SAPSTAIN MICROORGANISMS ASSOCIATED WITH SELECTED COMMERCIALLY IMPORTANT TIMBERS OF KERALA AND THEIR POSSIBLE CONTROL

THESIS
SUBMITTED
IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
OF THE COCHIN UNIVERSITY OF SCIENCE
AND TECHNOLOGY

By

E.J. MARIA FLORENCE M.Sc.

DIVISION OF FOREST PATHOLOGY
KERALA FOREST RESEARCH INSTITUTE
PEECHI-680 653, KERALA

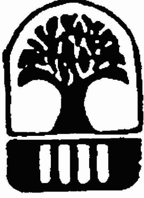
MAY 1996

DECLARATION

I hereby declare that this thesis entitled **Sapstain microorganisms associated with selected commercially important timbers of Kerala and their possible control**, has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Peechi
May 15, 1996

E.J. MARIA FLORENCE



KERALA FOREST RESEARCH INSTITUTE
PEECHI - 680 653
THRISSUR DIST.
KERALA STATE
INDIA

Tel : Kannara - 0487 - 782365
Kannara - 0487 - 782037
- 782061
- 782062
- 782063
- 782064
FAX : 0487 - 782249
TGM : KEFORINST
TLX : 887-275 KFRI IN

15th May, 1996

CERTIFICATE

This is to certify that the thesis entitled **Sapstain microorganisms associated with selected commercially important timbers of Kerala and their possible control** embodies the result of original research work conducted by Mrs. E.J. Maria Florence, under our guidance and supervision. We further certify that no part of this thesis has previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles of this or other Institute or any University or Society.

Dr. J.K. SHARMA
(Research Guide)
Scientist-in-Charge
Division of Forest Pathology
KFRI, Peechi-680 653

Dr. R. GNANAHARAN
(Research Co-guide)
Scientist-in-Charge
Division of Wood Science
KFRI, Peechi-680 653

CONTENTS

DECLARATION

CERTIFICATE

ACKNOWLEDGEMENTS i

1. INTRODUCTION 1

1.1. MOULD 2

1.2. FUNGAL STAIN 3

1.2.1. Mode of Infection.................... 4

1.2.2. Factors Influencing Fungal Staining ... 4

1.2.3. Economical Significance of Sapstain ... 5

1.2.4. Effect of Sapstain on Wood Properties .. 5

1.3. OBJECTIVES OF THE STUDY 6

2. REVIEW OF LITERATURE.................... 8

2.1. SURFACE STAINING BY MOULD FUNGI 9

2.2. SAPSTAIN 10

2.2.1. Sapstain Fungi 11

2.2.2. Mode of Spread 13

2.2.3. Stain Development 13

2.2.4. Effect of Sapstain on Physical and Strength Properties of Wood 14

2.2.5. Effect of Sapstain on Chemical Properties of Wood.................... 15

2.2.6. Chemical Control of Sapstain 16

2.2.7. Biological Control of Sapstain 19

3. FUNGI ASSOCIATED WITH SAPSTAIN OF VARIOUS TIMBER SPECIES 22

3.1. INTRODUCTION 22

3.2. MATERIALS AND METHODS 22

3.2.1. Selection of Timber Species 22

3.2.2. Collection of Wood Samples 24

3.2.3. Isolation and Identification of Microorganisms 26

3.2.4. Artificial Inoculation Trials of Fungal Isolates to Confirm Their Ability to Cause Stain 28

3.3. RESULTS 28

3.3.1. Isolation and Identification of Microorganisms 28

3.3.2. Artificial Inoculation of Fungal Isolates to Confirm their Ability to Cause Stain . 38

3.4. DISCUSSION 39

4. FACTORS AFFECTING GROWTH AND COLONIZATION OF *BOTRYODIPLODIA THEOBROMAE* 43

4.1. INTRODUCTION 43

4.2. MATERIALS AND METHODS 44

4.2.1. Influence of Moisture Content of Wood on the Growth of *Botryodiplodia theobromae* 44

4.2.2. Effect of Ambient Humidity, Temperature and Moisture Content of Rubber Wood on the Growth of *Botryodiplodia theobromae* 45

4.2.3. Comparative Growth of *Botryodiplodia theobromae* on *Hevea brasiliensis* and *Alstonia scholaris* 47

4.2.4. Temperature Tolerance of *Botryodiplodia theobromae* 48

4.2.5. Comparison of Weight Loss in *Hevea brasiliensis* Caused by *Botryodiplodia theobromae* with that of *Alstonia scholaris* 49

4.3. RESULTS 50

4.3.1. Influence of Moisture Content of Wood on the Growth of *Botryodiplodia theobromae*. 50

4.3.2. Effect of Ambient Humidity, Temperature and Moisture Content of Rubber Wood on the Growth of *Botryodiplodia theobromae* 51

4.3.3. Comparative Growth of *Botryodiplodia theobromae* on *Hevea brasiliensis* and *Alstonia scholaris* 55

4.3.4. Temperature Tolerance of *Botryodiplodia theobromae* 57

4.3.5. Comparison of Weight Loss of *Hevea brasiliensis, Ailanthus triphysa* and *Alstonia scholaris* Caused by *Botryodiplodia theobromae* 59

4.4. DISCUSSION 61

5. EVALUATION OF CHEMICALS FOR THE CONTROL OF MOULD AND SAPSTAIN. 66

5.1. INTRODUCTION 66

5.2. MATERIALS AND METHODS 66

5.2.1. Screening of Fungicides for Controlling Mould and Sapstain 66

5.2.2. Poisoned-food Method 66

5.2.3. Evaluation of Fungicides on Sterile Wood Blocks 69

5.2.4. Efficacy of Sodium Azide Against Stain and Mould Fungi. 70

5.3. RESULTS 72

5.3.1. Screening of Fungicides for Controlling Mould and Sapstain 72

5.3.2. Poisoned-food Method 72

5.3.3. Evaluation of Fungicides Using Sterile Wood Blocks 72

5.3.4. Efficacy of Sodium Azide Against Stain and Mould Fungi. 77

5.4. DISCUSSION 82

6. BIOLOGICAL CONTROL OF MOULD AND SAPSTAIN 86

6.1. INTRODUCTION 86

6.2. MATERIALS AND METHODS 87

6.2.1. Isolation of Biological Control Organisms 87

6.2.2. Testing of Antagonistic Activity 87

6.2.3. Wood Block Test 90

6.2.4. Field Test 90

6.3. RESULTS 93

6.3.1. Isolation of Antagonistic Microorganism 93

6.3.2. Testing of Antagonistic Activity 94

6.3.3. Wood Block Test 96

6.3.4. Field Test 100

6.4. DISCUSSION 105

7. CHEMICAL ANALYSIS OF SAPSTAINED RUBBER WOOD 109

7.1. INTRODUCTION 109

7.2. MATERIALS AND METHODS 110

7.2.1. Alcohol-benzene Solubility. 110

7.2.2. Estimation of Holocellulose 111

7.2.3. Estimation of Lignin 112

7.2.4. Estimation of Ash 113

7.2.5. Determination of Carbohydrates 114

7.3. RESULTS 115
7.3.1. Alcohol-benzene Solubility. 115
7.3.2. Estimation of Holocellulose 115
7.3.3. Estimation of Lignin 115
7.3.4. Estimation of Ash 115
7.3.5. Determination of Carbohydrates 120
7.4. DISCUSSION 120

8. STRENGTH AND ANATOMICAL PROPERTIES OF SAPSTAINED RUBBER WOOD 125
8.1. INTRODUCTION 125
8.2. MATERIALS AND METHODS 126
8.2.1. Density 126
8.2.2. Compressive Strength 127
8.2.3. Static Bending Strength 127
8.2.4. Anatomy of Rubber Wood Stained by *Botryodiplodia theobromae* 128

8.3. RESULTS 129
8.3.1. Density 129
8.3.2. Compressive Strength 129
8.3.3. Static Bending Strength 129
8.3.4. Anatomy of Rubber Wood Stained by *Botryodiplodia theobromae* 132
8.4. DISCUSSION 132

SUMMARY 137
LITERATURE CITED. 146

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to Dr. J.K. Sharma, Scientist-in-Charge, Division of Forest Pathology, Kerala Forest Research Institute, for suggesting the research problem, his valuable guidance and advice throughout the course of this work and preparation of the manuscript. I express my gratitude to Dr. R. Gnanaharan, Scientist-in-Charge, Division of Wood Science for his supervision, constructive criticism and encouragement during the course of this investigation.

I am thankful to Dr. P.M. Ganapathy, Dr. S. Kedharnath and Dr. S. Chand Basha, former Directors of KFRI for encouragements. I am indebted to Dr. C.T.S. Nair, former Director, KFRI for permitting me to register as part-time scholar in the Cochin University of Science and Technology and Dr. K.S.S. Nair, Director, KFRI for his constant help and encouragement.

My sincere thanks are also due to Ms. P. Rugmini, Scientist, Division of Statistics and Ms. K.A. Mercy, Assistant Professor, Kerala Agricultural University for advice in statistical analysis of the data.

I am thankful to Dr. M. Balasundaran, Scientist, Division of Pathology for providing help and useful suggestions.

I record my thanks to Shri. J.D. Fernandez, Deputy General Manager and Ms. Aleyamma Philipose and Ms. Mariyakutty Suresh of Hindustan Newsprint Ltd., Velloor for providing necessary help in carrying out chemical analyses. Thanks are also due to the Director, Central Institute of Fisheries Technology, Cochin for extending facilities for testing the strength properties of wood samples.

Thanks are also due to Mr. P.V. Santhosh Kumar, Ms. K. Annapoorni and Mr. K.G. Rizon for typing the manuscript.

My thanks are also due to all my colleagues in the Forest Pathology Division for their cooperation.

I also wish to thank M/s. Evershine Packing Industries, Ollur for carrying out the sawmilling work and preparation of test wood samples.

CHAPTER 1

INTRODUCTION

With the rapid increase in population, demand for timber by various sectors has increased considerably. Also, supply of conventional timbers has reduced due to deforestation. The growing demand for timber can be met to some extent by utilizing alternative species and increasing the timber production through intensive management. Prevention of wastage of wood by adopting appropriate methods is an area where much attention need to be paid since a considerable amount of wood and wood products are destroyed due to the activity of biological agents and chemical factors.

The major destructive agencies to which wood is exposed to, while in service are fire, wear and tear, chemicals, insects, marine borers and microorganisms. Among the microorganisms, fungi cause different types of damages to timber, namely decay, soft rot, mould and stain which are common in tropics. Biodeterioration by bacteria may result in decay and degradation. Subramanian (1983) classified wood inhabiting fungi into the following three categories: 1. moulds and stain fungi that normally do not degrade lignified cell walls and derive their nutrition from contents of dead cells, 2. soft rot fungi capable of limited enzymatic degradation of lignified cell walls and 3. decay fungi with high ability to degrade wood causing either brown or white rot. Of these, stain fungi and surface moulds are quite common in Kerala due to the conducive hot-humid climatic conditions prevalent in the State, situated between latitudes 8°18' and 12°48'N and longitudes 74°52' and 77°22'E. The State has typical tropical climate with average annual rainfall varying from 750-4000 mm, mean monthly temperature ranging from 17 to 30°C and mean relative humidity varying from

75 to 92 per cent. Most of the wood-based industries distributed throughout the State utilize different timber species of available soft woods for various purposes. The major problem in the utilization of these species is their susceptibility to fungal sapstain and surface mould growth which impart a blotchy appearance to the wood. The discoloured wood is not usually preferred for making packingcases due to hygienic reasons.

1.1 MOULD

Mould occurs on the surface of wood and generally, it has woolly or powdery appearance. Moulds cause superficial staining due to the development of masses of coloured spores on the wood surface. The mould fungi can penetrate into the wood, and their presence can be detected only by microscopic examination as the hyphae are colourless. But the discolouration of the wood occurs mostly near the surface. Predominant colours of moulds are shades of black, green, orange and yellow. But black colour is the most common; occasionally other light shades are also seen. The discolouration imparted by spore masses of fungi can generally be removed by planing or even by brushing. On hardwoods, moulds often cause shallow staining in addition to the surface discolouration. Profuse mould growth is seen during rainy season and also in wet stage. Moulds also occur abundantly on green timber, sawn wood and veneers.

Most of the mould fungi belong to the group Deuteromycetes (Fungi Imperfecti). Some of the common moulds are the species of *Trichoderma*, *Gliocladium* and *Penicillium*; all of them colour the surface with their green spores. Species of *Alternaria*, *Rhizopus* and *Aspergillus* are responsible for black colour; *Monilia* spp. cause orange discolouration. The moulds attack mainly the more utilizable material of the wood, the parenchymatous elements, starch and sugar, affecting only the appearance and permeability of wood.

1.2 FUNGAL STAIN

Fungal staining of wood is a serious problem which has attracted much attention during the last few years. Since the fungi responsible for this problem derive their nourishments from the reserve food materials stored in the living cells, they normally affect the sapwood and the stain is thus named as 'sapstain'. Sapstaining fungi have pigmented hyphae or they secrete soluble pigments which diffuse into and colour the cell wall of the wood. As the colour imparted to the wood by these fungi is frequently bluish-grey, the stain is often referred to as "blue stain". Blue stain or sapstain, mostly caused by fungi with dark coloured hyphae, is the most prevalent and economically important of all the fungal stains.

Stains may be superficial or deep seated, penetrating up to several layers of wood tissues. The latter type of stains are caused by fungi that have dark coloured hyphae or those produce pigments diffusing into the wood tissues. The colouration caused by fungi is not a stain in the true sense of the word. The presence of numerous dark-coloured hyphae in the translucent cells of the wood produces the tinting visible on the surface. The colouring matter present in the dark-coloured hyphae which is responsible for blue stain has been shown by Kitamura and Kondo (1958) to be a melanin-based pigment (Zink and Fengel, 1988; 1989; 1990).

The fungi causing stain belong to the groups Deuteromycetes and Ascomycetes. Even though stain caused by dark bluish hyphae is the common type of sapstain, bright-coloured stains, in shades of red, purple green and yellow, are also seen (Scheffer and Lindgren, 1940; Singh, 1974). The common blue stain found in tropics is caused by *Botryodiplodia theobromae* Pat. (Cartwright and Findlay, 1958; Olofinboba, 1974; Hong, 1976). This fungus is known to cause sapstain in rubber wood (Sujan *et al.*, 1980; Florence, 1991; Hong and Wong, 1993).

1.2.1 Mode of infection

The spread of sapstain infection in wood is reported to be through air currents, insects and sawmilling machinery (Verrall, 1941). Air in sawmills contains large number of spores of the stain fungi and also fragments of mycelium from the dust formed during the sawing of stained logs. Spores of many sapstain fungi are sticky and they are distributed through rain drops splashing from board to board (Dowding, 1969). Bark beetles also play an important role in disseminating the spores (Rumbold, 1936; Dowding, 1970). While feeding on timber, beetles carry the spores of stain fungi and spread the infection from one log to another. Mites also act as agents to transport ascospores of coniferous blue stain fungi (Moser, 1985).

1.2.2 Factors influencing fungal staining

Four conditions are necessary for the successful establishment of stain fungi in wood. They are, 1. sufficient moisture content of wood, 2. food source in the form of starch and sugars stored in cell cavities, 3. suitable temperature and 4. oxygen obtained from the air. Among these, moisture content of wood is an important factor influencing the infection by sapstain fungi. The moisture content of the sapwood in a vigorously growing tree is too high to permit the growth of staining fungi. But when the tree is felled, the moisture content at the cut ends of the logs and portions from which the bark has been peeled off or damaged during felling, gets reduced and the exposed portions become susceptible to infection by spores of staining fungi.

Under favourable conditions, spores germinate within hours and penetrate the wood through ruptured vessels or tracheids and exposed wood rays. The hyphae then rapidly colonize the parenchyma cells in the wood rays or longitudinal parenchyma by direct pit penetration. Stain fungi can grow up to 0.5 mm in tangential plane, 1 mm radially and 5 mm longitudinally over a 24 hour period

under ideal conditions (Lindgren, 1942). The rapid fungal growth rate results in extensive colonization of freshly sawn wood.

1.2.3 Economical significance of sapstain

Although significant loss in wood quality due to stain has been reported worldwide, the exact cost of this loss is rather difficult to assess. Scheffer (1973) estimated that annual loss due to stain exceeded 50 million dollars in the United States. Stain loss generally increases with increasing sapwood content. Percentage of loss would be higher in a climate conducive to fungal growth. Sapstain is responsible for deterioration of large quantities of susceptible timbers. Their value is highly reduced for decorative purposes and if heavily stained, the wood becomes unfit for paint finishing. For wood meant for packingcases, and containers intended for foodstuffs or drinks, bright clean appearance is considered essential; sapstain seriously reduces the market value of wood intended for such packingcases. Besides spoiling the appearance, staining fungi and moulds may actually damage the stored goods in contact with the wood of the container. Fungal stain of pulp chips is a concern for the pulp and paper industry because this results in loss of pulp yield and also quality of pulp (Shields and Unligil, 1968). Timbers, susceptible to sapstain attack, are to be processed at the earliest to avoid fungal infection and this urgency often causes practical difficulties to the manufacturers.

1.2.4 Effect of sapstain on wood properties

Although the most obvious effect of staining fungi is colouration, some stain fungi may also affect wood properties. Some fungi are known to reduce the toughness of wood. Findlay and Pettifor (1937; 1939) found that toughness was reduced by 30- 40 per cent of its original value. Stained wood is not generally recommended for structural purposes where strength is critical. Conditions favourable for stain development are also conducive to decay initiation. In

addition to strength, staining fungi increase wood permeability by degradation of ray parenchyma cell walls (Lindgren and Scheffer, 1939).

1.3 OBJECTIVES OF THE STUDY

In Kerala State, small-scale timber industries such as packingcase, plywood and match units utilize a large quantity of wood from miscellaneous tree species. At the end of 1987, there were 2915 registered industrial units which include sawmills and units which produce plywood, splints and veneers, furniture and fixtures, packingcase, pulp and paper etc. (Department of Factories and Boilers, Thiruvananthapuram, 1987). The major timber species employed in the match and packingcase industries are *Ailanthus triphysa* (Dennst.) Alston, *Alstonia scholaris* (Linn.) R. Br., *Anacardium occidentale* Linn. *Bombax ceiba* (Linn.), *Mangifera indica* Linn., and other suitable soft wood species available. With the fast depletion of natural forest resources, it has become necessary to depend on alternative timber sources to meet the current needs of wood. Rubber wood, *Hevea brasiliensis* Hbk. Muell Arg. with several positive attributes merits consideration when we look for an alternate cheap timber. In India, at present rubber wood is mainly used as firewood, and for making packingcases and plywood. Studies in Malaysia have established the suitability of rubber wood for furniture and panel products (Wong, 1979; Wong and Ong, 1979). With the increase in the production of rubber wood, it is becoming increasingly popular as a source of timber particularly for making furniture (Gnanaharan, 1984). But its susceptibility to sapstain and mould fungi, aggravated by the tropical warm-humid climate of Kerala, is posing a serious problem in the proper utilization of not only the rubber wood but also other timber species used for making packingcases, match veneers and splints and plywood.

In India, except for a few isolated studies, not much work has been done to find out the fungi associated with sapstain and their effect on wood. Bakshi (1953)

studied the cause of staining of timbers in general and its prevention. The cause of discolouration of rosewood (*Dalbergia latifolia*) (Roxb.) veneers was investigated by Ananthanarayanan (1971). Singh (1974) described several species of *Chlorosplenium* which imparted green colouration to the infected wood. Since no information is available on the spectrum of microorganisms responsible for causing sapstain in various commercially important timbers of Kerala and the quantum of damage these cause to the timber, the present study was undertaken with the following objectives:

1. to find out the fungi responsible for causing sapstain in selected economically important timbers of Kerala,

2. to study the effect of humidity, temperature and moisture content of rubber wood on colonization of *Botryodiplodia theobromae*,

3. to study the effect of sapstain on the chemistry of stained wood,

4. to ascertain changes in the physical and strength properties of stained wood and

5. to examine chemical and biological control of sapstain.

CHAPTER 2

REVIEW OF LITERATURE

Wood is deteriorated mainly by the activity of biotic agents, namely microorganisms and insects. The former, a major factor for biodegradation, accounts for serious loss of wood in quality as well as quantity. The decay and staining caused by fungi and to a lesser extent by bacteria are the main causes of timber loss. Fungi are unique organisms that have systems to penetrate, invade, digest and absorb soluble constituents from complex wood tissues with the help of enzymes (Zabel and Morrell, 1992).

Wood inhabiting fungi are classified into three categories depending on the type of damage such as moulds and stains, decay and soft rot (Subramanian, 1983). Of these, staining due to superficial moulds or sapstain or blue stain fungi is the most serious problem in humid tropics as well as temperate climatic regions affecting a large array of timbers. Zabel and Morrell (1992) reported that fungal stains are worldwide in distribution, but sapstain becomes a more serious problem when wood containing high percentage of sapwood is harvested and dried under warm, humid conditions, suitable for rapid fungal growth.

Sapstain of softwood timber was first recorded by Unger (1847) on spruce, but it was quickly realized that pines were more susceptible than other tree species. The observation that sapstain in softwood is caused by a fungus possessing dark-brown hyphae is generally attributed to Hartig (1878). Schrenk (1903) and Hedgecock (1906) were the first to describe sapstain in the United States of America on pines and sweet gum.

2.1 SURFACE STAINING BY MOULD FUNGI

Surface stain, caused by the superficial growth of mould fungi, occurs most frequently on sawn timber and logs which are exposed to rain and high humidity. The colour is mainly imparted due to coloured spore masses produced on the surface of the timber. The common mould fungi belong to Ascomycetes and Deuteromycetes (Fungi Imperfecti) (Scheffer, 1973). *Aspergillus* spp. and *Penicillium* spp. are the common mould fungi found on most of the tropical hardwoods (Cartwright and Findlay, 1958). Thapa (1971) reported *Penicillium* spp. and *Mucor* spp. as the common saprophytes growing on the surface of Shorea species. *Aspergillus* sp., *Penicillium* sp. and *Mucor* sp. also cause discolouration of rosewood (*Dalbergia latifolia* Roxb.) veneers in India (Ananthanarayanan, 1971). In Peninsular Malaysia, *Penicillium* spp., and *Trichoderma* spp. cause superficial stain on *Dyera costulata* Hk. f., a valuable light-coloured hardwood tree species (Hong, 1976). *Penicillium* sp. and *Aspergillus* sp. were also reported to be the common mould fungi occurring on the surface of rubber wood (Sujan *et al.*, 1980). Recently, Masuka and Kariwo (1992) reported the prevalence of *Penicillium* spp. as mould on pine logs.

Trichoderma spp., especially *T. viride* Pers. are another group of common mould fungi which colonize on wood surface (Cserjesi, 1967; Toole, 1971). *T. viride* was one of the most prevalent mould on the surface of pine logs in Zimbabwe (Masuka and Kariwo, 1992).

The mould fungi enter into the wood tissue through ruptured cells, vessels and exposed rays and spread from one cell to another cell via pits (Zabel and Morrell, 1992). As they infect the pit membrane, the wood becomes more receptive to the flow of fluids. Treatment with moulds had been proposed as a method of improving permeability of wood to preservative solutions (Lindgren and Wright, 1954; Schulz, 1956).

Since *Trichoderma* spp., *Penicillium* spp. and *Aspergillus* spp. are the most dominant fungi causing surface colouration, they are accepted as the test fungi in fungicidal evaluations against moulds (Hong, 1981; Drysdale and Preston, 1982; Cserjesi and Johnson, 1982; Plackett, 1982; 1984; Plackett and Chittenden, 1986; Presnell and Nicholas, 1990). These fungi are reported to be tolerant to some fungicidal formulations as they were re-isolated from the treated wood (Leightley, 1985; 1986; Drysdale, 1986; Drysdale and Keirle, 1986; Balasundaran and Gnanaharan, 1990). This is because moulds have been shown to detoxify wood preservatives (Verrall, 1949; Brown, 1953; Stranks and Hulme, 1975).

Colonization of wood by *Trichoderma* spp. inhibit the growth of stain and decay fungi on pine pulpwood during storage (Lindgren, 1952; Hulme and Shields, 1972). The potential of *Trichoderma* as a biological control agent has been explored (Bruce and King, 1983; Bruce *et al.*, 1984; 1989; Bruce and Highley, 1989).

2.2 SAPSTAIN

Stain caused by the growth of fungi on wood is commonly referred to as fungal stain. When the fungal growth is confined to sapwood, such stain is commonly known as sapstain. Sapstain is also known as 'blue stain' because the colour of the stained wood is frequently blue or bluish-grey. According to Zink and Fengel (1988; 1989; 1990) the staining of the wood may be caused either by the presence of coloured fungal hyphae, or by the secretion of coloured melanin-based pigments in the cell. Stain fungi can utilize the reserve food materials stored in the cells of the sapwood.

2.2.1 Sapstain fungi

Hedgecock (1906) was the first mycologist who described a number of new species of *Ceratostomella* and Fungi Imperfecti causing stain in pine and sweet gum. Subsequently, there were several reports on fungal sapstain of wood in the U.S.A. (Rumbold, 1929; 1931; 1936; 1941; Davidson, 1935; 1944; Verrall, 1939). Bakshi (1951) revived an older name *Ceratocystis* for the sapstain causing fungi previously included under *Ceratostomella* and presented details of three new species of *Ceratocystis* causing sapstain in the U.K. A very detailed study on taxonomy, artificial dispersal of spores, spore survival and ecology of *Ceratocystis* causing sapstain was made by Dowding (1968).

Kaarik (1980) compiled a useful comprehensive list of all staining fungi. In temperate zones *Ceratocystis* spp. were the predominant sapstain fungi (Kaarik, 1980; Miller and Goodell, 1981) causing stain in *Picea abies* Karst. (Benko, 1983), *Alnus rubra* Bong. (Morrell, 1987) and *Pinus* spp. (Troya and Navarrete, 1989).

In tropical hardwoods, the staining is caused principally by *Diplodia* spp., and in particular *Botryodiplodia theobromae* Pat. (Cartwright and Findlay, 1958; Findlay, 1959; Olofinboba and Lawton, 1968). *B. theobromae*, considered to be a known pathogen for numerous tropical and subtropical plants of economic importance (Punithalingam, 1980), causes stain in *Bombax buonopozense* P. Beauv. (Umezurike, 1969) and in poplar (Pinheiro, 1971). Thapa (1971) carried out investigations on the occurrence of black stain of commercially important trees in a dipterocarp forest of Sabah in Malaysia and found that the stain causing fungus was *B. theobromae*: the fungus was introduced into the wood by an unknown shot hole borer. Heavy economic loss in *Antiaris africana* Engl., an important tropical white wood (Olofinboba, 1974), and in Jelutong (*Dyera costulata* Hk. f.) in Malaysia (Hong, 1976) was reported on account of sapstain caused by *B. theobromae*. Umezurike (1978) studied various aspects of sapstain

due to *B. theobromae* in *Gossweilerodendron balsamiferum* (Verm.) Harms., a forest tree used for constructional purpose in Nigeria. Heavy loss of more than 40 per cent was recorded in Abachi wood (*Triplochiton scleroxylon* K. Schum.) due to sapstain caused by *B. theobromae* (Tabirih and Seehann, 1984). An extensive study on sapstain in peat swamp forests was undertaken in Sarawak and it was found that Ramin (*Gonystylus bancanus* (Miq.) Kurz.) was seriously infected by the sapstain fungus, *B. theobromae* (Hon, 1989). The major problem in the utilization of rubber wood (*Hevea brasiliensis*), now utilized for the manufacture of furniture and other wood products after preservative treatment (Yoichi, 1993), is sapstain caused by *B. theobromae* (Hong *et al.*, 1980; Sujan *et al.*, 1980; Tsunoda *et al.*, 1983; Florence and Sharma, 1990). Masuka (1991) reported *B. theobromae* as the most important fungus causing stain of pines in Zimbabwe.

In addition to dark colours (greyish-black), stains of other colours also occur in different timbers. Kaarik (1980) reported various fungi causing different colours of sapstains. Pink stain, both in sap and heartwood of North American softwoods, has been reported to be caused by *Geotrichum* sp. (Chidester, 1936). Purplish-pink stains are often caused by a species of *Fusarium*, while orange red by *Penicillium* spp. (Findlay, 1959). Fritz (1952) observed a chocolate brown stain by a *Cytospora* sp. in red pine sapwood. Brick red stain caused by *Ascocybe grovesii* Wells. was observed in sitka spruce (Davidson and Lombard, 1954). In India, Singh (1974) reported three species of *Chlorosplenium* causing green stain. *Confertobasidium olivaceo-album* (Bourd. & Galz.) Julich. was reported to cause golden-orange stain in the heartwood of lodgepole pine (*Pinus contorta* Dougl. ex Loud.) (Eslyn, 1981). Pink stain caused by *Arthrographis cuboidea* (Sacc. et Ellis) Singler was observed in red oak treated with sodium pentachlorophenoxide (NaPCP) (Schmidt and Dietz, 1985). Recently, Balasundaran and Gnanaharan (1990) reported a reddish stain caused by *Fusarium decemcellulare* Brick on preservative treated rubber wood.

2.2.2 Mode of spread

The spores of the staining fungi are known to be disseminated by air currents, insects and milling machinery (Verrall, 1941). Bakshi (1951) isolated *Ceratocystis piceae* (Munch) Bakshi from the gallery of the ambrosia beetle indicating that beetles were the agents of tree to tree dissemination of fungal spores. Role of the ambrosia beetles in the transmission of fungal stain had also been reported by Browne and Laurie (1968); Dowding (1969; 1970). Dowding (1969) reported that the spore dispersal of some species of *Ceratocystis* was through water splash. Mites are known to transport ascospores of coniferous blue stain fungi (Moser, 1985). Micro-arthropods are also vectors to introduce sapstaining fungi to freshly sawn pine sapwood boards (Powell *et al.* 1994).

2.2.3 Stain development

Under favourable conditions, the spores of staining fungi germinate and penetrate the wood surface through ruptured vessels and within two days the fungus colonizes heavily the surrounding parenchyma cells (Olofinboba, 1974). Sapstain fungi can grow up to 0.5 mm in the tangential plane, 1 mm radially and 5 mm longitudinally within 24 hours under ideal conditions (Lindgren, 1942).

While studying the effects of *B. theobromae* attack on Abachi wood (*Triplochiton scleroxylon* K. Schum.), Tabirih and Seehann (1984) reported that components of the protoplasm in the parenchyma cells were supposed to constitute the dominant source of nutrients for the stain fungus. Umezurike (1969) observed that *B. theobromae* made perforations in the wood of *Bombax buonopozense* P. Beauv. and formed elongated cavities with pointed ends in the secondary wall. Furthermore, in a similar manner to soft rot fungi, this fungus also metabolized cellulose. Umezurike (1978) also found that the sapstain fungus *B. theobromae* was capable of degrading some components of wood, particularly after utilization

of starch and other soluble carbohydrates. But Fougerousse (1985) reported that the actual components of wood cell wall such as cellulose and lignin were not significantly degraded by stain fungi. Recently, Encinas and Daniel (1994), with the help of SEM and TEM, found that *B. theobromae* was able to destroy the cell wall of tracheid fibres of birch (*Betula verrucosa* Ehrh.) but not that of Caribbean pine (*Pinus caribaea* var. *hondurensis* Barr. and Golf) and Scots pine (*Pinus sylvestris* L.). Detailed microscopic examination showed *B. theobromae* to cause incipient soft rot with S_2 cell wall, delamination of birch fibres and total destruction of parenchyma cells (Encinas and Daniel, 1995).

As the stain fungi grow through the wood, the pigmented hyphae discolour the wood (Findlay, 1959; Zabel and Morrell, 1992). Liese (1970) supported this through his electron microscopic observations on the growth of the stain fungi within wood tissues. Earlier, Kitamura and Kondo (1958) found melanin as the colouring matter. Recently, Zink and Fengel (1988; 1989; 1990) confirmed that hyphae of the stain fungi produced dark melanin- based pigments which imparted the dark colour to the wood.

2.2.4 Effect of sapstain on physical and strength properties of wood

Costa (1955) reported no significant decrease in the bending or impact strength of wood samples of *Pinus radiata* D. Don that had been exposed to infection by *Diplodia* sp. for 12 weeks. Thapa (1971) observed that strength of timber did not reduce significantly due to fungal stain. Tabirih and Seehann (1984) found that there was no significant effect on density, compression and bending strength, impact bending and modulus of elasticity of *Triplochiton scleroxylon* due to the infection of stain fungus, *B. theobromae*. However, in one of the earlier studies, Findlay and Pettifor (1937) reported that only toughness was affected to a level of practical importance and stained timber had to be rejected only when timber of exceptional toughness was required. They further observed that toughness of

the heavily stained softwood was reduced by more than 25 per cent and bending strength by 20 per cent than the normal unaffected wood. Pinheiro (1971) reported that blue stain fungi were responsible for the decrease in the static and dynamic bending properties of poplar wood.

2.2.5 Effect of sapstain on chemical properties of wood

Chemical properties of stained wood have not been studied much. Umezurike (1969) reported that the stain fungus, *B. theobromae*, utilized the cellulose of *Bombax buonopozense* similar to soft rot fungi. The study indicated that *B. theobromae* used starch and other saccharides present in the wood as initial substrates before degrading the cellulose and hemi-cellulose components of the wood; however, it did not degrade the lignin. Tabirih and Seehann (1984) also studied the effects of *B. theobromae* attack on wood of *T. scleroxylon* and found that soluble sugars like mannose and fructose were totally metabolized after 16 weeks of growth. Basic cell wall material, namely cellulose and lignin remained untouched, and hemicellulose was consumed only in insignificant amounts. Gao *et al.* (1994) reported the utilization of wood lipids in lodgepole pine sapwood by the staining fungus, *Ophiostoma piceae* (Munch) Sydow. He could isolate, identify and quantify the lipids utilized by staining fungi. Breuil *et al.* (1995) recently reported that staining fungi, *Alternaria tenuis* Nees., *Ophiostoma* spp. and *Trichoderma harzianum* Rifai produced proteinases and aminopeptidases during their growth. Encinas (1995) reported the cell wall degradation of softwood tracheids of birch and pine infected by *B. theobromae.* Encinas (1996) in another study noted that in addition to the consumption of soluble carbohydrates and extractives, major changes in the ultra structure of fibre cell walls was apparent with rapid attack of the gelatinous layer in aspen and rubber wood by *B. theobromae.*

2.2.6 Chemical control of sapstain

Chemicals have been employed for preventing sapstain since the early 1900s when aqueous solutions of sodium carbonate and borax were applied to wood (Findlay, 1959). In 1930s significant wood loss due to fungal stain resulted in the use of chlorinated phenols and organic mercury compounds (Scheffer and Lindgren, 1940; Verrall and Mook, 1951). In 1960s due to concern for human and environmental safety use of mercury compounds was stopped and sodium tetra or pentachlorophenoxide (NaPCP) was recommended for stain prevention (Zabel and Morrell, 1992). However, extensive studies were initiated all over the world to find out an alternative to NaPCP (Butcher, 1973; Cserjesi and Roff, 1975; Hulme and Thomas, 1979), since PCP dioxins found to pose health hazards (Dickson, 1980). Thus the use of pentachlorophenol and its derivatives were completely restricted in 1986 by the Environmental Protection Agency.

Butcher (1973) reported captafol (cis-N-((1,2,2-, tetra- chloroethyl)thio-4-cyclohexane-1,2-dicarboximide) as an alternative to widely used penta-chlorophenol. Butcher and Drysdale (1974; 1978) further advocated captafol as an alternative to NaPCP/borax to control sapstain and decay in sawn timber. They claimed that captafol was more effective than NaPCP/borax to control sapstain and it was equally good for the control of decay.

Of the 23 agricultural fungicides of low mammalian toxicity screened by Unligil (1976; 1979), Benlate (Methyl 1- (butylcarbamoyl)-2-benzimidazole carbamate) performed equally well or better than NaPCP for controlling sapstain in the laboratory. Quarternary ammonium compounds mixed with sodium carbonate were also effective anti-sapstain preservatives in the laboratory (Hulme and Thomas, 1979). Butcher (1980) tested commercially available anti-sapstains in New Zealand and TCMTB (2-thio-cyano-methyl-thio) benzothiazole) proved to be the best. In a laboratory study Cassens and Eslyn (1981) screened 22 fungicides

which had mammalian toxicity lower than NaPCP and several of them were found effective against sapstains.

In Malaysia, for the protection of rubber wood, Hong (1981) tested 18 preservatives of which Busan 30 (TCMTB), Benomyl (Benlate), Brassicol (Pentachloro-nitro-benzene), Fennotox S2 (Thiophanates, Thiocarbamates, Sodium nitrate), Mitrol PQ1C (chemical name not revealed) and Mitrol PQIL were found promising. In Brazil, 11 commercial biocidal formulations were screened for *Pinus elliottii* Engeln. and 2-(Thiocyanomethyl thio) benzothiazole (TCMTB) was found to be the best against sapstain and mould (Milano, 1981). Eleven anti-sapstain preservatives were tested in Sweden by Edlund and Henningsson (1982) and Cuzol, a methylene-bis-thiocyanate + boric acid product showed good effect against sapstain both in field and laboratory tests. Gnanaharan and Mathew (1982) found a simple momentary dip treatment of rubber wood in 1.67 per cent boric acid equivalent solution (1% each of borax and boric acid) and 0.5 per cent NaPCP, to be very effective against fungal and insect attack. Even though a number of anti-sapstain chemicals were evaluated by Gnanaharan (1983, 1984, 1986), only a few of them were found to have potential to replace NaPCP, but they were not cost- effective. In New Zealand, Drysdale and Preston (1982) screened 32 chemicals, but none either individually or in mixture, which controlled sapstain, were cost-effective for the New Zealand market when compared with the standard treatments of 0.2 per cent a.i. Captafol or 0.5 per cent NaPCP + 1.5% borax pentahydrate.

To find out a suitable alternative to NaPCP, Cserjesi and Johnson (1982) evaluated 44 anti-sapstain formulations and methylene-bis- thiocyanate and sodium tribromophenate proved to be the best formulations giving protection from sapstain and mould fungi for a period of four months. Among the seven commercial formulations tested, Plackett (1982) achieved more than 98% control of sapstain by PQ-8 (Copper-8-quinolinolate), Busan 30 (TCMTB) and BL2398

(TCMTB and MBT). Field tests on the effectiveness of Busan 30 (TCMBT), Captan (N-(trichloromethylthio)-4-cyclohexane- 1,2-dicarboximide) and Folpet (N-trichloromethylthio) phthalimide) against sapstain and mould in *Pinus elliottii* indicated Folpet as a promising replacement fungicide to pentachlorophenol (Milano and Neto, 1982).

In the U.S.A., Hansen (1984) reported IPBC (3-iodo-2-propynyl butyl carbamate) as a potential low toxic alternative to pentachlorophenol. Of the various sulfonium compounds evaluated against sapstain fungi in New Zealand by Plackett (1984), dodecyldimethyl sulfonium methosulphate gave positive results. In Australia, Captafol (0.2% a.i.) performed well among the 11 formulations tested by Leightley (1985). Drysdale (1986) found Haipen (Captafol) as an effective formulation for long-term protection of pine wood in the field. Out of seven new anti- sapstain chemicals including Busan-30 (30 per cent active 2-(thiocyanomethylthio) benzothiazole) as a 2 per cent solution) and Busan-1009 (10 per cent active methylene bisthiocyanate plus 10 per cent active 2-(thiocyanomethylthio) benzothiazole as a 2 per cent product solution), Busan-30 prevented the stain in pine wood in the field up to three months (Drysdale *et al.*, 1986). In long-term protection trials, Busan-30 was more effective up to six months than Busan-1009 (Drysdale and Keirle, 1986). In India, Jain *et al.* (1986) evaluated various agricultural pesticides and insecticides for protection of green timber against sapstain and termites and none proved to be an alternative to conventional NaPCP.

Drysdale (1987) updated the anti-sapstain chemicals available in New Zealand and found that Hylite 20 F (carbendazim), Mitrol PQ 375 (copper-8-quinolinolate) and Busan-1009 (MBT and TCMTB) were suitable for short-term and Haipen (Captafol) for long-term protection. Furthermore, incorporation of water repellents along with anti-sapstain chemicals increased the rate of protection of wood against sapstain than antisapstain chemicals alone (Drysdale and Plackett, 1987).

Chlorothalonil alone and in combination with other fungicides was screened against mould and sapstain fungi by Micales *et al.* (1989) and it was found that the most promising treatments were chlorothalonil supplemented with CCA or copper-8-quinolinolate. Three different formulations of chlorothalonil were tried on red pine wood and found that the emulsifiable concentrate performed the best against mould, sapstain and decay (Laks *et al.*, 1991). Laboratory and field studies conducted by Presnell and Nicholas (1990) revealed that low hazard biocides like Busan 110, Busan 1009, Busan 80 (2-(thiocyanomethylthio) benzothiazole) and Skane M-8 were not cent per cent effective when compared to NaPCP. Recently, a new formulation, Defence anti-stain (DAS), a combination of azaconazole (effective against stain fungi) and carbendazim (effective against mould) has been evaluated in various countries such as Indonesia, Ivory Coast, Japan, Portugal, Belgium, the Netherlands and Spain as it is found to be a promising alternative to NaPCP (Rustenburg *et al.*, 1990). While continuing their research on alternate wood preservatives, recently, Jain *et al.* (1986) found a combination of sodium trichlorophenoxide and Traetex-225 (organic cyclic compound) effective against sapstain fungi and termites.

The search for an effective and economical anti-sapstain formulation is still continuing as none of the chemicals provide fool-proof or acceptable level of protection against sapstain.

2.2.7 Biological control of sapstain

Biological control, defined as "the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man" (Baker and Cook, 1974), is a well known concept in agriculture. Now, biological control is considered seriously for wood protection also. The general feasibility of bioprotection systems on wood products was investigated by Dick and Hutchinson (1966), Hulme and Shields (1975), Ricard *et al.* (1969), Shields and Atwell (1963) and Toole (1971) to prevent or control

decay in laboratory and field tests. The first bioprotection against decay fungi in forest products was reported by Ricard and Bollen (1968) using *Scytalidium* sp. against *Antrodia carbonica* (Overh.) Ryv. et Gilbn in Dougles fir poles. Even though considerable amount of work has been done on the biological control of decay (Bruce and King, 1983; Greaves, 1970; Highley, 1990; Ricard and Bollen, 1968), very little work has been done on sapstains and moulds. However, recent health and environmental concerns with regard to the safe use of sodium pentachlorophenoxide and other chemicals for the protection of wood against sapstain fungi stimulated the studies on bioprotectants. Klingstrom and Johansson (1973) showed the activities of various *Scytalidium* species against blue stain fungus *Leptographium lundbergii* Lagerb. & Melin. Stranks (1976) reported that several antibiotics including scytalidin, hyalodendrin and cryptosporiopsin were effective in controlling blue stain in pine sapwood. Subsequent research revealed that *Bacillus subtilis* (Ehrenberg) Cohn. could inhibit growth of sapstain fungi both on nutrient medium and on wood (Bernier *et al.*, 1986; Seifert *et al.*, 1987).

White rot fungi had been reported to be parasitic on sapstain fungi in culture, but because of the damage caused to the wood by these parasites, they will not be suitable as biocontrol agents (Benko and Henningsson, 1986). Johnson (1986) studied the effects of polyoxin, an antibiotic that inhibits the synthesis of chitin, a major component of cell walls, on eight species of sapstain and mould fungi and concluded that it would not be economical as a protective agent because of the high concentration required. Benko (1987) demonstrated that crude culture filtrate of some antibiotic producing mycorrhizal fungi prevented the growth of several sapstaining fungi on pine blocks. Seifert *et al.* (1988) screened 88 fungi in agar culture as biological control agents and found that *Hypocrea gelatinosa (Tode) Fr.*, *Nectria cinnabarina (Tode ex Fr.) Fr.*, *Trichoderma viride* Pers., *T. polysporum* (Link ex Pers.) Rifai and

Gliocladium roseum were capable of preventing fungal stain caused by *Ophiostoma piliferum*, (Fr. Fr.) Syd. & P. Syd. *Ophiostoma* sp. and *Alternaria* sp.

Benko (1988a) evaluated several bacteria and actinomycetes and found that *Pseudomonas* spp. had strong antagonistic activity against blue stain fungi, *Aureobasidium pullulans* (de Barg) Arnaud, *Ceratocystis coerulescens* (Munch) Bakshi and *Ceratocystis* spp. Based on further investigations, Benko (1989) concluded that *Pseudomonas cepacia* (Burkholder) Palleroni and Holmes was a very strong antagonist of blue stain fungi in pure culture. *P. cepacia* was also successfully used as a biological control agent on wood samples in the laboratory against brown and white rot fungi and found very effective (Benko and Highley, 1990). Highley *et al.* (1991) and Croan and Highley (1992) found that *Streptomyces rimosus* Sobin *et al.* was the most effective against sapstain fungi affording good protection with one hour soaking in filter sterilized preparation. Freitag *et al.* (1991) reviewed the work of various workers on biological control of stain and decay fungi during the past two decades and discussed the prospects and research needs in this field. Kreber and Morrell (1993) studied the ability of 15 bacterial and fungal isolates to inhibit fungal stain in Ponderosa pine sapwood and found that *B. subtilis* was the most effective bacterium against fungal stain. Recently, Behrendt *et al.* (1995) demonstrated the biological control of blue stain caused by *Ophiostoma* spp. by a colourless strain of *O. piliferum* which is commercially available under the trade name Cartapip-97.

The literature search on biological control of mould and stain fungi suggests that there is ample scope for finding out suitable biological control organism which can be used effectively in the control of stain and mould.

CHAPTER 3

FUNGI ASSOCIATED WITH SAPSTAIN OF VARIOUS TIMBER SPECIES

3.1 INTRODUCTION

Wood-based industries located in different parts of Kerala require a large quantity of wood of different tree species for manufacturing various types of wood products such as plywood, packingcases, match boxes and splints. Sapstain infection of wood aggravated by the tropical warm-humid climate, poses a serious problem in the utilization of various soft wood species in Kerala. Since no information was available on the microorganisms causing sapstain of economically important timbers of the state, especially low density hardwood which are used by the wood-based industries in manufacturing the above wood products, a systematic study was undertaken to detect such microorganisms.

3.2 MATERIALS AND METHODS

3.2.1 Selection of timber species

Several wood-based industries in Trichur District were visited periodically for sapstain damage in various timber species which are commonly used for making plywood, packingcases, match box, veneers and splints. Based on the economic importance of the timber and extent of sapstain damage, the following eight timber species were selected for the study. The vernacular name and trade name are given in brackets.

1. *Ailanthus triphysa* (Dennst.) Alston, (Matty; Maharukh) - Simaroubaceae

Ailanthus triphysa is commonly grown in plantations and homesteads. It was introduced as a plantation species in Kerala during 1960's for its valuable soft wood which is used in match industry. The wood is mainly used for making match splints, boxes, slate frames and toys.

2. *Alstonia scholaris* (Linn.) R.Br., (Ezhilampala; Shaitan wood) - Apocynaceae

The tree is distributed in southern moist mixed deciduous and moist teak bearing forests. The timber, a soft wood, is mainly used for manufacturing plywood, match veneers and splints, packingcases, and wooden footwear.

3. *Anacardium occidentale* Linn. (Kasumavu; Cashew wood) - Anacardiaceae

Cashew is extensively cultivated throughout Kerala in plantations as well as in homesteads for cashewnuts and timber. The timber, a soft wood, is used for making low-cost furniture, fibre board, packingcases and charcoal.

4. *Bombax ceiba* Linn. (Mullilavu; Semul) - Bombacaceae

It is distributed throughout India, but occurs especially in moist deciduous forests. Being an important soft wood species, it is raised in plantations as well as in homesteads and farmlands. Timber of this species is utilized for making plywood, match veneers, packingcases and boxes, fishing boats, toys and frames for pencil slates.

5. *Erythrina stricta* Roxb. (Murukku; Coral tree) - Papilionaceae

Erythrina stricta is distributed naturally in southern moist deciduous forests and also grown commonly in homesteads as support for pepper vines. The soft

wood of this tree is used for manufacturing lacquer boxes, picture frames, toys, insulator boards and match splints.

6. *Hevea brasiliensis* (HBK.) Muell. Arg. (Rubber wood) - Euphorbiaceae

Rubber trees are raised extensively throughout Kerala in plantations for extraction of latex. After 30-35 years, when the latex yield is reduced, they are felled for planting the area with high yielding varieties. Hence, rubber wood is available in plenty in the State. The wood is moderately hard and used for manufacturing packingcases, fibre boards, particle boards, match splints and boxes. Studies have shown that rubber wood is suitable for furniture and panel products (Wong, 1979; Wong and Ong, 1979; Ser and Lim, 1980; Sharma *et al.*, 1982; Sonti *et al.*, 1982).

7. *Macaranga peltata* (Roxb.) Mueller - Argaau (Vatta) - Euphorbiaceae

This is found in deciduous and moist deciduous forests. Timber is soft, highly perishable and used mainly for making packingcases, match boxes and picture frames.

8. *Mangifera indica* Linn. (Mavu; Mango tree) - Anacardiaceae

Mango trees, extensively cultivated in Kerala for timber as well as mango fruits, are distributed in west-coast tropical evergreen and semi-evergreen forests. Wood is moderately hard and mainly used for ceiling boards, window frames, plywood, furniture and packingcases.

3.2.2 Collection of wood samples

For isolating the microorganisms causing sapstain, samples of stained wood of all the eight timber species were collected at monthly intervals for a period of one year from 15 wood-based industries in Trichur District (Table 3-1). Samples of other miscellaneous timber species, which were available during the collection

Table 3-1. Wood-based industries in Trichur District surveyed for sapstain and mould of the products

Sl. No.	Name of the wood-based industry	Place	Name of the products
1.	Ajantha Match Industries	Aloor	Match veneers and splints
2.	Arikkat Timber Industries	Aloor	Pc
3.	Evershine Packing Industries	Ollur	Pc
4.	Janatha Match Industries	Aloor	Match veneers and splints
5.	Kizhakkoot Industries	Arimpoor	Match veneers and splints
6.	Popular Timber Industries	Kallattumkara	Tea chest
7.	Rose Star Packers and Furniture	Ollur	Pc
8.	Sree Muruga Timber Industries	Chiyyaram	Pc
9.	Sree Ranjini Timbers	Marathakkara	Pc
10.	St. Joseph Sawmill and Timber Industries	Kodakara	Pc
11.	Sylvan Plywood Mills	Kallattumkara	Plywood
12.	Thirumal Velikkaran Match Industries	Kodakara	Match veneers and splints
13.	Timpeck Plywood Mills	Irinjalakuda	Plywood
14.	United Wood Industries	Ollur	Pc, furniture
15.	Usha Match Factory	Arimpoor	Match veneers and splints

Pc = Packingcases

period, were also collected. To ascertain the spectrum of stain causing organisms associated with different timbers in Kerala, one-time collection of stained wood samples was also made from other districts such as Cannanore (*H. brasiliensis, A. occidentale, A. triphysa*), Calicut (*H. brasiliensis, M. peltata, A. occidentale, B. ceiba*), Malappuram (*H. brasiliensis, A. scholaris, E. stricta, M. peltata, M. indica*), Ernakulam (*H. brasiliensis, A. scholaris, A. triphysa, M. indica, A. occidentale*), Kottayam (*H. brasiliensis, A. triphysa, M. indica, B. ceiba, E. stricta*), Alleppey (*H. brasiliensis, A. scholaris, A. occidentale, M. indica, B. ceiba, M. peltata*), Quilon (*H. brasiliensis, M. indica, A. occidentale, B. ceiba, E. stricta*) and Trivandrum (*B. ceiba, H. brasiliensis, A. occidentale, E. stricta, M. indica, A. scholaris*) (Fig. 3-1). All the wood samples were carried in separate clean polythene bags to the laboratory and stored in a refrigerator. To avoid development of any saprophytic growth on the specimens, isolations of stain causing microorganisms were made within two days of collection. Only under unavoidable circumstances, the specimens were stored in a refrigerator for longer duration.

3.2.3 Isolation and identification of microorganisms

For isolating the fungal/bacterial organisms associated with sapstain, small pieces of wood (2 mm^2) were cut from freshly sawn wood planks showing stained area. These pieces were surface sterilized with 0.1 per cent $HgCl_2$ solution, washed thoroughly in several changes of sterile distilled water and plated on potato dextrose agar (PDA) medium. For moulds, a small mass of fungal spores from the surface of wood was removed aseptically with sterile forceps and inoculated on PDA medium. For each sample, five replicate Petri dishes were maintained. The inoculated plates were incubated at $28 \pm 2^{\circ}C$ and observed for fungal/bacterial growth. After 7 days of incubation, the fungus/bacterium growing from the wood sample was isolated, purified and maintained in PDA slants for further studies. For bacterial isolates, nutrient agar (NA) was used as the culturing medium.

Based on cultural and morphological characteristics of the isolates the identification of microorganisms was attempted up to generic level. For confirming the identity of isolates, cultures were referred to the CAB International Mycological Institute, U.K. These cultures were periodically subcultured on PDA or nutrient agar medium as required and stored at 20 ± 2°C for further investigations.

3.2.4 Artificial inoculation trials of fungal isolates to confirm their ability to cause stain

The ability of the fungal isolates to stain healthy wood was confirmed through artificial inoculation trials. Each of the fungal isolates was inoculated on the wood of the sample species from which it was initially isolated. For inoculation, unstained sapwood blocks (50 x 10 x 70 mm) were cut from fresh green timber and steam sterilized for 15 minutes at 100 kPa. An agar disc of 8 mm diameter taken from the edge of an actively growing culture of different test fungi using a sterile cork borer was kept over the surface of each block. The inoculated blocks were then placed on glass rod supports over two moistened sterile filter papers in sterile Petri dishes to maintain high humidity inside the dishes and incubated at 28 ± 2°C for 15 days. The staining ability of each isolate was tested on five replicate blocks of the respective timber from which it was isolated; controls were also maintained with plain agar disc. After the incubation period, the inoculated blocks were examined regularly for fungal growth, initially on the surface and later inside the block by splitting them open.

3.3 RESULTS

3.3.1 Isolation and identification of microorganisms

The results of the survey indicated that there was no definite pattern in the occurrence of mould and sapstain in different timber species collected from various wood industries in different localities (Table 3-2).

Table 3-2. Severity of sapstain and mould growth on different timbers collected from various wood-based industries in Trichur District

Month of collection	Timber species	Place of collection	*Severity of sapstain and mould growth
January	*A. scholaris* *H. brasiliensis* *M. indica*	Chiyyaram Kodakara Ollur	0 ++ +
March	*A. scholaris* *B. ceiba* *H. brasiliensis* *M. indica*	Marathakkara Kodakara Ollur Chiyyaram	+ + +++ +
April	*A. occidentale* *A. scholaris* *A. triphysa* *B. ceiba* *E. stricta* *H. brasiliensis* *M. indica* *M. peltata*	Pudukkad Marathakkara Ollur Kodakara Pudukkad Irinjalakuda Ollur Chiyyaram	++ + ++ ++ + +++ ++ +
May	*A. scholaris* *B. ceiba* *E. stricta* *M. peltata*	Kodakara Pudukkad Aloor Pudukkad	+ ++ + +
June	*A. scholaris* *B. ceiba* *E. stricta* *H. brasiliensis* *M. indica*	Aloor Ollur Arimpoor Chiyyaram Arimpoor	+ ++ + +++ ++
July	*A. occidentale* *A. scholaris* *B. ceiba* *E. stricta* *M. indica* *M. peltata*	Palappilly Marathakkara Ollur Marathakkara Kodakara Kallatumkara	++ + ++ + ++ +

Table 3-2 contd.

Table 3-2 contd. from the previous page

Month of collection	Timber species	Place of collection	*Severity of sapstain and mould growth
August	*B. ceiba*	Pudukkad	++
	H. brasiliensis	Kallattumkara	+++
	M. indica	Aloor	++
	M. peltata	Ollur	+
September	*A. occidentale*	Chiyyaram	++
	A. scholaris	Kodakara	++
	A. triphysa	Marathakkara	++
	B. ceiba	Ollur	+
	H. brasiliensis	Chiyyaram	+++
	M. indica	Kodakara	+
	M. peltata	Arimpoor	+
November	*A. occidentale*	Chiyyaram	++
	A. scholaris	Manaloor	+
	A. triphysa	Arimpoor	++
	H. brasiliensis	Arimpoor	++
	M. indica	Ollur	++
	M. peltata	Arimpoor	0
December	*A. scholaris*	Arimpoor	+
	A. triphysa	Manaloor	+
	B. ceiba	Kodakara	+
	M. indica	Irinjalakuda	0
	M. peltata	Pudukkad	+

* 0 = Nil, + = Low, ++ = Medium, +++ = High

The surface moulds isolated from different timbers were *Absidia corymbifera* (Cohn) Sacc. & Trotter, *Alternaria alternata* (Fr.) Keissler, *Aspergillus flavus* Link ex Gray, *A. niger* Link., *Cladosporium* sp., *Memnoniella echinata* (Riv.) Galloway, *Mucor* sp., *Penicillium* spp., *Syncephalastrum racemosum* Cohn ex Schrot and *Trichoderma viride* Pers. (Table 3-3). Of these, *Aspergillus* spp., *Penicillium* spp. and *T. viride* were the dominant ones growing mostly on all timber species. Among all the timbers surveyed, timbers like *H. brasiliensis*, *M. indica* and *A. scholaris* had severe surface growth of different mould fungi. Comparatively little mould growth was recorded on *A. triphysa*, *E. stricta* and *M. peltata*.

Absidia corymbifera, *Mucor* sp., and *Syncephalastrum racemosum* which are some of the common saprophytic Phycomycetes were found to grow on timber surfaces of *A. scholaris*, *A. occidentale*, *E. stricta*, *H. brasiliensis* and *M. indica*. *Cladosporium* sp., causing a greenish-black surface discolouration, was isolated only from *A. occidentale*. Black surface growth recorded on all timbers was caused by *A. niger*, *Mucor* sp., *M. echinata*, *S. racemosum* and *Alternaria* sp. *M. echinata* was observed more commonly on rubber wood than other timbers (Fig. 3-2). The wood surface was stained mainly by the presence of heavy growth of fungal spores of mould fungi of different colours.

The common sapstain causing fungi isolated from different timber species were *Botryodiplodia theobromae* Pat., *Ceratocystis fimbriata* Ellis & Halst., *Acremonium strictum* W. Gams, *Scytalidium lignicola* Pesante, *Fusarium solani* (Mart.) Sacc., *F. roseum*, *F. equiseti* (Corda) Sacc. and *F. palledoroseum* (Cooke) Sacc. (Table 3-4). Among these, *B. theobromae* was the dominant one causing bluish-black stain on the surface as well as inside the wood (Fig. 3-3). The other stain fungi imparted various shades of different colours to the surface as well as internal tissues of the wood.

Acremonium strictum, though not a common stain fungus, caused greyish-brown stain in *A. triphysa*, *B. ceiba* and *H. brasiliensis*. Different species of

Table 3-3. Fungi causing superficial staining (mould) in selected timbers

Tree species	Colour	Fungus	Severity of occurrence*
Ailanthus triphysa	Yellowish-green	*Aspergillus flavus*	++
	Black	*A. niger*	++
Alstonia scholaris	Black	*A. niger*	++
	Yellowish-green	*A. flavus*	++
	Black	*Mucor* sp.	+
	Greyish-black	*Syncephalastrum racemosum*	+
	Bluish-green	*Penicillium* sp.	+++
Anacardium occidentale	Black	*A. niger*	+
	Yellowish-green	*A. flavus*	++
	Black	*Mucor* sp.	+
	Greenish-black	*Cladosporium* sp.	++
Bombax ceiba	Black	*Alternaria* sp.	+
	Yellowish-green	*A. flavus*	++
	Black	*A. niger*	++
	Bluish-green	*Trichoderma viride*	+++
Erythrina stricta	Bluish-green	*Penicillium* sp.	+++
	Black	*Mucor* sp.	++
	Bluish-green	*T. viride*	+++
Hevea brasiliensis	Yellowish-green	*A. flavus*	+++
	Black	*A. niger*	+++
	Grey	*Absidia corymbifera*	+
	Bluish-green	*T. viride*	+++
	Greyish-black	*S. racemosum*	++
	Black	*Memnoniella echinata*	+++
Macaranga peltata	Yellowish-green	*A. flavus*	+
	Black	*A. niger*	+
	Bluish-green	*T. viride*	++
Mangifera indica	Black	*A. niger*	++
	Yellowish-green	*A. flavus*	+++
	Yellow	*Aspergillus* sp.	++
	Greyish-black	*S. racemosum*	+
	Bluish-green	*T. viride*	+++
	Bluish- green	*Penicillium* sp.	+++

* + = Low
++ = Medium
+++ = Heavy

Table 3-4. Fungi causing different types of stain on selected timbers

Tree species	Colour	Fungus	Severity of occurrence*
Ailanthus triphysa	Bluish-black Greyish-brown	*B. theobromae* *A. strictum*	+++ +
Alstonia scholaris	Bluish-black Pinkish-yellow	*B. theobromae* *F. roseum*	+ +
Anacardium occidentale	Bluish-black Yellow	*B. theobromae* *F. solani*	+++ +
Bombax ceiba	Greyish-brown Bluish-black Greyish-black Pinkish-Yellow	*A. strictum* *B. theobromae* *C. fimbriata* *F. equiseti* *F. solani*	+ ++ ++ + +
Erythrina stricta	Bluish-black	*B. theobromae*	+
Hevea brasiliensis	Greyish-brown Bluish-black Greyish-black Yellow Bluish-black	*A. strictum* *B. theobromae* *C. fimbriata* *F. solani* *S. lignicola*	+ +++ +++ + +
Macaranga peltata	Bluish-black Yellow	*B. theobromae* *F. solani*	+ ++
Mangifera indica	Bluish-black Greyish-black Greenish-yellow Yellow	*B. theobromae* *C. fimbriata* *F.palledoroseum* *F. solani*	++ ++ + ++

* + = Low
++ = Medium
+++ = Heavy

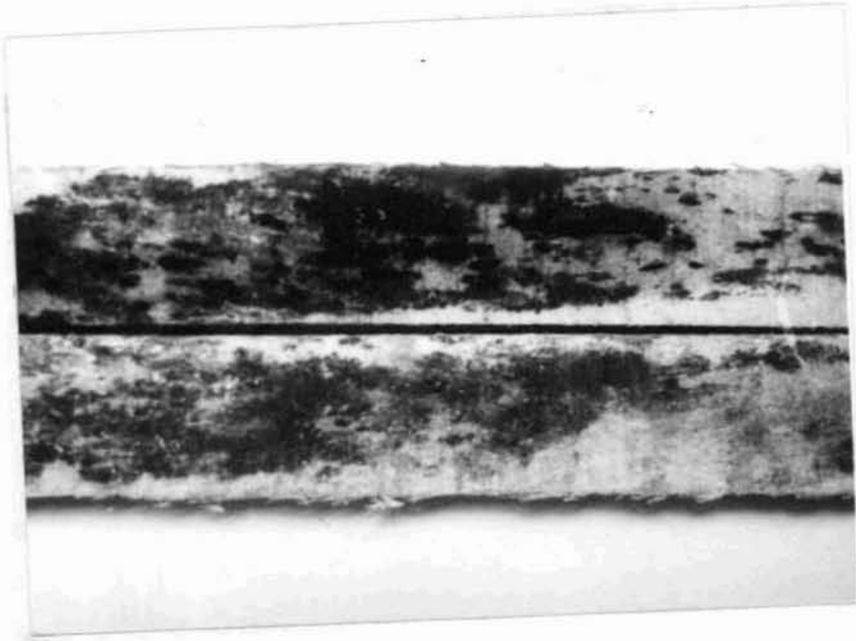


Fig. 3-2 Mould fungi growing on the surface of *Hevea brasiliensis*. Black colour shows the growth of *Memnoniella echinata*.

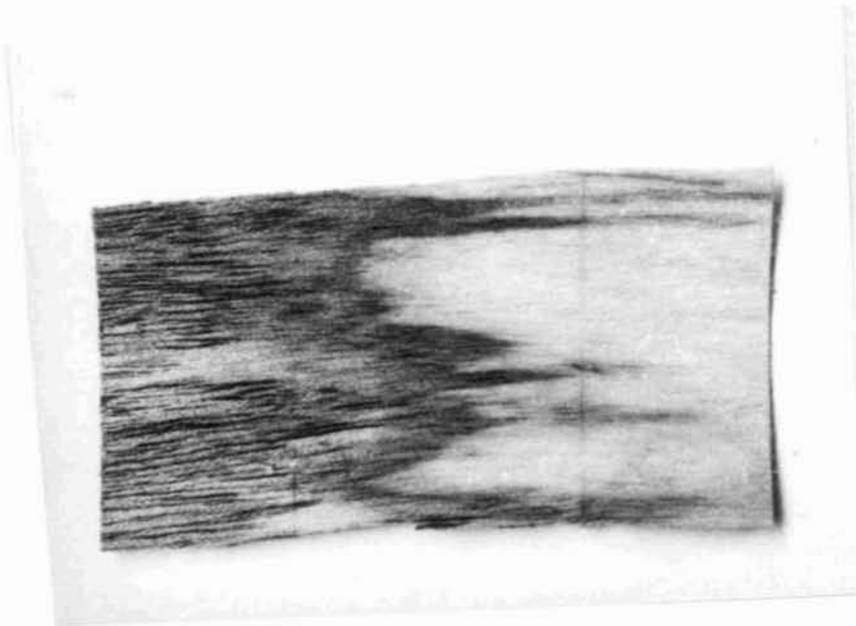


Fig. 3-3 Sapstain caused by *Botryodiplodia theobrome* on rubber wood.

Fusarium such as *F. equiseti*, *F. palledoroseum*, *F. roseum* and *F. solani* caused shades of yellow and pink colours to the sapwood of *A. scholaris*, *A. occidentale*, *B. ceiba*, *H. brasiliensis*, *M. peltata* and *M. indica*. The stain caused by *Fusarium* spp. did not penetrate deep as in the case of *B. theobromae*. Among the different species of *Fusarium*, *F. solani*, a soil pathogen was found to be more common than others. *M. indica* was found to be susceptible to different species of *Fusarium*. The maximum number of stain fungi was observed on *H. brasiliensis* and *M. indica*.

Ceratocystis fimbriata caused stain in *B. ceiba*, *H. brasiliensis* and *M. indica*. The severity of stain by *C. fimbriata* was medium; but heavy infection of this fungus was found only in rubber wood.

Scytalidium lignicola, producing a bluish-black discolouration, was isolated only from *H. brasiliensis*. This stain fungus was not of common occurrence.

Stains caused by various fungi other than *B. theobromae* and *C. fimbriata* were not of common occurrence and economic importance.

The results of the monthly survey conducted in various wood-based industries in Trichur District revealed that all the timber species were found to be susceptible to sapstain by *B. theobromae*. Among the timbers *H. brasiliensis* was the most severely and frequently affected. Based on the frequency of isolation, timbers such as *H. brasiliensis*, *A. occidentale* and *A. triphysa* were the most susceptible to *B. theobromae* while *B. ceiba*, *E. stricta*, and *M. indica* were moderately susceptible; least susceptible timbers were *A. scholaris* and *M. peltata* (Table 3-5). The monthly survey indicated that there was no definite pattern of attack of *B. theobromae* on rubber wood. The incidence of infection was cent per cent, except in January, April, May and December (Table 3-6, Fig. 3-4).

Table 3-5. Frequency of *Botryodiplodia theobromae* in fungal isolations made from various timber species

Timber species	No. of months samples collected in a year	Total No. of isolations	Frequency of infection (%)
Ailanthus triphysa	7	13	70
Alstonia scholaris	6	11	18
Anacardium occidentale	6	13	77
Bombax ceiba	7	43	40
Erythrina stricta	3	5	60
Hevea brasiliensis	11	66	81
Macaranga peltata	4	22	27
Mangifera indica	9	35	57

Table 3-6. Frequency of isolation of *Botryodiplodia theobromae* from rubber wood samples collected from various places in Trichur District

Locality	Month	Per cent isolation of *B. theobromae*
Ollur	January	67
Chiyyaram	March	100
Arimpoor	April	70
Aloor	May	50
Ollur	June	100
Kodakara	July	100
Kalletumkara	September	100
Ollur	October	100
Chiyyaram	November	100
Marathakara	December	70

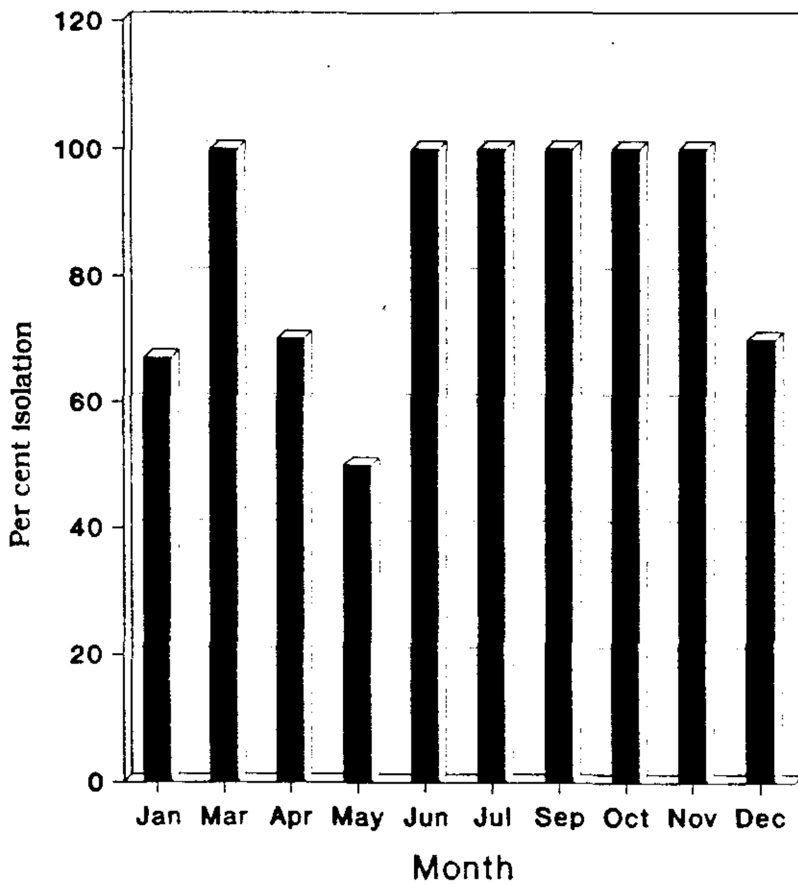


Fig. 3-4. Frequency (%) of isolation of *Botryodiplodia theobromae* from rubber wood samples collected from various places in Trichur district for period of one year.

One-time collection of sapstained timber made in various districts other than Trichur revealed that *B. theobromae* caused sapstain of various timbers. The incidence of the stain was severe in all the districts except for Cannanore and Alleppey, where it was medium (Table 3-7).

Table 3-7. Severity of sapstain caused by *Botryodiplodia theobromae* on different timbers sampled from various districts of Kerala other than Trichur

Districts	Timber species sampled*	Severity**
Alleppey	Hb, As, Ao, Mi, Bc, Mp	++
Calicut	Hb, Mp, Ao, Bc	+++
Cannanore	Hb, At, Ao	++
Ernakulam	Hb, As, At, Mi, Ao	+++
Kottayam	Hb, At, Mi, Bc, Es	+++
Malappuram	Hb, As, Es, Mp, Mi	++
Quilon	Hb, Mi, Ao, Bc, Es	+++
Trivandrum	Bc, Hb, Ao, Es, Mi, As	+++

* Ao = *A. occidentale* ; As = *A. scholaris* ; At = *A. triphysa*
Bc = *B. ceiba*; Es = *E. stricta*; Hb = *H. brasiliensis*
Mi = *M. indica* ; Mp = *M. peltata*
** ++ = Medium, +++ = Severe

3.3.2 Artificial inoculation of fungal isolates to confirm their ability to cause stain

In artificial inoculations, the test fungus grew out of the culture disc and the mycelium spread over the upper surface of the wood block within 5 days. After 15 days of incubation the whole block was covered by fungal mycelium. When the superficial mycelial mass was scraped off using a scalpel blade, the surface of test blocks of different timbers was found to be stained in different colours. In the case of *B. theobromae*, the whole block was stained dark imparting a

bluish-black colour to the wood. The staining penetrated to a depth of 4-8 mm inside the wood as seen after splitting the block. The control blocks, did not show any fungal growth and staining.

3.4 DISCUSSION

The peculiar climatic conditions prevailing in Kerala State appear to be very congenial for the development and spread of sapstain and mould growth on various timbers. This is very evident from the survey conducted in the present study. All the timbers surveyed were prone to both sapstain and mould infection; but the severity varies from timber to timber. Even though mould growth is only superficial, wooden planks with thick mycelial growth bearing spore masses of *Aspergillus*, *Penicillium* and *Trichoderma* are often rejected by the users. Among the various stains, blue stain due to *B. theobromae* causes a serious problem in the utilization of rubber wood which is the major source of timber for small-scale wood industry in the State.

Mould fungi isolated from various wood species belong to Phycomycetes and Fungi Imperfecti. *Penicillium* spp. and *Aspergillus* spp. are the most commonly encountered moulds. This finding is in agreement with those of other investigators (Cartwright and Findlay, 1958; Ananthanarayanan, 1971; Hong, 1976). Sujan *et al.* (1980), while studying the fungal degradation of rubber wood, reported that surface moulds such as *Aspergillus* spp. and *Penicillium* spp. occur on the surface of wood together with blue stain fungi. Balasundaran and Gnanaharan (1990) also isolated *Aspergillus terreus* Thom., *Aspergillus sydowii* (Bainier & Sastry) Thom. & Church and *Penicillium citrinum* Thom., from the surface of rubber wood treated with borax + boric acid and borax + boric acid + NaPCP. All these reports further confirm that *Aspergillus* spp. and *Penicillium* spp. are the common moulds found on timbers along with other stain fungi. The abundance of mould growth on timbers possibly reflects the conducive micro and macro-climatic conditions, especially RH prevalent in Kerala (Table 3-8).

Table 3-8. Weather data for 1989 at Trichur

Months	Mean Max.	Temp (^{o}C) Min.	Mean RH Max.	(%) Min.	Monthly rainfall (mm)
January	33.4	21.1	86	48	0(0)
February	36.6	20.8	96	42	0(0)
March	37.4	22.6	100	49	20(1)
April	37.1	24.2	95	56	54(1)
May	34.6	23.1	98	67	122(3)
June	30.0	21.7	100	78	668(20)
July	29.5	22.1	99	82	504(14)
August	30.1	22.5	98	76	298(10)
September	31.3	22.7	100	76	186(6)
October	32.3	22.4	100	76	329(9)
November	32.2	22.0	91	62	13(1)
December	32.3	21.7	93	61	24(1)

Note: The figures in parentheses indicate number of rainy days when rainfall was >10 mm

Among the various stain fungi isolated from different timbers *B. theobromae* is the dominant one causing bluish-black discolouration on all timber species surveyed; the most susceptible species is *H. brasiliensis*. The sapstain is present in almost all the timbers throughout the year in Trichur and other districts.

B. theobromae belonging to Hyphomycetes, a known wound pathogen causing diseases of various tropical and subtropical plants of economic importance (Punithalingam, 1980), is also a blue stain causing fungus of wide occurrence (Kaarik, 1980). It is frequently reported as the blue stain causing organism in tropical hardwoods (Cartwright and Findlay, 1958; Hong, 1976). Olofinboba (1974) reported *B. theobromae* as a blue stain causing fungus in *Antiaris africana*, an economically important tropical white wood. In Malaysia, rubber

wood is known to be very susceptible to sapstain caused by *B. theobromae* (Sujan *et al.*, 1980). In Sabah (Malaysia), the commercial Dipterocarp trees are also known to be stained by *B. theobromae* (Thapa, 1971). Pinheiro (1971) carried out investigations to study the blue stain fungi in Portuguese hardwoods and found that *B. theobromae* was the main pathogen causing blue stain in poplar wood. In Africa, staining by *B. theobromae* on *Triplochiton scleroxylon* (abachi) caused excessive damage to sapwood (Tabirih and Seehann, 1984). In Japan, Tsunoda *et al.* (1983) also isolated sapstaining fungus *B. theobromae* from rubber wood. Balasundaran and Gnanaharan (1990) also isolated *B. theobromae* from rubber wood treated with different wood preservatives. All these reports clearly indicate that *B. theobromae* is capable of causing blue stain in various timber species, especially under humid-tropical climate.

Ceratocystis fimbriata, the second dominant fungus causing greyish-black stain, belongs to Ascomycetes. It causes stain mainly by its pigmented hyphae and ascocarps. This fungus prominently occurs in rubber wood causing stain mostly in June, July and August which are the rainy months in Kerala. The present findings confirm the earlier reports that *Ceratocystis* spp. is responsible to cause blue stain in coniferous sapwood (Dowding, 1970). In temperate countries, *Ceratocystis* is the major sapstain fungus. In Britain, four species of *Ceratocystis* caused stain of coniferous timber (Bakshi, 1951). In southern U.S.A, *Ceratocystis coerulescens* is reported as the most important stain fungus on hardwoods (Verrall, 1941). In Russia, *Ceratocystis* spp. are responsible for discolouration of sap wood (Vanin, 1932). *C. picea* (Munch) Bakshi, normally a nonstaining fungus on conifers in western U.S.A, caused reddish purple stain in *Alnus rubra* (Morrell, 1987). In Spain, several species of *Ceratocystis* were reported to cause stain on pine wood (Troya and Navarrete, 1989). Benko (1983) reported different species of *Ceratocystis* causing stain in coniferous timber in Yugoslavia.

Bluish-black stain was also produced by *Scytalidium lingnicola* only in rubber wood. Even though it is not a very common sapstain fungus, Kaarik (1980) also reported *S. lignicola* causing dark blue black stain on *Pinus* and *Picea*.

In addition to *B. theobromae* and *Ceratocystis* sp. *Fusarium* spp. also caused yellow and pink stain on *M. indica*, *B. ceiba* and *H. brasiliensis*. The sapstain caused by *Fusarium* spp., is not as serious as those of *B. theobromae* and *C. fimbriata*. There are only few reports indicating that species of *Fusarium* are sapstain causing fungi. Findlay (1959) reported that purplish-pink stains are often caused by species of *Fusarium*. On preservative treated rubber wood, Balasundaran and Gnanaharan (1990) also reported a slightly reddish stain produced by *F. decemcellulare*.

Due to the warm-humid tropical climate of Kerala, the growth of stain and mould fungi on various economically important timbers is a serious problem in the utilization of soft wood species for different purposes. The stain caused by *B. theobromae* and mould growth on rubber wood are very common and economically important since the stained timber is usually rejected by the buyers. A detailed study on various aspects of growth and colonization of *B. theobromae* is dealt within the next few chapters.

CHAPTER 4

FACTORS AFFECTING GROWTH AND COLONIZATION OF *BOTRYODIPLODIA THEOBROMAE*

4.1 INTRODUCTION

Since *Botryodiplodia theobromae* (Bt) is the dominant fungus causing sapstain in timbers, a detailed study on the factors affecting its growth and colonization in wood was carried out.

B. theobromae is an important ubiquitous, facultative wound pathogen widely distributed in tropics and subtropics. It is reported to cause several types of diseases such as damping-off, seedling blight, die-back, stump rot, root rot, leaf spot and pre - and post-harvest fruit rots either alone or in combination with one or more microorganisms (Punithalingam, 1980). It belongs to the Fungi Imperfecti, order Sphaeropsidales with immersed to erumpent or superficial, simple or often aggregated pycnidia up to 5 mm wide, in which typical ellipsoidal two-celled dark brown spores are formed; conidia have characteristic longitudinal striations. A noticeable feature of Bt is that it often exhibits considerable variation in colony and pycnidial characters including colour, growth rate, pigment production, complexity of pycnidia and setae. Mycelium is hyaline when young, rapidly becomes greyish black thus giving to the infected wood a blue colour resulting in a well known phenomenon of light diffraction.

Infection and growth of fungi in wood, in general, are governed by external factors, namely presence of inoculum in the form of spores, air and moisture balance in the wood, ambient temperature, atmospheric humidity, pH of the

wood and also by internal factors like inherent properties of the wood species (Bakshi, 1988). However, very little is known about the external factors like temperature, humidity and moisture content of wood which influence the growth of *B. theobromae* in wood.

4.2 MATERIALS AND METHODS

Since rubber wood is highly susceptible to sapstain caused by Bt, all the experiments described in this chapter were conducted using rubber wood. Rubber trees of 30-year-old were selected for the study. One metre long billet above the ground level of the tree was cut. After debarking the log, small wood blocks of the required size were prepared and stored in a refrigerator.

4.2.1 Influence of moisture content of wood on the growth of *Botryodiplodia theobromae*

Generally, sapstain fungi are known to grow only when the timber is in green condition. An experiment was conducted using rubber wood blocks with 10 different moisture contents to determine the optimum moisture content of rubber wood required for maximum growth of Bt. Three hundred wood blocks (50 x 10 x 70 mm), prepared from freshly sawn rubber wood, were kept in an incubator at 35°C to reduce the moisture content gradually. Thirty sample blocks were removed every day from day-1 for 10 consecutive days from the incubator and steam sterilized for 15 minutes at 100 kPa. After cooling to room temperature (28 ± 2°C), from the 30 wood blocks, 10 were taken and the initial weight determined using an electronic digital balance. These 10 blocks were then oven-dried at 103 ± 2°C for 3 days and after cooling in a desiccator, determined the final weight and calculated the moisture content. Of the remaining 20 blocks, 10 were inoculated at the centre with a 8 mm diameter mycelial disc of an actively growing culture of Bt and the rest with plain agar disc to serve as control. The inoculated and the control blocks were then placed

over glass rod supports in sterile Petri dishes as mentioned in Chapter 3 (3.2.4) and incubated at 28 ± 2°C for 15 days. After the incubation period, the growth of the fungus on the surface of the inoculated blocks was assessed visually using the rating index given in Table 4-1.

Table 4.1. Rating index for assessing the fungal growth

Extent of growth	Rating
Nil	0
Trace	1 (0.1 - 1)
Light	2 (1.1 - 2)
Medium	3 (2.1 - 3)
Heavy	4 (3.1 - 4)

Data on the growth of Bt at different moisture content of wood were subjected to analysis of variance and mean comparing test. (Snedecor and Cochran, 1967).

4.2.2 Effect of ambient humidity, temperature and moisture content of rubber wood on the growth of *Botryodiplodia theobromae*

To find out the optimum conditions for growth and colonization of *B. theobromae* on rubber wood, effect of range of humidity (RH), temperature (T) and moisture content (MC) of timber was studied. Different levels of MC, T and RH used in the experiments were: MC (%): 50, 75 and 100; T (°C): 20, 30 and 40; RH (%): 80, 90 and 100. Different RH levels (Table 4-2) were maintained using saturated salt solutions (O'Brien, 1948).

The salt solutions were prepared in sterilized Horlicks bottles, each containing 30 ml of the appropriate salt solution. For maintaining 100 per cent RH, 30 ml

Table 4-2. Relative humidity over saturated salt solutions at various temperature (after O'Brien, 1948)

Salt solution/water	Relative humidity (%)	Temperature (°C)
Water	100.0	20
$ZnSO_4 7H_2O$	90.0	20
NH_4Cl	80.0	20
NH_4NO_3	67.4	20
$Ca(NO_3)_2 4H_2O$	56.0	20
Water	100.0	30
KNO_3	91.5	30
$(NH_4)_2SO_4$	81.0	30
$CO(NH_2)_2$	72.9	30
NH_4NO_3	60.0	30
Water	100.0	40
KNO_3	89.4	40
KCl	82.0	40
$NaNO_3$	70.5	40
$CO(NH_2)_2$	68.7	40

of sterile water was used at all temperatures. Fresh, randomly selected, and oven dried rubber wood blocks (25 x 25 x 40 mm size) were immersed in water for appropriate periods in order to attain approximate moisture content of 50 per cent, 75 per cent and 100 per cent. These wood blocks were then steam sterilized for 15 minutes at 100 kPa. After attaining room temperature the blocks were inoculated at the centre with mycelial disc of 8 mm diameter taken from an actively growing 7-day-old culture of *B. theobromae*, using a sterile cork borer. The blocks were suspended over the salt solution using stainless steel wire as described in the Pint Jar method (Schmidt and French, 1979) (Fig. 4-1). The control blocks were inoculated with plain agar disc. The inoculated and control

wood blocks were incubated for 3 months at three different temperature regimes viz., 20, 30 and 40°C. Each treatment combination (MC x T x RH) had five replications including the control blocks. After the incubation period, the blocks were taken out from the bottles and the growth of the fungus over the blocks was assessed, using the rating index given in Table 4-1. The staining of the wood was assessed externally by scraping off the mycelium with a sharp scalpel blade and internally by splitting the blocks into two pieces. The data on rating index were subjected to analysis of variance.

4.2.3 Comparative growth of *Botryodiplodia theobromae* on *Hevea brasiliensis* and *Alstonia scholaris*

Influence of moisture content and temperature on the growth of *B. theobromae* on *Hevea brasiliensis*, the highly susceptible timber to sapstain, was compared with that of the least susceptible timber *Alstonia scholaris*. Test blocks (25 x 25 x 40 mm size) of *H. brasiliensis and A. scholaris* were prepared from freshly felled logs and 100 blocks of each species were selected randomly. The moisture content of these wood blocks were brought to two different levels i.e., treatment 'a' and 'b'. From the 100 wood blocks, 50 wood blocks of each of the two species were steam sterilized for 15 minutes at 100 kPa and allowed them to cool to attain the room temperature for moisture content 'a'. The remaining 50 blocks of each species were transferred to an oven maintained at 33 ± 2°C for 2 days in order to reduce their moisture content gradually to 'b'. The moisture contents of wood blocks of each species were determined using oven-dry weight method. For determining the moisture content, 10 each of these wood blocks were weighed initially, oven-dried at 103 ± 2°C and the oven-dry weight determined. Twenty each out of the remaining 40 blocks were inoculated with 8 mm diameter agar disc from actively growing culture of Bt and the rest with plain agar disc to serve as control. After two days the wood blocks of both the species kept at 33 ± 2°C were removed from the oven, their initial moisture content determined as described above and inoculated half of them with Bt and the other half with

plain agar. All the 40 inoculated and control blocks of each timber species were placed in sterile Horlicks bottles for maintaining humidity as described above (4.2.2). Twenty each of the wood blocks with moisture regimes 'a' and 'b' were incubated for 3 months at two different temperatures viz. 30°C and 40°C. Each moisture content treatment consisted of 10 Bt inoculated and 10 control wood blocks. After the incubation period, the growth of the fungus over the wood blocks was examined and assessed using the rating index given in Table 4-1. Superficial mycelium was scraped off from each inoculated block using a scalpel blade without removing the woody tissue and final moisture content of the wood blocks was determined on oven dry weight basis.

4.2.4 Temperature tolerance of *Botryodiplodia theobromae*

Temperature tolerance of Bt was studied by assessing its growth in potato dextrose agar (PDA) medium and over rubber wood blocks at different temperature regimes.

Fungal growth in potato dextrose agar medium: The growth of Bt was assessed on PDA employing agar plate method. A mycelial disc of 8 mm diameter taken from an actively growing culture of Bt was inoculated at the centre of a 90 mm diameter Petri dish containing 20 ml of PDA. The inoculated plates were incubated in incubators maintained at 30, 40, 50 and 60°C; minimum temperature of 30°C was selected as it is the average temperature encountered in Kerala. For each treatment, five replicate dishes were maintained. Growth of the fungus was measured at three radii every day up to 7 days (168 hours).

Fungal growth on wood blocks: Forty fresh rubber wood blocks (50 x 10 x 70 mm size) having a moisture content of 80-90 per cent (ideal MC for good growth of Bt) were steam sterilized for 15 minutes at 100 kPa and after cooling to room temperature inoculated them at the centre with a mycelial disc of 8 mm diameter, taken from the actively growing culture of Bt. The inoculated blocks

were placed over glass rod supports kept inside sterile Petri dishes and incubated separately at 30, 40, 50 and 60°C for 7 days. Growth of Bt on the surface of wood blocks was visually observed every day and assessed using the rating index mentioned in Table 4-1.

Survival of Bt in wood blocks: The influence of temperature on the survival of the fungus in wood blocks was studied using pre- infected wood blocks. One hundred and twenty five freshly prepared and unstained wood blocks (50 x 10 x 70 mm) were sterilized at 100 kPa and inoculated aseptically at the centre with Bt culture and incubated in sterile Petri dishes at 28 ± 2°C. After one month's growth, 105 blocks having good growth of Bt were selected and divided into three groups of 35 blocks each. These three groups of wood blocks were transferred to separate incubators maintained at different levels of temperatures namely, 40, 50 and 60°C; lower temperature of 30°C was omitted, since this was found to favour good growth of Bt as indicated in an earlier experiment. After 24 hours, a set of five blocks each was taken from the incubator and a bit of the mycelium growing over the wood block was removed aseptically using a sterile forceps and inoculated in PDA medium in Petri dishes. A small piece (5 mm) of wood tissue removed from the inner side of each block was also inoculated in PDA for ascertaining the survival of the fungus inside the wood block. Similar inoculations were continued on subsequent days (every 24 hours) up to 168 hours. All the inoculated dishes were incubated at 28 ± 2°C for 7 days. The positive growth of the fungus from the mycelial bit and wood tissue in the medium was recorded as the measure of temperature tolerance of the fungus over and inside the wood tissues at a particular temperature.

4.2.5 Comparison of weight loss in *Hevea brasiliensis* caused by *Botryodiplodia theobromae* with that of *Alstonia scholaris*

Weight loss in blocks of *H. brasiliensis* and *A. triphysa* which are very susceptible to sapstain infection of Bt was compared with that of *Alstonia scholaris*, the least

susceptible one for a period of 4 months following the procedure described by Stranks (1976). Eighty test wood blocks (15 x 5 x 50 mm) of each timber species were cut from defect-free fresh sapwood, oven dried at 103 ± 2°C till the weight became constant, cooled in desiccator and initial weight recorded. The sample blocks were then placed over wet filter paper in a damp chamber to bring them back to equilibrium moisture. Following steam sterilization, at 100 kPa for 15 minutes, 40 blocks were inoculated aseptically with 8 mm diameter mycelial disc taken from the edge of a 7-day-old actively growing culture of Bt. These blocks were incubated in round bottom tubes (140 x 25 mm) containing 15 ml of sterile water. The inoculated wood blocks were kept free of direct water contact by glass supports projecting above the water level in the test tube. The atmosphere around the inoculated block was humidified by keeping a sterile filter paper wick (100 x 25 mm) covering the entire length of the test block. The lower end of the wick was immersed in sterile water. The tubes were closed with cotton plugs (Fig. 4-2). The remaining 40 blocks were inoculated with plain agar disc which served as control. These set-ups were incubated at 28 ± 2°C. Every month, 10 blocks each from inoculated as well as control sets were taken out from the tubes. After carefully scraping off the mycelial growth using a scalpel blade, the blocks were oven dried at 103 ± 2°C till the weight became constant and final weight recorded. The percentage of weight loss was calculated based on the original oven-dry weight of the block.

4.3 RESULTS

4.3.1 Influence of moisture content of wood on the growth of *Botryodiplodia theobromae*

Results pertaining to the growth of *B. theobromae* on rubber wood at different moisture contents are presented in Table 4-3 (Fig. 4-3).

No growth of Bt occurred at and below 23.8 per cent moisture content of rubber wood blocks. At 25 per cent, it was only trace growth which increased to medium

at 27 per cent moisture content. Moisture content of 29 per cent and above was most favourable as heavy growth was observed on the wood blocks. ANOVA showed significant difference between MC and growth rating (Table 4-4).

4.3.2 Effect of ambient humidity, temperature and moisture content of rubber wood on the growth of *Botryodiplodia theobromae*

The results presented in Table 4-5 indicated that ambient RH, temperature and MC of rubber wood significantly influence the growth of Bt. The growth of Bt

Table 4-3. Growth of *Botryodiplodia theobromae* at different moisture contents (MC) of wood at 28 ± 2 °C (Mean of 10 observations)

MC of rubber wood blocks (%)	Average growth rating ± SE	Growth rating*
65.63	4.0 ± 0	4
46.05	3.8 ± 0.13	4
29.49	4.0 ± 0	4
26.02	2.4 ± 0.27	3
27.04	2.2 ± 0.21	3
24.79	1.4 ± 0.22	2
25.86	1.0 ± 0.15	1
23.83	0.0	0
16.18	0.0	0
15.85	0.0	0

* For growth rate index see Table 4-1

Table 4-4. Analysis of variance of growth of *Botryodiplodia theobromae* at different moisture contents (MC) of wood at 28 ± 2 °C (Mean of 10 observations)

Sources	DF	SS	MSS	F ratio
Between groups	9	1.8755	0.2084	48.6647**
Within groups	89	0.3811	0.0043	-
Total	98	2.2566	-	-

** Significant at P = 1%.

Table 4-5. Growth rate index of *B. theobromae* grown on rubber wood blocks of different moisture content (MC) incubated at various temperature (T) and relative humidity (RH) (mean of 5 observations)

Relative humidity %	Moisture content %	Growth rating ± SE*		
		Temperature °C		
		20	30	40
60	50	0	0	0
	75	0	0	0
	100	0	0	0
70	50	0	$0.2^a \pm 0.2$	0
	75	0	$0.4^a \pm 0.24$	0
	100	0	$0.8^a \pm 0.37$	0
80	50	0	0	0
	75	0	$1.4^a \pm 0.24$	$0.2^a \pm 0.2$
	100	0	$1.6^a \pm 0.24$	$1.6^a \pm 0.68$
90	50	$1.2^b \pm 0.37$	$2.8^b \pm 0.2$	$0.2^a \pm 0.2$
	75	$1.8^b \pm 0.2$	$3.4^c \pm 0.6$	$0.4^a \pm 0.25$
	100	$3.4^c \pm 0.24$	$3.0^b \pm 0.45$	$1.2^b \pm 0.37$
100	50	$3.4^c \pm 0.25$	$3.4^c \pm 0.24$	$0.2^a \pm 0.2$
	75	$3.4^c \pm 0.25$	$3.6^c \pm 0.6$	$1.0^a \pm 0.12$
	100	$3.8^c \pm 0.2$	$4.0^c \pm 0.45$	$2.8^b \pm 0.73$

Figures in a column superscribed by different letters are significantly different
* For growth rate index see Table 4-1

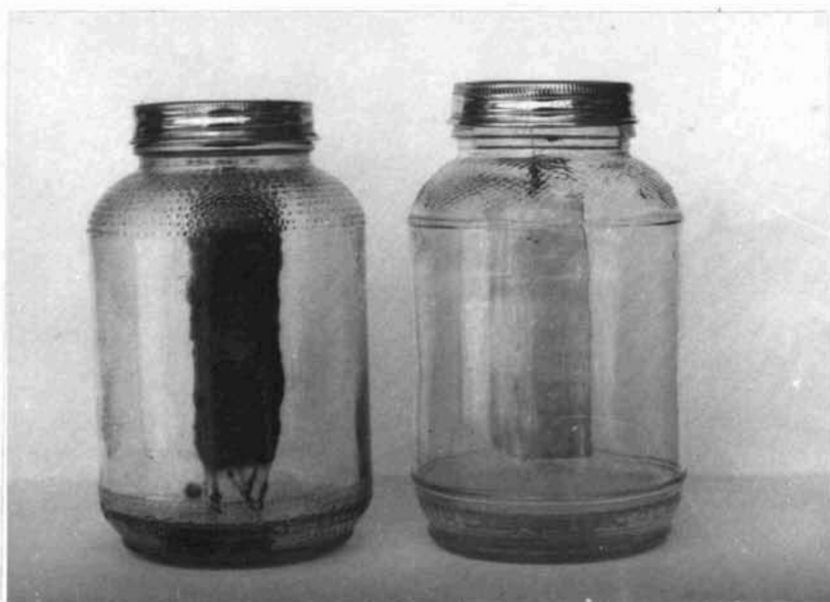


Fig. 4-1 Rubber wood blocks inoculated (left) and control (right) suspended inside Horlicks bottles and maintained at constant humidity.

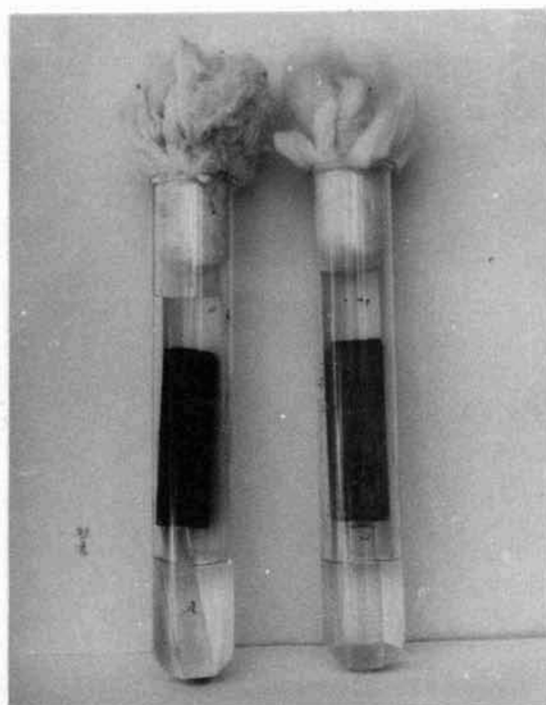


Fig. 4-2 Test tubes with rubber wood blocks inoculated with *Botryodiplodia theobromae* for assessing weight loss.

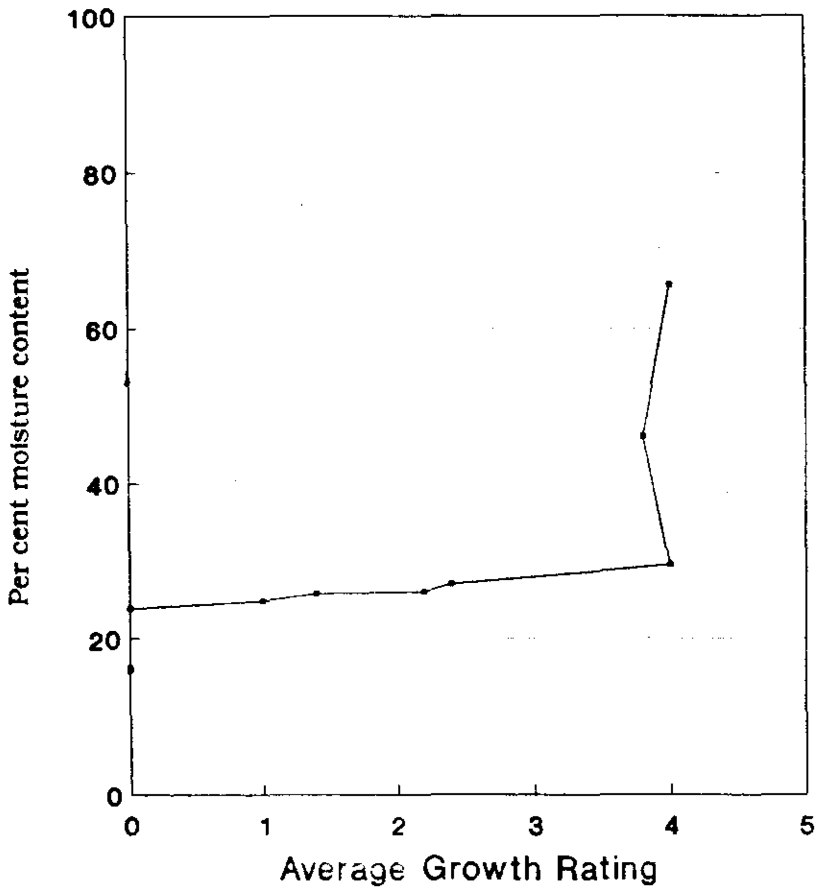


Fig. 4-3. Growth rate of *Botryodiplodia theobromae* at different moisture contents (%) of wood at 28 ± 2 °C.

was either negligible or nil at low RH (60, 70, 80%) irrespective of the range of temperature of 20, 30, 40°C and moisture contents (50, 75, 100%). At high RH (90-100%) in all the treatment combinations, the growth of Bt was better, but influenced differentially more by the temperature than MC of wood. High temperature (40°C) tended to inhibit the growth of Bt. Relative humidity was the most important factor which governed the favourable growth of Bt than the other factors. At 30°C and high ambient RH (90-100%) the maximum growth of Bt was found. Moisture content of wood did not affect the growth of Bt at high RH (100%) and at 20 or 30°C, However, either at low ambient RH (90%) or at high temperature (40°C) the growth was more at 100 per cent MC than at 50 or 75 per cent MC. High ambient temperature influenced the growth of Bt negatively even though high RH (100%) and MC of wood (100%) provided favourable regimes for fungal growth.

Results indicated that 30°C is the optimum temperature which promoted the best growth of *B. theobromae* at high RH (90-100%) irrespective of the MC of wood. Statistical analysis (Table 4-5) showed significant difference between the treatments. Treatment combinations such as 20°C, 100 per cent MC, 100 per cent RH; 30°C, 75 per cent MC, 100 per cent RH and 30°C, 100 per cent MC, 100 per cent RH were found to be significant as compared to all other treatment combinations.

4.3.3 Comparative growth of *Botryodiplodia theobromae* on *Hevea brasiliensis* and *Alstonia scholaris*

At both the moisture regimes i.e., (a) and (b) and both the temperatures (30°C and 40°C), heavy to light growth of Bt was observed on rubber wood blocks as compared to blocks of *A. scholaris*, where only traces of growth was recorded that too only in one combination at 30°C (Table 4-6).

Table 4-6. Comparative growth of *Botryodiplodia theobromae* in *Hevea brasiliensis* and *Alstonia scholaris*

Treatment of wood blocks	*H. brasiliensis*				*Alstonia scholaris*			
	Initial MC	Final MC	Incubation temperature °C	Mean growth rate index* ± SE	Initial MC	Final MC	Incubation temperature °C	Mean growth rate index ± SE
a	67.32	37.03	30	4.0 ± 0	111.19	71.14	30	1 ± 0.15
a	67.32	32.32	40	1.6 ± 0.16	111.19	39.77	40	0
b	27.53	29.42	30	4.0 ± 0	31.99	43.90	30	0
b	27.53	26.82	40	1.8 ± 1.3	31.99	41.39	40	0

* For growth rate index see table 4-1

In rubber wood heavy growth occurred at 30°C on wood blocks of both the moisture contents 'a' and 'b'. At 40°C, even though the initial moisture content of rubber wood was 67.32 per cent, the fungal growth was only trace because of the reduction of moisture content of wood blocks due to elevated temperature. From the results it is clear that 30°C is the ideal temperature for the luxurious growth of Bt on rubber wood. But in *A. scholaris*, even the conducive temperature did not have any influence on fungal growth of Bt.

4.3.4 Temperature tolerance of *Botryodiplodia theobromae*

Fungal growth on agar media: *Botryodiplodia theobromae* grew and covered the entire Petri dish within 48 hours at 30°C, whereas the growth was very much restricted at 40°C. No fungal growth was observed at high temperatures of 50°C and 60°C (Table 4-7).

Fungal growth on wood blocks: On wood blocks, no growth of Bt was observed from the inoculated disc at 50 and 60°C . At 30°C, even though the initial growth was very slow, heavy growth was observed in 120 hours. At 40°C, initially only traces of growth occurred around the inoculated agar disc on the wood blocks, but subsequently fungal growth stopped.

Survival of Bt in wood blocks: The results indicated that once Bt was established in the wood the mycelium was not killed on the surface as well as inside the block at 40°C and 50°C even after 168 hours of incubation. However, at 60°C, the fungus remained alive only for 72 hours (Table 4-8).

From this experiment, it became apparent that for the initial establishment and growth of Bt, optimum temperature (30°C) is required. Nevertheless, for survival, the fungus can tolerate still higher temperature if it is already established inside the blocks.

Table 4-7. Growth of *Botryodiplodia theobromae* on agar media and rubber wood blocks at different incubation temperatures (Mean of 15 observations on PDA and 10 observations of wood blocks)

Hours of incubation	30°C		40°C		50°C		60°C	
	Mean colony dia. on PDA	Mean growth rate index	Mean colony dia. on PDA	Mean growth rate index*	Mean colony dia. on PDA	Mean growth rate index*	Mean colony dia. on PDA	Mean growth rate index*
	cm ± SE	SE	cm ± SE	SE	cm ± SE	SE	cm ± SE	SE
24	4.3 ± 0.07	0.0 ± 0	0.96 ± 0.21	1.0 ± 0.28	0	0	0	0
48	9.0 ± 0	1.2 ± 0.25	1.72 ± 0.15	1.0 ± 0.47	0	0	0	0
72	9.0 ± 0	2.4 ± 0.16	3.20 ± 0.05	0	0	0	0	0
96	9.0 ± 0	3.4 ± 0.16	4.0 ± 0.03	0	0	0	0	0
120	9.0 ± 0	4.0 ± 0	4.5 ± 0.04	0	0	0	0	0
144	9.0 ± 0	4.0 ± 0	4.6 ± 0.06	0	0	0	0	0
168	9.0 ± 0	4.0 ± 0	4.7 ± 0.03	0	0	0	0	0

* For growth rate index see Table 4-1

Table 4-8. Survival of *Botryodiplodia theobromae* on rubber wood blocks for a period of 168 hours

Incubation period (hours)	Temperature of incubation		
	40°C	50°C	60°C
24	+	+	+
48	+	+	+
72	+	+	+
96	+	+	0
120	+	+	0
144	+	+	0
168	+	+	0

+ = Mycelium viable
0 = Mycelium non viable

4.3.5 Comparison of weight loss of *Hevea brasiliensis*, *Ailanthus triphysa* and *Alstonia scholaris* caused by *Botryodiplodia theobromae*

At the end of the fourth month of incubation, maximum weight loss occurred in rubber wood followed by *A. triphysa* and *A. scholaris* (Table 4-9, Fig. 4-4). In the latter, no weight loss was recorded for the first three months and it was only 4.5 per cent at the end of the fourth month. In rubber wood, initially the weight loss was 8 per cent which increased up to 12.2 per cent at the end of the experiment. In *A. triphysa*, the weight loss increased from 4.3 per cent to 10.1 per cent. No weight loss was recorded in control blocks.

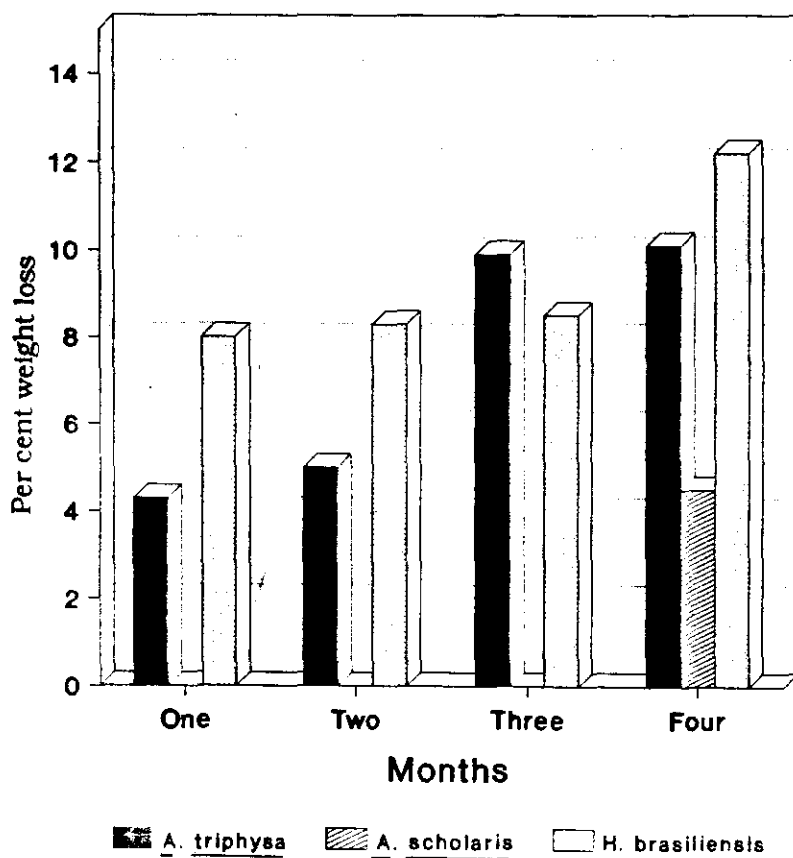


Fig. 4-4. Weight loss of *Ailanthus triphysa*, *Alstonia scholaris* and *Hevea brasiliensis* due to growth of *Botryodiplodia theobromae* for a period of four months.

Table 4-9. Weight loss in wood of different species due to growth of *Botryodiplodia theobromae* for a period of four months (mean of 10 observations)

Incubation period (month)	Weight loss percentage		
	Hevea brasiliensis	*Ailanthus triphysa*	*Alstonia scholaris*
	Mean ± SE	Mean ± SE	Mean ± SE
1	8.0 ± 0.25	4.3 ± 0.52	0.0 ± 0
2	8.3 ± 0.36	5.0 ± 0.32	0.0 ± 0
3	8.5 ± 0.55	9.9 ± 0.82	0.0 ± 0
4	12.2 ± 1.09	10.1 ± 0.84	4.5 ± 0.53

4.4 DISCUSSION

Water is a fundamental constituent of a living tree and the active system of water transport in a tree is restricted to sapwood. The moisture content of sapwood varies from tree to tree depending upon edaphic and various host factors. When the freshly felled timber is exposed to atmospheric conditions, it loses the moisture rapidly and, consequently, the moisture content of wood gradually comes to an equilibrium with the atmospheric relative humidity and temperature. This moisture content of wood varies with the temperature and relative humidity of the ambient atmosphere (Skaar, 1972). With the increase in temperature, the moisture content of wood decreases. Decrease in moisture content highly influences the rate of growth of sapstain fungi as observed for Bt in rubber wood. Bakshi (1988) reported high rate of spread of fungus in wood when it reached the fibre saturation point which varied from18 to 32 per cent moisture content based on oven dry weight. Furthermore, heavy growth of Bt on wood is favoured by 100 per cent RH and temperature of 30°C. Among all

the factors (RH, T, MC), relative humidity is the most important factor governing the extent of growth of the fungus on wood.

Similar studies were conducted by Colley and Rumbold (1930) who found that the lowest moisture content limit for staining Loblolly pine by *Ceratocystis pilifera* was about 24 per cent. Pinheiro (1971) reported that the lowest moisture content of poplar wood which permitted the growth of *B. theobromae* was 24 per cent. Bellmann and Francke-Grosmann (1952) also reported that the blue stain fungi did not survive in wood having less than 22 per cent of moisture. The results of this study support the earlier findings that the moisture content of wood less than 24 per cent does not favour colonization by *B. theobromae*. This is in agreement with the studies made by other workers. Viitanen and Paajanen (1988) found that the growth of moulds such as *Aspergillus versicolor*, *A. niger*, Aureobasidium pullulans, *Cladosporium sphaerospermum*, *Penicillium* spp. and *Trichoderma* sp., grew rapidly at high humidity (RH 96%), while the growth of the fungi was slow and could be detected only by microscope at RH 80 per cent. Bjurmann (1989) also reported that growth of mould, *Penicillium brevicompactum* was much reduced because of the fluctuations in the moisture content of sapwood of Scots pine (*Pinus sylvestris*) when adjusted with relative humidity of 80 per cent and 90 per cent.

The susceptible nature of rubber wood to sapstain caused by *B. theobromae* is very evident from the results of survey of sapstain fungi affecting various timbers. This is confirmed by the present study where the rubber wood harbours heavy growth of Bt at conducive climatic conditions. The results of the present study also confirm conclusively that *A. scholaris* is resistant to sapstain by *B. theobromae* under optimum temperature and RH and even a little decrease in the moisture content of wood will reduce the chance of sapstain infection. From this comparative study it is evident that even under favourable conditions such as sufficient moisture content of timber and optimum temperature, the

fungal growth on *A. scholaris* was very negligible whereas in *H. brasiliensis*, it was heavy. Possibly the chemical constitution of rubber wood promotes the growth of Bt whereas *A. scholaris* has some inhibitory properties which do not promote the growth of Bt.

The survival of Bt in wood in relation to temperature has greater significance in processing and kiln drying of timbers, especially in the context of managing sapstain. The results clearly show that in culture as well as on wood blocks the fungus can grow well at 30°C. Increase in temperature reduces the ability to establish on wood blocks. However, if the fungus had already established and grown in the wood block, it could tolerate still higher temperatures. Only 30°C favoured heavy fungal growth of Bt, both on agar medium as well as on wood blocks. Cessation of growth at 40°C was possibly due to the reduction of the moisture content of the wood as well as the drying up of mycelial agar disc. It is evident that, as the temperature increases, the timber gradually loses its moisture content and thus the reduced moisture content makes the timber unsuitable for the growth and establishment of Bt. Bakshi (1953) reported that moisture is the critical controlling factor of infection because the fungal spores can only germinate in free water or in an atmosphere of high relative humidity. In a similar study, Hong (1980) also tested the survival of Bt at various temperatures on agar as well as on wood blocks and reported that the growth of the fungus ceases after 2 days at 0°C and 50°C and after 3 days at 40°C on malt agar. On the contrary, this study reveals that there is no fungal growth in PDA at 50°C, because the optimum temperature for good growth of Bt in agar media is found to be 30°C, although on wood blocks the fungus can tolerate higher temperatures probably because it can penetrate and grow inside the wood for survival. In this study the fungus survives at 50°C whereas in Hong's experiment Bt could not survive above 40°C. This may be because the wood samples inoculated were much smaller than the wood blocks used in this study. It is expected that for bigger wood blocks longer time will be needed for the inner

most part of the wood sample to attain the higher ambient temperature. Therefore, the organism may survive at higher temperature in large wood samples.

Nevertheless there are reports indicating that certain staining fungi are fairly resistant to high temperatures. Fritz (1929) found a blue stain fungus alive after twelve hours at 49°C and 1 hour at 60°C. Lindgren (1942) found that some of the blue stain fungi tested, except *Ceratocystis ips* became non viable after two days. Findlay (1959) found that *Lasiodiplodia* spp. (Syn. *Botryodiplodia*) are more resistant to heat than other staining fungi and can survive for many hours after exposure to temperatures up to 65°C. Kaarik (1980) also subscribed to this finding and reported that Bt can withstand temperatures above 65°C.

This character of temperature tolerance of *B. theobromae* in timber is of practical value in adjusting the temperature inside the drying kiln and accordingly wood can be protected effectively from sapstain infection of Bt.

Weight loss caused by *B. theobromae* was more significant in rubber wood than *A. scholaris*; eventhough in *A. triphysa* the weight loss is less than that in rubber wood; it is quite significant. The weight loss recorded in rubber wood and *A. triphysa*, may possibly be due to the utilization of sugar and starch contents in the parenchymatous cells of the sample blocks. The high amount of carbohydrate present in rubber wood (Kadir and Sudin, 1989) may be the reason for higher loss in weight of rubber wood than the other two timbers. Earlier workers have also reported loss in weight of wood blocks by *B. theobromae* and other stain fungi. Tabirih and Seehann (1984) reported the weight loss caused by *B. theobromae* on *Triplochiton scleroxylon* and *Fagus sylvatica*. He observed that in *T. scleroxylon* the weight loss accounted was 8.6 per cent at the end of 16 weeks whereas in *F. sylvatica*, it was 3.6 per cent. The reason attributed for this weight loss was the presence of differential amount of parenchyma in *T. scleroxylon* (43%) and *F. sylvatica* (20%). Kaarik (1980) had also explained

the weight loss caused by different staining fungi. Investigations by Hong (1976) revealed that there was an initial weight loss of 7.51 per cent in *Dyera costulata* which increased up to 10.0 per cent by the end of fifth month due to infection by *B. theobromae*. Furthermore, decrease in weight loss was partly explained by the utilization of sugar and starch contained in the sample by the fungus, as reported earlier by Olofinboba and Lawton (1968). Umezurike (1969) had shown that *B. theobromae* was capable of degrading some components of wood, particularly after the utilization of starch and soluble carbohydrates. Later, Umezurike (1978) studied the mode of degradation of wood blocks of *Gossweilerodendron balsamiferum* (Verm.) Harms., a forest tree used extensively for constructional work in Nigeria, by *B. theobromae*. He recorded a weight loss of 5.7 per cent in the wood blocks and found that the pattern of attack of wood blocks by *B. theobromae* was similar to that of soft rot fungi (Krapivina, 1960; Levy, 1967). It is well known that both soft rot and blue stain fungi are capable of forming chains of cavities in the S_2 layer of the secondary cell wall of wood. Fougerousse (1985) also reported that susceptibility to blue stain depends on the predominance of parenchymatic tissue or the physiological condition like higher percentage of starch in the tree at the time of felling.

CHAPTER 5

EVALUATION OF CHEMICALS FOR THE CONTROL OF MOULD AND SAPSTAIN

5.1 INTRODUCTION

As soon as a tree is felled, it becomes prone to microbial degradation, especially by fungi. Wood can be protected against biodeterioration by making the wood unsuitable for the growth and development of fungi. This is achieved either by physical means such as reducing the moisture content by drying the wood or by applying appropriate chemicals as wood preservatives. So far, numerous chemicals have been evaluated as wood preservatives, but only a very few have been found to possess all the requisite qualities of a good preservative. In late 1960s, Sodium pentachlorophenoxide (NaPCP) was introduced for effective control of sapstain and mould. It was accepted as an antisapstain chemical throughout the world. However, over the years, concern for the toxic compounds and their related environmental problems have led to the reduced use of NaPCP. At present, the ban on the use of pentachlorophenol and NaPCP in most of the countries has led to the search for alternative anti-sapstain chemicals which are effective and equally environmentally safe.

5.2 MATERIALS AND METHODS

5.2.1 Screening of fungicides for controlling mould and sapstain

Different chemicals were screened for their efficacy in controlling mould and sapstain fungi in wood following various standard methods.

5.2.2 Poisoned-food method

The efficacy of some of the commonly available fungicides viz., carbendazim, captafol, carboxin, copperoxychloride, mancozeb and thiram was tested at

0.75, 0.5 and 0.25 per cent (a.i.) against sapstain and mould fungi. Also sodium azide, which was proved to be a good fungicide in an earlier study (Sharma and Mohanan, 1991) was also evaluated against stain and mould fungi (Table 5-1).

Initially, the efficacy of a fungicide is tested in Petri dishes (Richards, 1923). The rate of fungal growth in fungicidal agar medium was measured as a measure of chemical effectiveness. The results are then compared with the results of similar tests on accepted preservatives. This method provides only a relative measure of toxicity because the growth of fungi on artificial growth medium may be markedly different from the growth in wood. Weighed quantity of fungicides to make different concentration levels was mixed thoroughly in sterilized potato dextrose agar (PDA) medium while it was lukewarm. Each Petri dish contained 20 ml of the fungicidal agar medium. There were three Petri dishes for each concentration. After solidifying the agar, each Petri dish was inoculated at the centre with a mycelial disc of 8 mm diameter taken aseptically with a sterile cork borer from the margin of actively growing colonies of various mould fungi such as *Aspergillus flavus* (Af), *Aspergillus niger* (An), *Memnoniella echinata* (Me) and *Trichoderma viride* (Tv) and stain fungi such as *Acremonium recefei* (Ar), *Botryodiplodia theobromae* (Bt), *Ceratocystis fimbriata* (Cf), *Fusarium solani* (Fs) and *Scytalidium lignicola* (Sl). Control Petri dishes maintained simultaneously did not contain any fungicide in the agar medium. All the inoculated dishes were incubated at 28 $\pm$ 2°C till full radial growth was observed in control. Three diameter growth measurements were recorded in each Petri dish. The per cent inhibition of fungal growth in each fungicidal treatment and control was calculated by the following equation (Vincent, 1927).

$$I = \frac{100C-T}{C}$$

Where I = inhibition over control; C = growth in control and
T = growth in treatment.

The data on inhibition of fungal growth were analysed statistically.

Table 5-1. List of fungicides/chemicals evaluated against staining and mould fungi

Sl. No.	Trade name	Common name	Chemical name	% concentration tested (a.i.)
1	Fytolan	—	Copper oxychloride	0.25 0.50 0.75
2	Blue copper	—	Copper oxychloride	0.25 0.50 0.75
3	Bavistin	Carbendazim	Methyl-H-Benzimidazole-Z-yl-carbamate	0.25 0.50 0.75
4	Vitavax	Carboxin	5,6-dihydro-2-methyl-1, 4-Oxathiin-3-carboxanilide	0.25 0.50 0.75
5	Dithane-M-45	Mancozeb	Zinc iron+manganese ethylene-bis-dithiocarbamate	0.25 0.50 0.75
6	Thiride	Thiram	Tetramethyl thiuram disulphide	0.25 0.50 0.75
7	Ziride	Ziram	Zinc dimethyldithiocarbamate	0.25 0.50 0.75
8	Difoltan	Captafol	Cis-N-(1,1,2,2, tetrachloro ethyl thio)-4-cyclohexane-1, 2-dicarboximide	0.25 0.50 0.75
9	Sodium azide	—	Sodium azide	0.25 0.50 0.75
10	NaPCP + Borax	—	Sodium pentachloro-phenoxide + Borax	0.50 1.50

5.2.3 Evaluation of fungicides on sterile wood blocks

The efficacy of the fungicides was further evaluated using sterile wood blocks. Since the growth of the test fungus in artificial culture medium is expected to be markedly different from the growth in wood, a concentration of only 1 per cent was tested on wood blocks. Possibly the lower concentrations, which are effective in the agar media, may not be effective in wood. This is because, the sugars present in the sapwood can enhance the fungal growth and largely negate the protective value of some wood preservatives (Zabel and Morrell, 1992). All the fungicidal screening was done using rubber wood blocks as it was very susceptible to mould, sapstain and decay fungi (Sujan *et al.*, 1980; Gnanaharan, 1983).

Wood blocks were prepared from the bottom log of freshly felled 35-year-old rubber tree. After sizing the log into planks, clear defect free samples of 50 x 10 x 70 mm size blocks were prepared and selected randomly for the study, packed in polythene bags and stored in a refrigerator.

The desired concentration of preservative solution was prepared by dissolving 1 g (a.i.) of the fungicide in 100 ml of sterile distilled water. Wood blocks were steam sterilized for 15 minutes at 100 kPa, allowed to cool under room temperature for 10 minutes and individual blocks were immersed for 10 seconds separately in fungicidal solutions of carbendazim, captafol, carboxin, copperoxychloride, mancozeb, sodium azide, thiram and ziram with the help of sterile forceps. The excess solution was drained completely by keeping the blocks in a slanting position in sterile Petri dishes for 10 seconds. Subsequently, each block was placed individually over glass rod supports kept over two moistened sterile filter papers in each sterile Petri dish. For each fungus, six blocks were maintained in separate Petri dishes. All the treated blocks were inoculated individually with an 8 mm diameter mycelial disc taken from actively growing culture of the test fungi such as Af, An, Me and Tv (moulds fungi), Ar, Bt, Cf, Fs

and SI (stain fungi). Wood blocks dipped in sterile distilled water served as control and those treated (as above) with solution containing 0.5 per cent NaPCP and 1.5 per cent borax as standard (Butcher, 1980). All the treated blocks were incubated at 28 ± 2°C for 2 weeks and the fungal growth over the wood block was assessed visually using the rating index mentioned in Table 4-1. Data were analysed statistically.

As captafol had been found to be very effective against stain and decay fungi in other countries (Butcher, 1980; Hong *et al.*, 1980; Tan *et al.*, 1980), in addition to 1 per cent, two higher concentrations of this fungicide (1.5 and 2 per cent a.i.) were also evaluated. The higher concentrations were chosen as this fungicide was not found effective at 1 per cent in initial studies.

5.2.4 Efficacy of sodium azide against stain and mould fungi

In the initial evaluation, sodium azide proved to be a promising chemical for the control of stain and mould fungi, both in poisoned-food method as well as on wood block test. Hence, detailed studies on its efficacy to control sapstain and mould fungi at lower concentrations were attempted.

Effective concentration of sodium azide: Since sodium azide tested at 1 per cent was found to be effective in inhibiting the growth of all the stain and mould fungi, its efficacy was further tested on wood blocks at lower concentrations such as 0.75, 0.5 and 0.25 per cent. For standardizing the lowest effective concentration of sodium azide, wood blocks were again tested with 0.1, 0.05, 0.025, 0.01, 0.005 and 0.001 per cent concentrations of sodium azide.

Fresh unsterile wood blocks (FUS): In order to find out the efficacy of sodium azide in unsterile condition, rubber wood blocks were immersed individually for 30 seconds separately in a solution of 0.75, 0.5, 0.25 and 0.1 per cent. For each treatment 25 blocks were used. After draining the excess solution, blocks were

then placed individually inside unsterile Petri dishes on moistened filter papers to maintain humidity. After 4 weeks of incubation fungal growth over the blocks was recorded using the rating index mentioned in Table 4-1. The percentage control of fungal growth was calculated by counting the number of colonized and uncolonized wood blocks. The data were analysed statistically using Z-test.

Pre-incubated unsterile wood blocks (PUS): Twentyfive freshly cut rubber wood blocks were kept outdoors of a saw mill for an hour in order to expose them to air-borne spores of sapstain and mould fungi and then incubated in Petri dishes for 2 days to enhance the fungal growth on the blocks. These blocks were immersed for 30 seconds individually in different concentrations of sodium azide such as 0.75, 0.5, 0.25, 0.1 per cent. The wood blocks were incubated in Petri dishes for a period of 4 weeks. Observations were recorded at weekly interval and the fungal growth over the wood blocks was assessed visually using the rating index (Table 4-1). The efficacy of the chemical was calculated by counting the number of colonized and uncolonized wood blocks and the percentage inhibition of fungal growth at the end of four weeks was analysed statistically using Z test.

Effect of immersion time on the inhibition of fungal growth: To ascertain the effect of immersion time in sodium azide solution on its efficacy in controlling sapstain, freshly cut twenty five rubber wood blocks were immersed separately in 0.75, 0.5, 0.25 and 0.1 per cent solution for 10, 20 and 30 minutes. The excess solution was drained from the wood blocks. The blocks were incubated at 28 ± 2°C in Petri dishes for a period of 4 weeks. Control wood blocks were also treated in the same manner except that they were immersed in sterile distilled water. Weekly observations were recorded on the development of fungal growth on the wood blocks over a period of 4 weeks.

5.3 RESULTS

5.3.1 Screening of fungicides for controlling mould and sapstain

Various chemicals/fungicides were screened for their efficacy in controlling mould and sapstain fungi in wood following poisoned- food and wood block methods.

5.3.2 Poisoned-food method

In vitro evaluation of fungicides employing poisoned-food method indicated that sodium azide was the most effective fungicide that inhibited the growth of mycelium in all the three concentrations tested (Table 5-2 and 5-4). Carbendazim (Bavistin) was also found to be equally effective at three concentrations except for *S. lignicola* (Sl) and *F. solani* (Fs). All the three concentrations of mancozeb (Dithane M-45) inhibited *C. fimbriata* (Cf), *A. niger* (An) and *M. echinata* (Me), whereas it was not effective against *T. viride* (Tv) and *A. flavus* (Af). More than 70 per cent inhibition was observed in the case of *B. theobromae* (Bt), *F. solani* (Fs) and *S. lignicola* (Sl). Captafol was effective in inhibiting more than 90 per cent growth of Cf, Tv and Me. All the three different concentrations of copperoxychloride (Fytolan) were effective against *F. solani* (Fs) and *A. niger* (An) and *M. echinata* (Me) (>95 per cent control). When compared to all the fungicides, carboxin and ziram could not inhibit 100 per cent growth of any of the test fungi. Thiram inhibited 100 per cent growth of only *A. recefei* (Ar) and *M. echinata* (Me). The results of the two-way analysis of variance of the data related to fungicides, concentrations and their interactions in respect of various stain and mould fungi were highly significant (Tables 5-3 and 5-5).

5.3.3 Evaluation of fungicides using sterile wood blocks

Results of the evaluation of various fungicides on sterile wood blocks at 1 per cent (a.i.) revealed that a few fungicides inhibited the growth of the fungi

Table 5-2. Evaluation of fungicides against mould fungi using poisoned-food method

Fungicides	Concent-ration % (a.i.)	% of inhibition			
		Mould fungi*			
		Tv	An	Af	Me
Copper oxychloride (Fytolan)	0.25	81.89	100.00	79.79	97.35
	0.50	88.00	100.00	86.21	100.00
	0.75	79.21	100.00	100.00	100.00
Copper oxychloride (Blue copper)	0.25	25.88	100.00	100.00	100.00
	0.50	32.11	100.00	100.00	100.00
	0.75	41.33	100.00	100.00	100.00
Carbendazim (Bavistin)	0.25	100.00	100.00	100.00	100.00
	0.50	100.00	100.00	100.00	100.00
	0.75	100.00	100.00	100.00	100.00
Carboxin (Vitavax)	0.25	83.99	100.00	100.00	86.35
	0.50	85.32	98.55	85.21	87.35
	0.75	86.77	100.00	100.00	78.01
Mancozeb (Dithane-M-45)	0.25	67.67	100.00	0.00	100.00
	0.50	65.66	100.00	0.00	100.00
	0.75	59.22	100.00	0.00	100.00
Thiram (Thiride)	0.25	83.33	98.88	50.33	100.00
	0.50	87.33	92.32	52.22	100.00
	0.75	95.56	81.99	52.22	100.00
Ziram (Ziride)	0.25	74.33	90.88	88.55	97.85
	0.50	92.55	97.88	93.32	94.52
	0.75	91.32	97.77	94.77	100.00
Captafol (Difoltan)	0.25	96.88	64.77	66.99	94.52
	0.50	97.32	60.33	58.11	100.00
	0.75	92.52	54.44	59.77	100.00
Sodium azide	0.25	100.00	100.00	100.00	100.00
	0.50	100.00	100.00	100.00	100.00
	0.75	100.00	100.00	100.00	100.00

* Tv = *T. viride*, An = *A. niger*, Af = *A. flavus*
Me = *M. echinata*

Table 5-3. ANOVA of diameter growth of mould fungi evaluated against fungicides using poisoned- food method

Source	DF	Mould fungi@							
		Tv		An		Af		Me	
		MSS	F	MSS	F	MSS	F	MSS	F
Replication	2	0.029	1.38(ns)	0.003	0.17(ns)	0.024	1.33(ns)	0.005	1.00(ns)
Fungicide (F)	7	3.583	170.62**	2.587	143.72**	5.998	333.22**	0.449	89.80**
Concentration (C)	2	0.345	16.43**	0.798	44.33**	0.131	7.28**	0.008	1.60(ns)
F x C	14	0.181	8.62**	0.680	37.78**	0.082	4.56**	0.009	1.80*
Error	46	0.021		0.018		0.018		0.005	

** Significant at P = 0.01%
* Significant at P = 0.05%
ns Non-significant
@ Tv = *T. viride* , An = *A. niger* , Af = *A. flavus* , Me = *M. echinata*

Table 5-4. Evaluation of fungicides against sapstain fungi using poisoned-food method

Fungicides	Concentration % (a.i.)	% of inhibition				
		Stain fungi*				
		Bt	Cf	Fs	Sl	Ar
Copper oxychloride (Fytolan)	0.25	49.55	83.53	100.00	100.00	87.16
	0.50	88.88	84.66	96.65	95.55	85.75
	0.75	90.10	85.55	98.88	88.44	87.59
Copper oxychloride (Blue copper)	0.25	26.44	13.22	100.00	81.88	100.00
	0.50	24.11	32.55	100.00	86.99	100.00
	0.75	26.44	53.44	100.00	89.77	100.00
Carbendazim (Bavistin)	0.25	100.00	100.00	88.05	71.55	100.00
	0.50	100.00	100.00	92.94	72.33	100.00
	0.75	100.00	100.00	100.00	76.77	100.00
Carboxin (Vitavax)	0.25	67.55	69.66	67.80	56.88	64.30
	0.50	73.55	80.99	81.16	81.55	73.14
	0.75	74.33	79.88	79.13	88.66	74.59
Mancozeb (Dithane-M-45)	0.25	88.44	100.00	81.76	100.00	58.58
	0.50	70.88	100.00	91.16	98.55	95.89
	0.75	88.88	100.00	91.38	90.21	100.00
Thiram (Thiride)	0.25	84.55	89.99	86.08	86.44	100.00
	0.50	85.66	90.44	81.99	90.66	100.00
	0.75	87.88	100.00	84.25	93.55	100.00
Ziram (Ziride)	0.25	77.10	69.99	61.62	72.99	71.02
	0.50	74.33	94.21	78.90	91.10	100.00
	0.75	94.88	91.99	81.38	91.44	98.45
Captafol (Difoltan)	0.25	81.44	93.21	82.87	72.88	76.02
	0.50	87.32	92.55	85.21	87.99	73.50
	0.75	87.66	96.21	90.13	78.77	88.74
Sodium azide	0.25	0.00	0.00	0.00	0.00	0.00
	0.50	0.00	0.00	0.00	0.00	0.00
	0.75	0.00	0.00	0.00	0.00	0.00

*Bt = *B. theobromae* , Cf = *C. fimbriata* , Fs = *F. solani*
Sl = *S. lignicola*, Ar = *A. recefei*

Table 5-5. ANOVA of diameter growth of stain fungi evaluated against fungicides using poisoned-food method

Source	DF	Stain fungi[@]									
		Bt		CF		Fs		Sl		Ar	
		MSS	F	MSS	F	MSS	F	MSS	F	MSS	F
Replication	2	0.027	0.48(ns)	0.030	0.81(ns)	0.241	4.16*	0.010	0.24(ns)	0.021	1.11(ns)
Fungicide (F)	7	2.918	52.11**	3.686	99.62**	1.646	28.38**	1.168	28.49**	1.479	77.84**
Concentration (C)	2	0.624	11.14**	0.606	16.38**	0.385	6.64**	0.300	7.32**	0.198	10.42**
F x C	14	0.280	5.00**	0.125	3.38**	0.076	1.31(ns)	0.239	5.83**	0.383	20.16**
Error	46	0.056		0.037		0.058		0.041		0.019	

* Significant at P = 0.05%
** Significant at P = 0.01%
ns Non-significant
@ Bt = *B. theobromae*, Cf = *C. fimbriata*, Fs = *F. solani*, Sl = *S. lignicola*, Ar = *A. recefei*

selectively (Table 5-6). Sodium azide was effective against all the fungi tested. Carbendazim showed more than 85 per cent inhibition of all the fungi tested. Partial suppression of the growth of a few fungi (Ar, Cf, Fs, Me, Sl) was noted in the case of mancozeb, whereas it was not effective against Af and Tv. Nearly 80 per cent control of all the fungi except Fs was noted on wood blocks treated with thiram. Fytolan controlled the growth of Af, An, Bt, Fs, and Me (80-85 per cent), whereas blue copper was not effective against Bt, Cf, Sl and Tv. Carboxin was the only ineffective fungicide as it did not control the growth against any of the fungi tested. Mixture of NaPCP + borax, treated as standard was 100 per cent effective, whereas in control, 100 per cent fungal growth was observed.

Among the three different concentrations of captafol tested, 2 per cent (a.i.) was comparatively better than the others. Statistical analysis showed significant differences between all the fungi tested.

5.3.4 Efficacy of sodium azide against stain and mould fungi

Effective concentration of sodium azide: Table 5-7 indicated that sodium azide was 100 per cent effective at concentrations of 0.75, 0.5, 0.25 and 0.1 per cent in controlling the growth of all the mould and sapstain fungi tested on sterilized wood blocks. In control wood blocks, the fungal infection was 100 per cent. At lower concentrations of sodium azide, only 0.025 and 0.05 per cent were effective in preventing fungal growth on all wood blocks. The concentrations of sodium azide below 0.025 per cent were found to be ineffective.

Fresh unsterile, pre-incubated wood blocks: As the period of incubation increased, the efficacy of different concentrations of sodium azide decreased. At any given concentration the treatment was more effective in fresh unsterile blocks (FUS) than pre- incubated unsterile blocks (PUS). Over a period of 4 weeks' incubation, the maximum inhibition in fungal growth was 80 per cent in FUS, whereas in PUS it was only 60 per cent (Table 5-8). Neither treatment

Table 5-6. Mean of rating of fungal growth over wood blocks treated with different fungicides

Fungicides	Concentration % (a.i.)	Mean rating**								
		Tv*	An	Af	Me	Bt	Cf	Fs	Sl	Ar
Fytolan	1.0	2.17^{e}	0.83^{bc}	0.50^{ab}	0.5^{bcd}	0.50^{ab}	1.33^{d}	0.00^{a}	1.33^{cd}	1.83^{d}
Copper oxychloride	1.0	3.17^{f}	0.33^{ab}	0.17^{a}	0.33^{abc}	2.5^{f}	2.33^{e}	1.33^{cd}	4.00^{f}	0.67^{bc}
Carbendazim	1.0	0.33^{a}	0.67^{abc}	0.50^{ab}	0.00^{a}	0.00^{a}	0.00^{a}	0.67^{b}	0.50^{ab}	0.00^{a}
Carboxin	1.0	3.67^{fg}	2.67^{e}	2.67^{c}	3.00^{f}	2.33^{f}	3.50^{f}	3.50^{f}	2.83^{c}	1.67^{d}
Mancozeb	1.0	2.50^{e}	1.00^{cd}	2.33^{c}	0.17^{ab}	1.17^{cde}	0.17^{ab}	0.50^{ab}	0.67^{ab}	0.50^{b}
Thiram	1.0	0.67^{ab}	0.50^{abc}	0.83^{ab}	0.67^{cd}	0.67^{bc}	0.50^{bc}	1.33^{cd}	0.50^{ab}	0.50^{b}
Ziride	1.0	0.33^{a}	0.17^{a}	1.00^{b}	0.00^{a}	0.83^{bcd}	0.00^{a}	0.83^{bc}	0.17^{a}	0.00^{a}
Captafol	1.0	2.00^{de}	1.50^{d}	1.00^{b}	1.33^{e}	1.67^{e}	1.33^{d}	2.17^{e}	1.67^{d}	1.00^{c}
Captafol	1.5	1.50^{cd}	1.50^{d}	0.67^{ab}	0.83^{d}	1.33^{de}	0.83^{c}	1.67^{de}	1.67^{d}	0.33^{ab}
Captafol	2.0	1.00^{bc}	1.00^{cd}	0.5^{ab}	0.00^{a}	0.83^{bcd}	0.17^{ab}	1.33^{cd}	0.83^{bc}	0.00^{a}
Control	0.0	3.83^{g}	3.50^{f}	3.67^{d}	2.83^{f}	4.00^{g}	4.00^{g}	3.33^{f}	3.83^{f}	3.50^{d}

100% inhibition of fungal growth over wood block was observed in the case of sodium azide and NaPCP + Borax
Figures in a column superscribed by the same letters indicate non-significance
* Tv = *T. viride*, An = *A. niger*, Af = *A. flavus*, Me = *M. echinata*
Bt = *B. theobromae*, Cf = *C. fimbriata*, Fs = *F. solani*, Sl = *S. lignicola*
Ar = *A. recefei*
** For growth rate index see Table 4-1

Table 5-7. Mean rating of fungal growth over wood blocks treated with different concentrations of sodium azide

Concent-ration %	Fungal growth rating**																	
	Tv		An		Af		Me		Bt		Cf		Fs		Sl		Ar	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0.01	3.67	0.21	4.0	0	3.5	0.22	3.0	0	4.0	0	4.0	0	3.67	0.21	4.0	0	3.17	0.17
0.005	3.83	0.17	4.0	0	3.83	0.17	3.83	0.17	4.0	0	4.0	0	3.83	0.17	4.0	0	4.0	0
0.001	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0
Control	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0	4.0	0

* Concentration of 0.75, 0.5, 0.25, 0.1, 0.05 and 0.025% were 100% effective

** For growth rate index see table 4-1

Tv = *T. viride*, An = *A. niger*, Af = *A. flavus*, Me = *M. echinata*, Bt = *B. theobromae*, Fs = *F. solani*, Sl = *S. lignicola*

Ar = *A. recefei*

gave 100 per cent inhibition of fungal growth. In PUS treated with 0.1 per cent solution the stain and mould fungi colonized the wood blocks completely. The percent inhibition of fungal growth on wood blocks (FUS and PUS) during the second week was found to be statistically significant at 5 per cent level from all other treatments (Z = 1.99).

Table 5-8. Per cent inhibition over control of fungal growth on fresh unsterile and pre-incubated unsterile wood blocks treated with sodium azide solution

Sodium azide concentration (%)	Per cent inhibition over control							
	Period of incubation (in weeks)							
	1		2		3		4	
	FUS*	PUS**	FUS	PUS	FUS	PUS	FUS	PUS
0.1	20	12	20	12	12	0	12	0
0.25	32	28	32	12	32	12	28	12
0.50	32	56	32	48	32	28	32	28
0.75	88	68	88	64	80	60	80	60

* FUS = Fresh unsterile blocks
** PUS = Pre-incubated unsterile blocks

Effect of immersion time on the inhibition of fungal growth: Immersion time in sodium azide solution had a significant effect on the inhibition of fungal growth on wood blocks. The highest concentration of sodium azide i.e., 0.75 per cent was most effective in controlling fungal growth irrespective of immersion time (Table 5-9). Concentration of 0.5 per cent sodium azide was equally effective at all the three immersion times up to three weeks. However, during the fourth week only 30 minutes immersion provided complete control of fungal growth.

The lower concentration i.e., 0.1 per cent was effective up to the second week whereas the concentration of 0.25 per cent remained effective in controlling fungal growth up to the third week. Thereafter for all three immersion timings the colonization of blocks gradually increased. Statistical analysis of the data indicated that immersion in 0.5 per cent concentration for 20 minutes was significant at 5 per cent level (Z = 2.45) and for 30 minutes, highly significant at 1 per cent level (Z = 3.54).

Table 5-9. Per cent inhibition of fungal growth over control on wood blocks immersed in sodium azide solution for different durations

Immersion time (min.)	Increase in weight (g) of blocks due to absorption of solution (mean of 25 blocks)	Concentration of sodium azide (%)	% inhibition over control recorded at weekly intervals			
			1st week	2nd week	3rd week	4th week
10	1.31	0.1	100	100	80	40
		0.25	100	100	100	48
		0.50	100	100	100	60
		0.75	100	100	100	90
20	2.11	0.1	100	100	70	60
		0.25	100	100	80	80
		0.50	100	100	100	90
		0.75	100	100	100	100
30	3.45	0.1	100	100	72	60
		0.25	100	100	100	80
		0.50	100	100	100	100
		0.75	100	100	100	100

5.4 DISCUSSION

In tropical countries, sapstain and mould cause problems for the proper utilization of wood. Generally, mould and sapstain fungi are controlled by treating the wood with fungicides. Until recently, sodium pentachlorophenoxide was universally accepted as an anti-sapstain chemical, but due to its toxicity and human health hazards, in 1986 Environmental Protection Agency regulations restricted the use of pentachlorophenol and its derivatives. In the present study, an attempt was made to find out alternate chemicals/fungicides to control mould and sapstain.

Of the nine fungicides and chemicals screened only sodium azide was found to be effective against all the fungi such as *A. flavus, A. niger, A. recefei, B. theobromae, C. fimbriata, F. solani, M. echinata, S. lignicola,* and *T. viride* both on agar and wood blocks. Sodium azide (Loba Chemie, Bombay), is a colourless, crystalline chemical soluble in water, highly toxic, but having many properties of a good preservative. Among the various concentrations of sodium azide tested higher concentrations such as 0.75, 0.5, 0.25, were 100 per cent effective in both poisoned food method as well as wood blocks. All concentrations above 0.025 per cent were cent percent effective against mould and sapstain tested on wood blocks.

Excluding carbendazim (Bavistin) and sodium azide, the test fungi behaved in a manner different to fungicides both in poisoned-food method and on sterile wood blocks. The reason for this differential behaviour may be due to increase in the concentration of fungicides evaluated for wood blocks i.e., 1 per cent. From the results, it can be concluded that both carbendazim and sodium azide are effective fungicides against stain and mould fungi.

Carbendazim was effective against all test fungi, except for *S. lignicola,* a stain fungus. Laks *et al.* (1991) evaluated carbendazim at 0.04 and 0.1 per cent

against mould, sapstain and decay on red pine and reported that sapstain and decay were not controlled whereas mould growth was checked by 70-85 per cent. In the present study, 100 per cent control of the mould and sapstain growth was possible probably due to the fact that higher concentration of the solution was used than that of Laks *et al.* (1991). Though Carbendazim is an accepted agricultural fungicide used for controlling various plant diseases, not much studies have been carried out to test its effectiveness in protecting wood from stain and mould fungi.

Based on the poisoned-food and wood blocks method, carboxin was ineffective against sapstain and mould fungi tested. This indicates that carboxin cannot be recommended as an effective fungicide for the control of stain and mould fungal growth. The results of the present study confirm the findings of an earlier study made by Unligil (1979), that carboxin did not control 100 per cent of sapstain fungal growth on white pine even at a concentration of 4 per cent (a.i.).

Captafol was 70-90 per cent effective against all fungi tested in poisoned-food method, whereas it was not so effective on wood blocks. Even a concentration of 2 per cent (a.i.) was not 100 per cent effective against all fungi tested. This is because, the sugars present in sapwood of rubber tree possibly enhance the fungal growth and reduce the protective value of the fungicide (Zabel and Morrell, 1992).

Captafol has been reported widely as an effective anti-sapstain chemical in temperate countries. Butcher (1980) carried out tests on *Pinus radiata* in New Zealand and found that 0.2 per cent (a.i.) of captafol was equally effective as that of NaPCP solution (0.5 per cent NaPCP + 1.5 per cent Borax). In Australia, captafol at 0.2 per cent (a.i.) performed well against sapstain fungi (Leightley, 1985). But in tropical countries, much higher concentration of captafol is required for controlling sapstain since in the present study captafol inhibited the growth of stain and mould fungi on rubber wood blocks only at a higher

concentration of 2 per cent (a.i.). This is in agreement with the results of the studies carried out on rubber wood by Hong *et al.* (1980) and Tan *et al.* (1980) in Malaysia.

The growth of all the test fungi is inhibited in sterile wood blocks when compared to non-sterile ones. It is presumed that in sterile wood blocks, the efficacy of the chemical was more because some of the sugars and starch occurring in abundance in the parenchyma cells of rubber wood (Kadir and Sudin, 1989) which favour the growth of fungi, must have been removed during the process of sterilization. Apparently, this may be the reason for inhibition of fungal growth on sterilized wood blocks treated with sodium azide.

The inhibition of fungal growth on fresh unsterile (FUS) wood blocks is more than that of pre-incubated unsterile (PUS) blocks. This is possibly because in the FUS treatment, freshly cut blocks were immediately immersed in sodium azide solution thus avoiding chance of fungal colonization. In the case of PUS some of the stain and mould fungi already established on the surface and inside the blocks during the incubation period would continue to grow even after the effective treatment by the fungicide/chemical, though at a slower pace. It is apparent from this observation that the chemical is capable of inhibiting rather than killing the fungi and it cannot be used once the fungus has colonized the wood. Therefore, a prophylatic treatment of rubber wood with sodium azide immediately after felling the tree or conversion of timber will be better than treating the felled timber after time has elapsed.

The study on effect of immersion time on the inhibition of fungal growth indicates that 90 per cent inhibition is obtained by dipping in 0.5 per cent solution of sodium azide, for 20 minutes dipping whereas it is 100 per cent with 30 minutes dipping. The increase in inhibition of fungal growth with longer immersion times is possibly due to the absorption of greater quantities of chemical solution through the deeper wood tissues which is also corroborated from the quantity

of chemical absorbed per unit area. It implies that by increasing the time, the concentration of the chemical can be decreased to make it more cost effective.

Results of the present study indicate, sodium azide as a preservative for the control of sapstain and mould of rubber wood. Even though sodium azide is not a well known fungicide, it has been used to control effectively a few fungal diseases. Row *et al.* (1974) reported that it was effective against *Cylindrocladium* black rot of *Arachis hypogea* L. caused by *C. crotalariae*. Similarly, a 10 ppm solution of a related compound, has also been reported to inhibit the germination of microsclerotia of *Cylindrocladium floridanum* (Weaver, 1971). Recently, while studying the control of nursery diseases of eucalypts, Sharma and Mohanan (1991) found sodium azide to be very effective against several species of *Cylindrocladium* causing various diseases of eucalypt seedlings. In the laboratory evaluation, the chemical was cent percent effective at 0.05 and 0.1 per cent concentration. Dip applications of sodium azide and sodium fluoride have also been found to prevent kiln brown stain (Stutz, 1959; Stutz *et al.* 1961; Cech, 1966).

Even though the results clearly indicate that sodium azide is a promising chemical in the area of wood preservation, further detailed studies on its toxicity are required before it can be recommended for commercial utilization as a suitable wood preservative. Since it is an explosive, studies are also needed to be undertaken to nullify this unwarranted nature by mixing it with some other inert chemical.

CHAPTER 6

BIOLOGICAL CONTROL OF MOULD AND SAPSTAIN

6.1. INTRODUCTION

Preservation of wood and wood products against biological deterioration is one of the ways to prolong their service life. This is achieved mainly through treatment with chemical wood preservatives. Recent reports on health hazards and environmental pollution caused by the application of various toxic wood preservatives have brought out the necessity of adopting a new approach in this field for controlling the biodegradation. To avoid the use of chemicals altogether, one alternate method is to employ biological means such as the introduction of antagonistic organisms. Such organisms inhibit the growth of wood deteriorating microorganisms thereby reducing their inoculum potential and ability to invade wood to cause significant damage. This approach of control is termed as biological control, a well known concept in agriculture, which is yet to see extensive application in wood protection. During the past decade a few preliminary studies have been conducted on the biological control of sapstain by Bernier *et al.* (1986), Benko (1988 a) and Seifert *et al.* (1987; 1988). Recently, Kreber and Morrell (1993) studied the ability of various bacterial and fungal isolates as biological control agents and found that *Bacillus subtilis* had high potential to prevent fungal stain. In the present study, an attempt has been made to find out suitable biological control agents for protecting the selected timber species from the common mould and stain fungi.

6.2 MATERIALS AND METHODS

6.2.1 Isolation of biological control organisms

Wood chips of 0.5 x 0.5 mm size were removed from the surface as well as interior portion of wooden planks (40 x 200 x 600 mm size) of *A. scholaris*, *A. triphysa* and *H. brasiliensis* which had been sawn two days earlier. The wooden planks, collected from a saw mill located in Trichur district during August 1989, already had a medium growth of mould and sapstain fungi. After cleaning the sawdust, 25 wood chips of each timber species were cut from different portions of the plank using a sharp chisel. Five wood chips of each species, selected randomly, were immersed separately in 50 ml of sterile distilled water kept in 250 ml Erlenmayer flask and shaken vigorously in a horizontal shaker for 30 minutes in order to dislodge the conidia/spores of microorganisms colonizing the wood. One millilitre of this solution was transferred to sterile Petri dishes and lukewarm agar medium was poured mixing the solution thoroughly with the medium. Potato Dextrose Agar (PDA) and Nutrient Agar (NA) media were used to isolate fungal and bacterial populations, respectively. Three Petri dishes were used separately for each medium and tree species. The inoculated dishes were incubated at room temperature (28 ± 2°C). The representative bacterial and fungal colonies were isolated after an incubation period of seven days and maintained on agar slants for further studies.

6.2.2 Testing of antagonistic activity

The microorganisms obtained on PDA and NA were tested individually for antagonism to the selected mould and stain fungi. For testing the antibiotic activity, agar culture method and culture filtrate assay were followed (Johnson and Curl, 1972).

6.2.2 (i) Agar culture streak method

The most common method of determining the antibiotic activity is to place both the potential antagonist and the test organism in pure culture on the surface of an agar medium at a distance from each other.

All the fungal and bacterial organisms isolated from the wood chips were tested for their antagonistic activity in agar culture against four moulds and five stain fungi using agar culture method. For the inoculation of the antagonist, spore or conidial suspension was streaked over NA and PDA media at the centre of the Petri dish, respectively for bacteria and fungi. A mycelial disc of 8 mm diameter removed from the edge of an actively growing colony of test fungus was inoculated near the periphery on either side of the antagonist. Petri dishes were incubated at 28 $\pm$ 2°C and observed for the zone of inhibition for seven days. The following test fungi were used in the experiment.

Mould fungi

i. *Aspergillus flavus*
ii. *A. niger*
iii. *Memnoniella echinata*
iv. *Trichoderma viride*

Stain fungi

i. *Acremonium recefei*
ii. *Botryodiplodia theobromae*
iii. *Ceratocystis fimbriata*
iv. *Fusarium solani*
v. *Scytalidium lignicola*

The fungal or bacterial isolates which had developed an inhibition zone of more than 15 mm width were regarded as the potential antagonist. Such cultures were subcultured and maintained for further study.

To study the broad spectrum of antagonism, the potential antagonist was further tested against the same mould and stain fungi along with six common pathogenic fungi adopting the agar culture method. The zone of inhibition developed between the two colonies was measured at 2-day intervals for 15 days. The pathogenic test fungi used in the experiment were *Colletotrichum gloeosporioides* Penz., *Corticium salmonicolor* (Berk. & Br.), *Pestalotiopsis* sp., *Pythium* sp., *Rhizoctonia solani* Kuehn. and *Sclerotium rolfsii* Sacc.

6.2.2 (ii) Culture filtrate assay

In the culture filtrate assay, the liquid culture medium was tested for the microbial activity directly after removing the potential antibiotic-producing organism. The bacterial isolate(s) which was found to be antagonistic in agar culture was further tested by central well method using culture filtrate (Johnson and Curl, 1972). To prepare the culture filtrate of the bacterial isolate to be tested, Erlenmayer flask of 250 ml capacity containing 100 ml of sterile nutrient broth was inoculated with the spore suspension containing concentration of 1×10^6 CFU/ml and incubated for 10 days at $28 \pm 2^\circ C$. The flasks were shaken continuously over a platform shaker to obtain uniform growth of the organism. Culture filtrate was prepared by passing the broth through Whatman No. 1 filter paper and centrifuging at 10,000 rpm for 20 minutes. The supernatant of this filtrate was filter sterilized using a 0.45 μm millipore membrane. A well of 10 mm diameter was made at the centre of the PDA medium in assay plates by removing a plug of agar with a sterile cork borer. The well was then filled with filter-sterilized culture filtrate. The fungus to be tested was inoculated near the periphery of the Petri dish at two places on either side of the well. Three replicate plates were used for each test fungus and incubated for 7 days at room temperature ($28 \pm 2^\circ C$). The zone of inhibition developed between the colonies was measured on alternate days.

6.2.3 Wood block test

The efficacy of the potential antagonist in inhibiting the (growth of *B. theobromae*, the dominant stain fungus, was studied on the surface of wood blocks of *A. triphysa*, *A. scholaris* and *H. brasiliensis*. The spore suspension of the antagonist was prepared by inoculating 1 ml of a 1:10 dilution of a faintly turbid bacterial suspension into 100 ml of nutrient broth in a 250 ml Erlenmayer flask and allowed to grow for 48 hours with frequent shaking of the contents. Wood blocks (50 x 10 x 70 mm) of each timber species were steam sterilized for 15 minutes at 100 kPa and after attaining the ambient room temperature, immersed for 10 seconds in the suspension of the potential antagonist. After draining the excess suspension the wood blocks were placed in sterile Petri dishes, lined with two moistened sterile filter papers for maintaining high humidity. Control blocks were dipped only in sterile water. Treated and control blocks were inoculated with an 8 mm diameter agar disc of actively growing test fungus. All the Petri dishes were incubated at 28 ± 2°C and observations on fungal growth over the wood blocks recorded after two weeks.

6.2.4 Field test

Based on the laboratory results, the most effective antagonist in controlling the sapstain and mould fungi was selected and further tested for its efficacy in field trials. The experiment was conducted in the sawn wood stacking yard of M/s. Evershine Packingcase Industries, Ollur, Trichur District. Rubber wood planks (150 x 10 x 300 mm size) prepared from freshly felled rubber trees were used for the experiment. The experiment was conducted in the month of August, as the environmental conditions such as relative humidity and temperature were conducive for fungal growth.

Liquid culture of the bacterial antagonist was employed in each treatment. Nutrient broth was chosen as the medium for preparing the bacterial growth. Twenty Erlenmayer flasks of 1 litre capacity each were used for the preparation

of bacterial broth. Each flask containing 500 ml of sterile nutrient broth was incubated with 10 ml of 1:10 dilution of a faintly turbid suspension using a sterile pipette and incubated for 15 days; the suspension was the same used in an earlier experiment. For each treatment, 10 litre broth was prepared. The flasks were periodically shaken to obtain uniform growth of the antagonistic organism. The wooden planks were treated with the bacterial broth in four different ways as follows. Each treatment contained 16 treated planks.

1. **Dipping and close stacking**: The 10 litre bacterial broth prepared for the treatment, was taken in a clean plastic tray of 200 x 10 x 400 mm size. Rubber wood planks (150 x 10 x 300 mm) were sawn and the saw dust was removed using a clean brush. Each of the rubber wood plank was dipped individually for 10 seconds in the bacterial broth and kept in a slanting position for 10 seconds to drain the excess solution. All the treated planks were stacked closely one above the other without leaving any space between them. Each stack comprised of four individual planks and for each treatment, there were four replicated stacks. Control planks were dipped in tap water and stacked in the same manner.

2. **Dipping and open stacking**: After dipping the rubber wood planks in the bacterial broth for 10 seconds and draining, they were stacked one above the other, but separated from each other by a reeper of 60 x 10 x 150 mm size in order to provide air space in between the stacked planks. The reepers for separating the individual planks were also prepared from freshly sawn rubber wood and dipped in the bacterial broth for 10 seconds.

3. **Spraying and close stacking**: The third treatment involved spraying of planks with bacterial broth. Individual wood planks

were uniformly sprayed on both sides with the bacterial broth using a fine sprayer. Controls were sprayed with ordinary tap water. Treated planks were then stacked closely without leaving any space between planks as done under Treatment 1. Control planks were sprayed with tap water and stacked closely.

4. **Spraying and open stacking**: After spraying on either side individually with the bacterial broth, wood planks were stacked one above the other, but separated from each other using a reeper as mentioned in Treatment 2. The reepers were also sprayed with the bacterial broth. Control planks were sprayed with water and also stacked in the same manner as in the case of treated ones. All the treated and control planks were stacked on the ground under a shed. The four sides of the shed were half open so that, the planks were protected from rain from the sides. Temperature and relative humidity were recorded daily at the experiment site. Observations on the efficacy of the treatment in terms of fungal growth were recorded fortnightly for a period of two months. Destructive sampling procedure was adopted for recording the fungal growth in the treated and control planks. During each observation, four sets of samples were selected randomly from each treatment. Mould growth on both the surfaces of each plank was observed and the stained area marked using a pencil on either side. Later, the mould growth marked was copied to a paper. After recording the mould growth, the planks were planed and assessed for the extent of internal stain according to the rating index given in Table 4-1. The area covered by the fungal growth as drawn on the paper was then determined using 'LICOR' leaf area meter. The data were subjected to logarithmic transformation and analysed using ANOVA.

6.3 RESULTS

6.3.1 Isolation of antagonistic microorganism

The number of fungal and bacterial colonies isolated from different timber species is given in Table 6-1. The maximum number of bacterial colonies isolated were obtained from *H. brasiliensis*. Five bacterial isolates, which were different in cultural characters, were isolated from *H. brasiliensis*. From *A. triphysa* and *A. scholaris* only four fungal and four bacterial cultures each were obtained. The fungal cultures isolated from *H. brasiliensis* were *Aspergillus* spp., *Ceratocystis* sp., *Fusarium oxysporum*, *Memnoniella echinata*, *Penicillium* spp., and *Trichoderma viride*. Two species of *Aspergillus*, one of *Penicillium* and *Trichoderma viride* were isolated from *A. triphysa*. The fungal cultures such as *Penicillium* sp., *Aspergillus* sp., *Alternaria alternata* and *Trichoderma viride* were isolated from *A. scholaris*. Since the fungal cultures isolated from different wood species were the common mould/stain fungi colonized on wood, they were discarded. The bacterial cultures isolated were subcultured on nutrient agar slants for further studies.

Table 6-1. Number of fungal/bacterial colonies isolated from the wood chips of different timber species

Species isolated	*H. brasiliensis*	*A. triphysa*	*A. scholaris*
Aspergillus sp.	2	2	1
Aternaria alternata	0	0	1
Ceratocystis sp.	1	0	0
Fusarium oxysporum	1	0	0
Memnoniella echinata	1	0	0
Penicillium sp.	2	1	1
Trichoderma viride	1	1	1
Bacteria	5	4	4

6.3.2 Testing of antagonistic activity

Antagonistic activity was tested only for bacterial isolates. Studies on the antagonistic activity of a total 13 bacterial colonies isolated from *A. triphysa, A. scholaris* and *H. brasiliensis* against the test fungi showed varying degree of antagonism. The data pertaining to the extent of clear zone of inhibition shown by different bacterial isolates against the test fungi are given in Table 6-2.

Table 6-2. Antagonistic activity of different bacterial isolates against various test fungi in agar culture method

Colony No.	Mould fungi				Stain fungi				
	Tv*	Af	An	Me	Bt	Cf	Ar	Sl	Fs
A. scholaris									
1	++	+	++	-	+	-	+	++	-
2	-	-	+	+	+	+	-	-	-
3	+	-	-	-	-	-	-	-	+
4	-	+	+	-	-	-	-	+	-
A. triphysa									
1	-	-	-	-	-	-	+	-	-
2	-	+	-	+	-	++	-	-	-
3	-	-	++	-	+	+	++	-	+
4	+	-	-	-	-	+	-	+	-
H. brasiliensis									
1	-	-	-	-	+	-	-	-	-
2	-	+	+	-	+	++	-	+	+
3	-	-	-	-	++	-	-	++	-
4	-	-	-	+	-	-	+	+	-
5	++	+	+	+++	+++	++	+++	+++	+++

+ Inhibition zone <10 mm wide
++ Inhibition zone >10 mm wide
+++ Inhibition zone >15 mm

* Tv = *Trichoderma viride*; Af = *Aspergillus flavus*;
An = *Aspergillus niger*; Me = *Memnoniella echinata*
Bt = *Botryodiplodia theobromae*; Cf = *Ceratocystis fimbriata*
Ar = *A. recefei*; Sl = *Scytalidium lignicola*; Fs = *Fusarium solani*

6.3.2 (i) Agar culture streak method

Among the 13 bacterial isolates, only colony-5 isolated from *H. brasiliensis* showed strong antagonism against all the test fungi and the inhibition zone was 15 mm wide. None of the bacterial colonies isolated from *A. triphysa* showed any inhibition zone more than 15 mm wide. All the bacterial colonies isolated from *A. scholaris* and *A. triphysa* were weak antagonists because the width of the inhibition zone was either less than 10 mm or nil. All the bacterial cultures which showed very little antagonism were discarded. Preliminary identification of the bacterium was attempted and for authentic identification, the bacterial culture was sent to the CAB International Mycological Institute, U.K. The isolate was identified as *Bacillus subtilis* (Ehrenberg) Cohn.

The antagonistic activity of *B. subtilis* was further tested against six common pathogenic fungi along with four mould and five stain fungi and the zone width of inhibition measured is presented in Table 6-3 (Fig. 6-1). Among the mould fungi, *M. echinata* showed 13 mm wide zone of inhibition and *B. theobromae*, the stain fungus, 18 mm wide zone of inhibition (Fig. 6-2). Among all the pathogens the maximum clear zone observed was in *Pestalotiopsis* sp., followed by *C. salmonicolor*, *R. solani* and *S. rolfsii*. Among all the fungi tested, only *Pythium* sp. did not show any inhibition against *B. subtilis*.

6.3.2 (ii) Culture filtrate

The potential antagonist, *B. subtilis* which had shown maximum inhibitory zone was further tested using its culture filtrate. It was noted that the culture filtrate obtained from *B. subtilis* did not show any antagonistic effect against the test fungi which had shown inhibition in agar culture. All the test fungi inoculated on either side of the well grew and covered the entire area of the Petri dish within four days.

Table 6-3. Inhibitory zone produced by *Bacillus subtilis* against various fungi

No.	Fungi tested	Width of inhibition zone (mm)
Mould Fungi		
1.	*Aspergillus flavus*	6
2.	*A. niger*	7
3.	*Memnoniella echinata*	13
4.	*Trichoderma viride*	9
Stain fungi		
1.	*Acremonium recefei*	10
2.	*Botryodiplodia theobromae*	18
3.	*Ceratocystis fimbriata*	5
4.	*Fusarium solani*	14
5.	*Scytalidium lignicola*	11
Pathogenic fungi		
1.	*Colletotrichum gloeosporioides*	10
2.	*Corticium salmonicolor*	14
3.	*Pestalotiopsis* sp.	17
4.	*Pythium* sp.	0
5.	*Rhizoctonia solani*	14
6.	*Sclerotium rolfsii*	9

6.3.3 Wood block test

After the incubation period of two weeks, the wood blocks of three timber species, *A. scholaris*, *A. triphysa* and *H. brasiliensis*, treated with the suspension of *B. subtilis*, were found to be free from growth of *B. theobromae* (Figs. 6-3, 6-4). The control blocks had thick mycelial growth of *B. theobromae*. On splitting the control blocks, it was found that the fungus had also caused black stain of internal tissues.

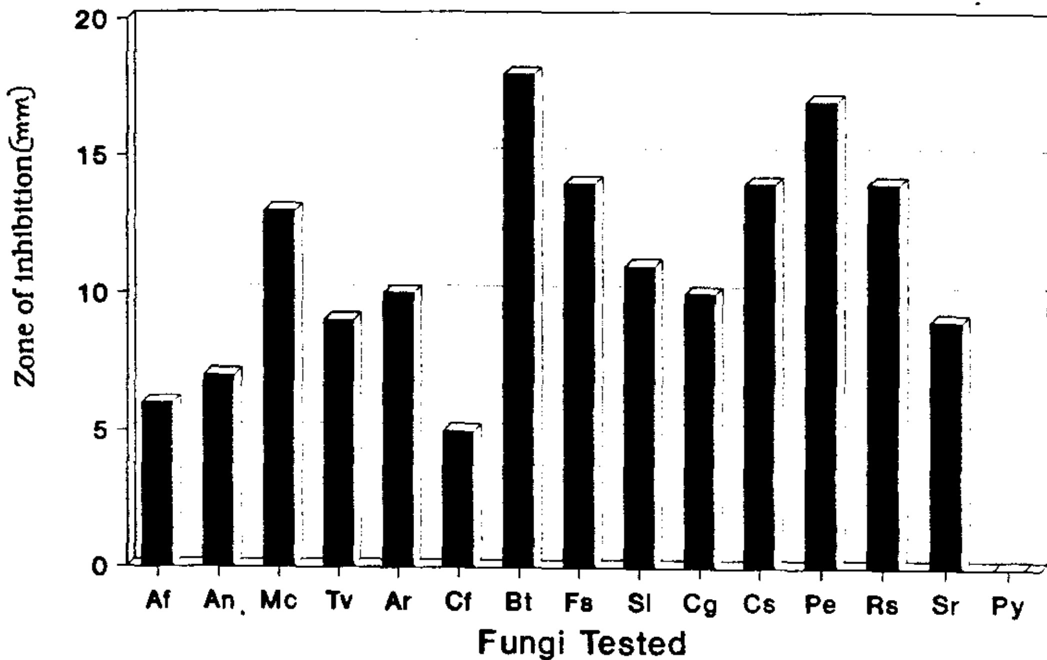


Fig. 6-1. Zone of inhibition produced by *Bacillus subtilis* against various fungi.

Af - *A. flavus*, An - *A. niger*, Mc - *M. echinata*, Tv - *T. viride*,
Ar - *A. recefei*, Cf - *C. fimbriata*, Bt - *B. theobromae*,
Fs - *F. solani*, Sl - *S. lignicola*, Cg - *C. gloeosporioides*,
Cs - *C. salmonicolor*, Ps - *Pestalotiopsis* sp., Rs - *R. solani*,
Sr - *S. rolfsii*, Py - *Pythium* sp.

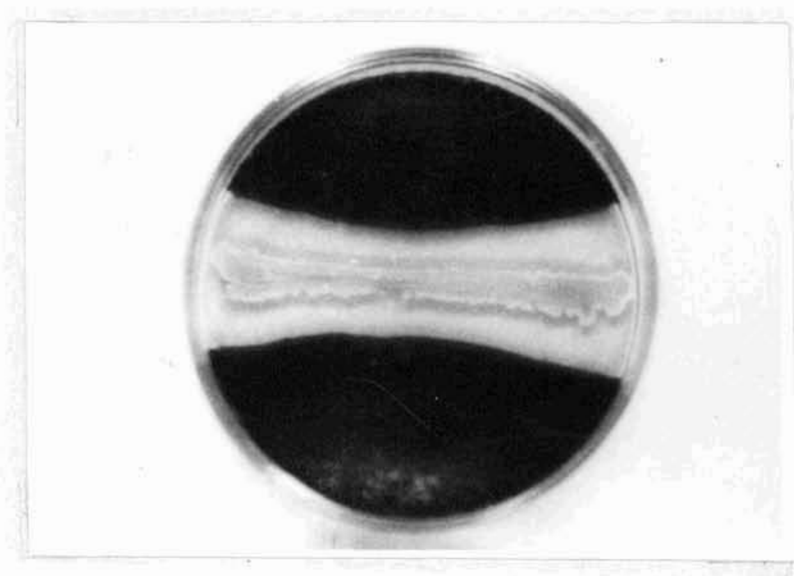


Fig. 6-2 The inhibition of growth of *Botryodiplodia theobromae* by *Bacillus subtilis* in agar culture streak method.

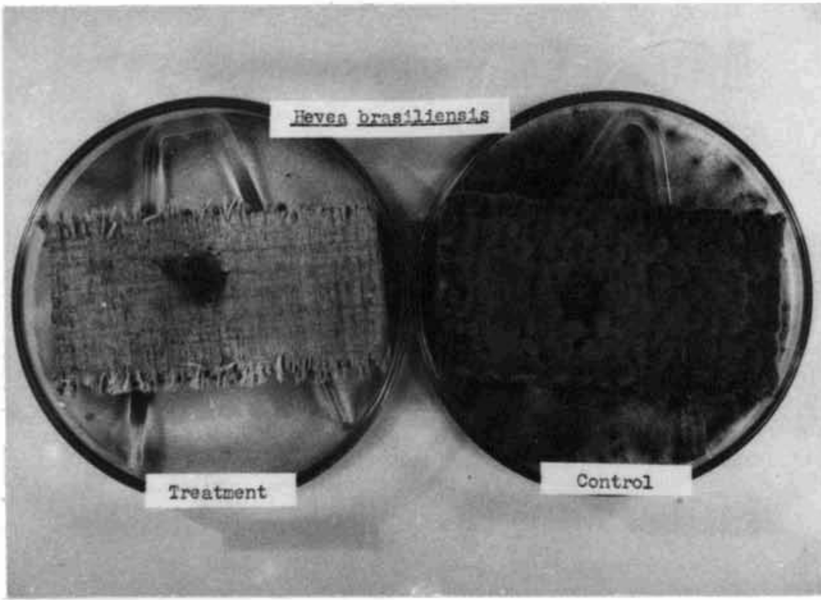


Fig. 6-3 Inhibition of growth of *Botryodiplodia theobromae* on wood blocks of *Hevea brasiliensis* by *Bacillus subtilis*; treated (left) and control (right).

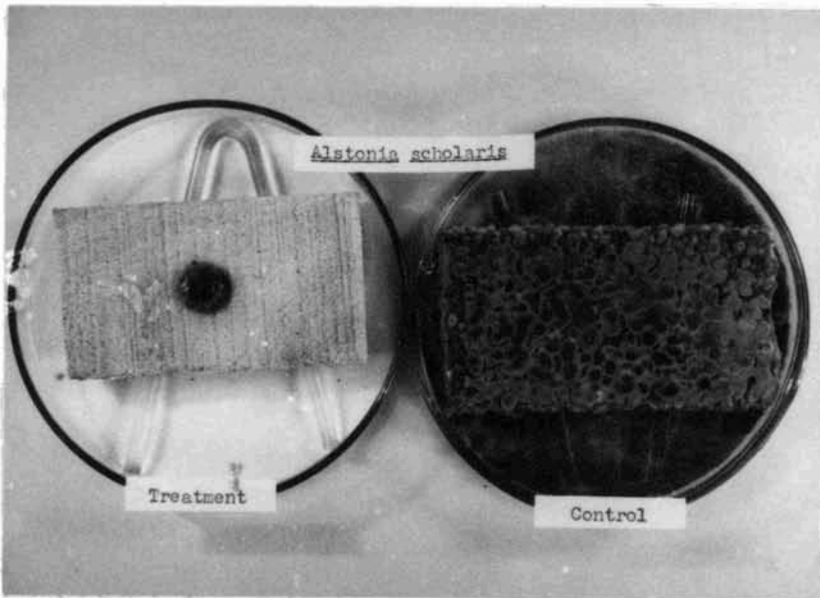


Fig. 6-4 Inhibition of growth of *Botryodiplodia theobromae* on *Alstonia scholaris* by *Bacillus subtilis*; treated (left) and control (right).

6.3.4 Field test

The efficacy of *B. subtilis* in controlling the growth of mould and stain fungi was evaluated in field experiment and the results are presented in Tables 6-4 and 6-5. During the incubation period, the temperature inside the storage shed varied between 25 ± 2°C and 28 ± 2°C, whereas the relative humidity ranged between 85 and 90 per cent. The range of temperature and RH encountered during the period was very conducive for the fungal growth.

1. **Dipping and close stacking (Treatment 1)**: The results presented in Tables 6-4 and 6-5 reveal that at the end of two weeks, the extent of sapstain in treated planks was less (17.61 per cent) whereas it was more (53.88 per cent) in control. At the end of four weeks, the infection percentage in the control planks increased considerably. Not much difference was noted in the infection percentage between the treated and control during the sixth week. However, at the end of the eighth week, the infection percentage in the treated planks (45.94 per cent) and control (74.04 per cent) was significantly different. The staining rate observed in the second week (0.88) gradually increased (2.0) in the incubation period. The maximum staining recorded for treatment planks was 2.0 whereas in control, it was 3.12.

2. **Dipping and open stacking (Treatment 2)**: It was observed that the infection in the treated planks gradually increased from the first observation (fourth week) to the last observation (eighth week) and not much difference in the inhibition percentage between treated and control planks was observed. In the treated planks, the staining rate gradually increased from 1.37 to 2.75. In control, severe staining was observed at the end of eighth week (3.75).

Table 6-4. Percentage of fungal growth on the surface of rubber wood planks under different treatments (Mean of 16 observations)

Duration (weeks)	Fungal growth (%)							
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	T*	C	T	C	T	C	T	C
2	17.61	53.88	20.84	37.26	10.64	33.92	15.14	35.90
4	17.39	86.41	27.66	24.56	19.33	39.97	14.48	22.09
6	50.96	53.89	39.32	44.21	19.77	62.41	19.83	34.45
8	45.94	74.04	53.29	52.83	16.19	62.98	25.47	21.16

* T = Treatment
C = Control

Table 6-5. Extent of fungal stain on rubber wood planks in different treatments (Mean of 16 observations)

Duration (weeks)	Fungal growth							
	Treatment 1		Treatment 2		Treatment 3		Treatment 4	
	T*	C	T	C	T	C	T	C
2	0.88	2.25	1.37	2.19	0.69	2.19	0.87	2.12
4	0.94	3.00	1.87	2.12	0.94	2.87	0.75	2.63
6	2.00	2.82	2.13	3.13	1.00	3.17	0.93	2.87
8	1.38	3.12	2.75	3.75	0.88	3.25	1.25	2.00

* For growth rate index see Table 4-1

T = Treatment; C = Control

3. **Spraying and close stacking (Treatment 3)**: Among all the treatments tried, this was found to be the best. The results presented in Tables 6-4 and 6-5 indicate that at the end of two weeks, the infection percentage in the treated planks was only 10.64; in control, it was 33.92 per cent. The percentage of infection in the treated planks increased gradually from 10.64 to 19.77. In the control planks, the infection percentage increased to 62.98 at the end of the experiment. Significant difference in staining between treatment and control was observed. After two weeks the staining rate was 0.69 in treated planks whereas in control it was 2.19. The maximum staining rate recorded was 1, but in control the staining score was 3.25.

4. **Spraying and open stacking (Treatment 4)**: From the results, it was observed that at the end of two weeks, the infection percentage of treated planks was 15.14 whereas in control it was 35.90. In the second and third observations, not much increase was noted in the infection percentage. But in the last observation (eighth week), the infection had increased to 25.47 per cent in the treated planks. In the control samples due to variation, infection was not consistent throughout the experiment. Inconsistency was also noted in the case of staining. Maximum staining was observed in the treated planks at the end of eighth week. In control, maximum staining was observed at the end of sixth week. However, significant difference between treatment and control was observed.

Results presented in Table 6-6 indicated that there were significant difference between the treatments. Treatment 3 was found to be significantly different from all the other treatments.

Table 6-6. Analysis of variance of data on inhibition of fungal growth on rubber wood planks tested with *B. subtilis* suspension

Sources	DF	2nd week			4th week			6th week			8th week		
		SS	MSS	F	SS	MSS	F	SS	MSS	F	SS	MSS	F
Between treatments	7	5.8987	0.8427	4.56**	7.5846	1.0835	9.85**	4.1754	0.5965	6.48**	4.7670	0.6810	10.60**
Error	117	21.5998	0.1846	-	12.4331	0.1100	-	10.7647	0.920	-	7.2632	0.0643	-
Total	124	27.4985	-	-	20.0176	-	-	14.9396	-	-	12.0302	-	-

** Significant at P = 0.01%

6.4 DISCUSSION

Excessive use of chemicals for protection or prevention of wood from the attack of biological organisms has become a health hazard and their far reaching consequences may be known only after sometime.

The antagonism observed between interacting microbes colonizing the exposed wood and the industrial exploitation of such antagonists as biological control agents against wood destroying organisms is an alternative to toxic preservative chemicals. The screening of antagonistic microorganisms to find out a potential microbe which can prevent/control the moulds and sapstain infection in wood can be very critical as its sensitivity will depend upon the testing methods and the test organisms used.

Among the several microorganisms colonizing wood, only one bacterium namely *B. subtilis* showed antagonistic activity. *B. subtilis*, a spore forming bacterium, surviving in air and soil is well documented for its production of antibiotics (Banerjee *et al.*, 1967; Katz and Demain, 1977; Korzybski *et al.*, 1978). There are several previous reports of the anti-fungal activity of *B. subtilis* and its use in controlling a number of plant pathogens (Aldrich and Baker 1970; Baker *et al.*, 1983; Dunleavy, 1955; Singh and Deverall, 1984; Swinburne and Brown, 1976). *B. subtilis* has also proved to be a promising biological control agent against mould and sapstain fungi as it shows broad spectrum antagonistic activity against several fungi. *Bacillus* spp. have also been reported as promising biocontrol agents of wood deteriorating fungi (Greaves, 1970; 1972) and mould and sapstain fungi (Bernier *et al.*, 1986; Seifert *et al.*, 1987; Kreber and Morrell, 1993).

The difference in the antagonism observed in the agar culture method and culture filtrate assay indicated that the degree of antagonistic effect varied based on the method used. The pronounced inhibitory effect of *B. subtilis* detected in

agar culture method may be due to the secretion of antibiotic substances and nutritional competition between the two microbes in culture (Johnson and Curl, 1972). Hsu and Lockwood (1969) also suggested that the appearance of the zone of inhibition in agar culture is due to nutrient deprivation of the test organism. The failure of the antagonists to show inhibitory effect in culture filtrate may be due to very little or no secretion of the antibiotic substance by the organism. Banerjee *et al.* (1967) reported that enrichment of media will enhance the production of antibiotic, mycobacillin by *B. subtilis*. In the present culture filtrate assay, the medium used for the bacterial growth was nutrient broth. Perhaps, nutrient broth may not be an ideal medium for the production of antibiotic. This may also be the reason for the failure of antagonism in culture filtrate. It is understood that the antagonism expressed in the present experiment may be with or without antibiotic production as reported by Johnson and Curl (1972).

In the laboratory test, the fungus *B. theobromae* failed to grow on rubber wood blocks dipped in bacterial suspension. It is presumed that the presence of living bacteria in the suspension only inhibited the growth of the fungus on wood blocks. Results of the present study are also in agreement with earlier reports on the efficacy of *B. subtilis* controlling sapstain and mould on various timbers in the laboratory. Bernier *et al.* (1986) and Seifert *et al.* (1987) reported the control of mould and stain fungi on pine blocks by dipping in *B. subtilis* suspension. By soaking spruce blocks in bacterial suspension of *B. subtilis* for 24 hours and inoculating with fungal cultures, Bernier *et al.* (1986) could attain good inhibition of fungal growth in wood blocks. It is understood that the bacterial cells present in the wood blocks inhibited the fungal growth on wood blocks.

In the field experiment, treatment 3 was found to be the best among all the treatments. Only 10.6-19.87 per cent infection was observed in the case of

surface fungal growth and the rate of stain caused by fungi was maximum. The bacterium could inhibit the fungal growth on treated planks to some extent when compared to control. However, 100 per cent control was not obtained. This may be due to insufficient quantity of bacterial cells on the suspension to inhibit the fungal growth.

In all the four different treatments, infection percentage was less than that of control. Inhibition of mould and stain fungi on rubber wood treated with bacterium varies with different treatments. Among all the treatments, treatment 3 was found to be the best. In this treatment, the surface fungal growth of the treated planks for the first observation was 10.64 per cent which increased up to 19.77 per cent at the end and the rate of stain produced by different fungi was more than 1 per cent. In a similar study conducted by Seifert *et al.* (1987) *B. subtilis* was found to be antagonistic to different staining fungi. The stain of *B. subtilis* (C186) evaluated by them produced antibiotics that were fungistatic to many fungi growing on the surface of pine. Even though *B. subtilis* C186 could control the stain fungi on pine blocks in the laboratory, staining was not sufficiently prevented in field tests. Bernier *et al.* (1986) also showed that an isolate of *B. subtilis* inhibited the growth of three sapwood- inhibiting fungi on agar and prevented colonization of spruce blocks on agar plates.

The reason for the inhibition of fungal growth in treatment 3 is possibly due to the presence of enough living bacterial cells on the surface of planks. Some degree of inhibition noted in all the treatments when compared to control confirms the fact that the control of stain and mould fungi in the treated planks are possibly due to the presence of bacterial cells on the planks.

In the present experiment, spraying the planks was found to give more protection than dipping. This may be due to the fact that planks absorb more moisture while dipping and this higher moisture content accumulated in the wood will help to enhance the growth of the fungus.

Though the bacterium was highly effective in controlling the fungal growth in the laboratory, due to uncontrolled micro- climatic conditions, the same results cannot be expected in field studies. Nevertheless, the bacterium holds promise in biological control for sapstain of rubber wood. However, further experimentation is necessary before *B. subtilis* is commercially viable as a biocontrol agent.

CHAPTER 7

CHEMICAL ANALYSIS OF SAPSTAINED RUBBER WOOD

7.1 INTRODUCTION

The important chemical constituents of wood are cellulose, hemicellulose and lignin of which the major component constituting approximately one half of the wood substance is cellulose (Browning, 1967). Cellulose is always accompanied by other polysaccharides commonly called as hemicellulose. Lignin is another important component of wood cell wall. Wood may also contain extraneous compounds such as extractives which are not considered as essential part of the cell wall.

Even though considerable progress has been made in the past few decades towards understanding the chemistry of decayed wood (Savory and Pinion, 1958; Kirk, 1971; 1973; 1975; Kirk and Highley, 1973; Highley, 1975; 1977; 1978), the chemistry of sapstained wood is yet to be studied in detail. Umezurike (1969) conducted some studies to find out whether chemical changes had occurred in the wood of *Bombax buonopozense* due to infection by *Botryodiplodia theobromae*. Tabirih and Seehann (1984) also studied the changes in the chemical structure of Abachi wood (*Triplochiton scleroxylon*) stained by *B. theobromae*. Since, in India no studies have been reported on this aspect, an attempt was made to study the chemical changes occurring in rubber wood stained by *B. theobromae*.

7.2 MATERIALS AND METHODS

Rubber trees, about 35-year-old, were selected from a rubber plantation located near Trichur. The butt log of the tree was used for preparing blocks of 20 x 20 x 80 mm size. After debarking, wood from the outermost 25 mm of the log and pith region were discarded. Forty defect-free wood blocks were selected randomly for the study and steam sterilized for 15 minutes at 100 kPa. After cooling, 20 blocks were inoculated with a mycelial disc of 8 mm diameter punched from the edge of an actively growing 7-day-old culture of *B. theobromae*. The remaining 20 blocks were inoculated with plain agar disc to serve as control. The individual blocks were suspended over water in bottles using stainless steel wire as described in pint jar method (Chapter 4, 4.2.2) to maintain humidity for fungal growth over the wood blocks. The inoculated and control blocks were kept in room temperature (28 $\pm$ 2°C) for 16 weeks. At the end of every four weeks, five control and five treated blocks were taken out, scraped off the external mycelia and then air-dried. The wood samples were powdered in a Wiley Mill and sieved using a No. 40 (425 μm) sieve. The estimation of each chemical component of wood powder was made on a dry weight basis correcting for the moisture content of the air-dried samples before and after extraction. This was obtained by oven-drying a small weighed portion of the sample at 105°C for 12 hours. The results obtained were expressed as percentage of the original oven dry weight of the powder.

7.2.1 Alcohol-benzene solubility

The alcohol-benzene solubility of stained wood was estimated according to ASTM D 1107-56. Five grams of the sieved wood powder was placed in soxhlet extraction apparatus having a tared soxhlet extraction flask. A small cone of fine-mesh wire screen was placed on top of the crucible to prevent loss of wood powder. The alcohol-benzene solution was prepared by mixing 33 ml of approximately 95 per cent ethyl alcohol with 67 ml of benzene. The wood powder

was extracted with 150 ml of alcohol-benzene solution for 6-8 hours, keeping the liquid boiling briskly using a heating mantle. After evaporating the solvent from the extraction flask, the contents along with the flask were dried in an oven for 1 hour at 105°C. The flask and the contents were cooled in a desiccator and weighed until the weight became constant. By substracting the weight of the flask alone, the amount of alcohol-benzene soluble matter in the wood powder was obtained. The percentage of alcohol-benzene soluble matter in the moisture free wood was calculated as follows.

$$\text{Percentage of alcohol-benzene} = \frac{\textit{Total weight of extract}}{\textit{soluble matter Oven-dry weight of sample}} \times 100$$

Data on percentage of alcohol-benzene extractives present in the stained and control rubber wood for a period of four months were analysed statistically.

7.2.2 Estimation of holocellulose

The holocellulose content in stained and control wood was estimated using the sodium chlorite method (Browning, 1967). Two grams of alcohol-benzene extracted sample was taken in a 250 ml beaker and 100 ml of 0.7 per cent sodium chlorite solution was added to the beaker. The solution was stirred well and three drops of glacial acetic acid were added so as to maintain pH below 4.5. The beaker was placed in a steam bath which was adjusted to a temperature of 70-80°C. The solution was intermittantly stirred with a glass rod. After one hour, the clear supernatant solution was filtered through a previously weighed G2 crucible. Then with the help of 100 ml of 0.7per cent sodium chlorite solution, the residue in the crucible was transferred to the beaker again and three drops of glacial acetic acid were added. The above procedure was repeated four or five times, till all the lignin was removed and the sample became pure white in colour. Finally, the residue was washed with water in order to make the sample free from sodium chlorite and then washed with alcohol and ether. The crucible with

the content was dried in a vacuum oven at 60°C and weighed. The weight of the dry residue was corrected for its ash content. This powder was used for determination of ash percentage in the holocellulose. The content of holocellulose (corrected for ash) in the moisture-free wood was calculated as follows.

$$\text{Percentage of holocellulose in wood} = \frac{A(100-Z)}{X(100-Y)} \times 100$$

where A = weight of holocellulose; Z = percentage of ash in the holocellulose; Y = percentage of moisture in the wood powder and X = wood powder weighed in grams.

Data on percentage of holocellulose present in stained and control rubber wood in each period was analysed statistically.

7.2.3 Estimation of lignin

The estimation of lignin content was carried out according to ASTM D 1106-56. About one gram of the alcohol-benzene extracted wood powder was taken in a 250 ml beaker and 15 ml of cold (12-15°C) 72 per cent sulphuric acid was added slowly with stirring. The sample was mixed well with sulphuric acid by stirring constantly for at least one minute. The mixture was allowed to stand for 2 hours with frequent stirring at a temperature of 18- 20°C. The mixture was then diluted to 3 per cent acid concentration with 360 ml of distilled water and boiled for 4 hours under a reflux condenser. The insoluble material was allowed to settle and filtered using a filtering crucible (G3) that had been dried at 105°C and weighed. The residue was washed with 500 ml of hot water in order to free the acid and then the crucible and contents were dried in an oven for 2 hours at 105°C. The crucible was cooled in a desiccator and the contents of the crucible weighed. The weighing procedure was repeated until the weight became constant. The ash correction of the lignin was done by transferring the lignin to

a tared platinum crucible and ashed by igniting at 900°C. The lignin content based on the weight of moisture-free wood was calculated as follows.

$$\text{Weight of ash corrected lignin} = \frac{\textit{Weight of lignin (100–ash \%)}}{100}$$

$$\text{Percentage of ash corrected lignin} = \frac{\textit{Weight of ash corrected lignin}}{\textit{Weight of oven–dry sample}} \times 100$$

Data on percentage of lignin present in stained and control wood for a period of four months were analysed statistically.

7.2.4 Estimation of ash

The estimation of ash content was carried out according to ASTM D 1102-56. The initial weight of the crucible was found by igniting the empty crucible in a muffle furnace at 600°C and cooling in a desiccator and then weighing to the nearest 0.1 mg. Two grams of the test wood powder which had been previously passed through No. 40 sieve were taken in the crucible and kept in the oven at 105°C to find out the initial dry weight of the wood powder. The crucible with the powder was cooled using a desiccator and the initial weight determined. The drying and weighing process was repeated till the weight became constant. The crucible with the content was placed in the muffle furnace adjusted to 580 to 600°C. The heating was started slowly to avoid flaming of the sample and continued until all the carbon was eliminated. After heating, the crucible was carefully removed and kept in a desiccator for cooling and final weight determined. The heating was repeated for 30-minute periods until the weight after cooling became constant. The percentage of ash based on the weight of the moisture free wood was calculated as follows.

$$\text{Percentage of ash} = \frac{W1}{W2} \times 100$$

where W1 = weight of ash and W2 = weight of oven dry sample.

Data on percentage of ash present in stained and control rubber wood for each period were analysed statistically.

7.2.5 Determination of carbohydrates

The anthrone method of Sadasivam and Manikam (1992) was followed to estimate the total carbohydrates present in the wood. A weighed portion (100 mg) of alcohol-benzene extracted wood powder was hydrolyzed with 5 ml of 2.5 N hydrochloric acid by keeping in a boiling water bath for 3 hours, then cooled to room temperature and neutralized with sodium carbonate. The solution was then made up to 100 ml and centrifuged at 5000 rpm. The supernatant solution was collected and 0.5 ml and 1 ml aliquots were taken in tubes for the analysis. The standards (glucose) were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard; '0' served as blank. The volume of standards was made up to 1 ml in all the tubes including the sample tubes by adding distilled water. Four millilitres of anthrone reagent was added in all the tubes and heated for 8 minutes in a boiling water bath. The solution was cooled rapidly and read the green to dark green colour at 630 nm using spectrophotometer. The standard graph was prepared by plotting concentration of the standard on the X-axis and absorbance on the Y-axis. From the graph, the amount of carbohydrates present in the sample tube was calculated as follows.

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \frac{glucose\ (mg)}{volume\ of\ the\ test\ sample\ (ml)} \times 100$$

Data on the percentage of carbohydrate present in stained and control rubber wood in each period was analysed statistically.

7.3 RESULTS

7.3.1 Alcohol-benzene solubility

In general, the amount of extractable substances dissolved in alcohol-benzene mixture did not differ significantly between sapstained and normal rubber wood (Table 7-1). At the end of one month, extractives present in the stained wood (mean = 3.62) were significantly higher ($P = 0.01$) than the normal wood (mean = 2.87). However, no significant difference in the amount of extractives was noted between stained and control wood at the end of second, third and fourth months.

7.3.2 Estimation of holocellulose

The amount of holocellulose present in the sapstained wood was almost same as that in unstained wood. At the end of first month the holocellulose content was 69.37 per cent (mean), which remained almost the same at the end of the second month. There was a little reduction in the holocellulose content at the end of third month, whereas at the end of fourth month, it was equal to the control. Statistical analysis showed no significant difference in the holocellulose content between treated and control wood samples (Table 7-2).

7.3.3 Estimation of lignin

The amount of lignin present in sapstained and normal wood was compared for four months and it was found that there was no difference in lignin content between stained and normal wood. Statistical analysis also showed no significant difference in the percentage of lignin between treated and normal wood (Table 7-3).

7.3.4 Estimation of ash

The ash content of normal and stained wood was compared. The results revealed that no considerable change occurred in ash content between the stained and normal wood (Table 7-4). Statistical analysis (t-test) also supported this result.

Table 7-1. Comparison of alcohol-benzene extractives present in stained and control rubber wood for a period of four months

	1st month			2nd month			3rd month			4th month		
	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t- value	Mean	SD	t-value
Treated	3.62	0.30		3.23	0.95		3.18	0.52		3.05	0.48	
			3.83**			0.84(ns)			1.28(ns)			0.79(ns)
Control	2.87	0.15		2.87	0.17		2.87	0.17		2.87	0.17	

** Significant at P = 0.01%
ns Non significant

Table 7-2. Comparison of percentage of holocellulose present in stained and control rubber wood for a period of four months

	1st month			2nd month			3rd month			4th month		
	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value
Treated	69.37	3.25		69.35	3.71		66.49	2.73		68.34	3.80	
			0.56(ns)			0.49(ns)			1.35(ns)			0.07(ns)
Control	68.46	1.65		68.46	1.65		68.46	1.65		68.46	1.65	

ns Non significant

Table 7-3. Comparison of percentage of lignin present in stained and control rubber wood for a period of four months

	1st month			2nd month			3rd month			4th month		
	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value
Treated	29.81	8.08		23.78	2.40		33.14	8.38		35.77	11.70	
			0.55(ns)			2.09(ns)			0.05(ns)			0.44(ns)
Control	32.85	9.38		32.85	9.38		32.85	9.38		32.87	9.38	

ns Non significant

Table 7-4. Comparison of percentage of ash present in stained and control rubber wood for a period of four months

	1st month			2nd month			3rd month			4th month		
	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value
Treated	2.36	0.65		1.58	0.50		1.46	0.50		2.04	0.85	
			1.36(ns)			0.95(ns)			1.29(ns)			0.38(ns)
Control	1.88	0.47		1.88	0.47		1.88	0.47		1.88	0.47	

ns Non significant

7.3.5 Determination of carbohydrates

Table 7-5 shows the amount of total carbohydrates present in the sapstained and normal wood. It was found that at the end of the first month, there was a reduction in the amount of total carbohydrates in the sapstained wood samples when compared to control. But at the end of second and third months, there was not much difference in the amount of carbohydrates present in control as well as in stained wood. However, at the end of fourth month, the amount of carbohydrates present in the sapstained wood was considerably lower than the normal unstained wood (Fig. 7-1). Statistical analysis revealed that there was significant difference in the amount of total carbohydrates present in sapstained and normal wood at the end of first month and fourth month.

7.4 DISCUSSION

On comparing the extractives of normal and stained rubber wood, it was observed that the quantity of extractable substances was more in stained wood than in inoculated wood. However, statistical analysis showed significance only at the end of first month. The slight increase in the quantity of extractives in stained wood at the end of first month may be due to the chemical substances produced during the growth period of stain fungus *B. theobromae* on rubber wood blocks. Gao *et al.* (1994) measured the growth rate of *Ophiostoma piceae* in lodgepole pine by ergosterol and found that the growth rate increased steadily during the first 2 weeks of incubation and then became stable. He corresponded this to the exponential growth phase. In this study the reason for the increase of extractives in the first month can be attributed to the exponential growth of the fungus. Further, the extractives produced by the mycelium might have also added along with the wood extractives. The present study also confirms the results obtained by Umezurike (1969) on *Bombax buonopozense* stained by *B. theobromae*. He found that the ethanol-soluble material increased during the first 14 days; later it was reduced.

Table 7-5. Comparison of percentage of carbohydrates present in stained and control rubber wood for a period of four months

	1st month			2nd month			3rd month			4th month		
	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value	Mean	SD	t-value
Treated	10.12	0.02		12.56	0.13		10.48	0.04		9.96	0.01	
			4.43**			0.56(ns)			2.70(ns)			5.96**
Control	15.38	0.02		15.38	0.02		15.38	0.02		15.38	0.02	

** Significant at P = 0.01%
ns Non significant

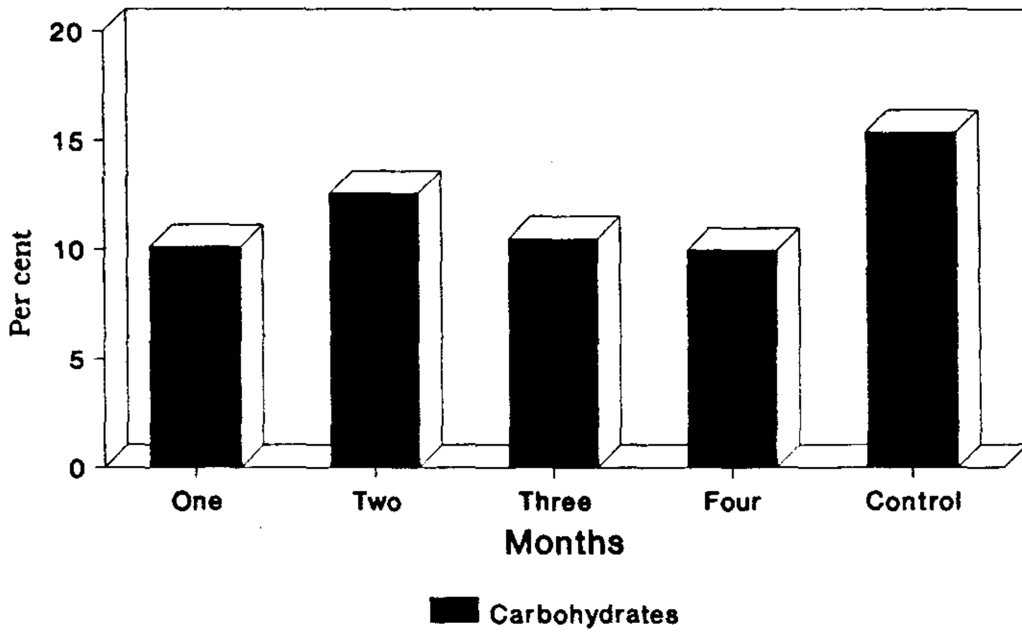


Fig. 7-1. The percentage of carbohydrate present in stained and control wood samples of *Hevea brasiliensis* for a period of four months.

The amount of holocellulose in the sapstained wood was almost same as that in normal wood. It is clear that there is no utilization of holocellulose in the rubber wood by *B. theobromae* during the period of 4 months. Studies on sapstain caused by *B. theobromae* on other wood species also confirm this aspect. Umezurike (1969) studied the cellulolyte activities of *B. theobromae* and found that the fungus used starch and other saccharides present in the wood of *B. buonopozense* as initial substrates and when the starch was depleted, the fungus attacked cellulose. It utilized cellulose in a manner similar to soft rot fungi. He also found a slow decline in the percentage of hemicellulose and cellulose than starch. On the contrary, in the present study, it was found that the holocellulose was not utilized by *B. theobromae*. This may be due to the presence of high amount of carbohydrates in rubber wood (Kadir and Sudin, 1989) which serve as the main source of food for the luxuriant growth of the fungus. Tabirih and Seehann (1984) also reported that on *Triplochiton scleroxylon*, the stain fungus *B. theobromae* did not utilize cellulose and lignin, but the hemicellulose was consumed only in insignificant amounts. These findings along with the results from the present study indicate that holocellulose is not the main constituent of wood preferred by the stain fungus *B. theobromae*. It is presumed that if the wood is in the infected condition for long time, there is every chance of utilization of hemicellulose or cellulose from the wood tissues as food material.

Lignin, one of the basic cell wall materials, was not utilized by the stain fungus *B. theobromae*, as it is evident from the results. There is no significant difference in the amount of lignin utilized between stained and normal rubber wood. The results of the present study are also in agreement with the general statement that lignin and cellulose are not utilized by the stain fungus. Few earlier works on this aspect also support this generally held view. Umezurike (1969) reported that *B. theobromae* did not degrade lignin component of the wood whereas it used starch and other saccharides present in the wood. Tabirih and Seehann (1984) also reported the inability of *B. theobromae* to utilize lignin component of wood.

Significant difference between stained and normal wood was observed in the utilization of total carbohydrates by *B. theobromae* at the end of first and fourth months. The fungus probably utilized more carbohydrates in the first month because of the exponential growth (Gao *et al.*, 1994). The results of the present study also confirm the finding of earlier workers. According to Ballard *et al.* (1982), sapstaining fungi are reported to be primary or initial wood colonizers. They invade and assimilate the easily available carbon and nitrogen nutrients present in ray parenchyma cells. Parenchyma cells are the only living tissues in the xylem of trees, and ray parenchyma is a tissue involved with food storage and radial translocation of sugars and water. *B. theobromae* is known to produce a variety of amylolytic, cellulolytic and pectinolytic enzymes (Umezurike, 1969 and 1978). This may explain why *B. theobromae* is a common pathogen of various sugar and starch-rich substances such as fruits and tubers and also it is found on wood (Encinas and Daniel, 1994). Rubber wood is also easily infected by *B. theobromae* since it contains high amount of carbohydrates (Kadir and Sudin, 1989). At the end of fourth month also, carbohydrates was reduced significantly. It is presumed that for a period of four months, the fungal mycelium inside the wood might have utilized most of the carbohydrates present in the wood. Umezurike (1969) while studying the cellulolytic activities of *B. theobromae* observed that the starch content of *B. buonopozense* decreased markedly during the first 14 days and after 25 days it had fallen to a constant level of 3 per cent dry weight. Tabirih and Seehann (1984) also indicated that the soluble sugars in *T. scleroxylon* wood were almost consumed after 16 weeks of fungal infection. From these studies, it is clear that *B. theobromae* utilizes more carbohydrates than cellulose and lignin.

CHAPTER 8

STRENGTH AND ANATOMICAL PROPERTIES OF SAPSTAINED RUBBER WOOD

8.1 INTRODUCTION

Strength refers to the ability of a material to resist external forces tending to change its size and alter its shape. The quality of a timber is normally decided based upon its strength properties. The different strength properties of wood are compressive strength, bending strength, tensile strength, hardness, etc. The most important single factor influencing the strength of timber is its density, but physical factors like knots, slope of grain and environmental factors like moisture content and temperature also play a significant role in determining the strength of wood.

Kerala accounts for a major share of rubber plantations in India over the past decade. Due to scarcity of conventional timbers, rubber wood has become the main source of industrial timber in Kerala. Although rubber wood is locally used for making packingcases, plywood, furniture, match veneers, etc., it has remained under-utilized mainly due to its poor durability and lack of sufficient information on its strength properties. Another important factor which limits the wider utilization of rubber wood is fungal and insect attack. Due to conducive climatic condition of Kerala, the sapstain and mould are the serious problem in the utilization of rubber wood. The study of the strength and anatomical properties of stained rubber wood is important as the wood is to be utilized properly for various structural purposes. Detailed study on this aspect have been carried out and the results are presented in this chapter.

8.2 MATERIALS AND METHODS

Rubber trees of 30 year-old were selected in a rubber plantation located near Trichur. One metre long billet above the ground level of the tree was cut for making wood blocks. After debarking, two planks of 200 x 10 x 1000 mm size were sawn for preparing wood blocks. Wood blocks of 10 x 10 x 40 mm size were prepared and stored in room temperature after air-drying. All the sample blocks were numbered. The numbering was done in such a way that two adjacent blocks were given the same number and one of them was used for treatment and other served as control.

8.2.1 Density

To determine the initial density, 160 air-dried wood blocks of size 10 x 10 x 40 mm were selected randomly. The weight of all the 160 blocks corrected to 0.001 g was taken using a top pan electronic balance. The density of 160 wood blocks was determined by water displacement method as described in The Indian Standard IS:1708-1982. A beaker of water was placed on the balance and the weight recorded. A test block suspended by a needle clamped on a stand, was lowered into the beaker and immersed completely in water and the weight recorded. The weight difference in grams was equal to the volume of the test block in cubic centimeters. The block was removed from the needle and dried in an oven kept at 103 ± 2°C to find out the oven dry weight. For the inoculation of the stain fungus, all the 160 oven-dry wood samples were kept in a moist chamber to bring them back to normal moisture equilibrium. Following steam sterilization at 100 kPa for 15 minutes, 80 blocks were inoculated aseptically with 8 mm diameter mycelial disc taken from the edge of 7-day-old actively growing culture of *B. theobromae*. The wood blocks were inoculated in test tubes as described earlier in Chapter 4 (4.2.2). The end-matched 80 wood blocks were served as control by inoculating them individually with plain agar disc. All the 160 wood blocks were incubated at 28 ± 2°C for 4 months. At the end of each

month, 20 treated and 20 control blocks were removed and final density was determined by water displacement method. The density was calculated as follows.

$$\text{Density} = \frac{\text{Oven-dry weight of the block}}{\text{Volume of the block in air-dry condition}}$$

Data on weight loss, initial density and final density of stained and control rubber wood blocks were compared statistically using t-test.

8.2.2 Compressive strength

After measuring the density, the samples were tested for compressive strength. Compression parallel to grain tests were carried out in a ZWICK Universal testing machine. The test was done as per the Indian Standard IS: 1708-1982. Data on compressive strength of stained and control wood blocks were subjected to statistical test.

8.2.3 Static bending strength

Wood blocks of 20 x 5 x 90 mm size were prepared from the same billet from which the blocks were also made for testing the compressive strength and air-dried. Fifty clear defect free blocks were selected and initial density estimated by water displacement method. The samples were dried at 103 ± 2°C and oven-dry weight determined. All the wood blocks were then kept in a moist chamber until the blocks gained the moisture equilibrium. Following steam sterilization, 25 blocks were inoculated at the centre with 8 mm diameter disc taken from a 7-day-old actively growing culture of *B. theobromae*. Control blocks inoculated with plain agar disc, were also maintained. Each wood block was placed individually in tubes of length 200 mm and 30 mm diameter as described in Chapter 4 (4.2.5) and incubated for 3 months at 28 ± 2°C. At the end of third month all the treated and control samples were taken out from the tubes. With

the help of a scalpel blade, the mycelial growth over the treated wood blocks was carefully removed without damaging wood tissues. The mass of mycelium was carefully transferred to a filter paper and the filter paper along with the mycelium dried in an oven set at 100°C for 2 days and the dry-weight of the mycelium recorded. The treated and control samples were air-dried and final density determined. All the wood blocks were dried at 103 ± 2°C and final oven-dry weight recorded to compare the weight loss of wood blocks due to the infection by *B. theobromae*. Static bending tests of the 50 samples were carried out in a ZWICK Universal testing machine. Data on static bending strength, initial density, final density, weight loss and mycelial weight of stained and control rubber wood blocks were analysed statistically.

8.2.4 Anatomy of rubber wood stained by *Botryodiplodia theobromae*

Anatomical changes occurred in the wood due to the infection by *B. theobromae* were studied in sapstained rubber wood blocks. Three clear, defect-free fresh wood blocks of size (20 x 5 x 90 mm) were prepared for the inoculation study. Following steam sterilization for 15 minutes, and after cooling in room temperature, the wood blocks were inoculated with 8 mm diameter disc of actively growing culture of *B. theobromae* and incubated in test tubes for 3 months. After the incubation period, the stained wood blocks were taken out from the tubes and the fungal mycelium growing over the wood blocks was removed. A small piece of 125 mm^3 was cut from the stained block and softened by boiling in water for one hour. About 15 to 20 µm thick transverse and longitudinal sections were taken on a sliding microtome. The sections were double stained using safranine and aniline blue. Permanent sections were made by mounting in Canada balsam and examined under Lietz Dialux microscope and photomicrographs were taken to examine the mode of infection of *B. theobromae* inside the wood tissues.

8.3 RESULTS

8.3.1 Density

When density of stained and unstained rubber wood blocks were compared at the end of first and fourth month it was observed that the treated samples had shown reduction in density. Statistical analysis (t-test) of the data showed significant difference between the density of treated and control blocks at the end of the first and fourth months (Table 8-1).

On a comparative study of the weight loss of the stained and normal wood it was observed that the weight loss in the first month was 9.83 per cent which increased up to 11.64 per cent at the end of the fourth month. Statistical analysis showed significant difference in weight loss between the treated and control samples at the end of first and fourth months. Comparison of density of the stained wood blocks also showed significant difference between first and fourth months (Table 8-2).

8.3.2 Compressive strength

No significant difference in compressive strength of stained and control samples was observed (Table 8-1). On comparing the compressive strength of wood blocks at the end of first and fourth months also, no reduction in compressive strength due to the attack of *B. theobromae* on rubber wood was observed.

8.3.3 Static bending strength

On comparison of static bending strength of control and stained rubber wood blocks, at the end of third month, it was observed that there was significant difference in bending strength between the treated and control samples. In the samples tested for bending strength, the final density of stained wood blocks was significantly different from the control wood blocks (Table 8-3).

Table 8-1. Comparison of weight loss, density and compressive strength between stained and control rubber wood blocks for first month and fourth month (Mean of 20 observations)

	1st month			4th month		
	Treated	Control	t-value	Treated	Control	t-value
Weight loss (%)	9.83	1.67	19.66**	11.64	2.26	44.34**
Initial density (Kg/m^3)	0.600	0.640	1.71(ns)	0.640	0.610	1.29(ns)
Final density (Kg/m^3)	0.540	0.620	9.04**	0.570	0.620	8.97**
Compressive strength (N/mm^2)	35.84	35.01	0.50(ns)	35.39	39.92	1.74(ns)

** Significant at P = 0.01%
ns Non significant

The weight loss recorded for the stained wood samples was 15.27 per cent, whereas in control samples it was 0.21 per cent (Table 8-4). It was also observed that weight of the mycelium which covered the wood block highly influenced the weight loss of the stained rubber wood samples. If the mycelial weight on the wood block was more, the weight loss recorded for that wood block was also more. On statistical analysis of the data, it was observed that there was strong correlation between the weight loss and mycelial weight ($r = 0.693$, $P = 0.01$).

Table 8-2. Comparison of density, weight loss and compressive strength of stained rubber wood between first month and fourth month (Mean of 20 observations)

	Stained wood blocks		
	1st month	4th month	t-value
Weight loss (%)	9.83	11.64	4.24**
Initial density (Kg/m^3)	0.600	0.640	1.56(ns)
Final density (Kg/m^3)	0.540	0.570	3.27**
Compressive strength (N/mm^3)	35.84	35.39	0.23(ns)

** Significant at P = 0.01%
ns Non significant

Table 8-3. Comparison of initial density, final density and bending strength between stained and control rubber wood blocks at the end of third month (Mean of 25 observations)

	Mean ± SE		
	Treated	Control	t-value
Initial density (Kg/m^3)	0.580 ± 0.003	0.580 ± 0.003	1.0(ns)
Final density (Kg/m^3)	0.540 ± 0.005	0.640 ± 0.007	11.93**
Bending strength (Kn/mm^2)	82.44 ± 3.01	95.11 ± 2.56	4.72**

** Significant at P = 0.01%

Table 8-4. Comparison of weight loss and mycelial weight of stained and control rubber wood blocks at the end of 3rd month (Mean of 25 observations)

Parameters	Mean ± SE	
	Treated	Control
Weight loss (%)	15.27 ± 0.330	0 ± 0
Mycelial weight (g)	0.21 ± 0.014	0 ± 0

8.3.4 Anatomy of rubber wood stained by *Botryodiplodia theobromae*

The surface of the stained wood appeared as bluish-black in colour. Microscopic observation revealed thick weft of highly branched mycelium in the parenchymatic tissues Fig. 8-1. Hyphal colonization was more in parenchyma cells in the wood rays or longitudinal parenchyma. The entry of mycelium (11.5-14.0 μm diameter) from one cell to another was through pits. Pit perforations were frequently associated with bulging of the hyphae at the tip (Fig. 8-2). The vessels were the first path of penetration. Fibres which lacked nutrients were not attacked and used for initial establishment of infection for spreading from one tissue to another. Observations showed that hyphae often crossed the parenchyma cells in a straight line (Fig. 8-3), constricting themselves to pass from one parenchyma to another through minute bore holes (Fig. 8-4), enlarging again when emerging out in the next lumen; and so on until reaching the parenchymatic area.

8.4 DISCUSSION

In the literature not much information is available on the changes occurred in strength properties of wood due to sapstain infection. The knowledge of strength properties of stained wood is essential for utilizing it for structural purposes. The results indicate significant difference in the final density of control and stained rubber wood samples at the end of first and fourth months incubation. Results also make clear that the growth of the stain fungus *B. theobromae* inside the wood is responsible for the changes inside the wood tissues. From the chemical analysis of stained wood (Chapter 7) it was apparent that although the fungus was not able to utilize any cellulose or lignin of the cell, it utilizes the carbohydrates. It is presumed that the utilization of high amount of carbohydrates present in the parenchyma tissues might have caused the reduction in density. Tabirih and Seehann (1984) have also found slight

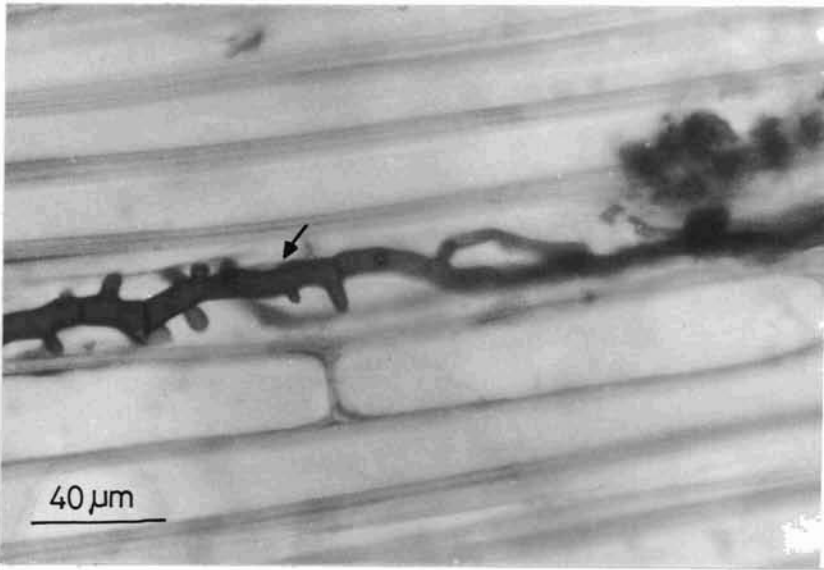


Fig. 8-1 Hyphae of *Botryodiplodia theobromae* growing in parenchyma cells.

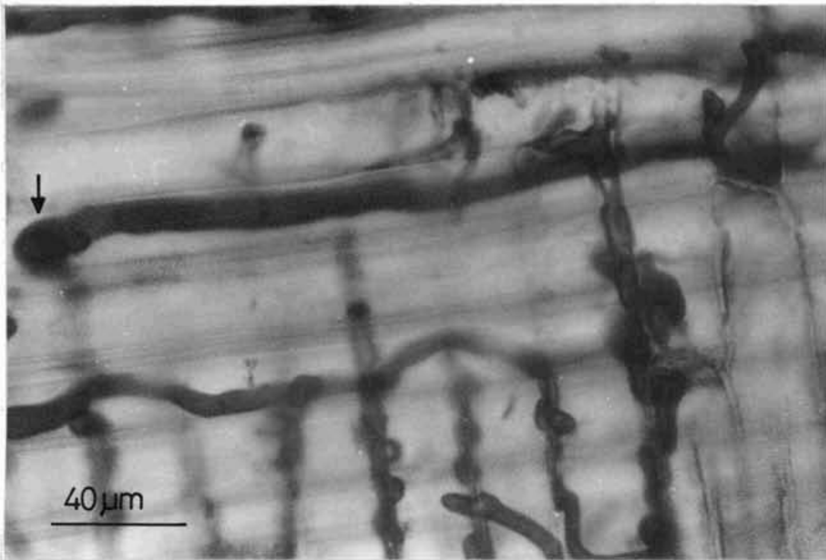


Fig. 8-2 Hyphae of *Botryodiplodia theobromae*. Note the bulging of hyphal tip.

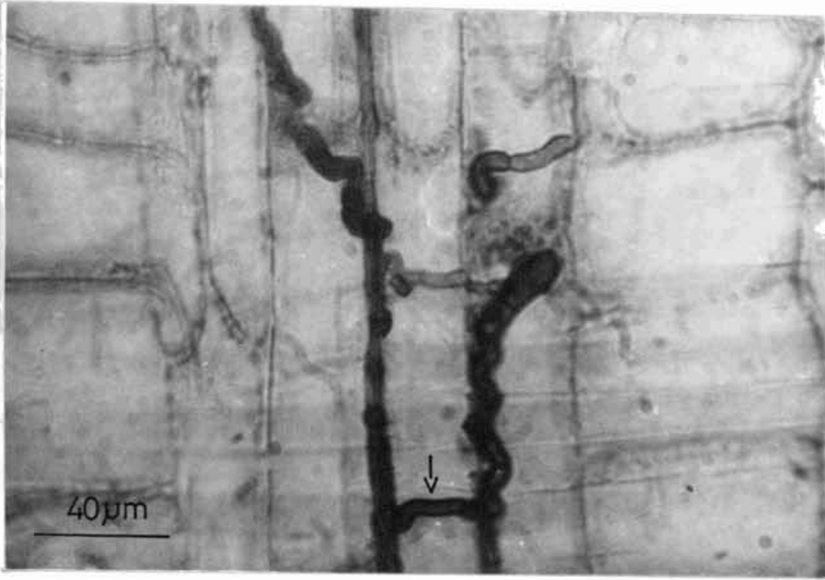


Fig. 8-3 Hyphae of *Botryodiplodia theobromae* crossing the ray cells in a straight line.

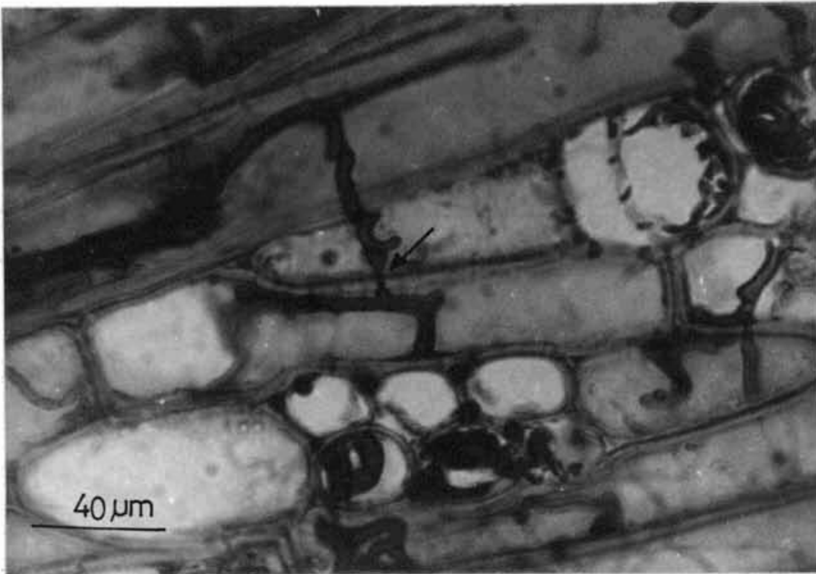


Fig. 8-4 Hyphae of *Botryodiplodia theobromae* passing from one parenchyma to another. Note the constriction of hyphae while crossing the cell wall.

reduction in density of *T. scleroxylon* wood stained by *B. theobromae* due to consumption of accessory compounds, especially starch in the parenchymatous tissue, by the fungus. Not much work is there on this aspect to support this finding. Umezurike (1978) documented that the blue stain fungus, *B. theobromae*, is capable of degrading some components of wood particularly after utilizing the starch and soluble carbohydrates. He also reported that the pattern of invasion of wood blocks by *B. theobromae* was similar to those of soft rot fungi by forming chains of cavities in the S_2 layer in the secondary cell wall of wood. Encinas and Daniel (1995; 1996) also reported the degradation of cell walls in birch, pine, aspen and rubber wood by *B. theobromae*. In the present experiment, it is not certain that the reduction in density is due to the attack of S_2 layer of the cell wall by *B. theobromae*.

It is evident from the results that there is no reduction in compressive strength due to infection of rubber wood by *B. theobromae*. This finding is in agreement with the work done earlier by Findlay and Pettifor (1939). They concluded that compressive strength of stained wood is never seriously affected, but the toughness of heavily stained softwood may be significantly lower than that of normal wood. Tabirih and Seehann (1984) also found no significant effect by fungus, *B. theobromae* on compression strength of Abachi and beech wood.

Static bending strength is reduced in the stained wood sample when compared to the unstained wood. Both density and weight loss are also reduced in the stained wood. Possibly the fungus utilizes carbohydrates present in tissues for its growth in the wood causing a reduction in weight as well as in density as it is also evident from the chemical analysis. This correlates well with the extent of mycelial growth over the rubber wood blocks. When the fungal mycelial weight is more, the reduction in weight of the wood block is also more. The reduction in both the weight and density may result in the reduction of bending strength of rubber wood. In 1939, Findlay and Pettifor also found that in heavily stained

soft tropical hardwoods of low density, toughness was reduced by 30-40 per cent and reduction in bending strength was by 20 per cent. Chapman and Scheffer (1940) also reported that although all strength properties of stained wood were reduced, only toughness was affected to a level of practical importance. A decrease in the resistance of static and dynamic bending of stained poplar wood caused by *B. theobromae* has also been observed by Pinheiro (1971). From the results of the present study and other reports cited it is very evident that the staining fungus can cause significant changes in the bending strength of wood.

The anatomical studies make it very clear that *B. theobromae* grows mainly in the parenchyma tissues which are the store house of carbohydrates. The reduction in the amount of carbohydrates in the stained wood, as it is observed in the chemical analysis strongly supports the fact that the fungus colonizes mainly in parenchyma cells.

The hyphae of *B. theobromae* spread from one cell to another through the pits. Tabirih and Seehann (1984) also observed that the entry of hyphae from one cell to another is through pits in the case of Abachi and beech wood stained by *B. theobromae*. Pit perforation has also been observed by Hong (1976); Olofinboba (1974) and Pinheiro (1971). But Findlay (1959) and Umezurike (1978) have also reported additional penetration by *B. theobromae* through the lignified cell walls including the thick walls of fibres. Possibly this is the result of great physiological variability shown by *B. theobromae* on different hosts.

The results of the present study indicate that the stain fungus, *B. theobromae* can cause reduction in weight, density and bending strength, but no reduction in compressive strength of rubber wood. The stained wood can be utilized for purposes for which density and bending strength are not of much importance.

SUMMARY

SUMMARY

Microorganisms cause various types of damages such as decay, soft rot, mould, stain and bacterial degradation on timber. Of these, mould and stain are common which affect the quality of timber from the aesthetic point of view. The discolouration imparted by fungal mycelium and fructifications growing on the surface of the timber is referred to as mould. Stain is caused either by the growth of fungi having coloured hyphae or by the production of coloured pigments like melanin in the hyphae walls. Since they normally affect only the sapwood, the stain is commonly known as sapstain.

In Kerala, the small-scale timber industry requires timber from miscellaneous tree species for the manufacture of packingcases, plywood, match splints and veneers. With the fast depletion of natural forest resources it has become necessary to depend on other alternate timber sources to meet the current needs of timber. Rubber wood, *Hevea brasiliensis* (Hbk.) Muell. Arg., with several positive attributes, merits consideration as an alternate timber. But the growth of mould and sapstain fungi aggravated by the tropical warm-humid climate of Kerala State is posing serious problem to the utilization of rubber wood and various other soft wood species.

COLLECTION OF STAINED WOOD SAMPLES

A preliminary visit was undertaken to different wood-based industries and eight economically important timber species namely, *Ailanthus triphysa*, *Alstonia scholaris*, *Anacardium occidentale*, *Bombax ceiba*, *Erythrina stricta*, *Hevea brasiliensis*, *Macaranga peltata* and *Mangifera indica* were selected for the study.

Stained wood samples of the selected eight timber species were collected at monthly intervals for a period of one year from 15 industries in Trichur District, to isolate the microorganisms causing sapstain. Wood samples were also collected from other miscellaneous timber species which were available during the collection period. To ascertain the spectrum of stain causing organisms associated with different timbers in Kerala, one-time collection of stained wood samples were also made from different districts of the State namely Cannanore (*H. brasiliensis, A. triphysa*), Calicut (*H. brasiliensis, M. peltata, A. occidentale, B. ceiba*), Malappuram (*H. brasiliensis, A. scholaris, A. occidentale, B. ceiba, M. indica*), Palghat (*A. scholaris, E. stricta, M. peltata, M. indica*), Ernakulam (*H. brasiliensis, A. scholaris, A. triphysa, M. indica, A. occidentale*), Kottayam (*H. brasiliensis, A. scholaris, M. indica, B. ceiba, E. stricta*), Alleppey (*H. brasiliensis, A. scholaris, A. occidentale, M. indica, B. ceiba, M. peltata*), Quilon (*H. brasiliensis, M. indica, A. occidentale, B. ceiba, E. stricta*) and Trivandrum (*B. ceiba, H. brasiliensis, A. occidentale, E. stricta, M. indica, A. scholaris*).

ISOLATION OF STAIN FUNGI

For isolating the fungal/bacterial organisms associated with sapstain, a small speck (2 mm^2) of wood, cut from freshly sawn wood plank having stained area, was surface sterilized with 0.1 per cent $HgCl_2$ solution, washed thoroughly in several changes of sterile distilled water and plated on potato dextrose agar (PDA) medium. For isolating mould, the fungal spores growing on the surface of wood were removed aseptically with sterile forceps and placed on PDA medium.

The fungus growing around the wood sample in the PDA medium was isolated, purified and maintained in PDA slants for further studies. For bacterial isolates nutrient agar was used as the culturing medium. After isolating the stain causing organisms in pure culture, identification was attempted up to generic level, based on cultural and morphological characteristics.

Aspergillus spp., *Penicillium* spp. and *Trichoderma viride* were the common moulds found to be growing on the surface, imparting stains on the timbers of *Ailanthus triphysa, Alstonia scholaris, Anacardium occidentale, Bombax ceiba, Erythrina stricta, Hevea brasiliensis, Macaranga peltata* and *Mangifera indica.*

The common stain causing fungi isolated from different timber species were *Acremonium strictum, Botryodiplodia theobromae, Ceratocystis fimbriata, Fusarium moniliformae, F. roseum F. solani, Geotrichum* sp., and *Scytalidium lignicola*,. Among these *Botryodiplodia theobromae* was the dominant one causing bluish- black discolouration on the surface as well as inside the wood. The most severely and frequently affected timber was *Hevea brasiliensis.* The sapstain infection was observed to be prevalent year-round.

Artificial inoculation trials of fungal isolates to confirm their ability to cause stain

The ability of the fungal isolates from various timbers to stain healthy wood was confirmed through artificial inoculation trials. For inoculation, unstained wood blocks (50 x 10 x 70 mm) were cut from freshly harvested timber and steam sterilized for 15 minutes at 100 kPa. An agar disc of 8 mm diameter taken from the edge of an actively growing culture of the different test fungi was placed over the surface of each block. The inoculated blocks were then placed inside sterile Petri dishes and incubated at 28 ± 2°C for 15 days. Each isolate was tested on five replicate blocks; controls were also maintained with plain agar disc. After the incubation period, the inoculated blocks were examined for the growth of fungi, first on the surface and then in the interior by splitting them open. In the artificial inoculation trial, the test fungus grew and spread over the entire block within five days. After 15 days the whole block was covered with fungal mycelium. Surfaces of test blocks of different timbers were found to be stained in different colours. In the case of *B. theobromae*, the dominant stain fungus,

the surface of entire wood block was turned into black colour. The fungus imparted a bluish-black colour inside the wood.

Effect of humidity, temperature and moisture content of rubber wood on colonization of *Botryodiplodia theobromae*

Factors such as humidity (RH), temperature (T) and moisture content of timber (MC) affecting the growth and colonization of *B. theobromae* on rubber wood was studied. The effect of varying moisture contents of wood such as approximately 50, 75, and 100 per cent and three different temperatures namely 20°C, 30°C and 40°C and five different relative humidity regimes such as 60, 70, 80, 90 and 100 per cent, which possibly govern the growth and spread of *B. theobromae* in the wood was studied. The results indicated that profuse growth of the fungus, *B. theobromae*, was favoured by high relative humidity (90% and 100%). Temperature above 30°C decreased the rate of fungal growth even though the wood contained 100 per cent moisture content. At 40°C, if the RH was low in the range of 60-80 per cent the growth of the fungus was considerably reduced, even though little growth was noted at cent percent RH and 100 per cent of moisture content of timber. It was also noted that the fungal growth was not influenced much by the moisture content of wood alone. Relative humidity was the most important factor which governed the favourable growth of *B. theobromae* than the other factors. Treatment combinations (T, MC, RH respectively), such as 20°C T, 100 per cent MC, 100 per cent RH; 30°C T, 75 per cent MC, 100 per cent RH and 30°C, 100 per cent MC, 100 per cent RH were found to be significantly different.

Effect of moisture content of rubber wood on the growth of *Botryodiplodia theobromae*

Generally, sapstain fungi are known to grow only when the timber is in green condition. An experiment was conducted using rubber wood blocks with 10 different moisture contents to determine the optimum moisture content

required for the maximum growth of *B. theobromae*. Three hundred wood blocks (70 x 50 x 10 mm), prepared from freshly sawn rubber wood, were kept in an incubator (maintained at 35°C) to reduce the moisture content gradually. Thirty sample blocks were removed each day for 10 consecutive days from the incubator and steam sterilized at 100 kPa for 15 minutes. In order to find out the moisture content, the initial weight and oven dry weight at 103 ± 2°C was recorded for 10 sterilized blocks. Of the remaining 20 blocks, ten were inoculated with a 8 mm diameter mycelial disc of an actively growing culture of *B. theobromae* and the rest with plain agar disc to serve as control. The moisture content of the inoculated blocks was determined by calculating their corresponding oven dry weight. The inoculated and the control blocks were then placed over glass rod supports in sterile Petri dishes and incubated at 28 ± 2°C for 15 days. After the incubation period, the growth of the fungus on the surface of the inoculated blocks was assessed.

Heavy growth of the fungus was observed above 29 per cent MC content. At 27 per cent MC, medium growth of *B. theobromae* was noted, whereas at 25 per cent only traces of fungal growth were recorded; below 25 per cent, the fungal growth was considerably reduced. Results clearly indicated that a moisture level of 29 per cent or more is required for the successful growth and development of sapstain fungus *B. theobromae* on rubber wood. Decrease in moisture content retarded the growth of the fungus . If the moisture content was reduced to less than 24 per cent, the colonization of wood by sapstain fungi can be minimized.

Optimum temperature requirement for the growth of *Botryodiplodia theobromae*

Maximum temperature which can be tolerated by *B. theobromae* for its active growth was studied by assessing its growth on potato dextrose agar medium as well as on wood blocks. *B. theobromae* survived in the wood even at a high temperature of 60°C provided the fungus was well established inside the wood

blocks. For the initiation of growth, the fungus required an optimum temperature of 30°C and at higher temperature ranges the establishment was very poor. At 30°C. the fungus grew well on wood blocks and Petri dish within 48 hours. But no fungal growth was observed at higher temperatures of 50 and 60°C. The knowledge of temperature tolerance of *B. theobromae* is of practical importance as it will be helpful in adjusting the temperature for drying and processing the timber in order to save wood from sapstain infection.

Evaluation of chemicals for the control of sapstain and mould fungi

The efficacy of some of the commonly available fungicides namely, carbendazim, carboxin, copper oxychloride, mancozeb, thiram and ziram, was tested at 0.25, 0.50, 0.75 and 1 per cent (a.i.) against various sapstain fungi in the laboratory. The efficacy of fungicides against numerous fungal organisms, was tested in Petri dishes using poisoned-food technique as well as on sterile rubber wood blocks. Among the fungicides/chemicals tested, sodium azide and carbendazim proved to be the best in controlling sapstain fungi.

Biological control of stain fungi

In order to minimize the hazards of environmental pollution, studies were conducted to find out a suitable biological control organism for preventing sapstain and mould growth. A bacterium namely, *Bacillus subtilis*, isolated from rubber wood was found to be highly antagonistic to all the stain fungi tested in the laboratory on agar media and wood blocks. The bacterium was found to be effective in controlling the growth of sapstain and mould fungi on PDA medium as well as on wood blocks. Based on the laboratory results, efficacy of bacterium was tested in controlling sapstain fungi at one of the wood industries at Ollur, Trichur District using rubber wood planks. Wood planks (150 x 10 x 300 mm size) from freshly felled rubber trees were used in different treatments. For each

treatment 10 litres of bacterial suspension was prepared by inoculating equal quantity of the bacterium in all the 20 flasks containing 500 ml PDA broth; the culture was incubated for 15 days. The treatments were: 1. dipping and close stacking, 2. dipping and open stacking, 3. spraying and close stacking and 4. spraying and open stacking. Observations on fungal growth were recorded fortnightly for a period of two months. At each observation four sets of four planks each from both the treatment and control samples were taken randomly and fungal growth on either side of the planks was measured. The area covered by fungal growth as marked on the paper was then determined using a 'LICOR' leaf area meter. After planing the planks the sapstain fungal growth was assessed using the rating index given in Table 4-1. The data obtained were subjected to analysis of variance. About 80-85 per cent inhibition of sapstain fungal growth was recorded when the bacterium was tested in the field on fresh rubber wood planks.

Effect of *Botryodiplodia theobromae* on the physical and strength properties of rubber wood

To ascertain the effect of *B. theobromae* on the physical properties of rubber wood, weight loss in rubber wood blocks due to infection and growth of *B. theobromae* for a period of four months was compared with that of *Ailanthus triphysa* and *Alstonia scholaris* which are less susceptible to sapstain infection.

It was found that the sapstain fungus *B. theobromae* also caused weight loss. The estimated weight loss in rubber wood was initially 8 per cent which increased up to 12.2 per cent by the end of the fourth month. The weight loss recorded in rubber wood was ascribed to the possible consumption of carbohydrates stored in the living cells of wood by the sapstain fungus.

CHEMICAL ANALYSIS OF SAPSTAINED RUBBER WOOD

Chemical properties such as alcohol-benzene extractives, lignin and holocellulose percentage, ash content and amount of carbohydrate present in the cells were estimated according to ASTM standards and compared with that of unstained wood. No significant change in the percentage of alcohol-benzene extractives, lignin, holocellulose and ash content was observed in stained wood samples when compared to unstained wood samples. There was significant reduction in the carbohydrate percentage of stained wood samples.

Strength and anatomical properties of rubber wood stained by *B. theobromae*

Density of rubber wood stained by *B. theobromae* and normal wood was determined using water displacement method as described in the Indian standard IS:1708-1982 and compared. It was found that the density of control and stained rubber wood samples at the end of first and fourth months was found to be significantly different. It is understood that the growth of stain fungus is causing changes inside the wood tissues. From the chemical analysis of stained wood it was clear that the fungus was not able to utilize any cellulose or lignin of the cell. But, the carbohydrates in the wood were utilized by the fungus and reduction in carbohydrate percentage was noted at the end of first and fourth months. The reduction in density was also noted at the end of first and fourth months. It is presumed that the utilization of high amount of carbohydrates present in the parenchyma cells might have caused the reduction in density.

From the anatomical studies, it was clear that, *B. theobromae* mainly grows on the parenchyma cells since they are the store house of carbohydrates. The infected wood appears to be black, as the colour of the mycelium is bluish- black. The reduction in the amount of carbohydrates in the stained wood, as it is

observed in the chemical analysis, supports the fact that the fungus colonizes mainly on parenchyma cells.

Compressive strength and static bending strength of control and stained wood was determined using 'SWICK' universal testing machine and compared. No reduction in the compressive strength of stained wood was noted whereas the bending strength was reduced when compared with that of control wood.

From this study, it may be concluded that sapstain caused by fungi is a serious problem affecting the quality of selected commercially important timbers of Kerala especially the rubber wood. Among the various microorganisms, *B. theobromae* is the major one causing sapstain in all the selected timber species. Microclimatic factors such as relative humidity, temperature and moisture content of wood highly influence the sapstain infection by *B. theobromae*. If the moisture content is reduced to less than 24 per cent, rubber wood can be protected from sapstain infection. Sapstain by *B. theobromae* causes a weight loss of 12.2 per cent in rubber wood. Among the various fungicides tested, sodium azide which was found effective in controlling sapstain in the laboratory appears to be a promising chemical worthy of testing in the field. The antagonistic potential of the bacterium, *Bacillus subtilis*, against sapstain fungi can be exploited for controlling the sapstain infection in the field rather than using hazardous poisonous chemicals. Even though there was reduction in the percentage of carbohydrate, other chemical constituents such as cellulose and lignin of the stained wood was not utilized by the sapstain fungus, *B. theobromae* infection. The results of the present study indicate that *B. theobromae* can cause reduction in weight, density and bending strength of wood; but no reduction in compressive strength. The stained wood can be utilized for purposes for which density and bending strength are not of much importance.

LITERATURE CITED

LITERATURE CITED

Aldrich, J. and Baker, R. 1970. Biological Control of *Fusarium roseum* f. sp. *dianthi* by *Bacillus subtilis*. *Plant. Dis. Reptr.* **54**: 446-448.

Ananthanarayanan, S. 1971. Investigations on the cause of discolouration of some rosewood (*Dalbergia latifolia*) veneers. *J. Ind. Acad. Wood Sci.* **2**(1): 27-31.

Baker, K.F. and Cook, R.J. 1974. *Biological Control of Plant Pathogens*. W.H. Freeman and Co. (First Indian Reprint 1979). S. Chand and Company Ltd., New Delhi. 433 pp.

Baker, C.J., Stavely, J.R., Thomas, C.A., Sasser, M. and MacFall, J.S. 1983. Inhibitory effect of *Bacillus subtilis* on *Uromyces phaseoli* and on development of rust pustules on bean leaves. *Phytopathology* **73**: 1148-1152.

Bakshi, B.K. 1951. Studies on four species of *Ceratocystis* with a discussion on fungi causing sapstain in Britain. *Mycological paper*, No. **35** Commonwealth Mycological Institute, U.K.

Bakshi, B.K. 1953. Decay and sapstain of timber, their causes and prevention. *Indian For.* **79**(2): 108-114.

Bakshi, B.K. 1988. Microbial Degradation of Wood. Pages 176-190. in: *Hand Book on Indian Wood and Wood Panels*, Oxford University Press, New Delhi. 313 pp.

Balasundaran, M. and Gnanaharan, R. 1990. Laboratory evaluation of preservative treated rubber wood against fungi. *J. Trop. For. Science* **2**(4): 303-306.

Ballard, R.G., Walsh, M.A. and Cole, W.E. 1982. Blue stain fungi in xylem of lodgepole pine: a light microscope study on extent of hyphal distribution. *Can. J. Bot.* **60**: 2334-2341.

Banerjee, A.B., Majumdar, S.K. and Bose, S.K. 1967. Mycobacillin. Pages 271-275. in: *Antibiotics Biosynthesis* Vol. II Gottilib, D. and Shaw, P.D. (eds.) Springer Verlag, Berlin. 466 pp.

Behrendt, C.J., Blanchette, R.A. and Farrell, R.L. 1995. Biological control of blue stain fungi in wood. *Phytopathology* **85**: 92-97.

*Bellmann, H. and Francke-Grosmann, H. 1952. Versuche Zur Transkfahigkeit verblauten Kiefernsplintholzes. Holz Roh. u. Werkstoff **10**: 456-463.

Benko, R. 1983. The Study of blue stain found in coniferous timber in SR Slovenia (Yugoslavia). *The International Research Group on Wood Preservation Document* No. IRG/WP/1177.

Benko, R. 1987. Antagonistic effect of some mycorrhiza fungi as biological control of blue stain. *The International Research Group on Wood Preservation Document* No. IRG/WP/1314.

Benko, R. 1988(a). Bacteria as possible organisms for biological control of blue stain. *The International Research Group on Wood Preservation Document* No. IRG/WP/1339.

---

* Original not referred.

Benko, R. 1989. Biological control of blue stain on wood with *Pseudomonas cepacia* 6253 laboratory and field test. *The International Research Group on Wood Preservation Document* No. IRG/WP/1380.

Benko, R. and Henningsson, B. 1986. Mycoparasitism by some white rot fungi on blue stain fungi in culture. *The International Research Group on Wood Preservation Document* No. IRG/WP/1304.

Benko, R. and Highley, T.L. 1990. Selection of media for screening interaction of wood attacking fungi and antagonistic bacteria. I. Interaction on agar. *Material u. Organismen* **25**: 161 - 171.

Bernier, R., Desrochers, M. and Jurasek, L. 1986. Antagonistic effect between *Bacillus subtilis* and wood staining fungi. *J. Inst. Wood Sci.* **10**: 214-216.

Bjurman, J. 1989. Effect of humidity fluctuations on mould growth on pine wood. *The International Research Group on Wood Preservation Document* No. IRG/WP/1412.

Breuil, C., Yagodnik, C. and Abraham, L. 1995. Staining fungi growing in softwood produce proteinase and aminopeptidases *Material u. Organismen* **29**(1): 15-25.

Brown, F.L. 1953. Mercury-tolerant *Penicillia* causing discolouration in northern white pine timber. *J. For. Prod. Res. Soc.* **3**: 67-69.

Browne, F.G. and Laurie, M.V. 1968. *Pests and diseases of Forest Plantation trees.* Clarendon Press, Oxford. 1330 pp.

Browning, B.L. 1967. *Methods of wood chemistry* Vol.II. Interscience publishers, John Wiley & Sons, Inc. New York, London. 882 pp.

Bruce, A., Austin, A. and King, W.J. 1984. Control of growth of *Lentinus lepideus* by volatiles from *Trichoderma. Trans. Br. mycol. Soc.* **82:** 423-428.

Bruce, A. and Highley, T.L. 1989. Effect of precolonization of blocks with common pole resident fungi on subsequent biological control of *Lepideus* by *Trichoderma* spp. *International Research Group on Wood Preservation Document* No. IRG/WP/1387.

Bruce, A. and King, B. 1983. Biological control of wood decay by *Lentinus lepideus* (Fr.) Produced by *Scytalidium* and *Trichoderma* residues. *Material u. Organismen* **18:** 171-181.

Bruce, A., King, B. and Highley, T.L. 1989. Decay resistance of wood removed from poles treated with *Trichoderma. International Research Group on Wood Preservation Document* No. IRG/WP/1386.

Butcher, J.A. 1973. Laboratory screening trials for new prophylactic chemicals against sapstain and decay in sawn timber. *Material u. Organismen* **8:** 51-70.

Butcher, J.A. 1980. Commercial anti-sapstain chemicals in New Zealand. *International Research Group on Wood Preservation Document* No. IRG/WP/3142.

Butcher, J.A. and Drysdale, 1974. Field trials with captafol an acceptable anti-sapstain chemical *For. Prod. J.* **24:** 28-30.

Butcher, J.A. and Drysdale, J. 1978. Laboratory screening trials with chemicals for protection of sawn timber against mould, sapstain and decay. *International Biodeterior. Bull.* **14:** 11-19.

Cartwright, K.St.G. and Findlay, W.P.K. 1958. *Decay of Timber and its Prevention*. Chemical Publishing Co., HMSO, London. pp. 282-320.

Cassens, D.L. and Eslyn, W.E. 1981. Fungicides to prevent sapstain and mold on hardwood lumber. *For. Prod. J.* **31**(9): 39- 42.

Cech, M.Y. 1966. New treatment to prevent brown stain in white pine. *For. Prod. J.* **16** (11): 23-37.

Chapman, A.D. and Scheffer, T.C. 1940. Effect of blue stain on specific gravity and strength of southern pine. *J. Agric. Res.* **61**: 125-134.

Chidester, M.S. 1936. A pink stain of wood caused by a species of *Geotrichum*. *Phytopathology* **30**: 530-533.

Colley, R.H. and Rumbold, C.T. 1930. Relation between moisture content of the wood and blue stain in loblolly pine. *J. Agric. Res.* **41**: 389-399.

Costa, E.W.B.Da. 1955. Effect of blue stain on the strength of *Pinus radiata*. *For. Prod. Newslett.* **209**: 1- 2.

Croan, S.C. and Highley, T.L. 1992. Synergistic effect of boron on *Streptomyces rimosus* metabolities in preventing conidial germination of sapstain and mould fungi. *The International Research Group on Wood Preservation Document* No. IRG/WP/1565.

Cserjesi, A.J. 1967. The adaptation of fungi to pentachlorophenol and its biodegradation. *Can. J. Microbiol.* **13**: 1243-1249.

Cserjesi, A.J. and Johnson, E.L. 1982. Mould and sapstain control: Laboratory and field tests of 44 fungicidal formulations. *For. Prod. J.* **32(10)**: 59-68.

Cserjesi, A.J. and Roff, J.W. 1975. Toxicity tests of some chemicals against certain wood staining fungi. *International Biodeterior. Bull.* **11**: 90-60.

Davidson, R.W. 1935. Fungi causing stain in logs and timber in the southern states including five new species. *J. Agric. Res.* **50**: 789-807.

Davidson, R.W. 1944. Two American hardwood species of *Endoconidiophora* described as new. *Mycologia* **36**: 300-306.

Davidson, R.W. 1985. Brown and red stain in ponderosa pine lumber. *Mycologia* **77**(3): 494-496.

Davidson, R.W. and Lombard, F. 1954. Brick red stain of sitka spruce and other wood substrata. *Phytopathology* **44**: 485.

Department of Factories and Boilers, 1987. Division-wise list of registered (2m) factories in Kerala. Government of Kerala, Government Press, Ernakulam.

Dick, C.M. and Hutchinson, S.A. 1966. Biological activities of volatile fungal metabolites. *Nature* **211**: 868.

Dickson, D. 1980. PCP dioxins found to pose health risks. *Nature* **283**: 418.

Dowding, P. 1968. The biology of blue stain fungi. *Ph.D. Thesis*, University of Cambridge, London.

Dowding, P. 1969. The dispersal and survival of spores of fungi causing blue stain in pine. *Trans. Br. mycol. Soc.* **52**(1): 125- 137.

Dowding, P. 1970. Colonization of freshly bared pine sapwood surfaces by staining fungi. *Trans. Br. mycol. Soc.* **55**(3): 399- 412.

Drysdale, J.A. 1983. Laboratory evaluation of potential anti- sapstain treatments for *Pinus radiata. The International Research Group on Wood Preservation Document* No. IRG/WP/3237.

Drysdale, J.A. 1986. A field trial to assess the potential of anti-sapstain chemicals for long-term protection of sawn radiata pine. *The International Research Group on Wood Preservation Document* No. IRG/WP/3375.

Drysdale, J.A. 1987. Commercially available anti-sapstain chemicals in New Zealand - an update. *The International Research Group on Wood Preservation Document* No. IRG/WP/3416.

Drysdale, J.A. and Hedley, M.E. and Butcher, J.A. 1986. An evaluation of chemical treatments for the protection of radiata pine logs from fungal degrade. *The International Research Group on Wood Preservation Document* No. IRG/WP/3377.

Drysdale, J.A. and Keirle, R.H. 1986. A comparative field test of effectiveness of anti-sapstain treatment on radiata pine roundwood. *The International Research Group on Wood Preservation Document No.* IRG/WP/3376.

Drysdale, J.A. and Plackett, D.V. 1987. A field trial of water repellents as anti-sapstain treatment additives. *The International Research Group on Wood Preservation Document* No. IRG/WP/3417.

Drysdale, J.A. and Preston, A.F. 1982. Laboratory screening trials with chemicals for the protection of green timber against fungi. *N. Z. J. F. Sci.* **12**(3): 457-466.

Dunleavy, J. 1955. Control of damping off of sugarbeet by *Bacillus subtilis*. *Phytopathology* **45**: 252-258.

Edlund, M.L. and Henningsson, B. 1982. Field and laboratory studies on anti-sapstain preservatives. *The International Research Group on Wood Preservation* Document No. IRG/WP/3205.

Encinas, O. and Daniel, G. 1994. Evidence for wood cell wall degradation by the blue stain fungus *Botryodiplodia theobromae* Pat. *The International Research Group on Wood Presveration Document* No. IRG/WP/10077.

Encinas, O. and Daniel, G. 1995. Wood cell wall biodegradation by the blue stain fungus *Botryodiplodia theobromae* Pat. *Material u. Organismen.* **29**: 255-272.

Encinas, O. and Daniel, G. 1996. Degradation of the Gelatinous- layer in aspen and rubber wood by the blue stain fungus *Lasiodiplodia theobromae. The Inernational Research Group on Wood Preservation Document.* No. IRG/WP/ (in press).

Eslyn, W.E. 1981. Ability of isolates of *Confertobasidium olivaceo-album* to stain and decay wood. *Can. J. For. Res.* **11**: 497-501.

Findlay, W.P.K. 1959. Sapstain of timber. *For. abstr.* **20**(1): 1-7.

Findlay, W.P.K. and Pettifor, C.B. 1937. The effect of sapstain on the properties of timber. I. Effect of sapstain on the strength of scots pine sapwood. *Forestry* **11**: 40-52.

Findlay, W.P.K. and Pettifor, C.B. 1939. Effect of blue stain on the strength of Obeche (*Triplochiton scleroxylon*). *Emp. for. J.* **18**: 259-267.

Florence, E.J.M. 1991. Sapstain fungi of some commercially important timbers of Kerala and their chemical control. *KFRI Research Report* **80**, 27 pp.

Florence, E.J.M. and Sharma, J.K. 1990. *Botryodiplodia theobromae* associated with blue staining on commercially important timbers of Kerala and its possible biological control. *Material u. Organismen* **25:** 193-199.

Fougerousse, M. 1985. Protection of logs and sawn timber. Pages 75-119. In: *Preservation of timber in the tropics.* Findlay, W.P.K. (ed.) Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands, 273 pp.

Freitag, M., Morrell, J.J. and Bruce, A. 1991. Biological protection of wood: status and prospects. *Biodeterior. abstr.* **5**(1): 1-13.

Fritz, C.W. 1929. Stain and decay in lumber seasoning yards with special reference to methods of prevention. *For. Ser. Circular*, Canadian department of the Interior. **27.**

Fritz, C.W. 1952. Brown stain in pine sapwood caused by *Cytospora* sp. *Can. J. Bot.* **30:** 349-359.

Gao, Y., Breuil, C. and Chen, T. 1994. Utilization of triglycerides, fatty acids and resin acids in lodgepole pine wood by a sapstaining fungus *Ophiostoma piceae. Material u. organismen* **28:** 105-118.

Gnanaharan, R. 1983. Preliminary note on the fungal problem of rubber wood. *International Research Group on Wood Preservation Document* No. IRG/WP/3246.

Gnanaharan, R. 1984. Evaluation of an alkyl ammonium compound as a fungicide to control sapstain and mould during diffusion storage. *International Research Group on Wood Preservation Document* No. IRG/WP/3282.

Gnanaharan, R. 1986. Anti-sapstain chemicals for diffusion treatment of rubber wood. *International Research Group on Wood Preservation Document* No. IRG/WP/3367.

Gnanaharan, R. and Mathew, G. 1982. Preservative treatment of rubber wood (*Hevea brasiliensis*). *KFRI Research Report* **15**, 16 pp.

Greaves, H. 1970. The effect of selected bacteria and actinomycetes on the decay capacity of some wood rotting fungi. *Material u. Organismen* **5**: 265-279.

Greaves, H. 1972. Influence of mixed microbial population on Basidiomycete decay. *Material u. Organismen* **7**: 11-25.

Hansen, J. 1984. IPBC - a new fungicide for wood protection. *The International Research Group on Wood Preservation Document* No. IRG/WP/3295.

*Hartig, R. 1878. *DieZerset. Zungserschcinungen des Holzes.* Verlag von Julius Springer, Berlin.

Hedgecock, G.C. 1906. Studies upon some chromogenic fungi which discolour wood. *Rep, Mo. Bot. Gdn.* **17**: 59-114.

Highley, T.L. 1975. Properties of cellulases of two brown-rot fungi and two white-rot fungi. *Wood and Fiber* **6**(4): 275-281.

Highley, T.L. 1977. Requirements for cellulose degradation by a brown-rot fungus. *Material u. Organismen* **12**: 25-36.

* Original not referred.

Highley, T.L. 1978. Degradation of cellulose by culture filtrates of *Poria placenta. Material u. Organismen* **12**: 161-174.

Highley, T.L. 1990. Laboratory studies on antagonism of *Scytalidium lignicola* to wood decay fungi. *Material u. Organismen.* **25**: 181-192.

Highley, T.L., Benko, R. and Croan, S. 1991. Laboratory studies on control of sapstain and mould on unseasoned wood by bacteria. *Inernational Research Group on Wood Preservation* No. IRG/WP/1493.

Hon, C.F. 1989. Sapstain - a preliminary study of fungal discolouration on the commercial swamp species of Sarawak. *Forest Research Report* No. FP6, 39 pp.

Hong, L.T. 1976. A blue stain organism of jelutong (*Dyera costulata* H.k.f.). *The Malay. Forester* **39**: 177-188.

Hong, L.T. 1980. Temperature tolerance and its significance in the control of sapstain caused by *Botryodiplodia theobromae. The Malay. Forester* **43**: 528-531.

Hong, L.T. 1981. Screening of preservatives against sapstain and mould fungi from tropical hardwoods. I. Laboratory tests against isolate from rubber wood. (*Hevea brasiliensis*) *The Malay. Forester* **44:** 116-121.

Hong, L.T., Tam, M.K., Daljeet Singh, K. and Arshad Omar 1980. The effectiveness of preservatives in the control of sapstain in rubber wood (*Hevea brasiliensis*) logs. *The Malay. Forester* **43**: 522-527.

Hong, L.T., Wong, A.H.H. 1993. Biodeterioration and preservation of rubberwood (*Hevea brasiliensis*). *The International Research Group on Wood Preservation Document* No. IRG/WP/10084.

Hsu, S.C. and Lockwood, J.L. 1969. Nutrient deprivation as a mechanism of inhibition of fungi in agar by streptomycetes. *Phytopathology* **43**: 403-404.

Hulme, M.A. 1975. Stain control in eastern white pine using ammoniacal zinc oxide in mill conditions. *For. Prod. J.* **25**: 36-39.

Hulme, M.A. and Shields, J.K. 1972. Effect of primary fungal infection upon secondary colonization of birch bolts. *Material u. Organismen* **7**: 177-188.

Hulme, M.A. and Shields, J.K. 1975. Antagonistic and synergistic effects for biological control of decay. Pages 52-63 in: *Biological transformation of Wood by Microorganisms* Liese. W. (ed.) German Federal Republic, Springer Verlag, Berlin. 203 pp.

Hulme, M.A. and Thomas, J.F. 1979. Control of fungal sapstain with alkaline solutions of quaternary ammonium compounds and with tertiary amine salts. *For. Prod. J.* **29**(11): 26-29.

Jain, J.K., Prem Chand, Sharma, R.P. and Kumar, S. 1986. Laboratory screening trials with agriculture pesticides and insecticides for protection of green timber against sapstain and termites. *J. timb. Dev. Assoc.* **32**(1): 33-43.

Johnson, B.R. 1986. Sensitivity of some wood stain and mould fungi to an inhibitor of chitin synthesis. *For. Prod. J.* **36**(3): 54-56.

Johnson, L.F. and Curl, E.A. 1972. Methods for research on the ecology of soil-borne plant pathogens. Burgess Publishing Co., Minneapolis, USA. 247 pp.

Kaarik, A. 1980. Fungi causing sapstain in wood. *The International Research Group on Wood Preservation Document* No. IRG/WP/199.

Kadir, A.A. and Sudin, R. 1989. Carbohydrates in Rubberwood (*Hevea brasiliensis* Muell.) *Holzforschung* **43**(3): 173-178.

Katz, E. and Demain, A.C. 1977. The peptide antibiotics of Bacillus, Chemistry, biogenesis and possible functions. *Biol. Rev.* **41**: 449-474.

Kirk, T.K. 1971. Effects of microorganisms on lignin. *Ann. Rev. Phytopathology* **9**: 185-210.

Kirk, T.K. 1973. Chemistry and Biochemistry of decay. Pages 149- 181 in: *Wood Deterioration and its Prevention by Preservative Treatments.* Vol. I *Degradation and Protection of Wood.* D.D. Nicholas (ed.), Syracuse University Press, Syracuse, New York. 380 pp.

Kirk, T.K. 1975. Effects of a brown-rot fungus, *Lenzites trabea,* on lignin in spruce wood. *Holzforschung* **29**(3): 99-107.

Kirk, T.K. and Highley, T.L. 1973. Quantitative changes in structural components of conifer woods during decay by white and brown-rot fungi. *Phytopathology* **63**(1): 1335-1342.

Kitamura, Y. and Kondo, T. 1958. Chemical Study on blue stained pine wood. On the pigments obtained from mycelia of blue stain fungi. *J. Jap. Wood. Res. Sco.* **4**: 51-55.

Klingstrom, A.E. and Johansson, S.M. 1973. Antagonism of *Scytalidium* isolates against decay fungi. *Phytopathology.* **63**: 473-479.

Korzybski, T., Kowszyk - Gindifer, Z. and Kurylowicz, W. 1978. *Antibiotics, Origin, Nature and Properties.* Vol. 3. *Am. Soc. Microbiol.*, Washington,DC. 2270 pp.

Krapivina, I.G. 1960. Destruction of the secondary layer of the cell wall by blue staining fungi. *Commonwealth Scientific and Industrial Research Organisation, Australia*, Translation No. 5329.

Kreber, B. and Morrell, J.J. 1993. Ability of selected bacterial and fungal bioprotectants to limit fungal stain in ponderosa pine sapwood. *Wood and Fiber Science* **25**(1): 23-34.

Laks, P.E., Dickens, J.B., Woods, T.L., Bell, P.J. and Nolf, J.M. 1991. Performance of chlorothalonil and chlorothalonil/biocide combinations in anti-sapstain tests. *For. Prod. J.* **41**(5): 23- 30.

Leightley, L.E. 1985. An appraisal of anti-sapstain chemicals in Queensland, Australia. *The International Research Group on Wood Preservation Document* No. IRG/WP/IRG/WP/3331.

Leightley, L.E. 1986. An evaluation of anti-sapstain chemicals in Queensland, Australia. *The International Research Group on Wood Preservation Document* No. IRG/WP/3374.

Levi, M.P. and Dietrich, R.L. 1976. Utilization of southern pine beetle-killed timber. *For. Prod. J.* **26**(4): 42-48.

Levy, J.F. 1967. Decay and degrade of wood by soft rot fungi and other organisms. *Records of the Annual Convention of the British Wood Preservation Association* 147-175.

Liese, W. 1970. Ultrastructural aspects of woody tissue disintegration. *Ann. Rev. Phytopathology* **8**: 231-258.

Lindgren, R.M. 1942. Temperature moisture and penetration studies of wood staining *Ceratostomella* in relation to their control. *Tech. Bull. U.S. Dep. Agric.* 807.

Lindgren, R.M. 1952. Permeability of southern pine affected by mould growth and other fungus infection. *Proc. Am. Wood Preserver's Assoc.* **48:** 158-174.

Lindgren, R.M. and Scheffer, T.C. 1939. Effect of blue stain on the penetration of liquids into air-dry southern pine wood. *Proc. Am. Wood Preserver's Assoc.* **35:** 325-326.

Lindgren, R.M. and Wright, E. 1954. Increased absorptiveness of molded Dougles fir Posts. *J. For. Prod. Res. Soc.* **4:** 162-164.

Masuka, A. 1991. Laboratory evaluation of the efficacy of antiblu and biocide in the control of sapstain and mould fungi on *Pinus elliotti. Eur. J. For. Path.* **21**: 185-188

Masuka, A.J. and Kariwo, P. 1992. Sapstain and mould in pine logs in Zimbabwe. *Comm. For. Rev.* **71:** 193-196.

Micales, J.A., Highley, T.L. and Richter, A.L. 1989. The use of chlorothalonil for protection against mould and sapstain fungi. I Laboratory evaluation. *The International Research Group on Wood Preservation Document* No. IRG/WP/3515.

Milano, S. 1981. Effectiveness of some microbiocides against the development of mould and sapstain in *Pinus elliotti. The International Research Group on Wood Preservation Document* No. IRG/WP/3169.

Milano, S. and Neto, J.A.A.V. 1982. Evaluation of the effectiveness of three microbiocides in the control of sapstain. *The International Research Group on Wood Preservation Document* No. IRG/WP/3212.

Miller, D.J. and Goodell, B. 1981. Blue staining in ponderosa pine sapwood at moderate and low temperatures. *For. Prod. J.* **31**(2): 54-59.

Morrell, J.J. 1987. A reddish purple stain of red alder by *Ceratocystis piceae* and its prevention. *For. Prod. J.* **37**(2): 18- 20.

Moser, J. 1985. Use of sporothecae by phoretic *Tarsonemus* mites to transport ascospores of coniferous blue stain fungi. *Trans. Br. mycol. Soc.* **84**(4): 750-753.

O'Brien, F.E.M. 1948. The control of humidity by saturated salt solutions. *J. Sci. Instruments* **25**: 73-76.

Olofinboba, M.O. 1974. Sapstain in *Antiaris africana* on economically important tropical white wood. *Nature* **249**: 860.

Olofinboba, M.O. and Lawton, J.R.S. 1968. An investigation into the biology of the blue stain organism in *Antiaris africana*. *J. Inst. Wood Sci.* **21**: 6-20.

Plackett, D.V. 1982. Field evaluation of alternative anti- sapstain chemicals. *The International Research Group on Wood Preservation Document* No. IRG/WP/3198.

Plackett, D.V. 1984. The Preliminary evaluation of selected sulfonium salts for use in wood preservation. *The International Research Group on Wood Preservation Document* No IRG/WP/3278.

Plackett, D.V. and Chittenden, C.M. 1986. Laboratory evaluation of water repellents as anti-sapstain treatment additives. *The International Research Group on Wood Preservation Document* No. IRG/WP/3382.

Pinheiro, A.C.A. 1971. Blue-stain in poplar wood. *Material. u. Organismen*. **6**: 93-100.

Powell, M.A., Eaton, R.A. and Webber, J.F. 1994. Insect transmission of fungal sapstain to freshly sawn unseasoned softwood lumber. *The International Research Group on Wood Preservation Document* No. IRG/WP/20025.

Presnell, T.L. and Nicholas, D.D. 1990. Evaluation of combinations of low hazard biocides in controlling mould and stain fungi on southern pine. *For. Prod. J.* **40**(2): 57-61.

Punithalingam, E. 1980. *Plant diseases attributed to Botryodiplodia theobromae* Pat. Bibliotheca Mycologica, Cramer Vaduz. 121 pp.

Ricard, J.L. and Bollen, W.B. 1968. Inhibition of *Poria carbonica* by *Scytalidium* sp., an imperfect fungus isolated from Dougles fir poles. *Can. J. Bot.* **46**: 643-647.

Ricard, J.L., Wilson, M.M. and Bollen, W.B. 1969. Biological control of decay in Douglas fir poles. *For. Prod. J.* **19**: 41 - 45.

Richards, C.A. 1923. Method of testing relative toxicity of wood preservative. *Proc. Am. Wood Preservers Assoc.* **79**: 207-212.

Row, R.C., Beute, M.K., Wells, J.C. and Wynne, J.C. 1974. Incidence and control of *Cylindrocladium* black rot of peanuts in North Carolina during 1973. *Plant Dis. Reptr.* **58**: 348-352.

Rumbold, C.T. 1929. Blue staining fungi found in the United States. *Phytopathology* **19**: 597-599.

Rumbold, C.T. 1931. Two blue staining fungi associated with bark beetle infestation of pines. *J. Agric. Res.* **43**: 847-873.

Rumbold, C.T. 1936. Three blue staining fungi including two new species associated with bark beetles. *J. Agric. Res.* **52**: 419-437.

Rumbold, C.T. 1941. A blue stain fungus, *Ceratostomella montium* and some yeasts associated with two species of *Dendroctonus*. *J. Agric. Res.* **62**: 589-601.

Rustenburg. Gb., Klaver, C.T. and Holland, N.V.H. 1990. Evaluation of the effectiveness of defence anti-stain on the control of sapstain in laboratory and field tests. *The International Research Group on Wood Preservation Document* No. IRG/WP/3593.

Sadasivam, S. and Manikam, A. 1992. *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Limited, New Delhi. 246 pp.

Savory, J.G. and Pinion, L.C. 1958. Chemical aspects of decay of beech wood by *Chaetomium globosum*. *Holzforschung* **12**(4): 99-103.

Scheffer, T.C. 1973. Microbiological degradation and the causal organisms. pages 31-106 in: *Wood deterioration and its Prevention by Preservative treatments*. D.D Nicholas (ed.) Syracuse University Press, Syracuse New York. 380 pp.

Scheffer, T.C. and Lindgren, R.M. 1940. Stains of sapwood and sapwood products and their control. *Tech. Bull. U.S. Dep. Agric.* 714.

Schmidt, E.L. and Dietz, M.G. 1985. *Arthrographis cuboidea* causing pink stain on sodium pentachlorophenoxide treated red oak. *Mycologia* **77**(2): 316-318.

Schmidt, E.L. and French, D.W. 1979. Two-day mold testing using a contact agar method. *For. Prod. J.* **29**(9): 42.

Schrenk, H. Von. 1903. The blueing and the red rot of the western yellow pine. *Bull. Bur. Pl. Ind., U.S.* Department of Agriculture **36:** 1-36.

Schulz, G. 1956. Exploratory tests to increase preservative penetration in spruce and aspen by mold infection. *For. Prod. J.* **6**(2): 77-80.

Seifert, K.A., Breuil, C., Rossignal, L., Best, M. and Saddler, J.N. 1988. Screening for microorganisms with the potential for biological control of sapstain on unseasoned lumber. *Material u. Organismen* **23**: 81-95.

Seifert, K.A., Hamilton, W.E., Breuil, C. and Best, M. 1987. Evaluation of *Bacillus subtilis* C186 as a potential biological control of sapstain and mould on unseasoned lumber. *Can. J. Microbiol.* **33:** 1102-1107.

Ser, C.S. and Lim, S.C. 1980. Steam bending properties of rubber wood. *The Malay. For.* **43:** 506-510.

Sharma, J.N., Gandhi, B.L., Kukreti, D.P. and Gairr, B.K. 1982. Shrinkage, hygroscopicity and steam bending property of rubber wood (*Heavea brasiliensis*). *J. Tim. Dev. Assoc. (India)* **28:** 19- 24.

Sharma, J.K. and Mohanan, C. 1991. Epidemiology and control of diseases of eucalypts caused by *Cylindrocladium* spp. in Kerala. *KFRI Research Report* **70,** 155 pp.

Shields, J.K. and Atwell, E.A. 1963. Effect of a mold *Trichoderma viride* on decay of birch by four storage rot fungi. *For. Prod. J.* **13**: 262-265.

Shields, J.K. and Unligil, H.H. 1968. Deterioration of softwood chips owing to outside storage in New Brunswick. *Pulp. Pap. Mag. Can.* Nov. 62-67.

Singh, H. 1974. *Chlorosplenium* in India. *Trans. Br. mycol. Soc.* **63**(2): 289-294.

Singh, V. and Deverall, J. 1984. *Bacillus subtilis* as control against fungal pathogens of citrus fruit. *Trans. Br. mycol. Soc.* **83**: 487-490.

Skaar, C. 1972. *Water in Wood.* Syracuse University Press, New York. 218 pp.

Snedecor, G.M. and Cochran, W.G. 1967. *Statistical methods.* Oxford and IBH Publication, New Delhi. 533 pp.

Sonti, V.R., Chatterjee, B. and Ashraf, M.S. 1982. The utilization of preserved rubber wood. *International Research Group on Wood Preservation Document* No.IRG/WP/3186.

Stranks, D. W. 1976. Scytalidin, Hyalodendrin, Cryptosporiopsin- Antibiotics for prevention of blue stain in white pine sapwood. *Wood Sci.* **9**: 110-112.

Stranks, D.W. and Hulme, M.A. 1975. The Mechanism of biodegradation of wood preservatives. *Material u. Organismen* **3**: 346-353.

Stutz, R.E. 1959. Control of brown stain in sugar pine with sodium azide. *For. Prod. J.* **9**: 59-64.

Stutz, R.E., Koch, P. and Oldlam, M.L. 1961. Control of brown stain in eastern white pine. *For.Prod.J.* **11**: 258-260.

Subramanian, C.V. 1983. *Hyphomycetes, Taxonomy and Biology.* Academic Press, London, 502 pp.

Sujan, A. Tan, A.G. and Stevens, M. 1980. Some studies on fungal deterioration of rubber wood. *The International Research Group on Wood Preservation Document* No. IRG/WP/2140.

Swinburne, T.R. and Brown, A.E. 1976. A comparison of the use of *Bacillus subtilis* with conventional fungicides for the control of apple canker, *Nectria galligena. Ann. Appl. Biol.* **82:** 365-368.

Tabirih, P.K. and Seehann, G. 1984. Effects of *Botryodiplodia theobromae* attack on Abachi wood (*Triplochiton scleroxylon* K. Schum.) *Material. u. Organismen* **19**: 9-21.

Tan, A.G., Chong, K.F. and Tam, M.K. 1980. Control of fungal attack in rubber logs. *The Malay. Forester* **43:** 516-521.

Thapa, R.S. 1971. Results of preliminary investigation on black stains in commercial trees in Dipterocarp forests of Sabah. *The Malay. Forester* **34**: 53-58.

Toole, E.R. 1971. Interaction of mould and decay fungi on wood in laboratory tests. *Phytopathology* **61:** 124-125.

Troya, M.T. and Navarrete, E.A. 1989. Blue stain fungi (*Ceratocystis* spp.) found in spain on pine wood. *The International Research Group on Wood Preservation Document* No. IRG/WP/1410.

Tsunoda, T., Takahashi, M. and Nishimoto, K. 1983. Studies on low toxicity anti-sapstain chemicals. (I) Chemical control to prevent sapstain and mould on rubber wood. *Wood Res. Tech. Notes* **17:** 122 -131.

Umezurike, G.M. 1969. Cellulolytic activities of *Botryodiplodia theobromae Pat. Ann. Bot.* **33:** 451-462.

Umezurike, G.M. 1978. Staining and degradation of wood blocks and agar media by *Botryodiplodia theobromae. Trans. Br. mycol. Soc.* **71**(2): 328-332.

*Unger, D.F. 1847. Uber einen in grosser Verbreitung as Nadelholzern beobachiteten Fadempilz. *Bot.Ztg.* **5:** 249-257.

Unligil, H.H. 1976. Prevention of fungal stain on pine timber Laboratory screening tests with fungicides. *For. Prod. J.* **26:** 32 -33.

Unligil, H.H. 1979. Laboratory screening tests of fungicides of low toxic hazard for preventing fungal stain of lumber. *For. Prod. J.* **29**(4): 55-56.

*Vanin, S.I. 1932. Materials relating to the study of the discolouration of wood caused by fungi and chemical effects (I)*Zvestija leningradskogo Instituta Borby vrediteljame v Sel'skom i Lesnom Hozjajstve* **3:** 3-37.

Verrall, A.F. 1939. Relative importance and seasonal prevalence of wood staining fungi in the Southern states. *Phytopathology* **29:** 1031-1051.

Verrall, A.F. 1941. Fungi associated with stain in chemically treated green lumber. *Phytopathology* 31: 270-274.

---

* Original not referred.

Verrall, A.F. 1941. Dissemination of fungi that stain logs and lumber. *J. Agri. Res.* **63**(9): 549-558.

Verrall, A.F. 1949. Some mould of wood favoured by certain toxicants. *J. Agric. Res.* 78(12): 695-703.

Verall, A.F. and Mook, P.V. 1951. Research on chemical control of fungi in green lumber. *Tech. Bull. U. S. Dep. Agric.* No. 1046, 1940-51.

Viitanen, H. and Paajanen, L. 1988. The critical moisture and temperature condition for the growth of some mould fungi and the brown rot fungus *Coniophora Puteana* on wood. *The International Research Group on Wood Preservation Document* No. IRG/WP/1369.

Vincent, J.M. 1927. Distortion of fungal hyphae in the presence of certain inhibitor. *Nature* **159:** 850.

Weaver, D.J. 1971. Control of *Cylindrocladium floridanum* with potassium azide in soil. *Plant Dis. Reptr.* **55**(12): 1094-1096.

Wong, C.N. 1979. Preliminary assessment of rubber wood (*Hevea brasiliensis*) for plywood manufacture. *The Malay. Forester* **42:** 30- 37.

Wong, W.C. and Ong, O.L. 1979. The production of particle board from rubber wood. The *Malay. Forester* **42**: 25-29.

Yoichi, T. 1993. Chemical characteristics of rubber wood damaged by *Sinoxylon conigern* Ger Stacker. *Bull. For. & For. Prod. Res. Inst.* **365:** 31-43.

Zabel, R.A. and Morrell, J.J. 1992. *Wood Microbiology: Decay and its Prevention.* Academic Press, San Diego, CA. 476 pp.

Zink, P. and Fengel, D. 1988. Studies on the colouring matter of blue-stain fungi. Part 1. General characterization and the associated compounds. *Holzforschung* **42**: 217-220.

Zink, P. and Fengel, D. 1989. Studies on the colouring matter of blue-stain fungi. part 2. Electron microscopic observation of the hyphae walls. *Holzforschung* **43**: 371-374.

Zink, P. and Fengel, D. 1990. Studies on the colouring matter of blue-stain fungi. part 3. Spectroscopic studies on fungal and synthetic melanins. *Holzforschung* **44**: 163-168.