. Baccifera* samples after Two- Step Treatment

4.6 Boron leached from *M. baccifera* samples treated with Step I (Copper sulphate - Boric acid) + Step II (Neem Oil Heat treatment).

Boron is a leachable element in the preservatives in the absence of a fixing agent. In this experiment effectiveness of the oil treatment (step II) in reducing leaching of boron was observed. It is evident from the Figure 4.9 that leaching of boron is reduced after step II treatment in all the portions of bamboo. Further, increase in duration of leaching process (from 24 hours to 120 hours) has shown decrease in leaching of boron from the treated samples. It is also important to note that greater amount of boron (up to 30 mg/l) was leached from the apical portion of bamboo before oil treatment compared to middle and basal portions (>10 mg/l). Oil treatment, however has reduced the leaching of boron to < 5 mg/l in all the portions of bamboo.

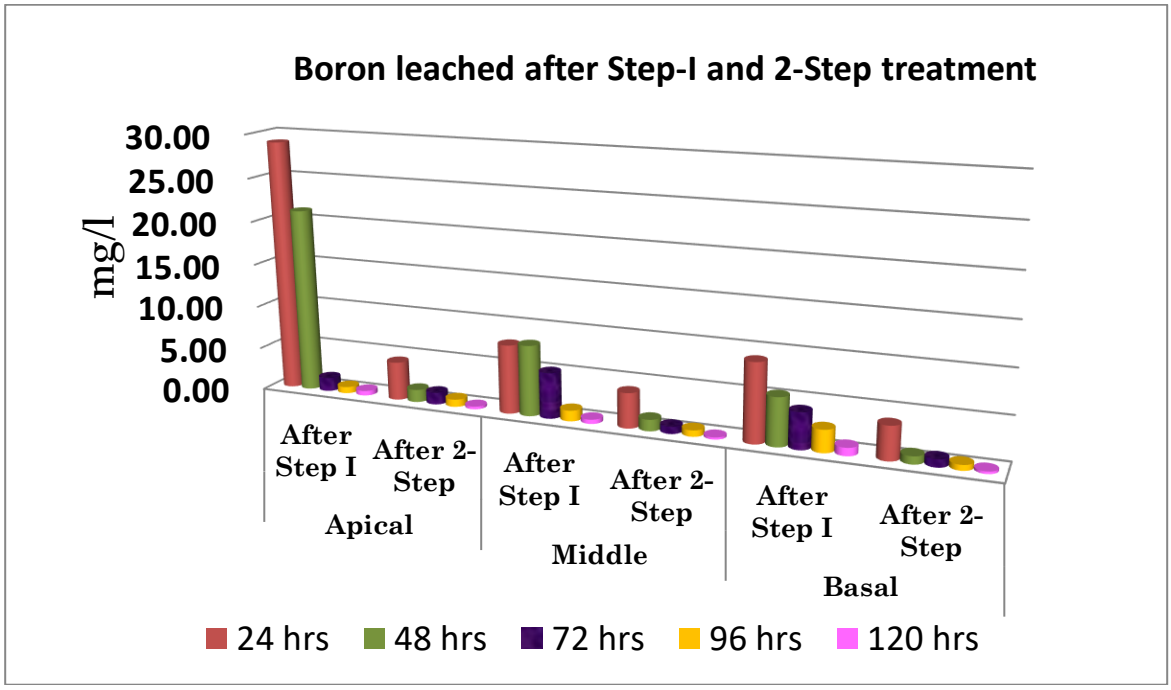

Figure 4.9 Boron leached from *M. baccifera* samples treated with Step I and treated with Step I (Copper sulphate : Boric acid) + Step II (Neem Oil Heat treatment)

4.7 Copper leached from *M. baccifera* samples treated with Step I (Copper sulphate - Boric acid) + Step II (Neem Oil Heat treatment)

Copper in the absence of chromium is also a leachable salt similar to boron. But, step II treatment with hot neem oil in the present study has considerably reduced the leaching of copper from the treated bamboo in all the three portions. From figure 4.10 it can be observed that although in basal and middle portions the leaching was less before hot oil treatment as compared to apical portion, after step II treatment, apical treated portions also showed significant reduction in the amount of leached preservative.

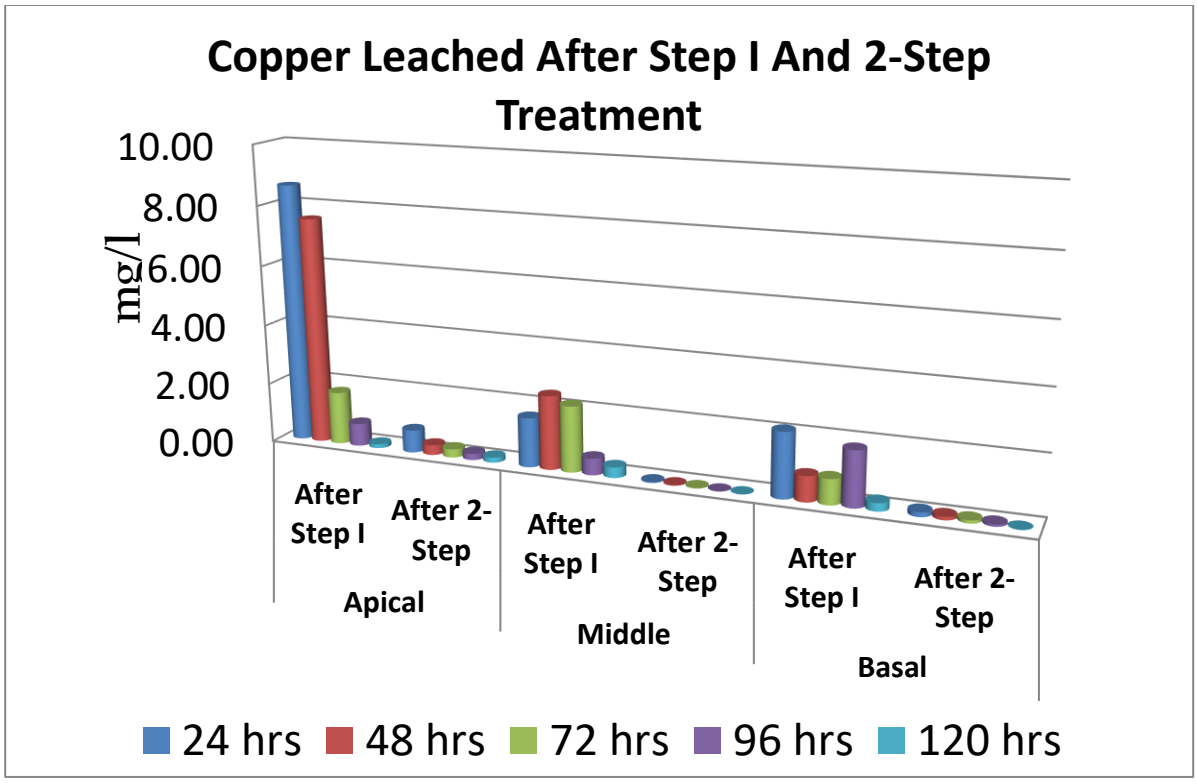

Figure 4.10 Copper leached from *M. baccifera* samples treated with Step I and treated with Step I (Copper sulphate : Boric acid) + Step II (Neem Oil Heat treatment)

4.8 Effect of Step I treatment and Two-Step treatment on Volumetric Swelling & Volumetric Shrinkage of *Melocanna baccifera*

In the present study, shrinkage and swelling are the properties indicating dimensional stability of the material. From Table 4.4 it can be observed that untreated bamboo samples have shown higher volumetric shrinkage as well as volumetric swelling. Among the untreated samples apical portion of bamboo showed higher swelling (20.2 %) and shrinkage (26.33 %). Middle and basal portions indicated similar values of shrinkage and swelling. Subsequently with step I treatment (1%, 4% and 7% concentrations of copper-boron mixture) there was gradual decrease in swelling and shrinkage. After step II treatment (Hot oil treatment) there was significant reduction in shrinkage but gradual but significant reduction in swelling. The basal portion of bamboo showed higher dimensional stability with reduction in shrinkage and swelling values down to 0.401 % and 1.97 % respectively after the hot oil treatment. Overall, the results revealed that step I treatment caused gradual decrease in shrinkage and swelling whereas step II treatment was effective in significantly reducing the same. Apical portion of bamboo was highly dimensionally unstable followed by middle and much stability was noticed in basal portion of the treated bamboo.

Table 4.4 Effect of Step I treatment and Two-Step treatment on Volumetric Swelling & Volumetric Shrinkage of *M. baccifera*

	Volumetric Swelling (%)				**Volumetric Shrinkage (%)**			
Portions (B)					**Portions (B)**			
Treatment (A)	**B1 (Apical)**	**B2 (Middle)**	**B3 (Basal)**	**Mean A**	**B1 (Apical)**	**B2 (middle)**	**B3 (Basal)**	**Mean A**
A1 (Control)	20.201	18.471	18.311	18.994	26.325	25.092	25.26	25.559
A2 (Step I at 1%)	19.3	17.295	17.413	18.003	22.191	20.686	19.736	20.871
A3 (Step I at 4%)	17.286	16.353	16.416	16.685	20.53	18.293	17.248	18.69
A4 (Step I at 7%)	16.102	15.086	14.391	15.193	17.1	16.317	17.12	16.846
A5 (Step I at 1% + Step II)	14.305	12.196	11.782	12.761	13.705	3.773	4.129	7.202
A6 (Step I at 4% + Step II)	12.402	8.707	8.265	9.791	1.844	6.143	2.333	3.44
A7 (Step I at 7% + Step II)	8.569	7.295	**1.978**	5.947	1.347	1.254	**0.401**	1.001
Mean B	15.452	13.629	12.651		14.72	13.08	12.318	
Factors	**C.D.$_{0.05}$**	**SE(d)**	**SE(m)**		**C.D. $_{0.05}$**	**SE(d)**	**SE(m)**	
Factor(A)	0.775	0.393	0.278		0.715	0.362	0.256	
Factor(B)	0.507	0.257	0.182		0.468	0.237	0.168	
Factor(A X B)	1.343	0.680	0.481		1.239	0.627	0.444	

4.8 Efficacy of two step treatment against the white rot fungi (*S. commune*)

Referring to the weight loss (%) of treated (Step I and II) and untreated (control) samples from Table 4.5 it can be deduced that the treatment A7 (Step I at 7% + Step II) resulted in significantly lower weight loss (5.438 %) in basal samples of Muli bamboo and on an average 6.24% considering all the portions of bamboo. Highest weight loss was observed in untreated samples A1 (Control) with 30.06% in apical portion and on an average 26.52 %. On an average the step I treatment at 7% concentration of copper boron mixture could resist the fungal decay reasonably bringing down the weight loss to 12.2 %. Table 4.6 shows the resistance category and life expectancy of Muli bamboo against white rot fungi.

While step 1 treatment at 7% concentration indicated the basal portion of bamboo to be “moderately resistant” with life expectancy of 3-6 years; the step II treatment in the basal portion indicated “resistant” category with life expectancy of 6-8 years. White rot is more serious decay compared to other types of decay. It is important to note that step I treatment alone at the highest concentration of copper and boron (7%) could only make the basal portion of treated bamboo moderately resistant, while Step II treatment along with the lowest concentration of copper boron (1%) could make the middle portion also moderately resistant and in subsequent concentrations make the bamboo resistant to white rot fungi. This clearly justifies the importance of fixation of the copper boron salt and also the efficacy of neem oil in enhancing the resistance of the Muli bamboo against the white rotting fungi (*S. commune*). Since the combination of

treatment is eco-friendly because of the use of natural product, this will highlight the significance of the results.

Table 4.5 Weight loss in *M. baccifera* samples treated with Step I and samples treated with Two-Step process against white rot *S. commune*

Treatment	**Weight loss (%)**			
	B1 (Apical)	**B2 (Middle)**	**B3 (Basal)**	**Mean A**
A1 (Control)	**30.061**	25.607	23.909	26.526
A2 (Step I at 1%)	27.447	24.734	23.424	25.202
A3 (Step I at 4%)	21.754	19.462	19.421	20.212
A4 (Step I at 7%)	14.692	11.415	10.686	12.264
A5(Step I at 1% + Step II)	21.072	8.763	6.141	11.992
A6 (Step I at 4% + Step II)	8.294	7.509	6.23	7.344
A7 (Step I at 7% + Step II)	6.531	6.757	**5.438**	**6.242**
Mean B	18.55	14.892	**13.607**	
Factors	**C.D.** $_{0.05}$	**SE(d)**	**SE(m)**	
Factor(A)	0.866	0.436	0.308	
Factor(B)	0.567	0.286	0.202	
Factor(A X B)	1.5	0.755	0.534	

Table 4.6 Resistance category and life expectancy of *M. baccifera* against *S. commune* based on weight loss

	Apical		**Middle**		**Basal**	
Treatment	**Resistance category**	**The expectancy of service life (years)**	**Resistance category**	**The expectancy of service life (years)**	**Resistance category**	**The expectancy of service life (years)**
A1 (Control)	Perishable	<2	Non-resistant	2-3	Non-resistant	2-3
A2 (Step I at 1%)	Non-resistant	2-3	Non-resistant	2-3	Non-resistant	2-3
A3 (Step I at 4%)	Non-resistant	2-3	Non-resistant	2-3	Non-resistant	2-3
A4 (Step I at 7%)	Non-resistant	2-3	Non-resistant	2-3	**Moderately resistant**	**3-6**
A5(Step I at 1% + Step II)	Non-resistant	2-3	**Moderately resistant**	**3-6**	**Moderately resistant**	**3-6**
A6 (Step I at 4% + Step II)	**Moderately resistant**	**3-6**	**Moderately resistant**	**3-6**	**Resistant**	**6-8**
A7 (Step I at 7% + Step II)	**Moderately resistant**	**3-6**	**Moderately resistant**	**3-6**	**Resistant**	**6-8**

4.9 Efficacy of Step I and Step II treatment in *M. baccifera* samples against brown rot fungi (*Coniophora puteana*)

Weight loss in untreated samples due to brown rot fungi (*Coniophora puteana*) was observed to be 39.7 %, 37.8 %, 35.7 % in apical, middle and basal portion of bamboo respectively (**Table 4.7**). Maximum inhibition/ lowest weight loss was observed in treatment A7 (Step I at 7% + Step II) with weight loss values of 3.3 %, 3.4 % and 4.9% in basal, middle and apical portions respectively. On an average considering all the portions of bamboo the step I treatment with 7% concentrations of copper boron was able to limit the weight loss to about 12 %. Among the portions weight loss was significantly lower ($p<0.05$) in basal portion followed by middle and apical portion. **Table 4.8** shows the resistance category and the life expectancy of treated Muli bamboo against the brown rot fungus. Untreated and step I (1 % Copper-Boron) were both ineffective and indicated the perishable category with life expectancy of <2 years where as step II (Copper boron 4% and 7% + Hot oil) showed resistance with life expectancy of 6-8 years. Pictures of samples of bamboo treated with step I and Step II incubated with both brown rot and white rot fungus are shown in Figure 4.11 and 4.12 respectively.

Table 4.7 Weight loss in *M. baccifera* samples treated with Step I and samples treated with Two-Step process against brown rot *C. puteana*

Weight loss %				
Treatments (A)	**B1 (Apical)**	**B2 (Middle)**	**B3 (Basal)**	**Mean A**
A1 (Control)	**39.702**	37.784	35.705	37.73
A2 (Step I at 1%)	31.315	30.85	29.677	30.614
A3 (Step I at 4%)	21.463	18.32	15.357	18.38
A4 (Step I at 7%)	14.456	11.369	10.48	12.102
A5(Step I at 1% + Step II)	11.028	8.001	7.447	8.825
A6 (Step I at 4% + Step II)	6.06	5.955	4.493	5.503
A7 (Step I at 7% + Step II)	4.942	**3.424**	**3.378**	**3.915**
Mean B	18.424	16.529	**15.22**	
Factors	**C.D. $^{0.05}$**	**SE(d)**	**SE(m)**	
Factor(A)	**0.885**	0.446	0.315	
Factor(B)	**0.579**	0.292	0.206	
Factor(A X B)	**1.532**	0.772	0.546	

Table 4.8 Resistance category and life expectancy of *M. baccifera* against *C. puteana* based on weight loss

	Apical		**Middle**		**Basal**	
Treatment	**Resistance category**	**Service life expectancy (years)**	**Resistance category**	**Service life expectancy (years)**	**Service life expectancy (years)**	**Resistance category**
A1 (Control)	Perishable	<2	Perishable	<2	Perishable	<2
A2 (Step I at 1%)	Perishable	<2	Perishable	<2	Perishable	<2
A3 (Step I at 4%)	Non-resistant	2-3	Non-resistant	2-3	Non-resistant	2-3
A4 (Step I at 7%)	Non-resistant	2-3	Non-resistant	2-3	Non-resistant	2-3
A5(Step I at 1% + Step II)	Non-resistant	2-3	**Moderately resistant**	**3-6**	**Moderately resistant**	**3-6**
A6 (Step I at 4% + Step II)	**Moderately resistant**	**3-6**	**Moderately resistant**	**3-6**	**Resistant**	**6-8**
A7 (Step I at 7% + Step II)	**Resistant**	**6-8**	**Resistant**	**6-8**	**Resistant**	**6-8**

Figure 4.11 Step I treated *M. baccifera* samples against *S. commune* & *C. puteana*

Figure 4.12 Two-Step treated *M. baccifera* samples against *S. commune* & *C. puteana*

4.10 Efficacy of step I and Step II treatment on sap stain fungus (*Alternaria alternata*)

Area coverage (%) of Sap stain fungi *A. alternata* on the bamboo is given in the Table 4.9. Untreated samples showed the complete (100%) coverage by the sap stain fungus. Step I containing Copper boron (7%) could reduce the sap stain attack up to 50 % in basal portion and 60 -65 % in middle and apical portions. Step II treatment (Copper boron + Hot Neem oil) restricted the sap stain attack to 5.167 % in basal portion of bamboo, whereas in middle and apical portions it was 19.67% and 50.33 % respectively. The results indicate there is significant susceptibility of apical and middle portions to sap stain when compared to basal portion. Table 4.10 shows the scoring of intensity of sap stain attack in which heavy to moderate attack can be found in all the portions of bamboo after copper boron treatment at different concentrations. After step II treatment apical and middle portions of bamboo were showing moderate to light attack. Minor attack was found only in the step II treated basal portions of the bamboo. The bamboo culm samples step I treated and step II treated and untreated are shown in the figures 4.13, 4.14 and 4.15 respectively.

Table 4.9 Area coverage (%) of *M. baccifera* samples treated with Step I and samples treated with Two-Step process by sap stain *A. alternata*

	B1 (Apical)	**B2 (Middle)**	**B3 (Basal)**	**Mean A**
A1 (Control)	100	100	100	100
A2 (Step I at 1%)	85.333	80	79.833	81.722
A3 (Step I at 4%)	70.333	65.333	64.5	66.722
A4 (Step I at 7%)	65.5	60.333	50.167	58.667
A5(Step I at 1% + Step II)	75	30.333	14.5	39.944
A6 (Step I at 4% + Step II)	60.333	25.167	9.667	31.722
A7 (Step I at 7% + Step II)	50.333	19.667	**5.167**	**25.056**
Mean B	72.405	54.405	**46.262**	
Factors	**C.D.** $^{0.05}$	**SE(d)**	**SE(m)**	
Factor(A)	1.662	0.837	0.592	
Factor(B)	1.088	0.548	0.387	
Factor(A X B)	2.878	1.45	1.025	

Table 4.10 Scoring of treated *M. baccifera* samples based on intensity of sap stain attack

Attack Category (Score)			
Treatment	**Apical**	**Middle**	**Basal**
Control	Heavy attack (4)	Heavy attack (4)	Heavy attack (4)
Step I at 1%	Heavy attack (4)	Heavy attack (4)	Heavy attack (4)
Step I at 4%	Heavy attack (4)	Heavy attack (4)	Heavy attack (4)
Step I at 7%	Heavy attack (4)	Moderate attack (3)	Moderate attack (3)
Step I at 1% + Step II	Heavy attack (4)	Light attack (2)	**Minor attack (1)**
Step I at 4% + Step II	Moderate attack (3)	Light attack (2)	**Minor attack (1)**
Step I at 7% + Step II	Moderate attack (3)	Minor attack (1)	**Minor attack (1)**

Figure 4.13 Step I treated *M. baccifera* samples against *A. alternata*

Figure 4.14 2-Step treated *M. baccifera* samples against *A. alternata*

Figure 4.15 Control samples of *M. baccifera* against *A. alternata*

CHAPTER 5

DISCUSSION

5 DISCUSSION

5.1 Preliminary screening of Neem Oil and Copper Sulphate-Boric acid formulation (1:1) against Test fungi

Neem oil with azadirachtin as the major component is an assured insect control (Kaur *et al*., 2016), and is reported to be effective against wood decaying fungi (Dhyani *et al*., 2008). Other workers too have tried either neem oil alone on different species like rubber wood (Venmalar and Nagaveni, 2005) or neem leaf extract with copper sulphate and boric acid on mango and rain tree (Islam *et al*., 2009) and have found different degrees of efficacy of neem seed oil in protectiong against both brown rot and white rot fungi.

In a study by Islam *et al*. (2009) neem leaf extract was tried as a fungicide on mango and rain tree. In line with the results of present study, the leaf extract treated wood showed higher resistance against the *S. commune*. At the same concentrations Machado *et al*. (2013) found neem oil to be effective against five species of decay fungi. Therefore, the efficacy shown by neem seed oil can be justified in the current study. Venmalar and Nagaveni (2005) observed similar efficacy of copper formulations against white rot fungi to protect rubberwood. Similar results about Neem oil and copper boron formulations were reported by Selvakumar (2007) and Dhyani (2008).

5.2 Penetration and retention of copper and boron

Basal portion followed by middle portion of bamboo culms showed significantly higher penetration with copper and boron in the current study. Baysal *et al*. (2016) found observed higher penetration of copper and boron in the basal portion of *Phyllostachys bambusoides* bamboo. Liese (1985) was of the opinion that vessels play important role in chemical solution distribution to the surrounding tissues. Untreated pockets, mainly in the parenchyma cells, are vulnerable to attacks. Baysal *et al*. (2016) found similar retention percentage of copper and boron in *Phyllostachys bambusoides*, however, among the other tested preservative chemicals also they found similar retention. Beraldo and Ferreira (2008) discussed that bamboo culms with greater vessel diameter will result in greater penetration.

5.3 Changes in properties of bamboo after heat treatment

In the current study it was observed that green bamboo lost about one third to half the moisture after the treatment whereas density was notably reduced to an extent of 0.1 (g/cm^3) after the treatment which can be considered a slight decrease. Heat treatment produces a material that is less hygroscopic than gently dried bamboo or wood. (Tjeerdsma *et al*., 2005; Obataya *et al*., 2000). There can be considerable variation among them with respect to the absolute values of hygroscopicity (Rowell and Banks, 1985). Salim *et al*. (2010) reported great reduction in density of *Gigantochloa scortechinii*. The reduction in density may be possibly due to the degradation of holocellulose. Metsä-Kortelainen *et al*. (2005), reported wood treated at high

temperature (130-230°C) loss some of its weight and at the same time reduced its density. Yang *et al*. (2016) also reported the decrement in the density of Moso bamboo (*Phyllostachys edulis*) after thermal modifications.

Weight loss percent was in the range of 25 % to 34 % due to the effect of treatment. Weight loss was caused by the hot oil treatment. In the basal portion of the bamboo the hot oil treatment caused a weight loss of 30 to 34 % irrespective of the step I treatment, whereas that in middle portion of the bamboo was in the range of 26 to 30 % and in apical portion it ranged from 25 to 30 %. Similar observations were made by Wahab *et al*. (2009) and Razak *et al*. (2007) in *B. vulgaris* and *G. scortechinii* respectively. The reasons for the percent weight loss of treated samples are same as that for basic density as mentioned earlier. Razak *et al*. (2005) reported improvement in basic density of Semantan bamboo after heat-treatment with palm oil. Manalo *et al*. (2009) tested physical and mechanical properties of three bamboo species *viz*., *Bambusa blumeana*, *B. vulgaris* and *Dendrocalamus asper* after treatment with virgin coconut oil. The improvement in dimensional stability and reduction in strength properties were correlated with temperature but duration seemed to have little or no effect on physical or mechanical properties. (Manalo and Acda, 2009). Rafidah S *et al*. (2010) studied the effect of oil heat treatment on physical properties of *Gigantochlora scortechinii.* These, study showed that the bamboo became less hygroscopic when subjected to higher temperature and longer heat treatment time. The oil heat treatment imparts the dimensional stability of the bamboo. Syrjanen (2001) found that heat treatment at temperature over 150°C reduced the shrinkage and swelling of wood and improved the equilibrium moisture

content. They also stated that the decrement in shrinkage can also be attributed to the reduction in the hemicellulose content, which would have possibly improved the dimensional stability of the wood. In a similar research, Tjeerdsma *et al*. (1998), found that the hygroscopicity of heat treated wood reduced to 60% compared to those untreated and the dimensional stability improved to 50%. Improvement of dimensional stability of heat treated wood was also reported by Seborg *et al*. (1953). They stated that the improvement was due to the loss of constitutional water in wood. Kamden *et al*. (2002) and Tjeerdsma *et al*. (2000) revealed that heat treatment enhanced cross-linking reactions of formaldehyde generated during the decomposition of wood organic acids and the phenol units of wood lignin. This theory may partially explain the dimensional stability of heat treated wood.

5.4 Fixation of copper and boron after two step treatment

Lahiry (1998) reported that leachability of copper preservative was accelerated just after the treatment but it is gradually lowered when the fixation took place. He has also mentioned that temperature accelerated the fixation process. Therefore, it can be justified that hot oil treatment has improved the fixation of copper and boron in the treated bamboo in the present study and as the duration increased from 24 hrs to 72 hrs, the fixation was also improved in all the three portions of the treated bamboo. Similar findings were reported by Jiang and Zhang (2008).

5.5 Efficacy of the treatment against decay fungi

In the current study the combination of neem oil and copper boron was highly effective against both white rot and brown rot fungi, moderately effective against sap stain fungi. Various plant extracts and eco friendly preservative chemicals such as neem leaf extract, oleander, guayule, paper mill effluent, bio oil, ZiBOC, palm oil etc have been tried in the past and found to be effective at varying degree against decay fungi as well as termites. These results are summarized in the **Table 5.1** and **Table 5.2** in comparison to the results of current study. Venmalar and Nagaveni (2005) reported that the formulations of copper with crude cashew nut shell liquid and neem oil gave complete protection to rubber wood (*Hevea brasiliensis*) against white rot fungus. They reported the weight loss was reduced from 60% to 10 % after treatment. In the current study, the weight loss came down to below 10 % with treatment A6 (4% copper-boron + Step II). Similar efficacy was reported by Islam *et al* (2009) and Machado *et al* (2013). Studies conducted by Venmalar and Nagaveni (2005) on other brown rotting fungi *Polyporus meliae undrew* also reported the combination of Neem oil, CNSL and Copper – Boron bringing down the weight loss from 43 % to below 10%, conforming to the results of current study. Brown rot fungi degrade polysaccharides in wood preferentially but also partially oxidize lignin (Eriksson *et al*., 2012). Tomak *et al.*(2012) had also shown that weight loss was lower in basal portions as compared to apical portions of bamboo culms affected by *C. puteana.* According to Wei *et al*. (2013) *C. puteana* caused weight loss of about 20% in wood and wood products.

Kim *et al.* (2011) have reported that *Alternaria alternata* is the most frequent isolate from the bamboo being decayed. They found the isolation frequency of *A. alternata* to be 23.8 %. Efficacy of copper and boron against mould and stain fungi is well documented already (Schmidt *et al.* 2013). From earlier studies by Dawson – Andoh *et al*. (2000) there are instances where *A. alternata* was fully inhibited by essential oils like Citronella, Geranium and Cedar. This confirms the efficacy of essential oils from plant extract against surface moulds and sap stain fungi.

Table 5.1 Plant extracts investigated for wood/ bamboo preservation

Formulations	**Fungal resistance**	**Termite resistance**	**Comments**
Guayule	Weight loss 4.64 % (Nakayama *et al.* 2000)	N/A	An acetone extracted Guayule was strongly antifungal
Neem leaf extract with copper sulphate and boric acid	Weight loss negligible (Islam *et al.* 2009)	Weight loss 3-5% (Islam *et al.* 2009)	Wood treated with this extract was buried in the field through 9 months of investigation
Neem seed oil with sodium chloride	N/A	0% (Himmi *et al.* 2013)	Neem seed oil dissolved into methanol and 5 % NaCl solution extracted with petroleum ether impacted complete resistance to termite attack when applied to rubber wood
Neem oil	Weight loss 25% (Kaur *et al* 2016 a)	Weight loss 100% in the field (Kaur *et al* 2016 b)	Neem oil was ineffective at protecting bamboo under laboratory as well as field conditions
Neem seed oil with copper oxide	Weight loss 5-7 % (Venmalar and Nagaveni 2005)	N/A	Neem oil alone gave good protection to rubber wood from fungal attack
Nerium oleander	Weight loss 6.33 % (Goktas *et al* 2007a)	N/A	An ethanol extract of powdered leaves and flowers impregnated into wood imparted fungal resistance
Chengal wood	Weight loss 9% (Yamamoto and Hang,	Termite mortality 100 %	The solution was effective at causing complete termite mortality

	1988)	(Kadir *et al* 2014)	
Cashew Nut Shell Oil	Weight loss < 10% (Venmalar and Nagaveni 2005)	Sound in the field till 24 months (Venmalar and Nagaveni 2005)	Wood samples impregnated with this solution showed on an average 7-8 times increase in service life
Cedar wood oil	Weight loss 3-19% Tumen *et al* 2013	N/A	Detailed information on field performance not available
Camphor leaf extract	Weight loss 6.6% Xu *et al* 2013	N/A	A mixture of camphor leaf extract with melamine modified UF resin was effective bamboo preservative
Neem leaf extract on bamboo	N/A	90% effective on most pests (Boateng and Kirabena, 2019)	3 weeks trial confirm effectiveness of neem leaf extract against most pests on various domestic products such as chopsticks, ladles, chopping boards and fruit trays made of bamboo
Palm oil	100 % inhibition of sap stain (Sunarta *et al*., 2011)	100 % resistance against termite after 5 days (Sunarta *et al*., 2011)	Palm oil was effective in complete inhibition against *A. alternate* (Sap stain) on Pine wood and against termites as well.
Lantana camara leaf extract	100 % inhibition of *Tremates versicolor* and *Oligophora placentus* (Tripathi *et al.,* 2009)	N/A	Complete inhibition was recorded against *Tremates versicolor* and *Oligophora placentas* in lab conditions
Copper incorporated oils of *Pongamia* and *Jatropha*	Moderate to good protection against decay fungi in rubber wood.	N/A	Slight amount of copper incorporation made Pongamia and Jatropha oils effective against decay fungi in both field and lab conditions

	(Venmalar, 2017)		
Present Study Copper - Boron (Step I) + Neem oil heat treatment (Step II)	5.4 to 6.7 % weight loss against *S.commune* (White rot) 3.3-4.9 % weight loss against *C. puteana* (Brown rot) 9.6 – 60.3 % area coverage against *A. alternata* (Stain fungi)	N/A	The combination of neem oil and copper boron was highly effective against both white rot and brown rot fungi, moderately effective against sap stain fungi.

Table 5.2 Eco-friendly chemicals used for enhancing the durability of wood / bamboo

Eco-friendly formulations	**Fungi and termite resistance**	**Field testing**	**Observations**	**Author/s**
Bio oil	Excellent decay resistance when not leached, activity is lost on leaching	N/A	Highly durable under laboratory condition	Mohan *et al*., 2008 Freel and Graham, 2002
Paper mill effluent	Provide complete protection	No insect attack in the field up to nine months of installation	-	Kaur *et al*., 2016
Tannin copper complex	High level protection of tannin-ammonia copper agents against termite	N/A	Fungal resistance not reported	Yamaguchi *et al*., 2002
ZiBOC	Provide 100% protection	N/A	Solution showed	Rawat *et al*, 2015

	against various fungi		encouraging results under laboratory condition	Tripathi, 2010
N-N- (1,8- napthalyl-hydroxyl amine) (NHA-Na)	Termite mortality can be improved by adding fixing agents	N/A	No weight loss of un leached samples was observed	Kose *et al*., 2009
Pyroligneous acids	Fairly effective against white rot and brown rot fungus	N/A	Soil block bioassay for bamboo and rubber wood showed 12 weeks of resistance	Theapparat *et al*., 2015
Present Study Copper – Boron solution	Complete inhibition was observed at 0.4 % concentration against *S.commune* (White rot) and *C. puteana* (Brown rot) where as against A. alternate (Sap stain) complete inhibition achieved at 0.2 % concentration.	N/A	Fungal toxicity is remarkable. Leachability is the cause of concern, therefore fixing by second step was undertaken	Kanchan Rawat

CHAPTER 6

SUMMARY AND CONCLUSION

6 SUMMARY AND CONCLUSION

Melocanna, like most of the bamboo species is naturally less durable (≤2 years) as it is prone to fungal attack. Preliminary screening of the neem oil against the test fungi showed as there was gradual increase in concentration of Neem oil the growth inhibition rate of each fungus was also increased. The 50% of growth inhibition was achieved in *S. commune* at 2.0% concentration of neem oil. Complete inhibition for *A. alternata* and *C. puteana* occurred at the highest concentration taken in the experiment i.e., 10.0% while for *S. commune* it was achieved at 8.0%. Similarly, the copper boron complex effected 100 % growth inhibition at 0.4 % concentration of Copper sulphate – boric acid (1:1) for *C. puteana* and *S. commune* where as for *A. alternata* it was observed at the concentration of 0.2 %. 50 % growth inhibition was observed for *C. puteana* and *S. commune* at 0.06 % concentration and that for *A. alternata* was observed at 0.08 %.

The 2-Step treatment process significantly improved dimensional stability of *M. baccifera* bamboo by reducing volumetric swelling and volumetric shrinkage. Among the portions of *M. baccifera*, basal portion was found to be significantly resistant to decay fungi as well as dimensionally stable followed by middle & apical portions. The Oil Heat Treatment dried the bamboo from green condition to 0.3-0.6 % moisture content in 30 minutes treatment duration. So it has an added advantage of faster drying. Neem oil Heat Treatment at 200°C for 30 minutes was effective in fixing the copper and boron. The 2-Step treatment process had significantly restricted the decay in *M. baccifera* caused by *S. commune, C. puteana* & *A. alternata.* As a major indication of the results of the current study, 2-Step treatment

(Copper – Boron + Heated neem oil) was promising to enhance the service life of bamboo by 5 to 10 times depending on the exposure.

Post harvest degradation of bamboo culms by fungi is a major challenge in bamboo utilization and preservation. In spite of versatile nature of bamboo material, it is considered as temporary material. Natural durability of bamboo varies from species to species; an untreated bamboo is believed to be degraded within three years of installation. Various fungi toxic formulations are known to control the decay across the world. At the same time some of these chemicals are harmful to the man, livestock and the environment and have been banned in various countries. Bio-control agents against these fungi are very limited and inconsistent. Therefore, the onus is on eco friendly, natural formulations which are safe to use and considerably effective in inhibiting the growth of decay and stain fungi. The current study was aiming to achieve two goals. One was to see if neem oil was effective against white rot, brown rot and sap stain fungi, secondly if it can fix the copper and boron in the treated bamboo (*M. baccifera*) to make the bamboo durable for longer durations.

Increase in durability reduces cost of frequent replacements. Therefore, awareness about preservation is needed among the agencies, industries and personnel involved in bamboo product manufacturing. When preservation is practiced in medium to large scale it is economically viable; even small scale enterprises when formed into cooperative or self help groups, can become economically viable. Bamboo has good carbon sequestration prospect but storage is only for short period. Therefore, preservation can be promoted for improving overall carbon management strategies also. Preservation can boost export potential of

bamboo products from NER to other parts of the country where bamboo resources are limited.

REFERENCES

Adsul, V. B., Khatiwora, E., Torane, R. and Deshpande, N. R. (2012). Antimicrobial activities of Ipomoea carnea leaves. *J. Nat. Prod. Plant Resour.*, *2*(5), 597-600.

Akter, M., Khan, M. A. I., Muhsin, M. D. A., Hamid, K., Ullah, M. O., Bulbul, I. J and Urmi, K. F. (2012). In vitro studies on antibacterial, antifungal, and cytotoxic properties of Leucas aspera. *Biology and Medicine*, *4*(4), 183.

Anagnost, S. E. and Smith, W. B. (1997). Comparative decay of heartwood and sapwood 01; Red Maple.

Anon., (2017). Department of Environment Forest and Climate Change, Govt. of Mizoram. https://forest.mizoram.gov.in/page/bamboo-resources-in-mizoram

Anon., (2008) Methods for laboratory testing of wood preservatives against fungi. Indian Standard: 4873–1968.

Arantes, V., Jellison, J., and Goodell, B. (2012). Peculiarities of brown-rot fungi and biochemical Fenton reaction with regard to their potential as a model for bioprocessing biomass. *Applied Microbiology and Biotechnology*, *94*(2), 323-338.

ASTM, (1980). Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. *American Standard for Testing and Materials, Annual Book of ASTM Standards–part 46 E729-80,* 279-280.

Bahadur, K. N. and Verma, J. (1980). Country Report India. In *Proceedings of the Workshop on Bamboo Research in Asia, Singapore (G. Lessard and A. Chorinard, eds.). IDRC, Ottawa, Canada* (pp. 19-46).

Baysal, E., Tomak, E. D., Topaloglu, E. and Pesman, E. (2016). Surface properties of bamboo and Scots pine impregnated with boron and copper based wood preservatives after accelerated weathering. *Maderas. Ciencia y tecnología*, *18*(2), 253-264.

Beraldo, A. L., and Ferreira, C. G. (2008). Chemical products traceability on treated bamboo. *Journal of Bamboo and Rattan*, *7*(3-4), 177-182.

BIS IS: 401 (2001). Code of Practice for Preservation of Timber. Bureau of Indian Standards New Delhi, India, p. 23.

BIS IS: 1902 (2006) Preservation of bamboo and cane for non-structural purposes. Bureau of Indian Standards, New Delhi

BIS IS: 9096 (2006) Preservation of bamboo and cane for non-structural purposes. Bureau of Indian Standards, New Delhi

Boateng, B. and Owusu, A. K. O. (2019). AZIDIRACHTA INDICA AND ITS POTENTIAL FOR THE PRESERVATION OF BAMBUSA VULGARIS IN GHANA. *European Journal of Social Sciences Studies*. 4 (4): 210-221

Chandra, A., Harsh, N. S. K., Tripathi, S. and Lepcha, S. T. S. (2010). Efficacy evaluation of Lantana camara for bamboo protection. *Indian Forester*, *136*(9), 1261.

Chee, A. A., Farrell, R. L., Stewart, A. and Hill, R. A. (1998, August). Decay potential of basidiomycete fungi from Pinus radiata. In *Proceedings of the New Zealand Plant Protection Conference* (Vol. 51, pp. 235-240).

Chen, Y. D., Qin, W. L., Li, X., Gong, J. and Gong, N. (1985). The chemical composition of ten bamboo species. In *Recent research on bamboo. Proceedings of the International Bamboo Workshop, Hangzhou, China* (pp. 6-14).

Daniel, G., and Nilsson, T. H. O. M. A. S. (1997). Developments in the study of soft rot and bacterial decay. In *Forest products biotechnology* (pp. 47-72). CRC Press.

Datar, V. V. (1995). Antifungal activity of neem (Azadirachta indica) leaves against some phytopathogenic fungi. *Neem for the Management of Crop Diseases. Associated Publishing Co., New Delhi*, 49-51.

Dawson-Andoh, B. E., Lovell, R. and Kamdem, D. P. (2000). Inhibitory and compatibility effects of essential oils on sapstain and biological control fungi. *Journal of Essential Oil Research*, *12*(4), 509-515.

Deka, P. C., Gayatri, B. and Mala, D. (1990). A preliminary investigation of disease of bamboo in north-east region of India. *Indian Forester*, *116*(9), 714-716.

Derbyshire, H., and Miller, E. R. (1981). The photodegradation of wood during solar irradiation. *Holz als Roh-und Werkstoff*, *39*(8), 341-350.

Desalegn, G., and Abegaz, M. (2012). Increasing the Service Life of Bamboo Culms as Construction and Furniture Material by Controlling Biodeterioration and Rational Utilization. *Forestry and Forest Products in Ethiopia*.

Dev, I., and Nautiyal, S. N. (2004). An eco-friendly wood preservative formulation (CRCNSL). *Journal-Timber Development Association of India.*, *50*(1/2), 19-25.

Dhyani, S. (2008). *Development of wood preservative from neem leaves and seeds* (Doctoral dissertation, PhD thesis, Indian Council of Forestry and Research Education, Forest Research Institute, Dehradun, India).

Eriksson, K. E. L., Blanchette, R. A. and Ander, P. (2012). *Microbial and enzymatic degradation of wood and wood components*. Springer Science and Business Media.

Fisher, C. W. (1968). Effluent handling for wood preserving plants environment health and safety section. Research Department, Koppers Co. *Inc., Pittsburg, pa*.

Freel, B. and Graham, R. G. (2002). *U.S. Patent No. 6,485,841*. Washington, DC: U.S. Patent and Trademark Office.

Gilbertson, R.L. (1981). North American wood-rotting fungi that cause brown rots. Mycotaxon 12:372–416

Gnanaharan, R. (2000). Preservative treatment methods for bamboo: A review. *Technical Rep*, *177*, 1-20.

Gogoi, J., Teron, R., and Tamuli, A. K. (2013). Blight disease of *Bambusa balcooa* Roxb.: A potential threat to village bamboo groves of Dimapur district in Nagaland state.

Goktas, O., Mammadov, R., Duru, E. M., Ozen, E., Colak, M. A., and Yilmaz, F. (2007b). Introduction and evaluation of the wood preservative potentials of the

poisonous Sternbergia candidum extracts. *African Journal of Biotechnology*, *6*(8). 982-986

Goktas, O., Mammadov, R., Duru, M. E., Ozen, E., and Colak, A. M. (2007a). Application of extracts from the poisonous plant, Nerium Oleander L., as a wood preservative. *African Journal of Biotechnology*, *6*(17). 2000-2003

Goyal, P. and Dev, I. (1982). Effect of extractives on durability of sal (Shorea robusta) vcooà. Journal of Timber Development Association of India 28: 12-1

Green III, F. and Clausen, C. A. (2005). Copper tolerance of brown-rot fungi: oxalic acid production in southern pine treated with arsenic-free preservatives. *International biodeterioration and biodegradation*, *56*(2), 75-79.

Green III, F. and Clausen, C. A. (2003). Copper tolerance of brown-rot fungi: time course of oxalic acid production. *International biodeterioration and biodegradation*, *51*(2), 145-149.

Gupta, P. and Dev, I. (1999). Studies on the Fungicidal Toxicity of Sal (Shorea Robusta) Heartwood Extractives. *JOURNAL-TIMBER DEVELOPMENT ASSOCIATION OF INDIA*, *45*(1/2), 16-24.

Gurung, D. and Singha, B. L. (2013). Carbon sequestration vs. bamboo preservative treatment. *TRADITIONAL KNOWLEDGE*, 88.

Gyansah, L. and Kwofie, S. (2011). Investigation into the performance of bamboo using the notched and the un-notched specimen. *Research Journal of Applied Sciences, Engineering and Technology*, *3*(4), 245-251.

Hassan, W. A., and Abdulkader, A. A. (2008) Soft rot and wood staining fungi and efficiency of their preservatives.

Hegde, N., Oka, K. Rawat, K and Upadhyay, K. K. (2017) Impact of varying temperature and duration on fungal decay resistance of hot oil treated *Dendrocalamus longispathus* (kurz). *Multilogic in Science* 8 (25): 77-81

Himmi, S. K., Tarmadi, D., Ismayati, M. and Yusuf, S. (2013). Bioefficacy performance of neem-based formulation on wood protection and soil barrier against subterranean termite, Coptotermes gestroi Wasmann (Isoptera: Rhinotermitidae). *Procedia Environmental Sciences*, *17*, 135-141.

Humar, M., Pohleven, F. and Žlindra, D. (2006). Influence of water properties on leaching of copper-based preservatives from treated wood. *Wood Research*, *51*(3), 69-76.

Hunt, G. M., and Garratt, G. A. (1967). Wood preservation. *Wood preservation.*, (3rd ed.).

Hyde, K. D., Zhou, D., and Dalisay, T. (2002). Bambusicolous fungi: a review. *Fungal Diversity*. 9: 1-14.

Iqbal, M. C. M., Jayasinghe, U. L. B., Herath, H. M. T. B., Wijesekara, K. B. and Fujimoto, Y. (2004). A fungistatic chromene fromAgeratum conyzoides. *Phytoparasitica*, *32*(2), 119.

Islam, M. M., Shams, M. I., Ilias, G. N. M., and Hannan, M. O. (2009). Protective antifungal effect of neem (Azadirachta indica) extracts on mango (Mangifera

indica) and rain tree (Albizia saman) wood. *International Biodeterioration and Biodegradation*, *63*(2), 241-243.

Izran, K., Razak, W., Zaidon, A., Abood, F., and Norhisham, A. R. (2012). The Effects of Crude Oil Boiling Treatment on Physical Properties of *Bambusa vulgaris* var. Striata (Buluh Gading). *Pertanika Journal of Tropical Agricultural Science*, *35*(4).

Jain, J. K. and Virendra, N. (1991). Arsenated borated cashew nut shell liquid: low cost environmentally safe preservatives. *J Timb Dev Assoc India*, *37*(2), 25-27.

Jha, L. K. and Lalnunmawia, F. (2003). Agroforestry with bamboo and ginger to rehabilitate degraded areas in North East India. *Journal of Bamboo and Rattan*, *2*(2), 103-109.

Jiang, M. L. and Zhang, Y. (2008). Leachability of Copper-Based Wood Preservatives [J]. *China Wood Industry*, *3*.

Jin, T., Wu, Y., and Wang, Q. (2012). The inhibitory effects of bamboo vinegar against bacteria and fungi. In *Information Technology and Agricultural Engineering* (pp. 451-457). Springer, Berlin, Heidelberg.

Joseph, K. V. (1958). Preliminary studies on the seasonal variation in starch content of bamboos in Kerala State and its relation tobeetle borer infestation. *J. Bombay Nat.Hist. Soc*.55, 221 – 227.

Kadir, R., Ali, N. M., Soit, Z. and Khamaruddin, Z. (2014). Anti-termitic potential of heartwood and bark extract and chemical compounds isolated from Madhuca

utilis Ridl. HJ Lam and Neobalanocarpus heimii King PS Ashton. *Holzforschung*, *68*(6), 713-720.

Kamden, D.P., Pizzi, A. and Jermannaud, A., (2002). Durability of heat-treated wood. Journal of Holz als Roh-und Wekstoff. Vol 60: 1-6. DOI 10.1007/s001007-001-0261-1.

Kapse, N.K. (1996). Formulation of biofungicidal preparation to control fungal decay of stored bamboos with particular emphasis on Trichoderma species. PhD thesis, Deemed University, Dehra Dun.

Kardam, V., Pant, K. K., Satya, S., and Naik, S. N. (2013). Scientific Investigation of Traditional Water Leaching Method for Bamboo Preservation. *Bamboo Science and Culture*, *26*(1).

Kaur, P. J., Pant, K. K., Satya, S., and Naik, S. N. (2016). Field investigations of selectively treated bamboo species. *European Journal of Wood and Wood Products*, *74*(5), 771-773.

Kaur, P. J., Satya, S., Pant, K. K., and Naik, S. N. (2016). Eco-friendly preservation of bamboo species: traditional to modern techniques. *BioResources*, *11*(4), 10604-10624.

Khalid, I., Wahab, R., Sulaiman, O., Mohamed, A., Tabet, T. A., and Alamjuri, R. H. (2010). Enhancing colour appearances of cultivated 15 year-old Acacia hybrid through oil heat treatment process. *International Journal of Biology*, *2*(2), 199.

Kim, J. J., Lee, S. S., Ra, J. B., Lee, H., Huh, N., and Kim, G. H. (2011). Fungi associated with bamboo and their decay capabilities. *Holzforschung*, *65*(2), 271-275.

Korkut, D., and Hiziroglu, S. (2014). Experimental test of heat treatment effect on physical properties of red oak (Quercus falcate Michx.) and southern pine (Pinus taeda L.). *Materials*, *7*(11), 7314-7323.

Köse, C., Terzi, E., and Kartal, S. N. (2009). Evaluation of decay and termite resistance of wood treated with copper in combination with boron and N′-N-(1, 8-naphthalyl) hydroxylamine (NHA-Na). *International Biodeterioration and Biodegradation*, *63*(6), 727-731.

Krisdianto. (2008). The effect of microwave heating on permeability of bamboo. *Journal of Tropical Wood Science and Technology* 6: 37–42.

Krishnayyaand, P. V., and Grewal, P. S. (2002). Effect of neem and selected fungicides on viability and virulence of the entomopathogenic nematode Steinernema feltiae. *Biocontrol Science and Technology*, *12*(2), 259-266.

Kumar, R., Tapwal, A., Pandey, S. and Rishi, R. (2013). Fungal diversity associated with bamboo litter from Bambusetum of Rain forest Research Institute, Northeast India. *Biodiversitas Journal of Biological Diversity*, *14*(2).

Kumar, S. Shukla, K.S. Dev, I. and Dobriyal, P.B. (1994). Bamboo Preservation Techniques: A Review. INBAR Technical Report No.3. INBAR, New Delhi and ICFRE, Dehradun. 59 pp.

Kumar, S., and Dobriyal, P. B. (2007). Treatability and Flow Path Studies in Bamboo Part I. Dendrocalamus Strictus Nees. *Wood and fiber science*, *24*(2), 113-117.

Kumar, S., and Kaushik, N. (2013). Endophytic fungi isolated from oil-seed crop Jatropha curcas produces oil and exhibit antifungal activity. *PloS one*, *8*(2), e56202.

Lahiry, A. K. (1998). CCA teachability of slow dried three major bamboo species of Bangladesh. *Bulletin of Materials Science*, *21*(2), 181-184.

Larnøy, E. (2006). *The use of chitosan as a wood protecting agent*. Sierke.

Leithoff, H. and Peek, R. D. (2001). *Heat treatment of bamboo. International research group on wood preservation*. IRG/WP/01-40216, Stockholm.

Lekounougou, S., Petrissans, M., Jacquot, J. P., Gelhaye, E., and Gerardin, P. (2009). Effect of heat treatment on extracellular enzymatic activities involved in beech wood degradation by Trametes versicolor. *Wood Science and Technology*, *43*(3-4), 331-341.

Liese, W. (1959). Bamboo preservation and soft-rot. *Food and Agriculture Organization Report to the Government of India*, *1106*, 36.

Liese, W. (1985). *Bamboos-biology, silvics, properties, utilization*. TZ Verlagsgesellschaft. pp. 196-208.

Liese, W. (1987). Research on bamboo. *Wood Science and Technology*, *21*(3), 189-209.

Liese, W. (1998). *The anatomy of bamboo culms* (Vol. 18). Brill.

Liese, W. and Kumar, S. (2003). Bamboo preservation compendium.

Machado, G. D. O., Cookson, L. J., Christoforo, A. L., Polito, W. L., da Silva, M. R., Junior, C. C., and Lahr, F. A. R. (2013). Wood preservation based on neem oil: Evaluation of fungicidal and termiticidal effectiveness. *Forest Products Journal*, *63*(5), 202-206.

Manalo, R. D., and Acda, M. N. (2009). Effects of hot oil treatment on physical and mechanical properties of three species of Philippine bamboo. *Journal of Tropical Forest Science*, 19-24.

Martawijaya, A. (1975). Laboratory testing on the resistance of wood against fungi. *Kehutanan Indonesia*, 775-777.

Metsä-Kortelainen, S., Antikainen, T., and Viitaniemi, P. (2006). The water absorption of sapwood and heartwood of Scots pine and Norway spruce heat-treated at 170 C, 190 C, 210 C and 230 C. *Holz als Roh-und Werkstoff*, *64*(3), 192-197.

Misdarti. 2006. Qualities of laminated bamboo of Toraja Regency, South Sulawesi. *Journal of Forest Products Research* 24: 183–189.

Mohan, D., Shi, J., Nicholas, D. D., Pittman Jr, C. U., Steele, P. H. and Cooper, J. E. (2008). Fungicidal values of bio-oils and their lignin-rich fractions obtained from wood/bark fast pyrolysis. *Chemosphere*, *71*(3), 456-465.

Mohana, D. C. and Raveesha, K. A. (2007). Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. *Journal of Agricultural Technology*, *4*(1), 119-137.

Mohanan, C. (1994). Diseases of bamboos and rattans in Kerala. *KFRI Researc h Report*, *98*.

Mohanan, C. (1997). An Illustrated manual of diseases of Bamboos in Asia. International network for Bamboo and Rattan, New Delhi.

Moline, H. E., and Locke, J. C. (1993). Comparing neem seed oil with calcium chloride and fungicides for controlling postharvest apple decay. *HortScience*, *28*(7), 719-720.

Nair, S.S. (2006). *Effectiveness of copper-boron diffusion treatments for wood*. M.Sc. Thesis, Department of Forest Products, University of Idaho, US, 91p.

Naithani, H. B. (1993). *Contributions to the taxonomic studies of Indian bamboos* (Doctoral dissertation, Ph. D. Thesis, Vol. IHNB Garhwal University, Srinagar, Garhwal).

Nakayama, F. S., Chow, P., Bajwa, D. S., Youngquist, J. A., Muehl, J. H., and Krzysik, A. M. (2000). Preliminary investigation on the natural durability of guayule (Parthenium argentatum) based wood products. In *[The International Research Group on Wood Preservation. Section 5, Environmental Aspects: 31st annual meeting, Kona, Hawaii, USA, 14-19 May 2000. Stockholm, Sweden: IRG Secretariat, 2000].: 9 p.*.

Nath, A. J., Das, G. and Das, A. K. (2008). Above ground biomass, production and carbon sequestration in farmer managed village bamboo grove in Assam, Northeast India. Bamboo Science and Culture, 21(1).

Obataya, E., Tanaka, F., Norimoto, M., and Tomita, B. (2000). Hygroscopicity of heat-treated wood, 1: Effects of after-treatments on the hygroscopicity of heat-treated wood. *Journal of the Japan Wood Research Society (Japan)*.

Olfat, A. M., Karimi, A. N., and Parsapajouh, D. (2007). Biological method to quantify progressive stages of decay in five commercial woods by Coriolus versicolor. *Pakistan Journal of Biological Sciences*, *10*(7), 1073-1077.

Onuorah, E. O. (2000). The wood preservative potentials of heartwood extract of Milicia excelsa and Erythrophleum suaveolens. *Bioresource Technology*, *75*(2), 171-173.

Purushotham, A., and Tewari, M. C. (1961). A preliminary note on the preparation of copper and zinc preservatives from cashew nut shell liquid. *Journal of Timber Preservation Association of India*, *7*, 8-10.

Rafidah, S., Zaidon, A., Samsi, H. W., Razak, W., and Roziela, H. A. (2010). Effect of oil heat treatment on physical properties of semantan bamboo (Gigantochloa scortechinii Gamble). *Modern Applied Science*, *4*(2), 107-113.

Rani, A., Jain, S., and Dureja, P. (2009). Synergistic fungicidal efficacy of formulations of neem oil, nicotinic acid and Ferula asafoetida with α, β-unsaturated carbonyl compounds against ITCC 5226 Sclerotium rolfsii and ITCC 0482 Macrophomina phaseolina. *Journal of Pesticide Science*, 0908030091-0908030091.

Rawat, M., Tripathi, S. and Pant, H. (2015). Laboratory evaluation el ZiBOC and CCA as an antisapstain on Populus deltoides. *Journal of Eco-friendly Agriculture*, *10*(1), 82-86.

Razak, B. W. (1998). *Effect of selected preservatives on the durability of Gigantochloa scortechinii* (Doctoral dissertation, Imperial College London (University of London)).

Razak, W., Janshah, M., Hashim, W. S., and Shirley, B. (2007). Morphological and anatomical characteristics of managed natural bamboo stands Gigantochloa scortechinii. *Journal of Bamboo and Rattan*, *6*(2), 115-122.

Razak, W., Mohamad, A., Samsi, H. W., and Sulaiman, O. (2005). Effect of heat treatment using palm oil on properties and durability of Semantan bamboo. *Journal of Bamboo and Rattan*, *4*(3), 211-220.

Rose, J. (1969). Copper in History and Distillation. Encyclopedia Brittanica, volume 6, Copper. http://www.aromaticplantproject.com/articles_archive/copper.html

Rowell, R. M., and Banks, W. B. (1985). Water repellency and dimensional stability of wood. *Gen. Tech. Rep. FPL-50. Madison, WI: US Department of Agriculture, Forest Service, Forest Products Laboratory; 1985: 24 pages*, *50*.

Salim, R., Ashaari, Z., Samsi, H. W., Wahab, R., Alamjuri, R. H., and Kinabalu, K. (2010). Effect of oil heat treatment on physical properties of semantan bamboo (Gigantochloa scortechinii Gamble). *Modern Applied Science*, *4*(2), 107-113.

Salim, R., Wahab, R., and Ashaari, Z. (2008). Effect of oil heat treatment on chemical constituents of Semantan bamboo (Gigantochloa scortechinii Gamble). *Journal of Sustainable Development*, *1*(2), 91-98.

Satish, K., and Dobriyal, P. B. (1990). Preservative treatment of bamboo for structural uses. In *Bamboos. Current research. Proceedings of the International Bamboo*

Workshop, Cochin, India, 14-18 Nov. 1988. (pp. 199-206). Kerala Forest Research Institute.

Schmidt, O. (2006) Wood and tree fungi. Biology, damage, protection, and use. Springer-Verlag. Berlin, Heidelberg, 334 pp.

Schmidt, O., Wei, D. S., Tang, T. K. H., and Liese, W. (2013). Bamboo and fungi. *J Bamboo Rattan*, *12*(1-4), 1-14.

Scurlock, J. M. O., Dayton, D. C., and Hames, B. (2000). Bamboo: An overlooked biomassresource? Biomass and Bioenergy, 19, 229–244.

Seborg, R. M., Tarkow, H., and Stamm, A. J. (1953). Effect of heat upon the dimensional stabilisation of wood. *Journal Forest Products Research Society*, *3*(9).

Selvakumar, G., Saha, S., and Kundu, S. (2007). Inhibitory activity of pine needle tannin extracts on some agriculturally resourceful microbes. *Indian Journal of Microbiology*, *47*(3), 267.

Seng, O.D. (1990). Specific Gravity of Indonesian Woods and its Significance for Practical Use. Communication No. 13. Forest Products Research and Development Centre, Bogor.

Sheoran, O. P. (2006). Online statistical analysis tool (OPSTAT).

Siddiqui, I., Bokhari, N. A., and Perveen, K. (2016). Antifungal ability of Nerium oleander against Fusarium oxysporum, Sclerotium rolfsii and Macrophomina phaseolina. *J. Anim. Plant Sci*, *26*(1), 269-274.

Singh, P. (1988). Current status of pests of bamboos in India. In: Rao, I.V.R., Gnanaharan, R; C. B. Sastry, (Eds). Bamboos: Current Research. Proc. of International Bamboo Workshop. 14-18 Nov. 1988. Cochin, India and IDRC, Canada.

Singh, S., Majumdar, D. K., and Rehan, H. M. S. (1996). Evaluation of anti-inflammatory potential of fixed oil of Ocimum sanctum (Holybasil) and its possible mechanism of action. *Journal of Ethnopharmacology*, *54*(1), 19-26.

Singh, U.P, Singh, H.B. and Singh, R.B. (1980). The fungicidal effect of neem (A. indica) extracts on somesoil born pathogens of gram (Cicer arietinum). Mycologia, 72: 1077-1093.

Singha, B. L., and Borah, R. K. (2017). Traditional methods of post harvest bamboo treatment for durability enhancement. *International Journal of Scientific and Engineering Research*, *8*(1), 518-522.

Soni, P. L. (1975). The Chemistry of Extractives of Dalbergia sissoo, PartI, The Occurrence of 3, 5 Dihydroxy-transstilbene in the Heartwood'. *J. Ind. Acad. Wood Sci*, *6*(2), 57-58.

Sorenson, J. R. J. (1976). Copper chelates as possible active forms of the anti-arthritic agents. *J. Med. Chem.* **19**: 135–148.

Standard, I. (2008). Method of tests for bamboo. *Timber and Timber Stores (IS 6874-2008), First Revision. New Delhi: Bureau of Indian Standards*.

Sulthoni, A. (1995). Shooting period of sympodial bamboo species: an important indicator to manage culm harvesting. In *Bamboo, People and the Environment.*

Proceedings of the Vth International Bamboo Workshop and the IVth International Bamboo Congress, Ubud, Bali, Indonesia, 19–22 June 1995, Volume 1: Propagation and Management (pp. 96-100). International Network for Bamboo and Rattan New Delhi, India.

Sunarta, S., Darmadji, P., Uehara, T., and Katoh, S. (2011). Production and characterization of palm fruit shell bio-oil for wood preservation. *Forest products journal*, *61*(2), 180-184.

Sundriyal, R. C., Upreti, T. C., and Varuni, R. (2002). Bamboo and cane resource utilization and conservation in the Apatani plateau, Arunachal Pradesh, India: implications for management. *Journal of Bamboo and Rattan*, *1*(3), 205-246.

Suprapti, S. (2010). Decay resistance of five Indonesian bamboo species against fungi. *Journal of Tropical Forest Science*, 287-294.

Suprapti, S., and Djarwanto, H. (2004). The resistance of five wood species against several wood destroying fungi. *J Penel Hasil Hut*, *22*, 239-246.

Swathi, D., Tripathi, S., and Dev, I. (2004). Preliminary screening of neem (Azadirachta indica) leaf extractives against Poria monticola–a wood-destroying fungus. *J Ind Acad Wood Sci,(NS)*, *1*(1), 2.

Syrjanen, T. (2001). Production and classification of heat treated wood in Finland. In *Proceedings of the special seminar held in Antibes, France, 2001*.

Tanaka, H., Itakura, S., and Enoki, A. (1999). Hydroxyl radical generation by an extracellular low-molecular-weight substance and phenol oxidase activity

during wood degradation by the white-rot basidiomycete Trametes versicolor. *Journal of Biotechnology*, *75*(1), 57-70.

Temiz, A., Kose, G., Panov, D., Terziev, N., Alma, M. H., Palanti, S. and Akbas, S. (2013). Effect of bio-oil and epoxidized linseed oil on physical, mechanical, and biological properties of treated wood. *Journal of Applied Polymer Science*, *130*(3), 1562-1569.

The Telegraph, (2019). https://www.telegraphindia.com/states/north-east/mizo-timber-plans-not-in-place-yet/cid/850298 Saturday, 30 November 2019. E- paper

Theapparat, Y., Chandumpai, A., Leelasuphakul, W. and Laemsak, N. (2015). Pyroligneous acids from carbonisation of wood and bamboo: their components and antifungal activity. *Journal of Tropical Forest Science*, 517-526.

Thompson, W.S., (1971). Contribution of the wood preserving industry to water pollution. In: W.S. Thompson (Ed.), Proceedings of the Conference on Pollution Abetement and Control in the Wood Preserving Industry. Forest Products Laboratory, Mississippi State University, Mississippi, pp. 50–75

Tjeerdsma, B. F., and Militz, H. (2005). Chemical changes in hydrothermal treated wood: FTIR analysis of combined hydrothermal and dry heat-treated wood. *Holz als roh-und Werkstoff*, *63*(2), 102-111.

Tjeerdsma, B. F., Boonstra, M., Pizzi, A., Tekely, P., and Militz, H. (1998). Characterisation of thermally modified wood: molecular reasons for wood performance improvement. *Holz als Roh-und Werkstoff*, *56*(3), 149.

Tjeerdsma, B. F., Stevens, M., and Militz, H. (2000). Durability aspects of (hydro) thermal treated wood. *International Research Group on Wood Preservation, Document no. IRG/WP 00-4*.

Tomak, E. D., Topaloglu, E., Gumuskaya, E., Yildiz, U. C., and Ay, N. (2013). An FT-IR study of the changes in chemical composition of bamboo degraded by brown-rot fungi. *International Biodeterioration and Biodegradation*, *85*, 131-138.

Tomalang, F.N., A.R. Lopez, J.A. Semara, R.F. Casin, and Z.B. Espiloy. (1980). Properties and utilization of Philippine erect bamboo. In: (G.Lessard and A. Chouinard, eds.). International Seminar on Bamboo Research in Asia. Singapore, May 28-30. Singapore: International Development Research Center and the International Union of Forestry Research Organization. pp. 266-275.

Tomar, J. M. S., Hore, D. K., and Annadurai, A. (2009). Bamboos and their conservation in North-East India. *Indian Forester*, *135*(6), 817-824.

Tripathi, S. (2010). Resistances of Chemically Treated Bambusa arundinacea against Fungi, Termite and Fire. *林產化學與工業*, *30*(4), 7-14.

Tripathi, S., Rawat, K., Dhyani, S., and Pant, H. (2009). Potential of Lantana camara Linn. weed against wood destroying fungi. *Indian Forester*, *135*(3), 403.

Tumen, I., Süntar, I., Eller, F. J., Keleş, H., and Akkol, E. K. (2013). Topical wound-healing effects and phytochemical composition of heartwood essential oils of

Juniperus virginiana L., *Juniperus occidentalis* Hook., and *Juniperus ashei* J. Buchholz. *Journal of medicinal food*, *16*(1), 48-55.

Venmalar, D. (2017). Screening of Oils of Pongamia pinnata Linn., Jatropha curcas Linn. and Simarouba glauca DC for Developing Eco-Friendly Wood Preservatives. In *Wood is Good* (pp. 261-268). Springer, Singapore.

Venmalar, D., and Nagaveni, H. C. (2005). Evaluation of copperised cashew nut shell liquid and neem oil as wood preservatives.

Wahab, R., and Samsi, H. W. (2004). Strength and durability of bamboo treated through an oil-curing process. *Journal of Biological Sciences (Pakistan)*.

Wahab, R., Khalid, I., Tabet, T. A., Mohamed, A., Sulaiman, O., Salim, R. M., and Ayob, F. W. (2012). Effectiveness of hot oil treatment on cultivated 15 year-old Acacia hybrid against Coriolus versicolors, Gloeophyllum trabeum and Pycnoporus sanguineus. *Sains Malaysiana*, *41*(2), 163-169.

Wahab, R., Mohamed, A., Mustafa, M. T., and Hassan, A. (2009). Physical characteristics and anatomical properties of cultivated bamboo (Bambusa vulgaris Schrad.) culms. *Journal of Biological Sciences*, *9*(7), 753-759.

Wahab, R., Sudin, M., and Samsi, H. W. (2005). Fungal Colonisation and Decay in Tropical Bamboo Species. *Applied Sci*, *5*, 897-902.

Wedge, D.E, Galindo, J.C.G. and Macias, F.A. (2000). Fungicidal activity of natural and synthetic sesquiterpene lactone analogs. Phytochemistry 53: 74

Wei, D., Schmidt, O., and Liese, W. (2013). Durability test of bamboo against fungi according to EN standards. *European Journal of Wood and Wood Products*, *71*(5), 551-556.

Xu, G., Wang, L., Liu, J., and Hu, S. (2013). Decay resistance and thermal stability of bamboo preservatives prepared using camphor leaf extract. *International Biodeterioration and Biodegradation*, *78*, 103-107.

Yamaguchi, H., Yoshino, K., and Kido, A. (2002). Termite resistance and wood-penetrability of chemically modified tannin and tannin-copper complexes as wood preservatives. *Journal of wood science*, *48*(4), 331-337.

Yamamoto, K., and Hong, L. T. (1988). Decay resistance of extractives from Chengal. *Journal of Tropical Forest Science*, *1*(1), 51-55.

Yang, T. H., Lee, C. H., Lee, C. J., and Cheng, Y. W. (2016). Effects of different thermal modification media on physical and mechanical properties of moso bamboo. *Construction and Building Materials*, *119*, 251-259.

Yudodibroto, H. 1987. Bamboo research in Indonesia. *Proceedings of the International Bamboo Workshop.* 6–14 October 1985, Hangzhou.

Zeelie, J. (1998). Effects of copper and zinc ions on the germicidal properties of two popular pharmaceutical antiseptic agents cetylpyridinium chloride and povidone-iodine. *Analyst*, *123*(3), 503-507.

Zhang, X., Yu, H., Huang, H., and Liu, Y. (2007). Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms. *International Biodeterioration and Biodegradation*, *60*(3), 159-164.

PARTICULARS OF THE CANDIDATE

NAME OF THE CANDIDATE	KANCHAN RAWAT
DEGREE	Ph. D.
DEPARTMENT	FORESTRY
TITLE OF THESIS	DEVELOPING ECO-FRIENDLY PRESERVATIVES And TREATMENT TECHNIQUES For *Melocanna baccifera* (ROXB.) KURZ.
DATE OF ADMISSION	01.08.2014
APPROVAL OF RESEARCH PROPOSAL	1. BOS : 08.05.2015 2. SCHOOL BOARD : 19.05.2015
REGISTRATION NO. & DATE	MZU/PhD/775 of 19.05.2015
EXTENSION (IF ANY)	NO

Head

Department of Forestry

BRIEF BIODATA OF THE CANDIDATE

Name Kanchan Rawat

Address Department of Forestry
School of Earth Sciences & NRM Mizoram University,
Tanhril, Aizawl-796 004

Mobile **+919774520069**

E-mail kanchanfri@gmail.com

Educational Qualifications

Degree	Institution	Year
Ph.D.	Mizoram University, Aizawl	2019
M.Sc.	Forest Research Institute, Dehradun	2008
B.Sc.	HNBGU, Srinagar, Uttarakhand	2006

Area of Interest/Specialization

Wood Science & Technology: Wood properties and Preservation, Bamboo preservation

Publications Profile

Number of publications: **04**

ABSTRACT

DEVELOPING ECO-FRIENDLY PRESERVATIVES AND TREATMENT TECHNIQUES FOR *Melocanna baccifera* (ROXB.) KURZ.

By

KANCHAN RAWAT

Department of Forestry

Submitted
in partial fulfillment of the requirement of the degree of Doctor of Philosophy in
Department of Forestry of Mizoram University, Aizawl

Use of bamboo for construction and house hold materials like basket, mat, pipes, furniture, musical instruments, pulp and paper, textile, tools, handles etc. is well documented as well as traditionally known. It has considerable strength properties even if it has many structural variations like hollowness, nodes, septa, tapering wall from base upwards etc.

North eastern states, Mizoram in particular are rich in bamboo resources both in abundance as well as in diversity. According to the Department of Environment, Forest and Climate change, Government of Mizoram, bamboo covers 57 % of the geographical area of the state and 27 species of bamboo have been so far reported of the 130 reported in the country (Anon., 2017). Moreover, Mizoram has been declared a timber deficit state because of scarcity of quality trees with considerable growth (The Telegraph, 2019). In Mizoram, National Bamboo Mission (NBM) is run as state bamboo mission through various nodal agencies. The objective is to utilize bamboo for locally useful products, promote local industries, employment generation and so on.

There are about 70 genera and 1200 species (Gyansah and Kwofie, 2011) of bamboo worldwide. India is second only to China in bamboo production. The North-Eastern region harbors more than one third of the Indian bamboo genetic resources having dense bamboo forests. Out of 136 bamboo plant species available in India, 89 taxa are in this region.

The chemical composition of bamboo is similar to that of wood. The main constituents of bamboo culms are cellulose, hemi-cellulose and lignin, which amount to over 90% of the total mass (Kumar *et al*. 1994; Wei *et al*. 2013). The minor constituents

of bamboo are resins, tannins, waxes and inorganic salts. Compared to wood, however, bamboo has higher alkaline extractives, ash and silica contents (Tomalang *et al*. 1980; Chen *et al*. 1985). The composition varies according to species, the conditions of growth, the age of the bamboo and the part of the culm. Because the bamboo culm tissue matures within a year when the soft and fragile sprout becomes hard and strong, the proportion of lignin and carbohydrates is changed during this period. However, after the full maturation of the culm, the chemical composition tends to remain rather constant (Leise, 1985).

Fungi are the most prominent bamboo decaying agents. Fungal attack can be seen at three stages of bamboo life cycle i.e. during planting, storing and service. A large proportion of culms can be attributed mainly to fungi which include soft rot, white rot and brown rot. Fungi attack only that bamboo tissue with sufficient moisture content, at least above fibre saturation point (20-22%), air dried bamboo is protected against fungal degradation. Among the different parts, the inner part of the culm is attacked faster than the outer one, which is attributed to the higher content of the nutritious parenchyma in the inner part. The starch content of the parenchyma cells influence to a larger extent for the susceptibility to attack by fungi, especially the blue stain fungi. Among different fungi experimental evidences show that white rot and soft rot cause more deteriorations in bamboo than by brown rot (Liese 1998). As many as 1100 species of fungi have been recorded from bamboo, comprising 630 Ascomycetes, 150 Basidiomycetes and 330 mitosporic taxa (Hyde *et al* 2002), most of which are reported from asia, with relatively fewer known from India and South Africa. *Fusarium*

moniliformae is the fungal pathogen associated with the rot of emerging culms. F. equiseta and *F. moniliforme* are the fungi associated with the rot of growing culms (Mohanan, 1997). As many as 42 fungi belonging to 23 genera have been recorded on seeds of bamboo from India (Mohanan, 1997).

Without any protective treatment, most bamboo species have an average natural durability of less than 2 years. Stored under cover, untreated bamboo may last for 4-7 years. These variations in bamboo durability strongly depend on the species, the length of the culm, the thickness of the wall and on the time of harvesting.

The lower portion of the bamboo culm is considered more durable, while the soft inner part of the wall deteriorates faster than the outer harder portion. This is related to the anatomical and chemical nature of the woody cells. Although some of the characteristics of bamboo resemble those of wood, its growth characteristics and microstructure is different (Kumar *et al*., 1994). Unlike timber species like teak, the structure of bamboo is void of toxic deposits. The large amounts of starch present in bamboo make it highly attractive to mould and fungi, termites and powder-post beetles. They cause much damage during drying, storage, and subsequent use. Tests have also shown that bamboo is more prone to soft rot and white rot attack than to brown rot. Bamboo consists of 50-70% hemicellulose, 30% pentosans, and 20-25% lignin (Chen *et al*. 1985). The lignin present in bamboos is unique, and undergoes changes during the growth of the culm. Bamboo is also known to be rich in silica (0.5 to 4%), but the entire silica is located in the outer layer (1 mm), with hardly any silica in the rest of the wall. Bamboos also have minor amounts of waxes, resins and tannins, but none of these have

enough toxicity to improve its natural durability. The conventional wood preservatives although are found to be very effective against wood destroying organisms, are said to cause environmental pollution and a few of them are hazardous to animals and human beings (Fisher, 1968; Thompson, 1971; Onuorah, 2000). The most common chemicals used by the industries and local artisans are copper chrome arsenate (CCA), Sodium penta chloro phenol, boric acid – borax, IPBC (3-ido-2-propanyl butyl carbonate) and synthetic pyrethroides, most of which have harmful effect on the environment. Over the past few decades, there has been substantial global awareness to develop eco-friendly wood preservatives and those, which do not cause any ill effect on the health of mammals (Onuorah, 2000). There is a continuous search for different methods of bio-control methods for wood preservation and to develop an ideal preservative.

Efforts have been made by many workers to use these plant products with the amendment of toxic metals and tested for durability against termites or fungi. The current study was undertaken involving the following objectives:

1. To standardize the minimum inhibitory concentration of the Neem oil and copper sulphate-boric acid (1:1) formulation against white rot, brown rot and sap stain fungus.

2. To determine the extent of retention and penetration of Neem oil and copper sulphate-boric acid formulation in *Melocanna baccifera* (Roxb.) Kurz treated by two-step process.

3. To estimate the leachability of preservative in treated samples.

4. To evaluate the resistance of treated culms of *M. baccifera* bamboo species against white rot, brown rot and sap stain fungus.

In the present study, step one treatment was done on *M. baccifera* with copper-boron solution using boucherie equipmenmt. After that the second step treatment was given by heated neem oil with an aim of fixing the copper-boron in the bamboo. Later, the treated samples were subjected to attack by *Schizophyllum commune* (White rot), *Coniophora puteana* (Brown rot) and *Alternaria alternata* (Sap stain) fungi. Fungal decay parameters were evaluated using standard procedures.

Melocanna, like most of the bamboo species is naturally less durable (≤2 years) as it is prone to fungal attack. Preliminary screening of the neem oil against the test fungi showed as there was gradual increase in concentration of Neem oil the growth inhibition rate of each fungus was also increased. The 50% of growth inhibition was achieved in *S. commune* at 2.0% concentration of neem oil. Complete inhibition for *A. alternata* and *C. puteana* occurred at the highest concentration taken in the experiment i.e., 10.0% while for *S. commune* it was achieved at 8.0%. Similarly, the copper boron complex effected 100 % growth inhibition at 0.4 % concentration of Copper sulphate – boric acid (1:1) for *C. puteana* and *S. commune* where as for *A. alternata* it was observed at the concentration of 0.2 %. 50 % growth inhibition was observed for *C. puteana* and *S. commune* at 0.06 % concentration and that for *A. alternata* was observed at 0.08 %.

The 2-Step treatment process significantly improved dimensional stability of *M. baccifera* bamboo by reducing volumetric swelling and volumetric shrinkage. Among the portions of *M. baccifera*, basal portion was found to be significantly resistant to decay fungi as well as dimensionally stable followed by middle & apical portions. The Oil Heat Treatment dried the bamboo from green condition to 0.3-0.6 % moisture content in 30 minutes treatment duration. So it has an added advantage of faster drying. Neem oil Heat Treatment at 200°C for 30 minutes was effective in fixing the copper and boron. The 2-Step treatment process had significantly restricted the decay in *M. baccifera* caused by *S. commune, C. puteana* & *A. alternata.* As a major indication of the results of the current study, 2-Step treatment (Copper – Boron + Heated neem oil) was promising to enhance the service life of bamboo by 5 to 10 times depending on the exposure.

Post harvest degradation of bamboo culms by fungi is a major challenge in bamboo utilization and preservation. In spite of versatile nature of bamboo material, it is considered as temporary material. Natural durability of bamboo varies from species to species; an untreated bamboo is believed to be degraded within three years of installation. Various fungi toxic formulations are known to control the decay across the world. At the same time some of these chemicals are harmful to the man, livestock and the environment and have been banned in various countries. Bio-control agents against these fungi are very limited and inconsistent. Therefore, the onus is on eco friendly, natural formulations which are safe to use and considerably effective in inhibiting the growth of decay and stain fungi. The current study was aiming to achieve two goals.

One was to see if neem oil was effective against white rot, brown rot and sap stain fungi, secondly if it can fix the copper and boron in the treated bamboo (*M. baccifera*) to make the bamboo durable for longer durations.

Increase in durability reduces cost of frequent replacements. Therefore, awareness about preservation is needed among the agencies, industries and personnel involved in bamboo product manufacturing. When preservation is practiced in medium to large scale it is economically viable; even small scale enterprises when formed into cooperative or self help groups, can become economically viable. Bamboo has good carbon sequestration prospect but storage is only for short period. Therefore, preservation can be promoted for improving overall carbon management strategies also. Preservation can boost export potential of bamboo products from NER to other parts of the country where bamboo resources are limited.