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STUDIES ON GROWTH AND PREVENTION OF SAPSTAIN FUNGUS BOTRYODIPLODIA THEOBROMAE IN RUBBER WOOD AND ITS EFFECT ON STRENGTH PROPERTIES

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ABSTRACT

Microorganisms cause various types of damages such as decay, soft rot, mould, stain and bacterial degradation to timber, Moulds and stains are common on rubber wood. The predominant fungal species causing sapstain in rubber wood is Botryodiplodia theobromae. Due to the environmental conditions favourable for fungal growth, sapstain is a serious problem preventing the utilization of rubber wood in Kerala. This report presents a detailed study on the factors affecting the growth and colonization of B. theobromae on rubber wood. The study has revealed that no growth of B. theobromae occurred in rubber wood with a moisture content at or below 23.8 per cent. Moisture content of 29.0 per cent or above was most favourable for heavy growth of the fungus. Temperature tolerance of B. theobromae showed that 30°C was the ideal temperature for the growth of the fungus on Potato Dextrose Agar medium. The results also indicated that once B. theobromae was established in the wood, the mycelium was not killed on the surface as well as inside the wood even after 7 days of incubation at 50°C. Weight loss of rubber wood due to B. theobromae infection was 8 per cent in the first month which increased to 12.2 per cent at the end of the fourth month. But in Alstonia scholaris. the weight loss was 4.5 per cent at the end of fourth month. There was also a reduction in the density of stained rubber wood at the end of fourth month. No reduction in the compressive strength of stained wood was noted, whereas the bending strength was reduced. In the field evaluation of different chemicals/fungicides for the control of sapstain and decay, Captafol even at 3 per cent did not prevent mould and sapstain fungi completely on rubber wood. In all the fungicidal treatments, better control was achieved by open stacking. Busan 1009 (1.0 per cent) mixed with boric acid (1.0 per cent) and borax (1.0 per cent) was effective in controlling fungal growth. Also, Hylite extra at 1.5 percent, and Chlorothalonil at 1 percent were equally effective in controlling sapstain. During rainy season, appropriate concentration of the fungicides has to be increased for better control of fungi growth.

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 and depletion of natural forests. The growing demand for timber can be met to some extent by utilizing alternative species and increasing the timber production through intensive management. Prevention ofwastage of wood by adopting appropriate measures 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 ofbiological agents and chemical factors.

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 (19831 classified wood inhabiting fungi into the following three categories: 1. moulds and stain fungi that normally do not degrade lignified cell walls but 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.

Most of the wood-based industries distributed throughout the State utilize different soft woods species available 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 malting packingcases due to hygienic reasons.

1.1 MOULD

Mould occurs on the surface of wood and generally it bas wooly or powdery appearance. Moulds cause superficial staining due to the development of masses of coloured spores on the wood surface.

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 '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.

1.2.1 Economical significance of sapstain

Although significant loss in wood quality due *to* stain has been reported worldwide, the exact economics of this loss are rather difficult to assess. Scheffer (1973)estimated that annual loss due to stain exceeded 50 million dollars in the United States. This loss generally increases with increasing sapwood content. Percentage of loss mould be higher in a climate conducive to fungal growth. Sapstain is responsible for deterioration of large quantities of susceptible timbers. Their value is reduced for decorative purposes and if heavily stained, the wood becomes unfit for paint finishing. For mood 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 pacltingcases. 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 in poor quality. of pulp (Shields and Unligil. 1968).Timbers. susceptible to sapstain attack, are to he processed at the earliest to avoid fungal infection and this urgeney often causes practical difficulties to the manufacturers.

1.2.2 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

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 mood. it is becoming increasingly popular as a source of timber particularly for making furniture (Gnanaharan, 1984).

Study conducted by Florence(l991) revealed that sapstain caused by *Botryodiplodia theobromae* Pat. is a serious problem in the proper utilisation of rubber wood in Keraia. The present study was undertaken with the following objectives.

1. to study the effect of humidity, temperature and moisture content of rubbcr wood on coionization of *Botryodiplodia theobromae.*

2. to determine the extent of weight loss due to *Botryodiplodia theobromae* on rubber wood.

3. to determine the cffcct of sapstain on the physical and strength properties of rubber wood.

4. to evaluate the efficacy of various chemicals to conrroi sapstain on rubber mood in the field.

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, account 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 degradation. 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).

2.1 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 rneianin-based pigments in the cells. Stain fungi can utilize the reserve food materials stored in the cells of the sapwood.

In tropical hardwoods. the staining is caused principally by *Diplodia* spp.. and in particular *Botryodipiodia theobromae* Pat. (Cartwright and Findlay 1958:Findlay. 1959: Olofinboba and Lawton. 1968).*B. theobromae.* a known pathogen on nuinerous tropical and subtropical plants of economic importance (Punithalingam, 1980). causes stain in *Bombax buonopozense* P. Beauv. (Umezurike. 1969) and in poplar (Pinheiro. 19711. 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 eccnomic loss in *Antiaris africana* Engl.. an important tropical white wood (Olofinboba. 1974).and in Jelutong (Dyera *costulata* Hk.f.) in Malaysia (Hang, 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 mas recorded in Abachi wood

(Triplochiton scleroxylon K. Schum.) due to sapstain caused by *B. theobromae* (Tabirih and Seehann. 19841. 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.

2.2 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 (1964) 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 (19371 reported that only toughness mas 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 soft-wood 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 dvnamic bending properties of poplar wood.

2.3 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 l930s the use of chlorinated phenols and organic mercury compounds was found to be an effective method to control fungal sapstain (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 of the United States.

Butcher (1973) reported captafol (cis-N-((1,2,2-, tetra- chloroethyl) thio-4-cyclohexane- 1.2-dicarboximide) as an alternative to widely used pentachlorophenol. Butcher and Drysdale (1974: 1978) further advocated captafoi 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

In Malaysia. for the protection of rubber wood. Hong (1981) tested 18 preservatives of which Busan 30 (TCMTB),Benomyl (Benlate),Brassicol (Pentachloro-nitro-benzcne), Fennotox S2 (Thiophanates. Thiocarbamates. Sodium nitrate), Mitrol PQlC (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 Tan *et al.*. (1980) and Gnanaharan (1983. 1984, 1986).only a few of them were found to have potential to replace NaPCP. but they were not cost-effective.

Among the seven commercial formulations tested, Plackctt (1982) achieved more than 98 per cent 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-(trichloromethvlthio)-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).

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-I009 [MBT and TCMTB) were suitable for short-term and Haipen (Captafol) for long-term protection.

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 it was 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.

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

3. EFFECT OF TEMPERATURE AND MOISTURE CONTENT OF RUBBER WOOD ON COLONIZATION OF *BOTRYODIPLODIA THEOBROMAE*

3.1 INTRODUCTION

Botryodiplodia theobromae (Bt) 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).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 mood which influence the growth of *B. theobromae* in wood.

3.2 MATERIALS AND METHODS

3.2.1 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 content levels 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 the blocks to room temperature (28 ± 2°C). 10 blocks were taken and their initial weight determined using an electronic digital balance. These

10 blocks were then oven-dried at 102 ±2°C for 2 days and after cooling them in a desiccator. their own-dry weight was determined. From these weight differencesthe moisture content was calculated. Of the remaining 20 blocks. 10 were inoculated at the centre with a 8 mm diameter inycelial disc of an actively growing culture of Bt arid 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 to maintain humidity. The Petri dishes were 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 1.

Table 1. Rating index for assessing the fungal growth

Extent of growth	Rating
Nil	0
Trace	l(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).

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

An earlier study made by Florence (1991) revealed that based on the frequency of isolation of *B. theobromae* on various timbers. *Hevea brasiliensis* was the most susceptible and *Alstonia* scholaris is the least susceptible timber.

influence of moisture content and temperature on the growth of *B. theobromae* on *H. brasiliensis,* the highly susceptible timber to sapstain. was rnmparcd with that of the least susceptible timber *A. scholaris.* Test blocks 125 x 25 x 40 mm size) of *H. brasiliensis* and *A. scholaris* were prepared trom 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 blocks of each of the two species were steam sterilized for 15 minutes at 100 kPa and allowed 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 (Fig. 1). The moisture contents of wood blocks of each species were determined using oven-dry weight method. For determining the moisture content, 10 blocks each were weighed initially, oven-dried at 103±2°C and the oven-dry weight determined. Twenty blocks each out of the remaining 40 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. Half of them were inoculated 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. In each bottle 30 ml of sterile water was placed for maintaining humidity. Twenty blocks each 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

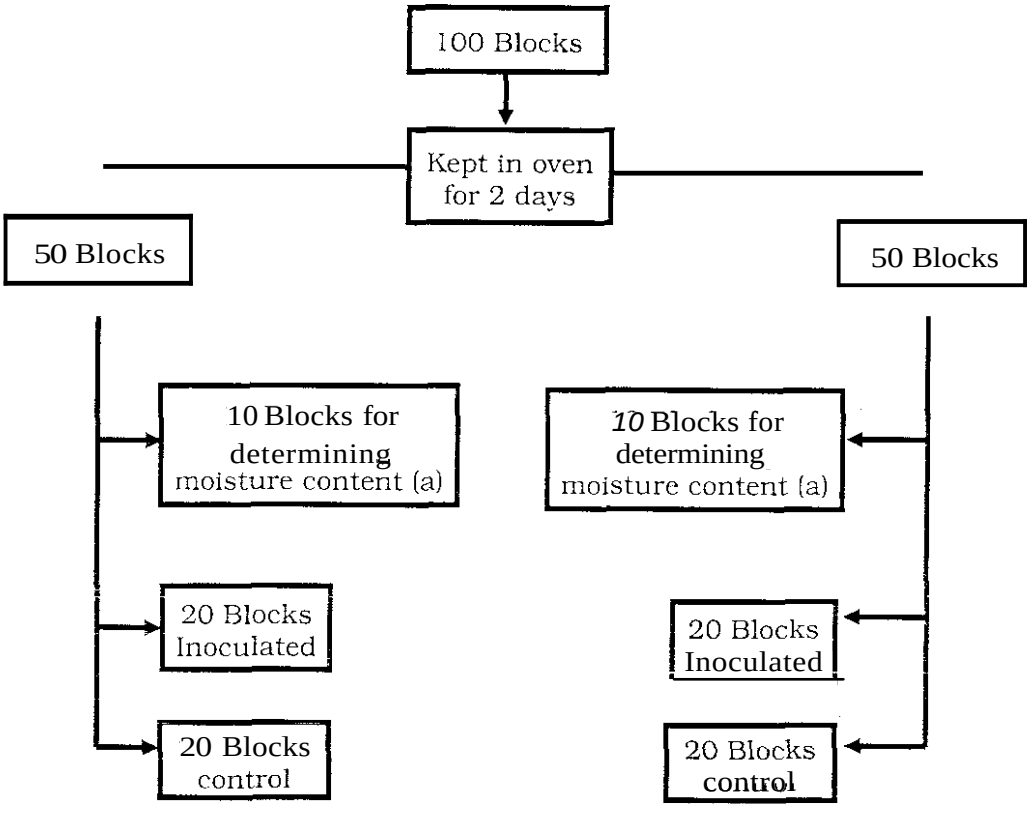

Fig. 1. Distribution of rubber wood blocks for assessing the comparative growth of *Botryodiplodia theobromae* on *Hevea brasiliensis* and *Alstonia scholaris*

period, the growth of the fungus over the wood blocks was examined and assessed using the rating index given in Table 1. Superficial mycelium was scraped off from each inoculated block using a scalpel blade without removing the woody tissue and the final moisture content of the wood blocks was determined on oven dry weight basis.

3.2.3 Temperature tolerance of *Botryodiplodia theobromae*

Temperature tolerance of Bt was studied by assessing its growth in potato dextrose agar (FDA) 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 FDA. 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 mas measured at three radii every day up to 7 days (168hours).

Fungal growth on wood blocks: Forty fresh rubber wood blocks (50 X 10 x 70 mm size) having a moisture content of 80-90 percent (ideal MC for good growth of Bt] were steam sterilized for 15 minutes at 100 kPa. After cooling them to room temperature they were inoculated them at the centre with a mycelial disc of 8 mm diameter. taken from the actively growing culture of Bt. The inoculated blocks mere 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 examined everv day and assessed using the rating index mentioned in Table 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 steam sterilized at 100 kPa and were 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 were taken out from the incubator and a bit of mycelium growing over the wood block was removed aseptically using a sterile forceps.Then they were 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 mood tissue in the medium was recorded as the measure of temperature tolerance of the fungus at a particular temperature.

3.2.4 Comparison of weight loss caused by *Botryodiplodia theobromae* in *Hevea brasiliensis* and *Alstonia* scholaris

Weight loss in blocks of *H. brasiliensis* which is 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 blocks (15 x 5 X 50 mm) of defect-free fresh sapwood were cut from each timber species. They were oven dried at 103± *2oC* till the weight became constant. cooled in desiccator and their 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 myceiial disc raken 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 matcr. The inoculated wood blocks were kept free of direcr contact with water using glass supports projecting above the watcr level in the test tube. The atmosphere around the inoculated blocks was humidified by keeping a sterile lilter paper wick (100 x 25 mm) covering the entire lenqth of the rest blocli. The lower end of the wick was immersed in srerile water. The tubes were closed with cotton plugs. The remaining 40 blocks were inoculated with plain agar disc which served as control. The procedure adopted for determining the weight loss described earlier (Florence. 1991) was used. 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 mere oven dried at 103±2°C till the weight became constant to record the final weight. The percentage of weight loss was calculated based on the original oven-dry weight of the block.

3.3 RESULTS

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

Results pertaining to the growth of *Botryodiplodia theobromae* on rubber wood at different moisture contents are presented in Table 2. No growth of

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

MC of rubber wood	Average growth rating ± SE	*Growth rating
65.63	4.0	4
46.05	3.8 ± 0.13	4
29.49	4.0	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 rating see Table I,

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 the most favourable as heavy growth was observed on the wood blocks. ANOVA showed significant difference between MC and growth rating (Table 3).

Table 3. 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	S S	MS	F
Between groups	9	108755	0.2084	48.6647**
Within groups	89	0.3811	0.0043	
Total	98	2.2566		

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

At both the moisture regimes i.e., (a)and (b)and both rhe temperatures (30°C and 40°C), heaw 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).

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 luxuriant growth of Bt on rubber mood. But in *A. scholaris*, even the conducive temperature did not have any influence on the growth of Bt.

3.3.3 Temperature tolerance of *Botryodiplodia theobromae*

Fungal growth on agar media: *Botryodipiodia 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 mas observed at high temperatures of 50°C and 60°C (Table 5).

Fungal growth on wood blocks: On wood blocks. no growth of Bt was found 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 bloclrs. bur subsequently the fungal growth stopped.

Survival of Bt in woodblocks: The results indicated that once Bt established in the wood. the mycelium mas 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 6).

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

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

Treatment of wood blocks	*H. brasiliensis*				**Alstonia** *scholaris*			
	Initial MC	Final MC	Temp. of incuba-tion °C	Mean growth rate indcx (SE)	Initial M C	Final MC	Temp. of incuba-tion °C	Mean growth rate index (SE)
a	67.32	37.03	30	4.0	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	31.99	43.90	30	0
b	27.53	26.82	40	1.8 (1.3)	31.99	4 1.39	40	0

* for growth rate index see table 1.

Table 5. Growth of *B. 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 (cm) (SE)	rate index	Mean colony dia. on PDA (cm) (SE)	*Mean growth rate index (SE)	*Mean colony dia. on PDA (cm) (SE)	*Mean growth rate index (SE)	Mean colony dia. on PDA (cm) (SE)	*Mean growth rate index (SE)
24	4.3 ± (0.07)	0.0 ± (0)	0.06 (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 ± (0.061	0	0	0	0	0
168	9.0 ± (0)	4.0 ± (0)	4.7 ± (0.031	0	0	0	0	0

for growth rate index see Table 4-1

Table 6. 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

3.3.4 Comparison of weight loss caused by *Botryodiplodia theobromae* in *Hevea brasiliensis* and *Alstonia scholaris*

At the end of the fourth month of incubation, maximum weight loss occurred in rubber wood (Table 71. In A. scholaris 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. No weight loss was recorded in control blocks.

Table 7. Weight loss in *H. brasiliensis* and *A. scholaris* due to growth of *Botryodiplodia theobromae* for a period of four months (mean of 10 observations)

Incubation period (month)	Weight Loss Percentage	
	Hevea brasiliensis Mean ± SE	*Alstonia scholaris* Mean ± SE
1	8.0 ± 0.25	0.0 ± 0
2	8.3 ± 0.36	0.00 ± 0
3	8.5 ± 0.55	0.0 ± 0
4	12.2 ± 1.09	4.5 ± 0.53

3.4 DISCUSSION

Water is a fundamental constituent of a living tree and the active 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 has got great influence on 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 from about 18 to 32 per cent moisture content.

Studies conducted by Colley and Rumbold (1930) showed 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*.

The survival of Bt in wood in relation to temperature has great 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 its ability to establish on wood blocks. However. if the fungus has already established in the wood block. it can tolerate still higher temperatures. A temperature of 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 during 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 germinate only 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 30°C on malt agar. On the contrary, this study reveals that there is no fungal growth in PDA at 30°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 innermost 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 years at 49°C and 1 hour at 60°C Lindgren (1942) found that some of the blue stain fungi tested, except *Ceralocystisips* became nonviable 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 pracricai value in adjusting the temperature inside the drying kiln and accordingly mood can be protected effectively from sapstain infection of Bt.

Weight loss caused by *B. theobromae* was more significant in rubber wood than in A. *scholaris:* eventhough in *A. triphysa* the weight loss is less than that in rubber wood: it is quite significant. The greater 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 (Kadirand Sudin, 1989) may be the reason for higher loss in weight of rubber wood as compared to other two timbers. Earlier workers have also reported loss in weight of wood blocks caused by the infection of *B. theobromae* and other stain fungi. Tabirih and Seehann (19841 reported the weight loss caused by *B. theobromae* on *Triplochiton scleroxylon* and *Fagus sylvatica.* He observed that in *T. scleroxy-*lon 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 per cent) and *F sylvatica* (20 per cent). Kaarik (1980) has 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) showed 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 by *B. theobromae* in wood blocks of *Gossweilerodendron balsamiferum* (Verm.) Harms.. a forest tree used extensively for constructional work in Nigeria. 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 lo that of soft rot fungi (Krapivina, 1960; Lew, 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. 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.

4. PHYSICAL AND STRENGTH PROPERTIES OF SAPSTAINED RUBBER WOOD

4.1 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 often 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 are also significant factors affecting the strength of wood.

Kerala accounts for a major share of rubber plantations in India. 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 paclcingcases, 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. Thus a major factor which limits the wider utilization of rubber wood is its susceptibility to fungal and insect attack. Due to conducive climatic condition of Kcrala, 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 has been carried out and the results are presented.

4.2 MATERIALS AND METHODS

Rubber trees of 30 years age were selected from 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 the other served as control.

4.2.1 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 volume of these wood blocks was determined by water displacement method as described in the Indian Standard IS: 1708-1982. The blocks were dried in an oven 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 10 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. 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 densitv was determined.

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

4.2.2 Compressive strength

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

4.2.3 Static bending strength

Wood blocks of 20 X 5 X 90 mm size were prepared from the same billet from which the blocks mere also made for testing the compressive strength. After air drying, fifty clear. defect-free blocks were selected and their initial density was estimated. All the wood blocks were kept in a moist chamber until the blocks gained the moisture equilibrium. Following steam sterilization, 25 blocks were inoculated 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 earlier and they were 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. The filter paper along with the mycelium was dried in an oven set at 100°C for 2 days and the dry-weight of the mycelium was recorded. The treated and control samples were air-dried and final density determined. All the wood blocks were dried at 103 ± 2°C and the final oven-dry weight recorded to compare the weight loss of wood blocks due to the infection by *B. theobromae*. Static bending tests of 50 samples was carried out in a ZWICK Universal testing machine. Data on static bending strength, initial density, final density, weight loss and myeelial weight of stained and control rubber wood blocks were analysed statistically.

4.3 RESULTS

4.3.1 Density

When density of stained and unstained rubber wood blocks was 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).

Table 8. 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 per cent level
ns non significant

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 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 the fourth months. Comparison of density of the stained wood blocks also showed significant difference between first and fourth months (Table 9).

Table 9. 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^2)	35.84	35.39	0.23(ns)

4.3.2 Compressive strength

No significant difference in compressive strength was found between stained and control samples.(Table 8). 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* was observed.

4.3.3 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.
the samples tested for bending strength, the final density of stained wood blocks was significantly different from that of control wood blocks (Table 10).

Table 10. 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 and SE		
	Treated	Control	t-value
Initial density (Kg/m^3)	580.00 ± 3	580.00 ± 3	1.0(ns)
Final density (Kg/m^3)	540.0 ± 5	640.00 ± 7	11.93**
Bending strength (Kg/mm^2)	82.44 ±3.01	95.11 ± 2.56	4.72**

** significant at P = 0.0lper cent. 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 11). It was also observed that weight of the mycelium which covered the wood block greatly 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 11. Comparison of weight loss and mycelial weight of stained and control rubber wood blocks at the end of 3rd month (Mean of 25 observations)

	Mean ±SE	
	Treated	Control
Weight loss (%)	15.27± 0.33	0 ± 0
Mycelial weight	0.21 ± 0.014	0 ± 0

4.4 DISCUSSION

In the literature not much information is available on changes 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 of

incubation. Results also make it clear that the growth of the stain fungus *B. theobromae* inside the wood is responsible for the changes inside the wood tissues. It is presumed that the utilization of high amount of carbohydrates present in the parenchyma tissues might cause the reduction in density. Tabirih and Seehann (1984)have also found slight 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 has been done 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. In the present cxpeiiment. 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.*

Recently, Encinas and Daniel (1994),with the help of SEM and TEM found that *B.theobromae* mas able to destroy the cell wall of birch *(Betula verrucosa* Ehrh.) but not the tracheids of Carribean pine (*Pinus caribaea* var. *hondurensis* Barr. and Golf) and Scots pine (*Pinus sylvestris* L.). Detailed microscopic examination showed that B. theobromae to cause incipient soft rot of S2 cell wall, delamination of birch fibres and total destruction of parenchyma cells (Encinas and Daniel. 1995).

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 due to the infection of *B. theobromae* on compressive strength of Abachi and birch 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. 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 greater. 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 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.

5. EVALUATION OF CHEMICALS FOR THE CONTROL OF SAPSTAIN

5.1 INTRODUCTION

As soon as a tree is felled. it is prone to microbial degradation, especially by fungi. Wood can be protected from biodeterioration by making it 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 preservative chemicals have been evaluated for their effectiveness but only a very few have been found to possess all the requisite qualities of a good preservative. In late 1960s. sodium penraclorophenoxide (NaPCP)was introduced for effective control of sapstain and mould. It was accepted as an antisapstain chemical throughout the world. However, over the years the growing concern regarding the toxic compounds and their related environmental problems has brought down the use of NaPCP. At present, the ban on the use of pentachlorophenol and NaPCP in most countries has led to the search for alternative anti- sapstain chemicals which are effective and environmentally safe.

5.2 MATERIALS AND METHODS

5.2.1 Screening of fungicides for the control of sapstain/ mould/decay

Different chemicals were screened for their efficacy in controlling sapstain caused by *B. theobromae* on rubber wood. The fungicides evaluated were Caprafol. Busan 1009. Hylite extra and Chlorothalonil.

5.2.2 Evaluation on wood blocks

The efficacy of Captafol (cis-N-{(1,1,2,2tetrachloroethyl thio}-4-cyclohexane-1.2-dicarboximide) was initially tested on sterile rubber wood blocks. Wood blocks were prepared from the bottom log of freshly felled 35-pear-old rubber tree. After converting the log into planks, samples of 50 x 10 x 70 mm blocks

were prepared and selected randomly for the study. Initially, three concentrations of Captafol such as 1.0, 2.0 and 3.0 per cent were tested in the laboratory. The different concentrations were prepared by dissolving weighed quantity of the fungicide in 100 ml of sterile distilled water. Wood blocks were steam sterilized for 15 minutes at 100 kPa and allowed to cool to room temperature. Individual blocks were immersed for 10 seconds separately in different concentrations of the fungicide. The excess solution was drained completely by keeping the blocks in a slanting position in sterile Petri dishes for 10 seconds. Subsequcntly, each block was placed aver glass rod supports kept over two moistened sterile filter papers in each sterile Petri dish. All the treated blocks were inoculated individually with an 8 mm diameter mycelial disc taken from actively growing culture of *B. theobromae*. Wood blocks dipped in sterile distilled water served as control. All the treated blocks were incubated at 28 ± 2°C for 2 weeks and the growth of the fungi aver the wood block was assessed visually using the rating index mentioned in Table 12.

Table 12. Rating index for assessing mould/stain/decay on rubber wood planks in the iield

Rating	Mould/stain/decay growth
1	<10%
2	10-25%
3	25-50%
4	50-75%
5	75-100%

5.2.3 Field evaluation

Various fungicides evaluated for the control of fungal growth in the field arc given in Table 13. For each rreaiment. rubber wood planks of different sizes were prepared from freshly felled rubber tree. Different concentrations of the chemicals were prepared by dissolving appropriate quantities in 251 of water and taken in a clean tray. Saw dust was removed and the planks were dipped individually for 10 seconds in the fungicidal solution and kept in a slanting position to drain the excess solution. After draining, the planks were stacked in different ways as described below.

Table 13. Fungicides evaluated against fungal growth on rubber wood planks in the field

Treat-ment No.	Fungicide	Concen-tration%	Size of planks (mm)	Months of treatment	Method of stacking
1.	Captafol Captafol	2.0 3.0	100 x 12 x 900	December 1994	Close and open (Figs.2a. 2b)
2.	Busan 1009 Busan 1009 Busan 1009 + Boric acid + Borax	1.0 1.5 1.0 + 1.0 + 1.0	100 x 12 x 900	July 1995	Close (Fig. 3)
3.	Hylite Extra Hylite Extra Hylite Extra	0.5 1.0 1.5	100 x 25 x 900	August 1995	Close (Fig. 4)
4.	Kavach Hylite Extra Hylite Extra + boric acid	1.0 1.5 1.0 + 1.0	75 x 25 x 900	September 1995	Close (Fig. 5)
5.	Kavach Kavach Hylite Extra + boric acid	0.5 1.0 0.5 + 1.0	100 x 25 x 900	December 1995	Close (Fig. 6)

Captafol: Field evaluation of captafol was conducted in the sawn wood stacking yard of M/s Evershine Packing case Industries, Ollur, Trichur District, Kerala. Two hundred and forty rubber wood planks were prepared for different treatments. Two concentrations of Captafol namely 2.0 and 3.0 per cent were used. Flanks were dipped in fungicidal solution and after draining, they were stacked in two different ways (Figs. 2a, 2b).

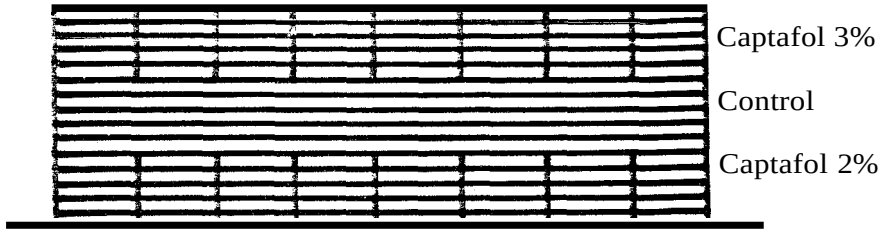

Fig. 2a. Close stacking of rubber wood planks treated with captafol

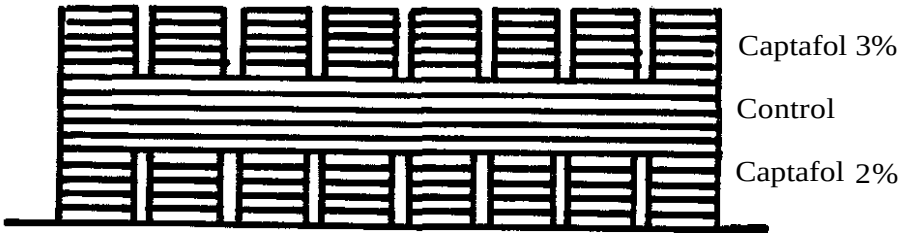

Fig. 2b. Open stacking of rubber wood planks treated with captafol

1. ***Close stacking:*** Of the 80 treated planks, 40 were used for close stacking. The lowest portion of the stack was piled with planks treated with 2.0 per cent Captafol and top portion with 3.0 per cent. In between the two treated piles. 40 control planks (untreated) were stacked. All the planks were closely stacked on the ground under a shed in eight rows of five each. without leaving any space in between planks.

2. ***Open stacking:*** The remaining 40 planks in each treatment were used for open stacking. The lowest part of the stack contained wooden planks treated with 2.0 per cent and the top with 3.0 per cent. Captafol. The planks were stacked in such a way that a space of 50 mm was left in between each plank. The control planks were also stacked separating the two treatments in the same manner as in the case of treated ones. Temperature, relative humidity and rainfall were recorded at the experimental site. Observations on the efficacy of the treatment in terms of fungal growth were recorded at the end of four weeks. Twenty samples each were drawn randomly from the treated as well as control sets. The surface of each plank was observed for mould growth and assessed according to the rating index given in Table 12. The wooden planks were planed and observed for internal sapstain and assessed using the above table. The data were analysed using ANOVA.

Busan 1009: The chemicalBusan 1009 (2-(Thiocyanomethylthio Benzathiazole))was procured from M/s Buckman Laboratories, Singapore. The chemical was tested alone and in combination with boric acid and borax. Field evaluation of Busan 1009 was conducted in the stacking yard of M/s Evershine Packing Industries, Ollur. A total of 200 rubber wood planks were prepared. Two different concentrations of Busan 1009 such as 1.0, 1.5 per cent and a mixture of Busan (1.0) per cent) + borax (1.0) per cent) + boric acid

(1.0 per cent) were prepared as described in section 5.2.3. Treated planks were stacked inside the shed as shown in Fig. 3. The lower portion of the stack consisted of planks treated with 1.0 per cent solution. The control planks were arranged in five rows and in each row there were eight planks. Above is set of eight planks each treated with Busan 1009 (1.0 per cent) + borax (1.0per cent) + boric acid (1.0per cent) stacked in criss-cross manner. The top most part of stack comprised planks treated with 1.5per cent Busan. Each treated pile was separated from the other by control planks in criss-cross manner. Both treated and control planks were stacked close by and kept for one month. The experiment was conducted in the month of July (1994) when there was high humidity and rainfall. congenial for the growth of fungi on wood. Observations on the efficacy of the treatment in terms of fungal growth were recorded at the end of four weeks. Twenty samples of wooden planks from control and treatment sets mere drawn randomly. The surface growth of mould over the planks was assessed according to the rating index given in Table 12. The internal sapstain of the planks was assessed after planing. The data on the rating index of mould and sapstain were analysed using ANOVA.

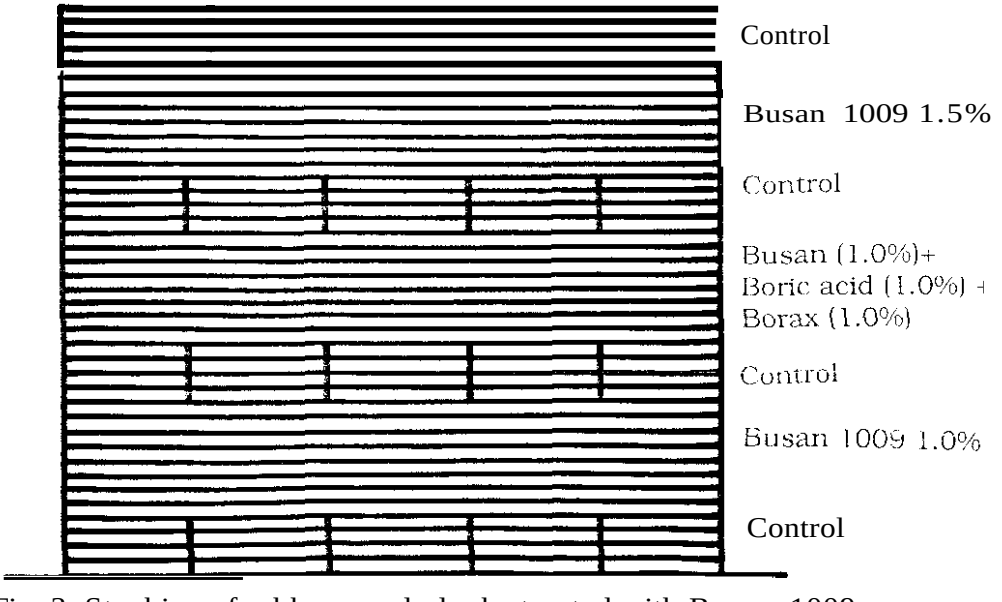

Fig. 3. Stacking of rubber wood planks treated with Busan 1009

Hylite Extra: The chemical Hylite extra (75 per cent carbendazim) was procured from M/s Chemicca, Auckland, New Zealand. The evaluation of the chemical was conducted in the stacking yard of M/s Evershine Packing Industries, Ollur. Three hundred and twenty planks were sawn for the treatment. Three different concentrations of Hylite extra, namely, 0.5. 1.0 and 1.5 per cent were prepared. All the treated and control planks were numbered individually and pooled together and stacked randomly using random numbers (Fig. 4). Of the eighty control planks, forty were arranged in the lowermost portion of the stack and the remaining forty. on the top of the stack over the treattd planks. Observations on the efficacy of the treatment in terms of fungal growth were recorded after one month. Ten samples from each set including control were drawn randomly and observed for the growth of mould. After planing, the intensity of sapstain and decay was assessed according to the rating index given in Table 12. The data were analysed statistically using ANOVA.

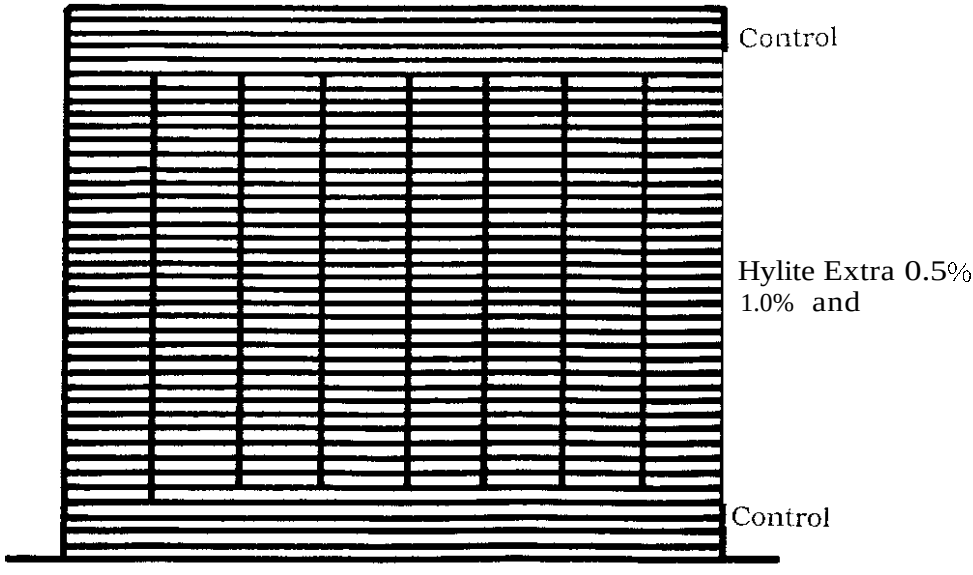

Fig. 4. Stacking of rubber wood planks treated with Hylite Extra

Kavach (Chlorothalonil 75 per cent): Kavach which is commonly used as a broad spectrum fungicide was procured from the local market. The efficacy of this fungicide was compared with that of Hylite extra and Hylite extra + boric acid combination. Field evaluation of this fungicide was conducted in the month of September 1995 at M/s Sitaram Son's Wood Compressors, Padavarad, Trichur. A total of 378 rubber wood planks were prepared and dipped individually in different fungicidal solutions such as Kavach 1.0. 1.5 per cent, Hylite Extra 1.0 per cent and a mixture of Hylite Extra (1.0 per cent) + Boric acid (1.0 per cent]. Seventy two planks were selected for each treatment concentration. Ninety untreated planks of the same size formed the control. Close stacking was done. (Fig. 51. The lower most portion of the stack was arranged with 18 planks (2 rows of 9 each) of control. Above this, planks treated with kavach 1.0 per cent (8 rows of 9 each) were arranged in a criss-cross manner followed by treatments Kavach 1.5 per cent, Hylite Extra 1.0 per cent and combination of Hylite Extra (1.0 per cent) + boric acid (1 .0 per cent). In between two sets of treated planks, 18 numbers of control planks were stacked. Top portion of the stack was also covered with 18 control planks. The stacking was done inside the shed and the observations on the efficacy of the treatment in terms of fungal growth were recorded at the end of one month. Ten samples each were drawn randomly from control as well

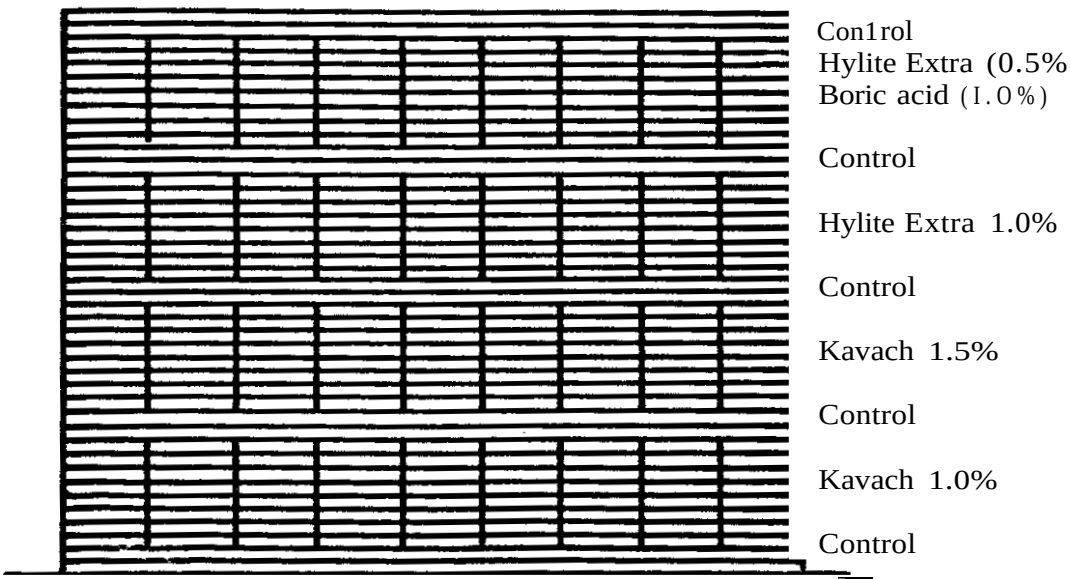

Fig. 5. Stacking of rubber wood planks treated with Kavach and Hylite Extra

as from different treatments. The extent of growth of mould over the surface was recorded and after planing the intensity of sapstain and decay was observed and assessed according to the rating index given earlier. The data were analysed statistically.

In addition to the above trial. there was another field trial conducted to compare lower concentrations of Kavach and Hylite Extra for the control of fungal growth over rubber wood. The experiment was conducted in the month of December 1995. Four different concentrations such as Kavach 0.5, 1.0: Hylite Extra 0.5 per cent and a combination of Hylite Extra (0.5 per cent) + Boric acid I1.0 per cent) were evaluated. In each treatment, 72 numbers of planks were used while a total of 80 planks were maintained as control. After the treatment, all the planks including those of control were numbered serially before stacking. The planks were close stacked inside a shed for one month (Fig. 61. The lowermost portion of the stack was of control planks arranged in eight rows of 2 planks each . Over these planks treated with Kavach 0.5 and 1.0 per cent were arranged using random numbers in a criss-cross manner. After stacking 72 planks of both Kavach 0.5 and 1.0 per cent. a layer of 16 control planks was also stacked. The remaining 72 planks of the above treatments were randomly arranged over the control. Over the

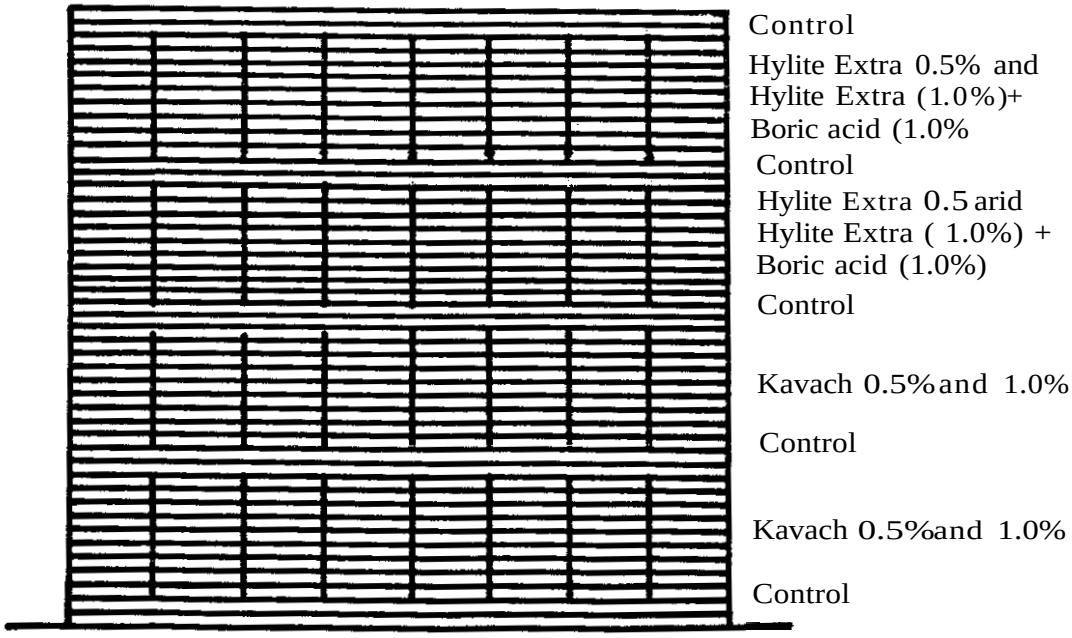

Fig. 6. Stacking of rubber wood planks treated with Kavach and Hylite Extra + Boric acid

control planks, 72 numbers of treated planks of Hylite Extra (0.5per cent) and Hylite Extra (0.5per cent) + boric acid (1.0 per cent) combination were mixed and stacked randomly in a criss-cross manner. The top most portion of the stack was covered by control planks. Observations on the efficacy of the treatment, in terms of fungal growth, were recorded at the end of one month. Ten samples from each treatment including control were drawn randomly and observed for the growth of mould. After planing the intensity of sapstain and decay was recorded and assessed according to the rating index given in Table 12. The data were analysed statistically using ANOVA.

5.3 RESULTS

5.3.1 Screening of fungicides for the control of sapstain/ mould/decay

The fungicide Captafol was tested both in the laboratory and in the field. Other fungicides were tested only in the field.

5.3.2 Laboratory Evaluation on wood blocks

Results of the evaluation of three different concentrations (1.0. 2.0 and 3.0 per cent) of Captafol using sterile wood blocks revealed that all the concentrations inhibited the growth of *B. theobromae* whereas in control. 100 per cent fungal growth was observed.

5.3.3 Field evaluation

Captafol: Results of the field evaluation of Captafol (Table 14)indicated that the percentage of stain and mould growth on the planks was less in the open stacking when compared lo close stacking. Among the two different concentrations of Captafol. 3.0 per cent solution showed maximum inhibition of stain and mould fungi. The ANOVA showed that there was no significant difference in mould growth among the concentrations in open and close stacking. However, when compared to control, significant difference was noted both in close and open stacking. In the case of stain also, no significant difference between 2.0 per cent and 3.0 per cent Captafol was noted for open and close stacking. But when compared to control, significant difference in the treated planks was noted both in open and close stacking. In the control. planks had severe stain and mould growth and there was no difference in the intensity of infection between close and open stacking.

Table 14. Field evaluation of Captafol for the control of mould and sapstain on rubber wood (Mean of 20 observations)

No.	Treatments	Concent-ration %	Mean Rating Index*	
			Mould	Stain
1.	Captafol close stacking	2.0	1.90^{b}	0.85^{b}
2.	Captafol open stacking	2.0	0.90^{a}	0.20^{a}
3.	Captafol close stacking	3.0	1.95^{b}	0.85^{b}
4.	Captafol open stacking	3.0	0.75^{a}	0.10^{a}
5.	Control open stacking	-	4.95^{d}	4.40^{c}
6.	Control close stacking	-	4.45^{c}	4.30^{c}

Figures in a column superscribed by different letters are significantly different.
* for growth rate index see Table 12.

Busan 1009: The results of the field testing (Table 15) indicated that among the three different treatments, Busan (1.0 per cent) + boric acid (1.0 per cent) + borax (1.0 per cent) to be the best in controlling the mould and stain growth over the planks, as it was statistically significant from the other treatments. But when compared to control, all the three treatments were significantly different. In the case of stain, 1.5 per cent of Busan 1009 was significantly different from all the other treatments.

Treatments	Concentration	Mean Rating Index*	
Busan 1009	1.0	2.35^{c}	1.55^{b}
Busan 1009	1.5	1.85^{b}	0.90^{a}
Busan 1009 + Boric acid + Borax	1.0 + 1.0 + 1.0	1.35^{a}	1.35^{b}
Control	-	4.42^{d}	3.67^{c}

Figures in a column superscribed by different letters are significantly different.
* for growth rate index see Table 12.

Hylite Extra: Mould, stain and decay infections were recorded and no significant difference between the three concentrations (0.5.1.0 and 1.5per cent) of the fungicide was noted in stain infection (Table 16); but control planks differed significantly from all treatments. Among the three different concentrations, 1.5 per cent of Hylite Extra was found to be the best in controlling stain and decay fungi.

Table 16. Field evaluation of Hylite extra for the control of mould, sapstain and decay (Mean of 10 observations)

No.	Treatments	Concent-ration %	Mean Rating Index'		
			Mould	Stain	Decay
1.	Hylite Extra	0.5	0.7^{a}	0.8^{a}	1.7^{ab}
2.	Hylite Extra	1.0	0.5^{a}	1.8^{b}	1.0^{a}
3.	Hylite Extra	1.5	0.6^{a}	1.3^{ab}	0.7^{a}
4.	Control	-	3.9^{b}	2.7^{c}	2.4^{b}

Figures in a column superscribed by different letters are significantlydifferent.
* for growth rate index see Table 12.

Kavach: When Kavach was compared with Hylite Extra, it was found that Hylite Extra +boric acid combination was the best in controlling mould. stain and decay fungi (Table 17). Hylite Extra (1.0per cent) was also found to be equally effective for the control of stain when compared to Hylite Extra + boric

Table 17. Field evaluation of Kavach for the control of mould, sapstain and decay (Mean of 10 observations)

No.	Treatments	Concent-ration %	Mean Rating Index*		
			Mould	Stain	Decay
1.	Kavach	1.0	1.1^{ab}	2.3^{bc}	0.3^{a}
2.	Kavach	1.5	2.7^{c}	3.3^{d}	0.4^{a}
3.	Hylite Extra	1.0	1.5^{b}	1.7^{ab}	0.5^{a}
4.	Hylite Extra + Boric acid	1.0 + 1.0	0.3^{a}	1.3^{a}	0.2^{a}
5.	Control	-	3.1^{c}	2.5^{c}	1.0^{a}

Figures in a column superscribed by different letters are significantly different.
* for growth rate index see Table 12.

acid combination. Both the concentrations of Kavach were found to be effective in controlling the stain. In the experiment, where lower concentrations of Kavach were compared with that of Hylite Extra, it was found that Kavach 0.5 and 1.0 per cent were equally effective in controlling fungi causing mould and stain. When compared to control all the four different treatments such as Kavach 0.5 and 1.0 per cent, Hylite Extra 1.0 per cent and Hylite Extra + boric acid combination were statistically significant (Table 18). But none of the fungicidal concentrations could give 100 per cent control of the fungal growth.

Table 18. Comparison of Kavach and Hylite extra for the control of mould, sapstain and decay (Mean of 10 observations)

No.	Treatments	Concentration %	Mean Rating Index'		
			Mould	Stain	Decay
1.	Kavach	0.5	0.6^{a}	1.5^{a}	0.0
2.	Kavach	1.0	1.0^{ab}	1.3^{a}	0.0
3.	Hylite Extra + boric acid	0.5 + 1.0	1.7^{b}	1.3^{a}	0.0
4.	Hylite Extra	0.5	1.8^{b}	1.1^{a}	0.0
5.	Control	-	4.4^{c}	4.4^{b}	0.0

Figures in a column superscribed by different letters are significantly different.
* for growth rate index see Table 12.

5.4 DISCUSSION

Captafol, a common fungicide used in agriculture. has been found to be effective in controlling sapstain and mould on timber in various tropical and temperate countries (Butcher, 1973: 1980: Hong *et al.*. 1980: Hong. 1981: Cassens and Eslyn. 1981: Leightley, 1985). Low concentration of Captafol such as 0.2 per cent (a.i.)was found to control 84 to 100 per cent of mould and stain on radiata pine wood. Also it was found effective against sapstain and mould fungi on Pinus elliotti in Queensland, Australia. (Leightley, 1985). In New Zealand also a concentration of 0.2 per cent (a.i.)of Captafol was recommended for fungal control in boron-diffused timber (Butcher, 1980). However, in a few cases Captafol was not found to be effective to give 100 per cent control of fungal growth on wood. In general, in tropical countries lower dosages of Captafol proved to be ineffective. In the present experiment also,

only a high concentration of 2.0 per cent controlled more than 80 per cent of mould and sapstain under open stacking. It was found that the fungal infection was much less in open stacking than in close stacking. The high fungal infection in close stacking could possibly be due to the accumulation of high moisture content of wood inside the stack, thus enhancing the fungal growth. In open stacking, due to the circulation of air the moisture content of wood tends to come down. It was also found that higher dosages of Captafol (3.0 per cent) could not control cent per cent fungal infection. This may be probably due to the presence of high amount of inoculum present in the atmosphere. and also conducive environmental conditions such as high humidity and low temperature during night favouring the fungal growth. Lower concentrations (0.5 and 1.0 per cent (a.i.) of Captafol were found not effective in controlling sapstain and mould in yellow poplar *Liriodendron tulipifera* L. (Cassens and Eslyn, 19811. Only a high concentration such as 2.0 per cent (a.i.) in Shellkote *3* was found to be effective in controlling mould and sapstain on rubber wood in Malaysia (Hong *et al.,* 1980).

Busan 1009. an emulsion formulation containing 10 per cent of 2-thiocyano methylthio-benzothiazole and 10 per cent methylene bis-thiocyanate is widely used in temperate countries for the control of stain and mould growth on wood (Plackett. 1984; Drysdale. 1983: 1986: 1987: Leightley, 1986: Drysdale *et al.*. 1986 and Drysdale and Plackett, 1987). In the present field trial, though Busan 1009 + boric acid combination controlled effectively mould and stain fungi on rubber wood but not cent per cent. However. Drysdale (1983) obtained almost 100 per cent control of fungal stain at a concentration of 1.0 per cent. Treatment of *Pinus radiata* pulp pellets with a lower concentration of 0.3 per cent of Busan 1009, the growth of stain and decay fungi was controlled whereas the mould growth was heavy. For long term protection, higher concentration of the chemical is required. Busan 1009 at 1.5 per cent was found to be very effective in the long-term protection of round wood of *Pinus radiata* (Drysdale, 1987). In this trial. a higher concentration was used because the smooth surfaces of the round wood take up less solution than rough-sawn timber and the round wood takes more time for drying. Drysdale *et al.* (1986) also revealed that for long term protection, higher concentration of the preservative is needed. In the present experiment, 100 per cent inhibition of various fungi was not achieved because the treatment was conducted at a period when there was high rain fall and high humidity (Table 19). Another reason for incomplete control also may be due to close stacking of the wooden planks. Possibly during rainy period a higher concentration of the chemicals will be needed whereas in dry period, concentration can be reduced. Furthermore. concentration of the fungicide can also be reduced, if the planks are open stacked because it will reduce the moisture content of planks thus reducing the fungal infection.

Table 19. Monthly averages of weather data for 1995 at Trichur

Month	Mean Temp. (°C)	Mean r.h. (%)			Rainfall (mm)
	Max.	Min.	Max.	Min.	
January	34.7	20.5	79.0	47.0	0 (0)
February	37.1	22.1	81.0	44.0	0 (0)
March	39.2	22.8	82.0	36.0	2 (0)
April	38.5	23.7	81.0	46.0	45 (1)
May	34.1	22.9	82.0	58.0	333 (10)
June	31.9	22.9	83.0	66.0	463 (15)
July	29.5	21.7	84.0	70.0	838 (22)
August	30.4	22.1	84.0	67.0	401 (9)
September	31.3	22.2	85.0	69.0	330 (11)
October	33.5	22.0	84.3	63.5	111 (3)
November	32.7	21.6	84.0	58.0	56 (3)
December	33.6	19.9	78.0	43.0	0 (0)

Note: r-.h.: Relative humidity; the ligures in parenthesis indicate the number of rainy days when rainfall was > = 10 mm.

Hylite 20 F (20 per cent Carbendazim) mas also tested as a wood preservative for the control of stain and mould in various countries (Drysdale. 1986: 1987 and Drysdale and Keirle, 1986). In the present field evaluation. Hylite extra (75 per cent Carbendazim). a modified formulation of Hylite 20 F, was evaluated at three different concentrations namely 0.5. 1.0 and 1.5 and 1.5 per cent was found to be more or less equally effective 165-80 per cent) in controlling mould, stain and decay. It is interesting to note that increased solution strength apparently gave no real improvement in performance. This may be due to the efficiency of the fungicide to control fungal growth at lower concentrations. Hylite 20 F at a concentration of 0.5 and 1.0 per cent has been reported to be effective up to 4 months but the treatments were ineffective after 6 months (Drysdale, 1986). In the present experiment, even 1.5 per cent of Hylite extra was not 100 per cent effective. This is because the concentrations used in temperate countries may not be sufficient for the control of fungal growth in tropical countries: the tropical warm-humid climatic conditions especially in Kerala. are congenial for the luxuriant growth of fungi on wood

The performance of Hylite Extra was compared with an agricultural fungicide Kavach (Chlorothalonil) and it was found to be equally effective. Kavach controlled the fungal growth over the planks up to 90 per cent. The effectiveness of Kavach was inconsistent in the two trials conducted in the field. Kavach 1.0 and 1.5 per cent are not able to control mould and stain when compared to untreated planks (Table 17) whereas Kavach 0.5 and 1.0 per cent were effective in controlling mould and stain fungi, respectively (Table 18). The reasons that can be attributed for this inconsistent behaviour are the different climatic conditions which prevailed during the two field trials. As there was continuous rain for 10 days after the first trial (Table 19) severe fungal growth might have occurred on the wooden planks. During the month of November, the rainfall was almost nil and hence there was control in the fungal growth. Chlorothalonil is a commercially important fungicide with many industrial and agricultural applications. It has a very low mammalian toxicity and is considered to be an environmentally safe chemical. The suitability of chlorothalonil for the control of sapstain was reported by Butcher and Drysdale (1978) Hayward *et al.* (1984) Micales *et al.* (1989) and Laks *et al.* (1991). Laks *et al.* (1991) reported that chlorothalonil at 1.0 per cent performed as good as NaPCP. Micales *et al.* (1989) also obtained very good control when the concentration of chlorothalonil was increased to 1.0 per cent. In the present experiment also comparatively better control of stain and mould is obtained by using 1 per cent solution of chlorothalonil.

It can be concluded that open stacking of the planks reduces the fungal infection. When the environmental conditions are favourable for fungal growth (during rainy season) possibly the planks can be treated with higher concentration of the fungicide. Similarly, during dry period, when the fungal infection is minimal, less concentration of the fungicide may be effective.

6. CONCLUSIONS

1. Sapstain caused by *B. theobromae* effects weight loss in rubber wood over a period of 4 months.

2. No growth of *B. theobromae* occurred at and below 23.8 per cent moisture content of rubber wood. Moisture content above 29 per cent favoured good growth of the fungus.

3. If the fungus is already established in rubber wood, it can tolerate higher temperatures of 40 and 50° C for 7 days.

4. No reduction in compressive strength of stained rubber wood was noted whereas density and bending strength was reduced when compared to control.

5. Busan 1009 (1 per cent) in combination with boric acid (1 per cent) was found to be effective in controlling sapstain.

6. Chlorothalonil (Kavach), a locally available fungicide, was also found to be effective in controlling sapstain.

7. Open stacking was found to reduce the fungal infection on the wooden planks.

8. During rainy season, the concentration of the fungicide has to be increased for better control of fungal growth.

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