Khulna University
Life Science School
Forestry and Wood Technology Discipline

Author(s): Jamila Khatun

Title: Natural decay resistance of *Mangifera indica* (Linn.), *Syzygium cumini* (Linn.) and *Artocarpus heterophyllus* (Lam.)

Supervisor(s): Dr Md. Ashaduzzaman, Associate Professor, Forestry and Wood Technology Discipline, Khulna University

Programme: Bachelor of Science in Forestry

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COURSE TITLE: PROJECT THESIS

COURSE NO: FWT-4114

This paper has been prepared for the partial fulfillment of the requirement of fourth-year B.Sc (Hon's) degree in forestry and wood Technology Discipline, Khulna University, Khulna, Bangladesh.

Supervisor

18/4/2016

Dr. Md. Ashaduzzaman
Associate Professor
Forestry and Wood Technology Discipline
Khulna University
Khulna-9208
Bangladesh.

Submitted by

Jamila
18.04.16

Jamila Khatun
Roll No:110530
Forestry and Wood Technology Discipline
Khulna University
Khulna-9208.
Bangladesh.

DEDICATED TO

MY BELOVED PARENTS

AND YOUNGER BROTHER AND SISTER

DECLARATION

I am Jamila Khatun, declare that this thesis is the results of my own works and it has not been submitted or accepted for acceptance degree in any other university.

I do hereby-giving for my thesis, it accepted, to be available for photocopying and for inter-library loan, for the title and summary to be made available to outside organization.

Candidate...Jamela.....

Date...18.04.16....

ACKNOWLEDGEMENT

First of all, I am very grateful to almighty Allah for his gracious glance upon me for the successful completion of this thesis paper.

I would like to express my special thanks to Dr. Md. Ashaduzzaman, Associate professor, Forestry and wood Technology Discipline, Khulna University, Khulna, for his supervision, guidance, valuable suggestion, criticism, inspiration and co-operation for preparing this paper.

I am also thankful to the people of wood Laboratory, Forestry and Wood Technology Discipline, Khulna University, Khulna who help me to prepare the sample.

My special thanks goes to my classmates kamona mondol,Nasrin Sultana, Hira Khanom and all of my friends and well-wishers for their technical assistance, suggestions and supports in the preparation process of this paper.

Finally, I would like to express my gratefulness to my family members who always sacrificed their happiness for my education at Khulna University.

Jamila Khatun

ABSTRACT

Natural decay resistance (natural durability) of wood helps to give sufficient reliability regarding end-use related predication of performance of wood. In this study Natural decay resistance of native wood species (i.e. *Mangifera indica, Syzygium cumini and Artocarpus heterophyllus*) were evaluated by agar block test method. The fungal test considered of incorporating standard size timber specimens with fungus which was already inoculated in jars and then data was recorded after twelve weeks to obtain weight loss. The average weight losses (%) of *Mangifera indica, Syzygium cumini and Artocarpus heterophyllus* due to decay of white rot fungus *Trametes versicolor* in sapwood and heartwood were 20.14%, 16.61%, 8.69% and 10.10%, 5.33%, 5.79% respectively. Again, the average weight losses (%) of *Mangifera indica, Syzygium cumini and Artocarpus heterophyllus* due to decay of white rot fungus *Ceriporiopsis subvermispora* in sapwood and heartwood were 6.89%, 15.53%, 6.99% and 5.19%, 3.96%, 5.35% respectively. From the analysis of variance it has been found that there was significant difference of weight loss (%) among the heartwood and sapwood of *M. indica, S. cumini and A. heterophyllus* caused by *both* white rot fungus *Trametes versicolor* and *Ceriporiopsis subvermispora.* Based on weight loss (%) of heartwood and in accordance to the European standard of natural durability classification system it was found that *S. cumini and A. heterophyllus* were durable and *M. indica was* Moderately durable.

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CHAPTER ONE
INTRODUCTION

1.1 Background of the study

Natural durability is meant the inherent ability to resist decay and other destructive agencies. Natural durability, therefore, conveys the idea of the extent of the heartwood portion only (FAO, 1986). Wood is one of the earth most valuable and renewable natural resources which can be used for indefinite period of time (Lihary, 2001). It is a material used by man of thousands of years without precise knowledge of its properties (Wangaard, 1981). According to the European standard EN 350-1, the natural durability of wood is defined as "the natural durability of wood destroying organisms". This standard provides guidance on test methods to determine the natural durability of wood against fungal decay using methods like EN-113 and ENV 807 (Acker *et al.,* 2003).

The decay of wood and termites is mainly caused by Basidiomycetes fungi, but Ascomycetes and fungi imperfecti also take part in wood decay. Decay fungi attack all woody parts of a tree giving rise to root, butt, heart, trunk or sap rot. Decay causes two types of rots white rot in which mainly lignin is utilized and brown rot in which cellulose is mainly utilized by the fungi (Puri *et al.*, 1968).But timber in its various form subjected to several types of deterioration (Hunt and Garratt, 1967). Because of its natural origin and its organic nature, it is an ecological habitat for a wider range of insects and fungi (Shrivastava, 1997). Some timber displays good resistant to many degrading processes, in the other hand, timber is not durable in damp while others have little resistance (Desch and Dinwoodie, 1996).

The durability of timber depends to a large extent on the environment under which it is used or stored in addition to the inherent characteristics of the timber in dry environment all timber species are more or less durable except certain species which are liable to attack by wood destroying borers. On the other hand, timber is not durable in damp environment. It perishes quickly when it is used in contact with the moist soil or exposed to humid atmosphere. This deterioration in damp condition is caused by wood decaying fungi and wood destroying termites (Hunt and Garratt, 1967).

Therefore the durability of timbers under similar condition is variable from species to species selection of a timber species for a specific use; therefore play an important role in achieving its most economic utilization (Latif *et al.*, 1978).

1.2 Justification of the study

Timber has varying degree of natural durability due to the variation of environment where it is exposed to (Panshin and De Zeeuw, 1980). The common environments where timber generally stays for a longer period of time are air, water and soil. These media contain a huge number of chemical agents and among these some are harmful to timber and its product that deteriorate its longevity of life (Panshin and De Zeeuw, 1980). In case of durability, it is the property of timber to remain in sound condition for a long time when exposed to the forces of nature in an exposed or underground condition (Aziz, 1995).

In Bangladesh timber has versatile uses and plays a vital role in national economy. There are about 500 timber tree species occurring in the forest and homesteads of Bangladesh most of which are hardwoods (Sattar, 1997). Throughout the country, besides composite timbers, solid timbers are used for internal domestic works such as in making doors, windows, furniture, cabinet, panel works, etc. For agricultural implements and boat building, localized timbers are traditionally used. It is thus important to understand the basic properties and their implications in order to be able to select and to design with confidence that the desired results will be obtained. Am *(Magnifera indica*) and Jam (*Syzgium cumini*) and Kanthal (*Artocarpus heterophyllus*) are abundantly grown and widely used timber species in Bangladesh. But the economic importance of those three species is different. Am *(Magnifera indica)*, Jam (*Syzgium cumini)* and Kanthal (*Artocarpus heterophyllus*) timber are also important for furniture making and other construction purpose (Sattar, 1997).

Mango *(Mangifera indica)* is also known as Am and found abundantly all over the country. It is a grey and moderately strong wood. Because of its moderate weight and hardness, it can be used for rough furniture, planking, packing boxes, shoe hells, toys and making boats. If treated with preservatives it can be used for door, window and constructional purposes. It also makes good veneer for the production of plywood (Hossain and Awal, 2012).

Jam (*Syzygium cumini)* is a medium dense and moderately strong timber. Its shrinkage capacity is high. It is a good constructional timber due to its heavy weight and may be used for door, window and furniture. It is also suitable for boat building, cart and railway sleeper (Hossain and Awal, 2012).

Jackfruit *(Artocarpus heterophyllus)* is locally known as Kanthal, which is the tree of national fruit of Bangladesh. The wood is strong, hard, durable and easy to saw, machine or carve. Its colour is yellow to light yellow. It is used for high quality furniture, house construction (doors, windows, and 70 roof rafters), implements, boat building, cabinet making and musical instruments such as violins (Hossain and Awal, 2012).

This study was therefore, undertaken to determine the life span of Am *(Mangifera indica*), Jam (*Syzgium cumini)* and Kanthal (*Artocarpus heterophyllus)* and their durability. These three species are mostly used timber species in Bangladesh. In the natural condition of Bangladesh these three species is analyzed and classify into the grade of natural durability in contact with white rot fungus.

1.3 Objective of the study

- To determine the natural decay resistance of Am (*Mangifera indica*), Jam (*Syzgium cumini)* and Kanthal *(Artocarpus heterophyllus)*.
- To classify these wood species according to standard natural durability classification.

CHAPTER TWO
LITERATURE REVIEW

2.1 Am (*Mangifera indica* Linn.) – An overview

2.1.1 General description

Mangifera indica is also known as mango. Mango belongs to genus *Mangifera* which consists of about 30 species of tropical fruiting trees (Shah *et al*., 2010). Mango belongs to the genus *Mangifera* of the family Anacardiaceae.The genus *Mangifera* contains several species that bear edible fruit. Most of the fruit trees that are commonly known as mango belongs to the species *Mangifera indica.* Mango has become accepted and adapted all over the tropics and subtropics. Mango is a common garden tree throughout the tropics. Although grown commonly, mangoes favor a warm, frost-free climate with a well-defined winter dry season. Rain and high humidity during flowering and fruit development decreases fruit produces. The tree generally flowers in mid to late winter; with fruit maturing in the early to mid-summer months. The dense canopy of the mango is a basis of shelter and shade for both animals and humans. Mangoes are well modified to cultivation and have been grown commercially for centuries. Today, mangoes are recognized and eaten throughout the world and are considered as one of the most widespread and respected tropical fruits (Bally, 2006). Density of Mango (*Mangifera indica)* wood species is 0.52 gm/cm^3 (Reys *et al.*, 1992).

2.1.2 Distribution

2.1.2.1 Native range

The genus *Mangifera* creates in tropical Asia, with the greatest number of species originates in Borneo, Java, Sumatra, and the Malay Peninsula. The most-cultivated *Mangifera* species, *M. indica* (mango), has its origins in India and Myanmar (Orwa *et al*., 2009).

2.1.2.2 Current distribution

Mango is now cultivated throughout the tropical and subtropical world for commercial fruit production, as a garden tree, and as a shade tree for stock. In the Pacific region, all mangoes were presented from other parts of the world. The original recorded introductions into Hawaii prior to most introductions to the Pacific islands have happened over the past 100 years. Few other *Mangifera* species are found in the Pacific. *Mangifera gedebe*, *M. minor* and *M. mucronulata* are found in the Solomon Islands (Orwa *et al.*, 2009).

2.1.3 Silvicultural characteristics

- Mango is a shade-bearer tree
- It prefers a deep well-drained loamy soil and a moist warm climate.
- It withstands normal frosts but suffers both from severe drought and frosts
- It is killed by girdling (Troup,1921)

Temperature above 45^{0}C accompanied by strong winds damage the fruit, requiring windbreak around orchards. Large tree withstand fire well (Luna, 1996). In unsuitable places young mango trees suffer from the sun. (Troup, 1921)

2.1.4 Phenology

The tree is truly evergreen. It renews its leaves irregularity in all seasons of the year (Krishnaswamy and Mathauda, 1954). The flowering process is entirely depends on climatic conditions prevailing in an area (Bose and Mitra, 1990).The flowering of mango in India takes place as early as November-December in Raylasema area of Andhra Pradesh earlier (Ghandhi, 1995), February to March in the northern India (Singh, 1967) and slightly earlier (January- February) in the eastern parts of the country. In Queen's land, Australia, Flowering occurs between June and October. In Fiji, Flowering starts in July and extends through September/October (Iqbal, 1982). The flowering of mango in Egypt occurs during November to January (Nakhal, 1980). Fruit ripens in Bangladesh during May-July (FAO/UNDP, 1989).

2.1.6 Uses

Timber: Heartwood is pale yellowish-brown to reddish-brown, darkening on exposure, not clearly demarcated from the pale yellowish-brown sapwood. Grain somewhat wavy, texture moderately coarse; recently cut wood is scentless. The wood is used for many purposes, including indoor construction, meat-chopping blocks, furniture, carpentry, flooring, boxes, crates and boat building (Orwa *et al.*, 2009).

Medicine: Well-cooked and powdered leaves make a plaster to remove warts and also act as a styptic. Seeds are used to treat determined colds and coughs, obstinate diarrhoea and bleeding piles. The bark is astringent, homeostatic and antirheumatic (Orwa *et al.*, 2009).

2.2 Jam (*Syzgium cumini* Linn.)-An overview

2.2.1 General description

Syzgium cumini is a very large evergreen tropical tree belonging to the family Myrtaceae (Wealth of Indian., 2010). A slow growing species and can live more than 100 years. At the base of the tree, the bark is rough and dark grey, becoming lighter grey and smoother higher up. The wood is water resistant. The flowers of are fragrant and small, about 5 mm in diameter. The pulp of the fruit, extracts from the bark and seeds is of great benefit when it comes to lowering of blood glucose level. Taking dried extract of the seeds orally greatly reduces the blood sugar and glucosuria. The leaves and bark are used for controlling blood pressure. Wine and vinegar are also made from the fruit. It has a high source in vitamin A and vitamin C (Orwa *et al.*, 2009). Density of Jam (*Syzygium cumini)* wood species is 0.70 gm/ cm^3 (Reys *et al.*, 1992).

2.2.2 Distribution

2.2.2.1 Native range

India, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand (Orwa *et al.*, 2009).

2.2.2.2 Exotic range

Algeria, Antigua and Barbuda, Australia, Bahamas, Barbados, Colombia, Cuba, Dominica, Dominican Republic, Ghana, Grenada, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Indonesia, Jamaica, Kenya, Martinique, Mexico, Montserrat, Nepal, Netherlands Antilles, Nicaragua, Panama, South Africa, St Kitts and Nevis, St Lucia, St Vincent and the Grenadines, Sudan, Tanzania, Trinidad and Tobago, Uganda, United States of America, Vietnam, Virgin Islands (US) (Orwa *et al.*, 2009).

2.2.3 Phenology

Seedlings grow slowly the first year, rapidly thereafter, and may exceeding 3 m in height after 2 years, reaching full size in 40 years. Flowering occurs February-March in Florida, USA, May-August in Sri Lanka, and July-August in Java, Indonesia, and the fruit ripens in April in French Polynesia, May-June in the Philippines, May-July in India and Florida, late summer and autumn in Hawaii, September-October in Java, and November-December in Sri Lanka Dry weather during flowering and fruiting will increase fruit production. Fruits need to be harvested by hand as they ripen, requiring several collections over the season, with a crops of 700 fruits possible from a 5-year-old tree, and the production of a large tree may be overwhelming to the average household (Morton, 1987).

2.2.6 Uses

Timber: The reddish-grey or reddish-brown heartwood is fine grained and is utilized in exterior joinery and carpentry. Wood is durable in water, resistant to termites, and although difficult to work, it saws and machines well and is used for construction, boat building, commercial tea and chest plywood, agricultural implements, tool handles, cart wheels, well curbs and troughs, sleepers, furniture and as props for shafts and galleries in mines. It is also used for building bridges and for making musical instruments, especially guitars (Orwa *et al.*, 2009).

Medicine: The seeds and bark are well known in the Far East for the treatment of dysentery and in control of hyperglycemia and glycosuria in diabetic patients. The astringent bark may be used as a gargle. Fruits are used as a relief for colic, while the wood yields a sulphate pulp that has medicinal uses (Orwa *et al.*, 2009).

2.3. Kanthal (*Artocarpus heterophyllus* Lam.) - An overview

2.3.1 General description

The *Artocarpus heterophyllus* is a species of tree of the mulberry family (Moraceae) is known by other names jackfruit (Eng.), Kanthal (Hindi). Jackfruit *(Artocarpus heterophyllus*) produces heavier yield than any other tree species, and bear the largest known edible fruit. The jackfruit tree has several uses. The tree is also known for its durable timber, which ages to an orange or reddish brown color, with anti-termite properties (Arung *et al.*, 2006). The leaves and fruit waste provide valuable fodder for cattle, pigs and goats. Jackfruit wood chips yield a dye, which is used to give the famous orange-red color to the robes of Buddhist priests. In addition, many parts of the plant, including the bark, roots, leaves and fruits have medicinal properties. It requires a soil which is well drained but moist, with a pH of 4.3 to 8.0 and with medium soil fertility. The optimum temperature is 19 to 29^0C, altitude at approx. 1600 meters above sea level and the annual rain fall between 1000 and 2400 mm (Roy *et al.*, 1996). Density of Khantal (*Artocarpus heterophyllus)* wood species is 0.60 gm / cm^3 (Reys *et al.*, 1992).

2.3.2 Distribution

2.3.2.1 Native range

Jackfruit is cultivated more or less everywhere in Bangladesh. It is cultivated all over the Bangladesh in plantations and orchards. In Bangladesh, Tangail, Meherpur, Kustia, Mymenshingh etc. are the more favored areas for Jackfruit cultivation (Muniruzzaman, 1988) .

2.3.2.2 Current distribution

Since ancient times, Jackfruit has been cultivated in many parts of the tropics, particularly Asia like India, Bangladesh, Burma, China, Sri Lanka, Malaysia, Indonesia,Thailand and the Philippines. It is also grown in parts of Africa, Brazil, Suriname, Florida and Australia. It has been introduced to many pacific islands since post-European contact and is of particular importance in Fiji, where there is a large population of Indian Descent (Elevitch and Manner, 2006).

2.3.3 Silvicultural characteristics

- The tree is a pronounced shade-bearer.
- It develops best with a fair amount of light and growing space
- It is decidedly frost-tender
- It coppices well
- The bark and leaves are eaten by elephants

During seedling stage it can stand a considerable amount of shade but develops best in full light and does not appear to mind the sun provided the soil is kept moist. It is sensitive to drought and frost tender. During first season its growth is slow to moderate, a height of 6-8 inch being ordinary attained by the end of the season (Troup, 1986).

2.3.4 Phenology

Irrespective of the method of propagation, the tree stars bearing from the seventh of eighth year after planting. The flower generally starts appearing in December to March (Troup,1986 and Samaddar,1990).Fruit ripens in summer (Samaddar, 1990). However, Troup (1986) notes that fruit ripen during the rainy season. At higher altitude fruit growth may continue up to September. Occasionally in rare cases, off-season flowering in September-October may be noticed (Samaddar, 1990).

2.3.5 Chemical compounds of wood

Chemical analysis of wood of the jackfruit tree reveals cellulose content of 56.74%, lignin content 28.76%, pentose content 18.64% (Komaryati, 1995). Lu and Lin (1994) isolated and identified morin and morin calcium chelate compound from heartwood of *Artocarpus heterophyllus.* Radhakrishnan *et al.* (1995) isolated two new flavones named norartocarpetin and artocarpesin from young heartwood of *Artocarpus heterophyllus.* Methanolic extracts obtained from of *Artocarpus heterophyllus.* There is only 3.3% tannin in the bark, which is occasionally made into cordage or cloth.

2.3.6 Uses

Medicinal uses

The ash of jackfruit leaves, burned with corn and coconut shells, is used along or mixed with coconut oil to heat ulcers. The dried latex yields artostenone, convertible to artosterone, a compound with marked androgenic action. Mixed with vinegar, the latex promotes healing of abscesses, snakebite and glandular swellings. The root is a remedy for skin diseases and asthma. An extract of the root is taken in cases of fever and diarrhea. The bark is made into poultices. Heated leaves are placed on wounds. The wood has a sedative property; its pith is sat to produce abortion (Morton, 1987).

Wood value

The wood is classified as a medium hardwood (specific gravity 0.6-0, 7) and is highly valued for building material, furniture and cabinet making and even for musical instruments. It is highly durable, resisting termites and decay, seasons easily, resembles mahogany in appearance, and takes a beautiful polish. As the wood ages, it turns from yellow or orange to red or brown. Although not as strong as teak (*Tectona grandis*), jackfruit wood is considered superior for many purposes including furniture, construction, turnery, masts, oars and musical instruments. The excavated roots of old trees are highly prized for carving and picture frames. There is only 3.3% tannin in the bark which is occasionally made into cordage or cloth. Branches and trunk are burned for fuel wood. In the province of Cebu, Philippines, the wood is highly prized for making guitars, ukuleles and other musical instrument. The inner bark can be made cordage or cloth (Elevitch and Manner, 2006).

2.4 General information on natural durability of wood

2.4.1 Natural durability

Disintegration or decomposition of wood by fungal attack or any other agencies called wood decay (Zabel and Morrell, 1992). Naturally durable woods have a variety of commercial uses. Natural resistance to decay has been evaluated in field and laboratory tests of wood from many species, but there are few places where commercial wood users can find comparative assessments of natural durability (Scheffer and Morrell, 1998).

Wood is a natural polymer containing primarily of cellulose, hemicellulose, and lignin in a matrix that provides structural support to the living tree and some resistance against microbial attack. Cellulose, because of its partial crystallinity, is somewhat resistant to microbial attack. Lignin is a heterogenous polymer of phenyl propane units and is extremely resistant to some decay fungi. However, other organisms have established the ability to attack one or more of the polymers in the wood cell wall. Some wood species have evolved to produce extractive compounds that can protect the wood; these are the principal source of decay resistance in all species. These compounds are produced as the living ray cells in the inner sapwood zone die, forming the nonliving heartwood. As the sapwood dies in wood species with durable heartwood, a series of reactions in the storage or parenchyma cells of the wood rays converts the stored sugars and starch into a wide array of fungi toxic compounds that become a constituent of the new heartwood. Sapwood of nearly all species has no natural durability (Toole, 1972 and Eslyn, 1979).

Heartwood of some species has a distinctive darker color, while in others it differs little in color from the sapwood. The decay resistance among woods may vary among tree species, among individual trees, and within individual trees. Variation in the inhibitory components of the heartwood (Schaffer, 1986). Fungal attack is the result of fungal attack, which leads to the breakdown and ultimate dissolution of the wood substance. Most of the wood destroying fungi belong to the Basidiomycetes. Some of the members of the Ascomycetes cause the troublesome dark stains in light colored wood. Fungi obtain nourishment by absorbing it through the walls of the hyphae. For this, they must first liquefy their food materials. All wood rotting fungi can breakdown cellulose. Those fungi which attack only the cellulose and polysaccharides cause wood to darken in color such rot called “brown rots”. There are other fungi; they can breakdown the lignin by means of oxidizing enzymes. These fungi because wood pale in color and may eventually reduce it to a fibrous whitish mass. Rots of this kind known as "white rots" (Zabel and Morrell, 1992).

The sap wood of almost all wood is readily susceptible to decay whereas the heartwood of many woods is resistant to such deterioration. The greater natural durability of heartwood over sapwood is attributed primarily to certain chemical changes that take place when and after the sapwood is transferred to heartwood (FAO, 1986).

The reason of natural durability of heartwood of some species is presence of toxic substances in the heartwood (Panshin and Dezeeuw, 1984). Natural decay of timber will depend on the conditions of use it may include resistant to fungal decay, insect attack and mechanical wear and in special circumstances chemical attack. Timber which is protected from these various forms of deterioration will last indefinitely (Shrivastava, 1997).

Natural durability is a result of density, permeability, moisture behavior and the presence of non- structural chemicals called extractives. As a broad generalization, the heartwood of dense dark colored timbers tends to be more resistance to decay than light colored ones (Shrivastava, 1997). In addition to that dense impermeable timbers tend to be more resistant than light porous species. Variation exit in decay resistance in timbers of the same species, the environment of the growing tree particularly important. In addition, certain softwood, larch, western red cedar and hardwood such as oaks, Africa mahogany and teak, have inner heart wood in the vicinity of the pit which is less durable than the other heartwood (Shrivastava, 1997). He also reported that despite these variations, durable timbers could be used successfully in conditions where perishable timbers would decay rapidly. Cost, size and shape required, and end use will determine whether a durable timber or a preservative treated non- durable will be chosen. The classification of natural decay resistance helps the timber users to arrive at a decision if they would use a particular timber species in its natural state or it requires protection by preservative treatment for a particular end use (Latif *et al.*, 1978).

2.4.2 Natural durability classification

Different natural durability classification system has been used in different parts of the world based on the service life and laboratory test methods. Some of them are given below:

Table 2.1. Natural durability classification of timber in temperate region (England) (Eaton and Hale, 1993).

Classification	Life expectancy in ground (Year)
Perishable	Up to 5
Non-durable	5-10
Moderately durable	10-15
Durable	15-25
Very durable	Over 25

Table 2.2. Different criteria during durability class based on mass loss mentioned in European Standard EN-350-1(1994).

Durability class	Description	Mass loss ML (%)
1	Very durable	$ML \leq 5$
2	durable	$5 < ML \leq 10$
3	Moderately durable	$10 < ML \leq 20$
4	Slightly durable	$20 < ML \leq 30$
5	Not durable	$ML > 30$

Table 2.3. Natural durability classification of timber according to laboratory soil block test (ASTM, 1989)

Resistant class	Weight Loss (%)
Very resistant	0-10
Resistant	11-24
Moderately resistant	25-44
Non-resistant	>44

Table 2.4. Natural durability classification of timber (Anon, 1972).

Durability class	Weight loss (%)
Very durable	<1
Durable	1-5
Moderately Durable	5-10
Non-durable	10-30
Perishable	>30

2.5 General information about white rot fungus *Ceriporiopsis subvermispora* and *Trametes versicolor*

2.5.1 White rot fungi

White rot fungi are Basidiomycetes that are capable of degrading the lignin component of lignocellulose substrates. There are other fungi capable of digesting lignocellulose, such as brown rot fungi, but they do not produce the same lignolytic enzymes and tend to concentrate their activities on the cellulose component. White rot fungi are so called because the degradation process results in a bleaching of the wood substrate (it's the polyphenolic lignin that provides most of the colour to native timber (Moore *et al.*, 2011). Relatively few white-rot fungi are regarded as important decay of commercial timbers when compared to the large number of brown rot fungi (Duncan and Lombard, 1965) which cause a greater degree of damage in a given period of time (Henningsson, 1967). White rot is a common feature of decay in smaller dimension woody material found in forest floor (Moore *et al.*, 2011).

Oxidative lignin breakdown depends on a panel of enzymes including:

- **Lignin peroxidase**, (a haem (Fe)-containing protein) which catalyses H_2O_2-dependent oxidation of lignin;
- **Manganese peroxidase**, which also catalyses H_2O_2-dependent oxidation of lignin;
- **Laccase** (a copper-containing protein) which catalyses demethylation of lignin components (Moore *et al.*, 2011).

The process of catabolic lignin degradation involves:

- Cleavage of ether bonds between monomers;
- Oxidative cleavage of the propane side chain;
- Demethylation;
- Benzene ring cleavage to ketoadipic acid which is fed into the tricarboxylic acid cycle as a fatty acid (Moore *et al.*, 2011).

2.5.2 Characteristics of white rot fungi

Lahiri (1995) reported some characteristics of white rot fungi, which are given below:

- The color of the affected wood is white and usually the wood is fibrous in texture.
- If the timbers are rotten, the shape and dimension will not change.
- White rot does not result in cubical cracking as in brown rot and the wood does not readily powdered when ground between the fingers.

2.5.3 *Trametes versicolor*

One of the most common fungi to be found in the woods is *Trametes versicolor*, the turkey tail fungus. The common name comes from the banding pattern on the fruiting bodies that resemble the tail of a strutting turkey. The colors of the bands can be quite variable, depending on the genetics of the organism and its environment. Most of the bands are dark to light brown in color, alternating with light colored bands of white to tan, with still more bands of blue, orange, maroon, and other. They are among the most easily found fungi. The species has a widespread distribution, having been found in nearly every state in the United States and in most other countries. (Thomas , 1997).

Ttrametes versicolor is a polypore, a member of the Polyporaceae. There are about 100 genera in this family. These pores can be very small, 10 per millimeter, or much larger, up to 2 mm per pore. Some of these fungi are highly valued because of their wood-degrading (and especially lignin-degrading) abilities. *Trametes versicolor* is one of the fungi that are used in bio pulping. That is, the fungus decays the lignin and leaves the cellulose behind (Thomas , 1997).

Trametes versicolor mycelia were immobilized in carboxymethyl cellulose, CMC, beads through entrapment, and the bead containing immobilized fungus spores were incubated at 30 ^{0}C for 3 days to attain uniform growth on the bead surface (Thomas , 1997).

2.5.4 *Ceriporiopsis subvermispora*

Ceriporiopsis subvermispora is capable of decomposing lignin without penetration of enzymes into wood cell walls. To elucidate the mechanism of ligninolysis at a site far from enzymes, peroxidation of low molecular mass compounds produced by this fungus was analyzed. *C. subvermispora produced* free 9, 12- octadecadienoic 9-octadecenoic, 11-octadecenoic, hexadecanoic and octadecanoic acides, predominantly at early stages of cultivation on wood meal cultures. In prolonged cultivation period after 2 weeks, the amount of intact fatty acids decreased with increasing organic hydroperoxide and TBARS production. These results suggest that lignin degradation by *C. subvermispora* is related to extracellular lipid peroxidation (Enoki *et al*., 1999).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Collection and preparation of wood sample

Mangifera indica, Syzgium cumini and Artocarpus heterophyllus wood were collected from sawmill of Rupsha, Khulna. The samples were selected from both sapwood and heartwood of the each wood species. The samples were cut to 20×20 cm cross-section and 1m long stick. The samples were free from defects.

3.1.1 Preparation of wood samples for fungal decay test

From each wood species, 24 samples were collected from both sapwood and heartwood. Among them 12 samples were sapwood and 12 samples were heartwood. 36 samples were used for *Trametes versicolor* fungus and 36 samples were used for *Ceriporiopsis subvermispora* fungus. The dimensions of wood blocks for the tests were 20 mm x 20 mm x 5 mm were be prepared for fungal decay test.

Figure 3.1: Preparation of samples

3.2 Pure fungus culture

When testing the efficiency of a fungus, it is necessary to select suitable strains of test fungi. Fungi are chosen on the basis of their economic importance, their resistance to disinfectants, their growth and decay rates and their ease of cultivation in the laboratory (Eaton and Hale, 1993).

A large number of the white rot fungi which have been isolated are important members of the microflora involved in natural decomposition and beneficial decay on the forest floor. On the other hand many cause decay problems of wood in storage and in service. White rot is a common feature of decay in smaller dimension woody materials found on the forest floor (i.e. 1-6 cm diameter) and many are rapid colonizers of unseasoned processed wood. *Trametes versicolor* and *ceripriopsis subvermispora* which occurs on standing trees of both hard and softwoods, common logs, stumps and on timbers in ground contact. Colonization of these fungi is rapid in tropical climates (Eaton and Hale, 1993).

The fungi, *Trametes versicolor* and *Ceriporiopsis subvermispora* were collected from Pathology and Microbiology Laboratory, Forestry and Wood Technology Discipline, Khulna University. To develop the growth of these on to malt agar media on petridishes, some intensive laboratory works were carried out.

3.2.1 Sterilization of laboratory glass wares

Jars were washed and dried well. The jars, beakers and fluxes etc. were sterilized by autoclaving for 20 minutes at 15 p.s.i at 121^0C temperatures. Other laboratory equipment such as knives, forceps, scalpels etc. were also sterilized by dipping in rectified sprit and then flaming over spirit lamp. The chamber (laminar flow) in which the sterilized inocula were placed and other transferring works were done was also sterilized by using rectified spirit. The bench was wiped with cotton soaked in rectified spirit up to the elbow.

3.2.2 Preparation of culture media

The selection of satisfactory media for stimulating growth and sporulation of particular fungus is important. Any rich carbohydrate source will support fungi growth but Potato Dextrose Agar (PDA), 2% Malt Agar (2% MA), Potato Sucrose Agar (PSA), Corn Media Agar, Oatmeal Agar etc. are the most commonly used media. In this study 2% MA media has been used for the preparation of 2% MA media 20 gm of malt, 20 gm agar powder and 1 litter water were used. At first the malt was added into the water and boiled until dissolved. Then agar powder was added into the suspension and then autoclaved for 20 minutes at 15 p.s.i at 121^{0}C temperature in an autoclave.

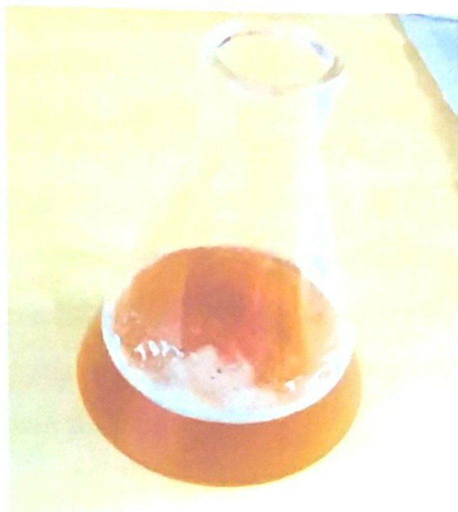

Figure 3.2: Preparation of culture media

3.2.3 Planting of inocula

After cutting the inocula from the pure culture, the inocula were placed on the sterilized media that was previously poured and solidified into the sterilized jar. All these works were done into the sterilized laminar airflow. The inocula were put on to the media by sterilized forceps about one cm from edge of the jars. In small jars three inocula were inoculated and big jars five inocula were inoculated. The jars were incubated in an incubator at Temperature 25±2^{0}C and 65±5 % Relative Humidity which is appropriate for fungal growth.

3.2.4 Observation of inocula

After 7 or 10 days the inoculated jars were observed for fungal growth. The inocula yielding fungal growth were carefully observed and then further followed at seven days interval up to fifteen days, which is the optimum range for maximum fungal growth after inoculation. The extent of growth and pattern of mycelia growth and color of the mycelia were used to distinguish the definite fungi i.e. *Trametes versicolor* and *Ceriporiopsis subvermispora.*

3.3. Agar block test

The samples were made from clear heartwood and sapwood and were free from knots, stain, decay, insect holes or other deformity or defects. At first, the samples were dried in oven at 103±2^{0}C to obtain constant weight (initial).

After oven drying of the test samples, these were kept jar in which fungus was growing. 6 samples were placed from heartwood and sapwood from each of the wood species on each of the fungal culture. The test samples were removed from jar after 12 weeks. Then samples were whipped carefully dried in oven at 103±2^{0}C to obtain constant weight (Final weight). From the initial and final weights, the percentage of deterioration (weight loss) was calculated and then data was recorded.

3.4 Determination of the weight loss

The formula which was used is given below

Loss of weight in wood (%) = $[(M_0-M)/ M_0] \times 100$ (Amusant *et al.*, 2005).
M_0 = Oven dry weight of wood prior to the decay test.
M= Oven dry weight of wood after the decay test.

3.5 Statistical analysis

The data we collected from agar block test for determining the performance of natural durability and analyzed by using Microsoft office excel 2010 and SPSS 16.

CHAPTER-FOUR
RESULTS AND DISCUSSION

4.1 Results

Deterioration of wood could be indicated by its weight loss due to fungal attack. It is considered that percentage losses in mass are a better indicator of loss of strength, which is the real indicator for performance in service (Acker *et al.*, 2003). In general, factors that influence wood resistance include growing site, growth rate, age of the trees, portion of wood (heartwood and sapwood), extractive content in wood and environment of the wood being exposed to (Suprapti, 2010).

The average weight losses (%) of sapwood of *M. indica, S. cumini and A. heterophyllus* wood species due to decay by *Trametes versicolor* are presented in figure 4.1.

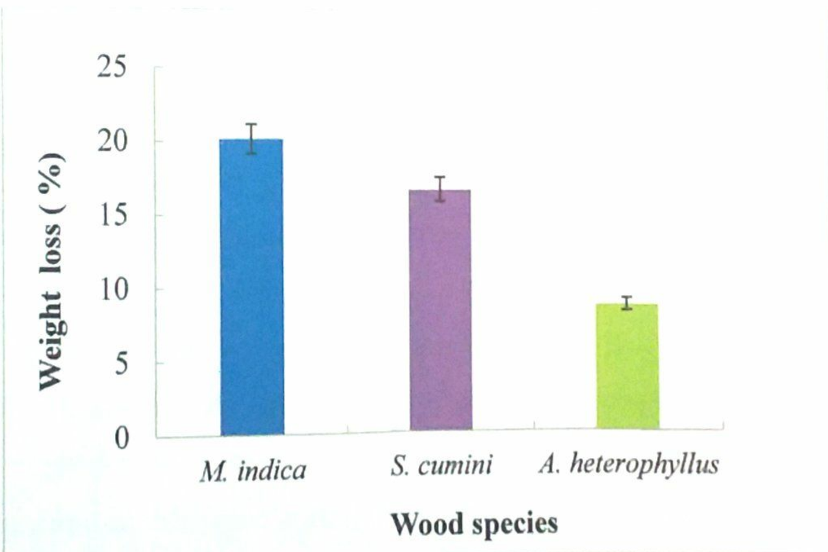

Figure 4.1: Average weight losses (%) of sapwood of *M. indica, S. cumini and A. heterophyllus species* due to decay by *Trametes versicolor.*

From the figure 4.1, the average weight losses (%) of *M. indica* sapwood is (20.14%), *S. cumini* sapwood is (16.61%) and *A. heterophyllus* sapwood is (8.69%) due to decay of *Trametes versicolor*. From the analysis of variance (Table 4.1) it has been found that there was significant difference among the weight losses (%) of *M. indica, S. cumini* and

A. heterophyllus sapwood. The weight loss (%) of *M. indica* sapwood is significantly higher than other two species. The weight loss (%) is significantly lowest in *A. heterophyllus* sapwood (Appendix-02).

The average weight losses (%) of heartwood of *M. indica, S. cumini and A. heterophyllus* wood species due to decay by *Trametes versicolor* are presented in figure 4.2.

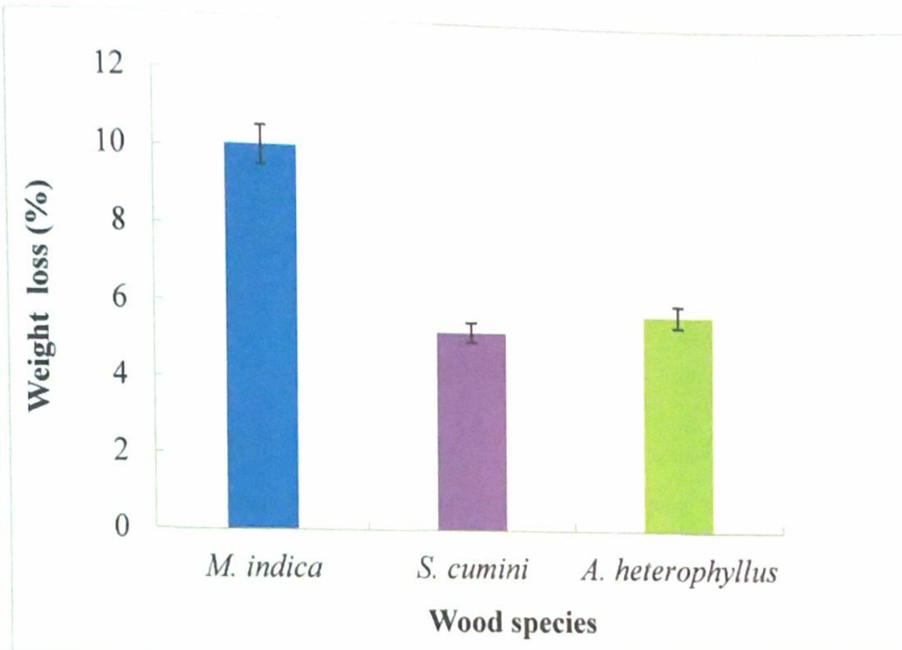

Figure 4.2: Average weight losses (%) of heartwood of *M. indica, S. cumini* and *A. heterophyllus species* to decay by *Trametes versicolor*.

From the figure 4.2, the average weight losses (%) of *M. indica, S. cumini* and *A. heterophyllus* heartwood are 10.12%, 5.33% and 5.79% respectively due to decay of *Trametes versicolor*. From the analysis of variance (Table 4.1) it has been found that there was significant difference among the weight losses (%) of *M. indica, S. cumini* and *A. heterophyllus* heartwood. But, there is no significant difference between *S. cumini* and *A. heterophyllus* heartwood. The weight loss (%) of *M. indica* heartwood is significantly higher than other two species. The weight loss (%) is lowest in *S. cumini* heartwood (Appendix-03).

The average weight losses (%) of sapwood of *M. indica, S. cumini and A. heterophyllus* wood species due to decay by *Ceriporiopsis subvermispora* are presented in figure 4.3.

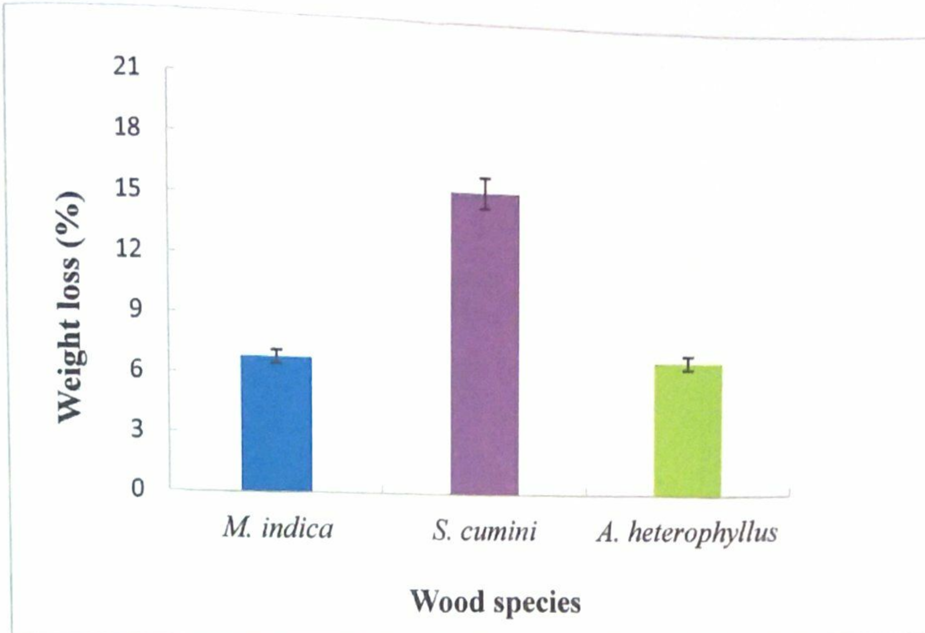

Figure 4.3: Average weight losses (%) of sapwood of *M. indica, S. cumini* and *A. heterophyllus* wood due to decay by *Ceriporiopsis subvermispora.*

From the figure 4.3, the average weight losses (%) of *M. indica* sapwood is (6.89%), *S. cumini* sapwood is (15.53%) and *A. heterophyllus* sapwood is (6.99%) *due to decay* of *Ceriporiopsis subvermispora*. From analysis of variance (Table 4.2) it has been found that there was significant difference among the weight losses of *M. indica, S. cumini and A .heterophyllus* sapwood. But, there is no significant difference between *A. heterophyllus* and *M. indica* sapwood. The weight loss (%) of *S. cumini* sapwood is significantly higher than other two species. The weight loss (%) is significantly lowest in *M. indica* sapwood (Appindix-04).

The average weight losses (%) of heartwood of *M. indica, S. cumini and A. heterophyllus* wood species due to decay by *Ceriporiopsis subvermispora* are presented in figure 4.4.

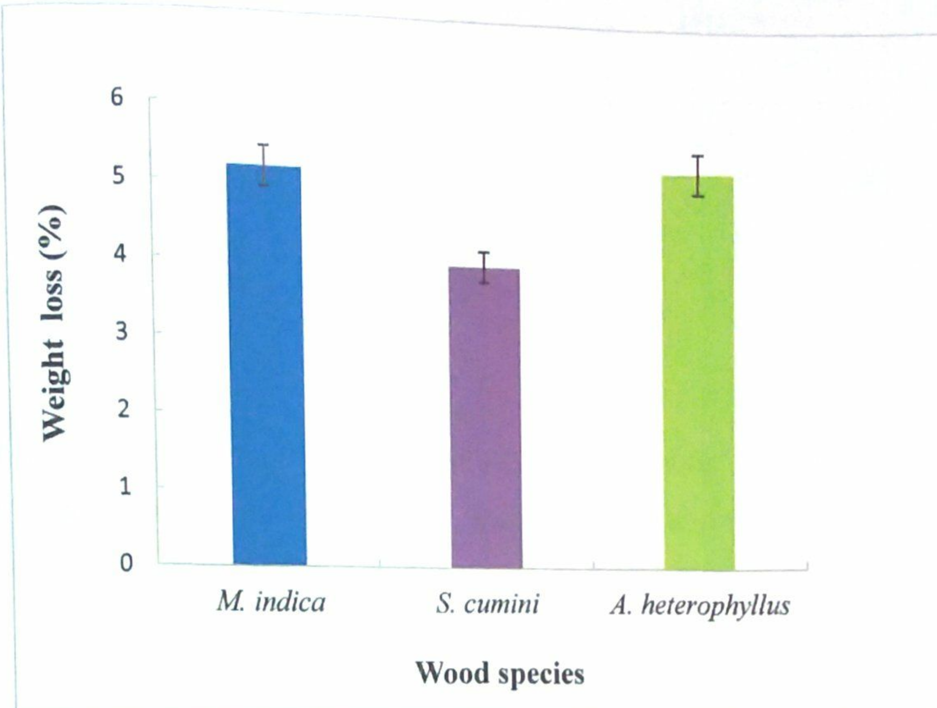

Figure 4.4: Average weight losses (%) of heartwood of *M. indica, S. cumini* and *A. heterophyllus species* due to decay by *Ceriporiopsis subvermispora.*

From the figure 4.4, that the average weight losses (%) of *M. indica*, S. *cumini A. heterophyllus* heartwood are 5.19%, 3.96% and 5.35% respectively *due to decay* of *Ceriporiopsis subvermispora*. From analysis of variance (Table 4.2) it has been found that there was significant variation among the weight loss of *M. indica, S. cumini and A. heterophyllus* heartwood. But, there is no significant difference between *A. heterophyllus* and *M. indica* heartwood. The weight loss (%) of *A. heterophyllus* heartwood is significantly higher than other two species. The average weight loss (%) is significantly lowest in *S. cumini* heartwood (Appendix-05).

Table 4.1: Summarize of ANOVA for weight losses (%) among *M. indica, S. cumini and A. heterophyllus* sapwood and heartwood caused by *Trametes versicolor.*

Source of variation	Sapwood	Heartwood
Wood species	*	*

Note: * indicates significant difference, $p<0.05$.

Table 4.2: Summarize of ANOVA for weight loss (%) among *M. indica, S. cumini and A. heterophyllus* both sapwood and heartwood caused by *Ceriporiopsis subvermispora.*

Source of variation	Sapwood	Heartwood
Wood species	*	*

Note: * indicates significant difference, $p<0.05$.

Discussion

The weight losses (%) among these three species are different in *Trametes versicolor* and *Ceriporiopsis subvermispora* because the decay mechanism of these two fungi is different. From the literature review, *Trametes versicolor* grows at 30^0C temperature for 3 days and *Ceriporiopsis subvermispora* grows after 2 weeks of cultivation. So the decay of *Trametes versicolor* is always higher than *Ceriporiopsis subvermispora* (Moore, 2011 and Enoki et *al*., 1999).

In this study, the weight loss (%) of heartwood was lower than the weight loss (%) of the sapwood for agar block test. In this study it has also been found that the weight losses (%) among *M. indica, S. cumini and A. heterophyllus* sapwood and heartwood were significantly different. The presence of reserve food in the parenchyma cells of sapwood may increase its susceptibility to decay and particularly to bacterial and fungal staining and the main reason of natural durability of heartwood of some species are presences of toxic substances in the heartwood (Panshin and De Zeeuw, 1984).

The decay resistance of heartwood is greatly affected by difference in the preservation qualities of the wood extractives, the attacking fungus and the condition of exposure (Anon, 1999). The natural durability of timber largely depends on the extent of heartwood formation with accumulation of extractable chemicals. These chemicals impart natural durability to the heartwood against decay and vary in quality in different timber species resulting in variability in natural durability (Akhter *et al.*, 1992).
Difference of mass losses between tree species could be explained by the variation in wood properties such as extractive content, color and density (Yamamoto *et al.*, 2003). Generally, weight loss of wood caused by fungal attack depends on wood species and also species and strain of fungi (Pildain *et al.*, 2005). The ability of fungi to cause weight loss in wood depends on their ability to degrade lignin (Harsh and Tiwari, 1990). White rot fungi grow better on wood samples dominated by broad-leaved wood species (hardwood) over conifer (softwood) (Schmidt, 2006).

Classification of natural durability is evaluated based on the weight loss of heartwood specimens by a fungus in the laboratory test and the weight loss ranges proposed differ somewhat in the literature. According to European Standard EN 350-1 (Anon, 1994) species with weight loss less than 5% is classified as very durable, 5 to 10% as durable, 10 to 20% as moderately durable, 20 to 30% as slightly durable and above 30% as not durable. Again, ASTM (1989) classify very resistant showing weight loss of 0 to 10%, resistant 11 to 24%, and moderately resistant 25 to 44%, non-resistant above 44%. On the other hand, according to Anon (1972) species showing weight loss of less 1% is classified as very durable, 1 to 5% as durable, 5 to 10% as moderately durable, 10 to 30% as non-durable and over 30% as perishable.

Table 4.3: According to European Standard EN-350 the heartwood and sapwood of *M. indica, S. cumini and A. heterophyllus* wood species durability classification due to decay of *Trametes versicolor* are given below:

species	Heartwood		Sapwood	
	Weight loss%	Durability class	Weight loss %	Durability class
M. indica	10.10	Moderately Durable	20.14	Slightly durable
S. cumini	5.33	Durable	16.61	Moderately durable
A. heterophyllus	5.79	Durable	8.69	durable

Table 4.4: According to European Standard EN -350 the heartwood and sapwood of *M. indica, S. cumini and A. heterophyllus* wood species durability classification due to decay of *Ceriporiopsis subvermispora* are given below:

species	Heartwood		Sapwood	
	Weight loss%	Durability class	Weight loss %	Durability class
M. indica	5.19	Durable	6.89	Durable
S. cumini	3.96	Very Durable	15.53	Moderately durable
A. heterophyllus	5.35	Durable	6.99	Durable

Classification of natural durability is evaluated based on the weight loss of heartwood specimens by a fungus in the laboratory test form different literature. From the above Table 4.3 and 4.4, in case of heartwood, it has been found that durability class of *S. cumini and A. heterophyllus* wood are durable due to decay of *Trametes versicolor*. Because the average weight loss (%) of these species is less than 10% at agar block test. But *M. indica* wood *is* moderately durable because the average weight loss of this species less than 20%. Otherwise, due to decay of *Ceriporiopsis subvermispora*, it has been found that durability class of *M. indica, and A. heterophyllus* wood are durable. Because the average weight loss (%) of these species are less than10% at agar block test. But, S. *cumini* species is very durable because the average weight loss of this species less than 5%.

CHAPTER-FIVE

CONCLUSION

Mangifera indica, Syzygium cumini and *Artocarpus heterophyllus* species are very common species in Bangladesh and the wood of these species are widely used. But the potential uses of this wood are hampered by due to wood destroying agents. The natural decay resistance determined by the agar block test using white rot fungus, *Syzygium cumini* and *Artocarpus heterophyllus* that can be classified as durable wood species and *Mangifera indica* can be classified as moderately durable. Wood products from *Mangifera indica, Syzygium cumini* and *Artocarpus heterophyllus* have a possibility for outdoor uses instead of those from high value durable species or treated wood wish preservatives. To fully understand the natural decay resistance of timbers it would be necessary for assessments of resistance to fungal decay using a wide range of species of wood rotting fungi. The performance of a wood in service is likely to be determined by those fungi which it is particularly susceptible. It also helps to economic utilization of timber species. Otherwise, Wood susceptible to attack with brown rots fungus, but no study has been conducted against brown rot fungi of the above mentioned species. So, further studies should be necessary to determine the decay resistance by brown rot fungi.

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Appendix

Appendix: 01

Table1: Average weight losses (%) of sapwood and heartwood of *Magnifera indica, Syzygium cumini and Artocarpus heterophyllus* wood due to decay by *Trametes versicolor.*

Species type	Type of wood	Average weight loss (%)
Mangifera indica	Sapwood	20.14(±1.58)
	Heartwood	10.10(±0.96)
Syzygium cumini	Sapwood	16.61(±0.93)
	Heartwood	5.33(±0.96)
Artocarpus heterophyllus	Sapwood	8.69(±1.37)
	Heartwood	5.79(±0.59)

Note: Values in parenthesis are standard deviation

Table 2: Average weight losses (%) of sapwood and heartwood of *Mangifera indica, Syzygium cumini and Artocarpus heterophyllus* wood due to decay by *Ceriporiopsis subvermispora.*

Species type	Type of wood	Average weight loss (%)
Mangifera indica	Sapwood	6.89(±0.52)
	Heartwood	5.19(±0.38)
Syzygium cumini	Sapwood	15.53(±0.93)
	Heartwood	3.96(±0.39)
Artocarpus heterophyllus	Sapwood	6.99(±0.94)
	Heartwood	5.35(±0.82)

Note: Values in parenthesis are standard deviation

Appendix: 02

ANOVA

Dependent variable: weight loss (%)

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	412.550	2	206.275	117.603	.000
Within Groups	26.310	15	1.754		
Total	438.860	17			

ltiple Comparisons

pendent variable: weight loss (%)

VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig	95% Confidence Interval Lower Bound	Upper Bound
ngifera indica TV sap wood	*Syzgium cumini* TV sap wood	3.53167*	.76463	.000	1.9019	5.1614
	Artocarpus heterophyllus TV sap wood	11.45000*	.76463	.000	9.8202	13.0798
zgium cumini TV sap wood	*Mangifera indica* TV sap wood	-3.53167*	.76463	.000	-5.1614	-1.9019
	Artocarpus heterophyllus TV sap wood	7.91833*	.76463	.000	6.2886	9.5481
tocarpus heterophyllus TV p wood	*Mangifera indica* TV sap wood	-11.45000*	.76463	.000	-13.0798	-9.8202
	Syzgium cumini TV sap wood	-7.91833*	.76463	.000	-9.5481	-6.2886

e mean difference is significant at the 0.05 level.

Appendix: 03

ANOVA

Dependent variable: weight loss (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.611	2	25.305	34.327	.000
Within Groups	11.058	15	.737		
Total	61.668	17			

Multiple Comparisons

Dependent variable: weight loss (%)

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mangifera indica TV heartwood	*Syzgium cumini* TV heart wood	3.76333*	.49571	.000	2.7067	4.8199
	Artocarpus heterophyllus TV heart wood	3.30667*	.49571	.000	2.2501	4.3633
Syzgium cumini TV heart wood	*Mangifera indica* TV heartwood	-3.76333*	.49571	.000	-4.8199	-2.7067
	Artocarpus heterophyllus TV heart wood	-.45667	.49571	.372	-1.5133	.5999
Artocarpus heterophyllus TV heart wood	*Mangifera indica* TV heartwood	-3.30667*	.49571	.000	-4.3633	-2.2501
	Syzgium cumini TV heart wood	.45667	.49571	.372	-.5999	1.5133

The mean difference is significant at the 0.05 level.

Appendix: 04

ANOVA

Dependent variable: weight loss (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	295.239	2	147.619	220.712	.000
Within Groups	10.033	15	.669		
Total	305.271	17			

ltiple Comparisons

pendent variable: weight loss (%)

VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
ngifera indica cs sapwood	*Syzgium cumini* cs sapwood	-8.64000*	.47217	.000	-9.6464	-7.6336
	Artocarpus heterophyllus sapwood	-.09833	.47217	.838	-1.1047	.9081
zgium cumini cs sapwood	*Mangifera indica* cs sapwood	8.64000*	.47217	.000	7.6336	9.6464
	Artocarpus heterophyllus sapwood	8.54167*	.47217	.000	7.5353	9.5481
tocarpus heterophyllus owood	*Mangifera indica* cs sapwood	.09833	.47217	.838	-.9081	1.1047
	Syzgium cumini cs sapwood	-8.54167*	.47217	.000	-9.5481	-7.5353

he mean difference is significant at the 0.05 level.

Appendix: 05

ANOVA

Dependent variable: weight loss (%)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.042	2	3.021	9.387	.002
Within Groups	4.828	15	.322		
Total	10.869	17			

Multiple Comparisons

Dependent variable: weight loss (%)

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Mangifera indica TV heartwood	*Syzgium cumini* TV heart wood	3.76333*	.49571	.000	2.7067	4.8199
	Artocarpus heterophyllus TV heart wood	3.30667*	.49571	.000	2.2501	4.3633
Syzgium cumini TV heart wood	*Mangifera indica* TV heartwood	-3.76333*	.49571	.000	-4.8199	-2.7067
	Artocarpus heterophyllus TV heart wood	-.45667	.49571	.372	-1.5133	.5999
Artocarpus heterophyllus TV heart wood	*Mangifera indica* TV heartwood	-3.30667*	.49571	.000	-4.3633	-2.2501
	Syzgium cumini TV heart wood	.45667	.49571	.372	-.5999	1.5133

The mean difference is significant at the 0.05 level.