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SAPSTAIN FUNGI OF SOME COMMERCIALLY IMPORTANT TIMBERS AND THEIR CHEMICAL CONTROL

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ABSTRACT

Sapstain caused by fungi results in considerable qualitative loss of wood products. Due to favourable environmental conditions such as high rainfall and humidity, sapstain is a serious problem in Kerala. A preliminary survey conducted in various wood based industries in different districts of Kerala State revealed that sapstain and mould growth were caused by a number of fungi. Eight commercially important timber species were selected for the study and *Botryodiplodia theobromae* was found to be the dominant fungus causing sapstain on all the timbers through out the year. Studies revealed that in rubber wood infected by *B. theobromae* there was a weight loss of 8.0% in the first month which increased to 12.2% by the end of fourth month. In *Ailanthus triphysa* the weight loss increased from 4.3% in the first month to 10.1% in the fourth month. But in *Alstonia scholaris* only 4% weight loss was recorded by the end of fourth month. The results clearly showed that rubber wood was easily susceptible to sapstain by *B.* theobronzae. Effect of wood moisture content and microclimatic factors on growth of *B. theobromae* was studied and it was found that the growth of the fungus was influenced by high relative humidity (90 and 100%). When the moisture content of timber was reduced to <24%, the fungal growth on timber was very much restricted. Among the various fungicides/chemicals tested, sodium azide proved to be the best to control both sapstain and mould fungi in the laboratory. The chemical was effective even at lower concentrations viz. 250 and 500 ppm. A bacterium viz. *Bacillus subtilis,* isolated from rubber wood, showed antagonism against several stain and mould fungi. The efficacy of this isolate was tested in laboratory and proved to be effective in controlling the growth of several stain fungi. In field tests also, the bacterium was effective in preventing the fungal growth on rubber wood up to 80%.

INTRODUCTION

Fungi cause different kinds of damages to timber viz. decay, soft rot, sapstain and mould. Mould occurs on the surface of the timber and generally has a woolly or powdery appearance. Moulds cause superficial staining due to the presence of mycelium as well as their coloured spore masses. Discolouration of sapwood caused by fungi is commonly known as sapstain. As the colour that these fungi impart to the wood is frequently bluish-gray, the stain is often referred to as blue stain. Stain due to fungi may be superficial or penetrating. Deep seated penetrating stains are caused by fungi that have dark coloured hyphae or that produce pigments diffusing into the wood tissues. Soon after the tree is felled, the cut ends of the logs and portions from which the bark has been removed, get exposed to infection by spores of staining fungi.

Discolouration of wood is one of the defects which causes considerable loss (in quality) of wood products. Sapstain, besides spoiling the appearance of the timber, affects the goods stored in contact with the stained wood. In paper industry, when stained timber is used, more chemicals are required to bleach the pulp. Due to the susceptible nature of some of the timbers to sapstain attack, they are processed at the earliest to avoid infection and this urgency often causes practical difficulties to the manufacturers.

In Kerala, small scale wood-based industries such as packing case, ply-wood and match units, utilize large quantity of wood from miscellaneous tree species. It is estimated that there are about 460 packing case, 81 plywood and 144 match units in the State. The major timber species employed in match industries in Kerala are Bombax ceiba (Linn.), *Ailanthus triphysa* (Dennst) Alston, *Alstonia scholaris* (Linn.) R. Br and some other soft woods available. In recent years, with the dwindling natural forest resources it has become necessary to depend on other alternative timber sources to meet the current and future needs of wood. *Hevea brasiliensis* (HBK.)Muell. Arg. (rubber wood) with some positive attributes, merits consideration when we look for an alternative and cheap timber. After extracting the latex, rubber trees are felled for replanting the area and the wood thus obtained is being utilized now-a-days as the main source of wood for manufacturing packing-cases.

In India, not much work has been done on the effect of sapstain fungi on wood. Some studies on decay and sapstain of timbers, their causes and prevention were conducted by Bakshi (1953). The cause of discolouration of rosewood *(Dalbergia latifolia* (Roxb.) veneers was investigated by Ananthanarayanan, (1971). Due to favourable environmental conditions such as high rainfall and humidity, sapstain is a serious problem in Kerala. However, no work has been done on sapstain of commercially important timbers in the State. This was the relevance and context of the present study, attempting to find out the microorganisms responsible for sapstain in selected timbers and possibilities of their chemical

control. Due to the commercial importance of rubber wood and its high susceptibility to fungal attack studies were carried out to find out the sapstain fungi particularly on this timber. Since *Botryodiplodia theobromae* Pat. was the dominant fungus causing stain on most of the timbers, studies were also undertaken to investigate various aspects of infection by this fungus on rubber wood.

MATERIALS AND METHODS

Survey

A preliminary survey was conducted in some of the wood-based industries such as plywood, packing case and match factories in Trichur District and eight timber species were selected for the study, viz., *Ailanthus triphysa* (Dennst.), Alston (Matty), *Alstonia scholaris* (L m.) R. Br. (Ezhilam-pala), *Anacardium occidentale* Linn. (Cashew), *Bombaxceiba* (Linn.) (Mullilavu), *Erythrina stricta* Roxb. (Murukku), *Hevea brasiliensis* (HBK.) Muell. Arg. (Rubber), *Macaranga peltata* (Roxb.) Muell. Arg. (Vatta) and *Mangifera indica* Linn. (Mango). Wood samples of these species were collected at monthly interval from wood based industries in Trichur District to isolate the mould and stain fungi growing on them. In addition, samples were also collected from Calicut, Malappuram, Palghat, Ernakulam, Kottayam, Alleppey, Quilon and Trivandrum Districts, but only once.

Isolation of microorganisms

A small piece of the stained wood was surface sterilized using 0.1% $HgCl_2$ solution, washed thoroughly in several changes of sterile distilled water and plated on potato dextrose agar (PDA). The plates were incubated at 28 ± 2^0C and observed for the growth of any fungus. After 5 days, the fungus growing around the wood sample was isolated, purified and maintained on PDA slants for further studies.

Identification of isolates

Cultural and morphological characteristics of isolates were studied and for the confirmation of their identity, cultures were referred to the CAB International Mycological Institute, Kew, UK. These cultures were periodically subcultured and stored for further investigations.

Susceptibility test

Fungi isolated from various timbers were evaluated for their ability to stain healthy wood. For inoculation, unstained sapwood blocks (7 x 5 x 1 cm) were cut from freshly harvested green timber and steam sterilized for 15 minutes. A disc of 8 mm dia taken from the edge of an actively growing culture of the test fungus was placed over the surface of each block. The ,inoculated blocks were

then placed on'glass rod supports over moistened sterile filter papers in sterile Petri dishes and these set-ups were incubated at 28 + 2°C for 15 days. Each isolate was tested on 5 replicate blocks; controls were also maintained with plain agar discs. After the incubation period, the inoculated blocks were examined for growth of the fungi on the surface and internal stain after splitting them open.

Weight loss due to *Botryodiplodia theobromae*

Weight loss in wood blocks of *Ailanthus triphysa, Alstonia scholaris,* and *Hevea brasiliensis* (widely used for making match splints/packing cases) due to the infection by Botryodiplodia *theobromae* Pat., the most dominant fungus causing sapstain was studied over a period of four months following the procedure described by Stranks (1976). Forty test blocks (5.5 x 1.5 x 0.5cm size) of each timber were cut from clear fresh sapwood, oven dried at 105°C for a few days till the weight became constant and their initial dry weight recorded. The sample blocks were then placed over a wet filter paper in a closed damp chamber to bring them back to normal moisture equilibrium. Following steam sterilization for 15 min twenty blocks were inoculated with 8 mm dia agar disc taken from the edge of a 5-day-old culture of B. *theobromae.* These blocks were incubated in large test tubes (14.0 x 25.0 cm) containing 15ml of sterile water. The atmosphere around the inoculated blocks was humidified by inserting a paper wick (2.5 x 10 cm) covering the entire length of the block. Each tube contained 15 ml of sterile water in which the lower end of the wick was immersed; the inoculated wood blocks were kept free of direct water contact by glass supports projecting above the water level. The tubes were closed with cotton plugs (Fig. 1). The remaining blocks were inoculated with plain agar discs and these blocks were used as control. The set-ups were incubated at 28 + 2°C. Every month, five blocks each from inoculated and control sets were taken out from the tubes. After removing the mycelial growth under running tap water, the blocks were dried at 105°C for a few days and their weight recorded.

Effect of wood moisture content and microclimatic factors on the growth of *B. theobromae*

Effect of varying moisture content (MC) of rubber wood on the growth of *Botryodiplodia theobromae* was studied at three different temperatures (T) and five different relative humidity (RH) regimes. Different RH levels were maintained using saturated solutions of various salts (O'Brien, 1948). The salt solutions used for maintaining different relative humidities were prepared in sterile water and each sterile Horlicks bottle contained 30 ml of solution (Table 1). For maintaining cent percent humidity, 30 ml of sterile water was used in the bottles. Fresh oven dried rubber wood blocks (3.3x 4.0 x 2.5 cm) were immersed in water for appropriate periods so that they attain approximate MC of 50, 75 and 100%. These wood blocks were then steam sterilized for 15 minutes and inoculated with an 8 mm dia disc.

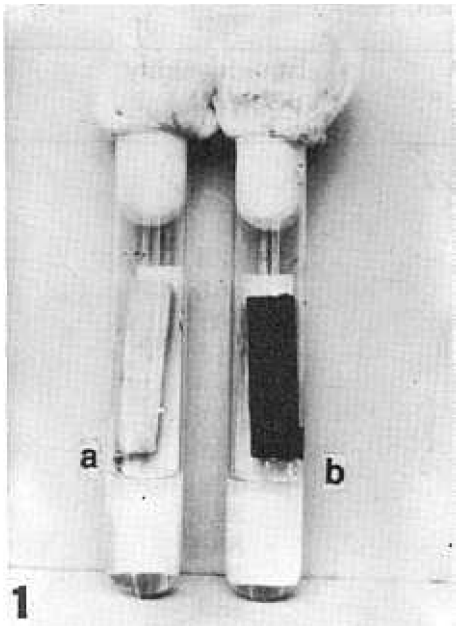
Table **1** Relative humidities over saturated solutions at various temperatures (after O'Brien, 1948).

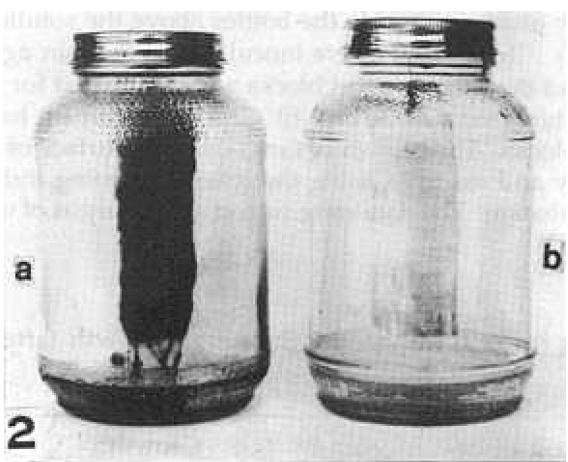
Salt	Relative humidity percentage	Temperature ^{0}C
Water	100	20
$ZnSO_4$ $7H_2O$	90	20
NH_4CI	80	20
NH_4NO,	67.4	20
$Ca(NO_3)_2$ $4H_2O$	56.0	20
Water	100	30
KNO_3	91.5	30
$(NH_4)_2SO_4$	81.0	30
$CO(NH_2)_2$	72.9	30
NH_4 NO_3	60.0	30
Water	100	40
KNO_3	89.4	40
KC1	82.0	40
$NaNO_3$	70.5	40
$CO(NH_2)_2$	68.7	40

taken from an actively growing *B. theobromae* culture. The wood blocks were selected randomly and hung inside the bottles above the solution using stainless steel wire (Fig 2a). The controls were inoculated with plain agar discs (Fig. 2b). The inoculated and the control wood blocks were incubated for 3 months at three different temperatures viz. 20,30 and 40 ^{0}C. Each treatment had five inoculated and five control blocks. The growth of fungi over the surface of wood blocks was examined visually and assessed using the following rating index (Table 2) after four weeks of incubation. The data were subjected to analysis of variance (Snedecor and Cochran, 1967).

Table **2** Rating index for assessing the fungal growth (after Plackett, 1982).

Rating	Growth
0	No growth
1	Trace growth
2	Light growth
3	Medium growth
4	Heavy growth





Figs. 1 - 2. 1a. Control block inoculated with plain agar disc. 1b. Test tubes **with rubber wood blocks inoculated with *B. theobromae* for assessing weight loss. 2a. Rubber wood block inoculated with *B. theobromae.* 2b. Control block inoculated with plain agar disc.**

Optimum moisture content of wood for the growth of B. theobromae

Optimum moisture content of rubber wood required for maximum growth of B. *theobromae* was determined. Two hundred wood blocks (7.0 x 5.0 x 1.0 cm) made from freshly sawn rubber wood were kept in an incubator maintained at 35°C. Twenty sample blocks were removed each day for 10 consecutive days from the incubator and steam sterilized for 15 minutes. The initial weight of 10 sterilized blocks was recorded and blocks were oven dried at 105°C for the estimation of final weight. Of the remaining 10 blocks, five were inoculated with 8 mm dia disc of an actively growing culture of *B. theobromue* and the rest with plain agar disc to serve as control. All the blocks were incubated in sterile Petri dishes for 2 weeks at 28 + 2°C. Moisture content of the inoculated blocks was determined by calculating the corresponding oven dry weight of blocks. After the incubation period, the inoculated blocks were observed for the growth of the fungus on the surface. The growth was assessed visually using the rating index as given under Table 2.

Chemical control of sapstain and mould growth

(a) Evaluation of various fungicides using sterile wood blocks

The efficacy of some of the commonly available fungicides viz. carbendazim, carboxin, copper oxychloride, mancozeb, sodium azide, thiram and ziram were tested at 1% a.i. against stain as well as mould fungi. Further, the efficacy of sodium azide was tested at concentrations of 0.1, 0.25, 0.5 and 0.75 %. Since higher concentrations were found to be effective in controlling stain and mould fungi, the chemical was also tested in lower concentrations. (10, 50, 100, 250 and 500 ppm). All fungicidal evaluations were done using freshly sawn rubber wood blocks (7.0 x 5.0 x 1.0 cm size) as rubber wood was very susceptible to mould, sapstain and decay fungi (Ali et al., 1980).

Sterile wood blocks were dipped in fungicidal solution for 30 seconds and excess solution drained completely before placing inside the Petri dishes for inoculation. The test wood blocks were inoculated with 8 mm dia disc from actively growing cultures of *Botryodiplodia theobromae, Cerutocystisfimbriata* Ellis & Halstead, *Acremoniunr* spp., *Fusarium* sp., *Scytalidium lignicola* Pesante, *Aspergillus niger* Van Tieghem, *Aspergillus flavus* Link ex Gray, *Trichoderma viride* Pers. ex Gray, and *Memnoniella echinata* (Riv.) Galloway. Wood blocks dipped in distilled water served as control and blocks dipped in solutions containing 0.5% sodium pentachlorophenoxide (NaPCP) plus 1.5% borax as standard (Butcher, 1980). For each fungicidal treatment, there were six replicate wood blocks. The Petri dishes were incubated at 28 + 2°C for 2 weeks. The fungal growth observed on the blocks was visually assessed using the rating index (Table 2) and the percentage inhibition of fungal growth over control calculated.

As captafol had been found to be very effective against stain and decay fungi (Butcher, 1980) three different concentrations (1.0%, 1.5% and 2.0% a.i.) of this fungicide were also tested.

(b) Evaluation of sodium azide against stain and mould fungi using fresh nonsterile wood blocks

Since sodium azide has proved to be a promising chemical for the control of stain and mould fungi even at very low concentrations, some more detailed studies involving this chemical were attempted. The efficacy of sodium azide to protect fresh nonsterile rubber wood blocks from fungal growth was studied over a period of one month. Twenty five freshly cut unstained rubber wood blocks were dipped in sodium azide solution of 0.1,0.25,0.5 and 0.75% concentration for 30 seconds and kept inside nonsterile Petri dishes moistened with filter papers to maintain humidity. Fungal growth over the blocks was recorded after four weeks. The percentage of control of fungal growth was calculated by counting infected and healthy wood blocks.

(c) Evaluation of sodium azide against stain and mould fungi using preincubated nonsterile wood blocks

The effect of sodium azide to control mould and stain fungi was tested using nonsterile preincubated wood blocks. Twenty five freshly cut rubber wood blocks were kept in open air at the saw mill for an hour. These blocks were then transferred to a humid chamber for 2 days so that the spores of the mould as well as stain fungi fallen over the blocks grew and established over the wood blocks. These wood blocks were dipped in sodium azide solutions of 0.25,0.5, and 0.75% concentration for 30 seconds and incubated in Petri dishes. Observations were taken at weekly intervals for one month. The growth of the fungus over the blocks was assessed visually. The efficacy of the chemical was calculated by counting the number of infected and healthy wood blocks and the data on the percentage of inhibition of fungal growth at the end of the fourth week tested by standard normal deviate test.

(d) Effect of dipping time on the control of fungal growth over the wood blocks

Freshly cut rubber wood blocks were dipped in sodium azide solutions of 0.1, 0.25, 0.5 % concentration for 10, 20 and 30 minutes. The excess solution was drained off from the wood blocks and incubated in Petri dishes for four weeks. Control blocks were dipped in sterile water for 10, 20 and 30 minutes. Weekly observations on fungal growth over the wood blocks were taken for a period of one month. The percentage of control of fungal growth was calculated by recording the number of infected and noninfected wood blocks and the data analysed statistically using standard normal deviate test.

Biological control

(a) Testing of the bacterium on culture medium

The bacterium, *Bacillus subtilis* (Ehrenberg) Cohn which had earlier been evaluated as a potential biocontrol agent for sapstain and mould growth on unseasoned timbers (Seifert et al., 1987) was found to colonise the surface of rubber wood. The antagonistic ability of the bacterial isolate against common mould, stain and pathogenic fungi such as *Aspergillus flavus,* A. *niger, Trichoderma viride, Botyodiplodiu theobroniue, Acremonium* sp., *Scytalidium lignicolu, Ceratocystis fimbriata Fusarium solani* (Mart.) Sacc., *Pestalotiopsis* sp., *Colletotrichum gloeosporioides* (Penz.) Sacc., *Sclerotiuiti rolfsii* Saccardo, *Corticium salmonicolor* Berk & Br., *Pythium* sp. and *Rhizoctonia solani* Kuhn. was evaluated using the agar plate technique. A streak of the bacterium was made in the centre of a 9 cm dia Petri dish containing 20 ml of PDA and the test fungus was inoculated on either side of the streak (Figs. 7, 8). The control Petri dishes were inoculated with the fungus alone. Radial growth of the inoculated fungus with and without bacterium was measured at 3-day intervals for two weeks. The distance between the mycelial tips and the margin of the bacterial colony was also measured at each observation.

(b) Testing of the bacterium on wood blocks

The efficacy of *B. subtilis* in inhibiting the fungal growth on the surface of wood blocks of rubber, *A. triphysa* and *A. scholaris* was studied. Wood blocks (7.0 x 5.0 x 1.0 cm size) of each species sterilized for 15 minutes were dipped for 5 seconds in bacterial suspension. The suspension was prepared from a 48-hr-old culture grown in nutrient broth. The excess suspension was drained off before placing the blocks in sterile Petri dishes, moistened with sterile filter paper for maintaining huqidity. Treated and control wood blocks were inoculated with an 8 mm dia disc of actively growing test fungi. The Petri dishes were incubated at $28 \pm 2\ ^0C$ (Figs. 9/10) and observations on fungal growth over the wood blocks recorded after two weeks.

(c) Field testing the efficacy of bacterium

Based on the laboratory results, the efficacy of bacterium in controlling sapstain and mould fungi was tested in Evershine Packing case Industries, Ollur, Trichur District using rubber wood planks. Wood planks (30 x 15 x 1 cm size) from freshly felled rubber trees were used in different treatments. For each treatment 10 litres of bacterial suspension was prepared by inoculating a loopfull of the bacterium in each of 20 flasks containing 500 ml of PDA broth; the culture was incubated for 15 days. The treatments were carried out as follows.

Treatment 1 : Dipping and stacking without space

The rubber wood planks were dipped in bacterial suspension for 10 seconds, drained completely to remove excess solution and then piled one above the other. Each pile comprised of four individual planks and for each observation there were four replicates. Control planks were dipped in water and stacked in the same manner.

Treatment 2 : Dipping and stacking with space

After dipping in bacterial suspension the planks were stacked one above the other, but separated from each other by a reeper of size 15 x 6 x 1.0 cm so as to have some air space in between the planks. Controls dipped in water were also stacked in the same way.

Treatment 3 : Spraying and stacking without space

The wood planks were uniformly sprayed with the bacterial suspension using a sprayer. Controls sprayed with water were also maintained. Planks were then stacked without any space in between.

Treatment 4 : Spraying and stacking with space

Planks were stacked one above the other after spraying with bacterial suspension, but separated individually using a reeper. Controls were maintained by spraying with water.

Observations on fungal growth were recorded fortnightly for a period of two months. At each observation, samples were taken randomly and outline of fungal growth on either side of the planks was marked and drawn on a paper. The area covered by fungal growth as marked on the paper was then determined using a 'Licor' leaf area meter. The data obtained at the end of the fourth week were subjected to analysis of variance.

RESULTS AND DISCUSSION

The common stain causing fungi isolated from different timber species such as *Hevea brasiliensis, Ailanthus triphysa, Bombax ceiba, Alstonia scholaris, Mangifera indica, Anacardium occidentale, Macaranga peltata* and *Erythrina stricta* were *Botryodiplodia theobrontae, Ceratocystis fimbriata, Acremonium* spp., *Scytalidium lignicola* and *Fusarium* spp. of which B. *theobromae* was the most dominant on causing stain (Figs. 3, 4). B. *theobrontae* was reported as the most prevalent sapstain fungus on tropical woods (Cartwright and Findlay, 1958; Olofinboba, 1974; Hong, 1976). It was also known to cause sapstain in rubber wood in Malaysia (Ali et al., 1980).

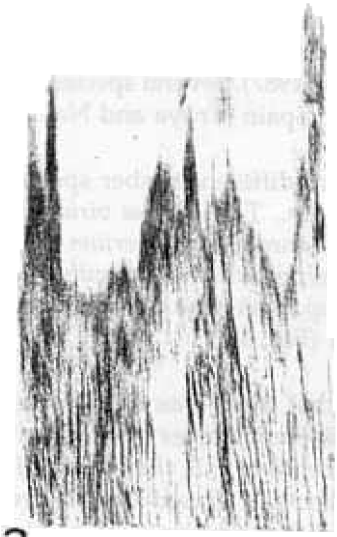
Ceratocystis fimbriata was prominently found causing stain in rubber wood during rainy season in Kerala. *Ceratocystis coerulescens* was reported as the most important stain fungus on hardwoods in the southern USA (Verrall, 1941). In Russia, *Ceratocystis* spp. were responsible for the discolouration of the wood (Vanin, 1932). *C. picea,* normally a nonstaining fungus on conifers in the western USA, caused stain in red Alder *(Alnus rubra)* (Morrell, 1987). Several species of *Ceratocystis* were reported to cause stain on pine wood in Spain (Troya and Navarrete, 1989).

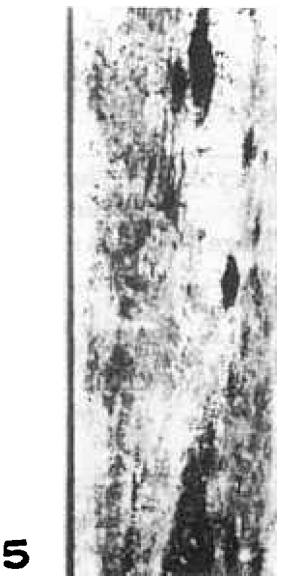
The fungi isolated from the surface of different timber species surveyed were *Aspergillus niger, A. flavus, Penicillium spp., Trichoderma viride, Memnoniella echinata, Syncephalastrum racemosum* Cohn ex Schrot., *Cladosporium* sp., *Mucor* sp., and *Absidia corymbifera* (Cohn) Sacc. & Trotter, of which *Aspergillus* spp., *Penicillium* spp., T. *viride,* S. *racemosum* and *Mucor* sp., were the dominant ones (Fig. 5). *M. echinata* was seen more on rubber wood (Fig. 6).

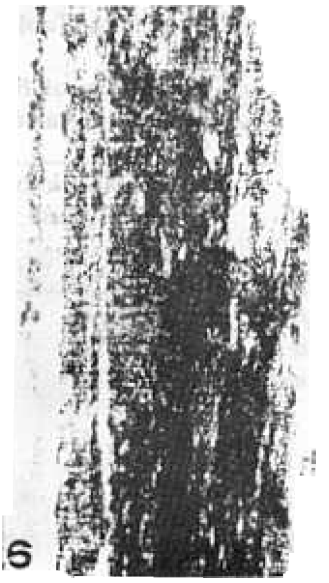
The results of the survey indicated that there was no definite pattern in the occurrence of mould and sapstain on different timber species collected. All timber species surveyed were found to be susceptible to B. *fheobromae;* H. *brasiliensis* being the most severely and frequently affected. Based on the frequency of isolation of B. *theobromae,* it can be concluded that timbers such as H. *brasiliensis, A. occidentale, Artocarpus* spp. and *A. triphysa* were the most susceptible ones while M. *indica, B. ceiba* and E. *stricta* moderately susceptible; least susceptible timbers were *A. scholaris,* and *M. peltata* (Table 3).

Table 3. Frequency percentage of *Botryodiplodia theobromae* in fungal isolations made from various timbers of Kerala.

Timber species	Total No.of isolations	Frequency of infection percentage	Number of months samples collected in a year
Ailanthus triphysa	13	70	7
Alstonia scholaris	11	18	6
Anacardium occidentale	13	77	6
Artocarpus spp.	11	75	4
Bombax ceiba	43	40	7
Ceiba pentandra	7	42	5
Erythrina stricta	5	60	3
Hevea brasiliensis	66	81	
Macaranga peltata	22	27	4
Mangifera indica	35	57	9







Figs. 3 - 6 3. Sapstain caused by *B. theobromae* on *Bombax ceiba*.
4. Sapstain caused by *B. theobromae* on rubber wood.
5. Mould fungi growing on the surface of rubber wood.
6. *Memnoniella echinata* growing on the surface of rubber wood.

A survey conducted in various wood industries of Trichur District revealed that there was no clear pattern of attack of B. *theobromae* on rubber wood throughout the year. The incidence was observed to be more than 50% in all the months of collection (Table 4). Even in drier months cent percent infection was noted.

Table 4 Percentage isolation of *Botryodiplodia theobromae* on rubber wood from various places in Trichur District during the year 1987.

Locality	Month	Percent isolation of *B. theobromae*
Ollur	January	67
Chiyyaram	March	100
Pudukkad	April	100
Alur	May	50
Ollur	June	70
011ur	July	100
Kodakara	August	100
Kalletumkara	September	100
Ollur	October	100
Chiyyaram	November	100
Marathakara	December	70

Susceptibility test

In the artificial inoculation trial, the test fungus grew from the culture disc and spread over the upper side of the wood block within five days. After fifteen days, the whole block was covered by fungal mycelium. When the superficial mycelia were scraped using a scalpel blade, surfaces of the test blocks of different timbers were found to be stained. In the case of *B. theobromae,* the staining penetrated to a depth of 4 to 8 mm inside as seen after splitting the blocks. The control samples remained unstained.

Weight loss due to *Botryodiplodia theobromae*

Studies revealed that there was a weight loss in rubber wood in the first month which increased to 12.2% by the end of fourth month (Table 5). In the case of *Ailanthus triphysa* the weight loss increased from 4.3% in the first month to 10.1% in the fourth month. In *Alstonia* scholaris there was no weight loss noted in the first three months, but at the end of fourth month a weight loss of 4.5% occurred. The results clearly showed that rubber wood is the most susceptible to sapstain caused by *B. theobromae* while *A. scholaris* is the least. These results also confirm the findings of the survey.

Table 5 Percentage of weight loss due to *B. theobromae* on different wood species for a period of four months.

Months of incubation	*A.triphysa*	*A.scholaris*	*H.brasiliensis*
1	4.3	0.0	8.0
2	5.0	0.0	8.3
3	9.9	0.0	8.5
4	10.1	4.5	12.2

The attack of sapstain fungi on the physical and strength properties of wood has not been studied earlier in detail. Findlay and Pettifor (1939) reported that a loss of 30-40% in toughness and 20% in bending strength was noted in obechi *(Triplochitonscleroxylon)* stained heavily with B. *theobromae.* Pinheiro (1971) also recorded a decrease in bending strength of poplar wood due to the attack by the same organism. Umezurike (1969) showed that the blue stain fungus, B. *theobroniae* was capable of degrading cellulose in wood of *Bombax buonopozense* P. Beauv., particularly after utilizing starch and soluble carbohydrates. The mode of degradation of wood blocks of *Gossweilerodendronbalsamiferum*(Verm.). Harms., a tropical forest tree extensively used for construction work in Nigeria, by B. *theobromae* has been studied under laboratory conditions by Umezurike (1978). He recorded a 5-7% weight loss of infected wood blocks. The pattern of invasion of wood blocks by *B. theobromae* has been found to be similar to those of soft rot fungi (Krapivina, 1960; Levy, 1967; Umezurike, 1969). As both soft rot and blue stain fungi are capable of forming chains of cavities in the S2 layer of the secondary cell wall of wood, the main difference between these two groups of fungi lies, apparently, in the ability of blue stain fungi to synthesise pigment material by secondary metabolism. The weight loss of rubber wood and *A. triphysa* reported here may be due to the degradation of cellulose. The susceptibile nature of rubber wood to *B. theobromae* may be either due to high percentage of starch content in the tissues or the presence of any chemical which promotes the fungal growth. However, this was not verified. Fougerousse (1985) reported that susceptibility to blue stain depends on the predominance of parenchymatic tissues or the physiological condition like higher percentage of starch in the tree at the time of felling.

Effect of wood moisture content and microclimatic factors on the growth of *B. theobromae*

At relative humidity (RH) 60,70 and 80% no fungal growth was recorded at 20 ^{0}C (T) even at cent percent moisture content (MC) of timber. But fairly good growth occurred at 100% RH in three moisture content levels. At 30^{0}C, profuse growth of the fungus was noted at 90% and 100% RH; the fungal growth was not much influenced by the moisture content of timber. At 40^{0}C, in all combinations

of RH and MC, the growth of the fungus was not very much restricted, but comparatively better growth was noted at 100% RH. It is presumed that the growth of the fungus on wood blocks was influenced by higher percentage of relative humidity. Statistical analysis showed significant difference between the treatments (Table 6). Treatments (T, MC, RH), 20°C, 100%, 100%; 30°C, 75%, 100% and 30°C, 100%, 100% were found to be significant as compared to all other treatments. Hong (1980) studied the temperature tolerance of B. *theobromae* on agar medium as well as on wood blocks. He found that the optimum temperature for growth of B. *theobromae* on malt agar was very close to 30°C. But on wood blocks the fungus could survive at higher temperatures. In the present study also, 30°C was found to be the ideal temperature for good growth of the fungus. Viitanen and Paajanen (1988) studied the growth of mould fungi such as *Aspergillus versicolor, Cladosporium sphaerospermum, Penicillium* sp. and *Aureobasidium pullullans* in different RH and temperature. He found that the moulds grew rapidly in higher humidities (RH > 96%) and the lowest humidity for slow growth was 80%. Generally most of the fungi grow well in higher humidities (95% or more) (Cochrane, 1958). From the present study, it was clear that when the humidity was high, the wood blocks having low moisture content, absorbed moisture from the surroundings and enhanced the growth of B. *theobromae* on wood blocks.

Table 6 Growth rate index of *B. theobromae* grown on rubber wood blocks of different moisture contents (MC) incubated at various temperatures and relative humidity (RH).

	200			300			400		
	Moisture content			Moisture content			Moisture content		
	50	75	100	50	75	100	50	75	100
60	0^{a*}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
70	0^{a}	0^{a}	0^{a}	0.2^{a}	0.4^{a}	0.8^{a}	0^{a}	0^{a}	0^{a}
80	0^{a}	0^{a}	0^{a}	0^{a}	1.4^{a}	1.6^{a}	0^{a}	0.2^{a}	1.6^{a}
90	1.2^{b}	1.8^{b}	3.3^{c}	2.8^{b}	3.4^{c}	3.0^{b}	0.2^{a}	0.4^{a}	1.2^{b}
100	3.4^{c}	3.4^{c}	3.8^{c}	3.4^{c}	3.6^{c}	4.0^{C}	0.2^{a}	1.0^{a}	2.8^{b}

* Values superscribed by the same letter are not statistically different

Optimum moisture content for the growth of *B. theobromae*

Growth rating of B. *theobromae* on rubber wood at different moisture contents is given in Table 7. Above 29% moisture content, heavy growth of the

Table 7 Growth rating of *B.theobromae* at different moisture contents (MC) of wood

S1.No.	MC%	Rating*
1	65.63	4
2	46.05	3.8
3	29.49	4
4	27.02	2.4
5	27.04	2.2
6	25.79	1.4
7	25.86	1.0
8	23.83	0.0
9	16.18	0.0
10	15.85	0.0

* For explanation see Table 2.

fungus was seen on the wood blocks. In 27% moisture content, light growth of B. ***theobromae*** was noted where as in 25% only traces of fungal growth was observed. Below 25% the fungal growth was found to be very restricted. This study has confirmed earlier reports on the lowest moisture content of wood essential for the fungal growth. Pinheiro (1971) studied the maximum and minimum moisture contents of poplar wood which favoured blue stain attack by B. ***theobromae.*** He found that the lowest moisture content which permitted the growth of the fungus was 24%. Colley and Rumbold (1930) also found that the lower limit for stain development in loblolly pine by ***Ceratocystis pilifera*** was 24%. From this study also it was clear that if the moisture content of the wood is reduced to <24% the timber can be protected from fungal sapstain.

Chemical control

a) Evaluation of various fungicides using sterile wood blocks

The results of the fungicidal evaluation (Table 8) indicated that only sodium azide at 1% a.i. inhibited the growth of all fungi while carbendazim and carboxin were effective against a few fungi only. Captafol 1.0% a.i. was effective in inhibiting all fungal growth except ***T. viride,*** *B.* ***theobromae*** and ***C.fimbriata.*** The same trend was also noted in the tests with higher concentration of captafol. None of the three concentrations inhibited 100% growth of *B.*

Table 8 Evaluation of fungicides for the efficacy against various staining and mould fungi (Percent inhibition).

Chemicals	Concentration % a.i.	*Af*	*An*	*Me*	*Tv*	*Bt*	*Cf*	*Fs*	*As*	*Sl*
Carbendazim	1	00	90	00	00	00	00	85	90	50
Sodium azide	1	100	100	100	100	100	100	100	100	100
Carboxin	1	00	00	100	00	100	25	100	00	100
Thiride	1	00	50	90	00	90	00	50	00	00
Copperoxychloride	1	00	00	80	00	00	00	00	50	25
Ziram	1	00	00	90	00	90	00	00	50	50
Mancozeb	1	00	00	100	00	50	00	00	00	00
Captafol	1	90	90	100	00	10	00	90	90	100
"	1.5	95	95	100	00	00	00	90	90	100
"	2	95	95	100	10	00	00	90	90	100
NaPCP+Borax	0.5+1.5	100	100	100	100	100	100	100	100	100
Control	00	00	00	00	00	00	00	00	00	00

Af - *Aspergillus flavus* *An* - *Aspergillus niger* *Me* - *Memnoniella echinata*
Tv - *Trichoderma viride* *Bt* - *Botryodiplodia theobromae* *Cf* - *Ceratocystis fimbriata*
Fs - *Fusarium solani* *As* - *Acremonium strictum* *Sl* - *Scytalidiuni lignicola*

Sodium pentachlorophenoxide (NaPCP) is widely used as a preservative for the control of sapstain fungi, moulds and decay fungi. A concentration of 0.4 to 0.5% a.i. is suggested to control sapstain (Anon, 1972). During the last few years much work has been carried out to find out a suitable substitute for NaPCP which has high mammalian toxicity. In the field tests conducted in Brazil, Busan, a fungicide at 1.5% a.i. proved to be effective against sapstain and mould fungi (Milano, 1981). In the field tests for a period of five months Plackett (1982) proved that Busan 1.2% a.i. was effective against sapstain and mould fungi. Under various trade names, copper-8-quinolinolatehas been tested in the laboratory or in the field. In laboratory tests against several sapstain and mould fungi Cassens and Eslyn (1981) obtained rather poor results with 0.02% a.i. on yellow poplar *(Liriodendron tulipifera)* at 0.02% a.i., the highest tested concentration; while Butcher (1980) found it effective on *Pinus radiata* against sapstain, mould and decay fungi at 0.025% a.i. concentration.

Many studies have been reported on the efficacy of preservatives for the control of B. *theobromae* causing sapstain (Tan et al., 1980; Hong, 1981). Ali et al., (1980) have screened 11 fungicides against B. *theobromae, Aspergillus* sp., and *Penicillium* sp., on rubber wood. They found that captafol was effective to all the three fungi at 1-2% a.i. Gnanaharan (1983) made tests on rubber wood boards treated

by the boron diffusion process and found that 0.5% NaPCP was effective against sapstain and moulds. Butcher and Drysdale (1974) screened various chemicals against sapstain in New Zealand and reported that captafol 0.2 to 0.3% a.i. was superior to NaPCP. Later, Butcher (1980) found that 0.15% captafol was adequate for long-term protection of pine timber. But in the present study captafol even at 2% was not at all effective against B. *theobromae* in the laboratory screening.

Evaluation of sodium azide at 0.1, 0.25, 0.5 and 0.75% revealed that the chemical was 100% effective at all concentrations tested on sterile wood blocks. When further tested the chemical was effective at 250 and 500 pprn only (Table 9).

b) Evaluation of sodium azide against stain and mould fungi using fresh nonsterile and preincubated wood blocks.

The results indicated that 80% control of fungal growth was noted at the end of the 4th week in fresh nonsterile wood blocks dipped in sodium azide solution of 0.75%, whereas in preincubated wood blocks only 60% inhibition of fungal growth was noted (Table 10). These two treatments were found statistically significant from all other treatments. It is presumed that, in preincubated wood blocks some fungi which might have established over the wood blocks prior to the treatment, continued to grow during the incubation period even after the treatment. But in fresh nonsterile ones, since the dipping was done immediately after cutting, the fungi might not have got favourable conditions or chance to establish. From this it is clear that a prophylatic treatment immediately after felling or cutting will always be better rather than controlling the stain after the infection has occurred.

Table 9 Efficacy of sodium azide against staining and mould fungi at different concentrations (Percentage of inhibition).

Sodium azide concentration	*Af*	*An*	*Me*	*Tv*	*Bt*	*Cf*	*Fs*	*As*	*Sl*
10 ppm	00	00	00	*00*	00	00	00	00	00
50 ppm	00	00	00	00	*00*	00	00	00	0
100 ppm	00.	00	00	00	00	00	00	00	00
250 ppm	85	100	100	100	90	100	100	100	100
500 ppm	100	100	100	100	100	100	100	100	100

Af *- Aspergillus flavus* ***An*** *- Aspergillus niger* ***Me*** *- Memnoniella echinata*
Tv *- Trichoderma viride* ***Bt*** *- Botryodiplodia theobromae* ***Cf*** *- Ceratocystisfinibriata*
Fs *- Fusarium solani* ***As*** *- Acremonium strictum* ***Sl*** *- Scytalidium*

Table 10 Percent control of fungal growth on unsterile and preincubated wood blocks dipped in sodium azide solution.

	Concentration %	Period of incubation (weeks) Ist	2nd	3rd	4th
Fresh nonsterile	0.25	32	32	32	28
	0.5	32	32	32	32
	0.75	88	88	80	80
	control	0	0	0	0
Preincubated	0.25	28	12	12	12
	0.5	56	48	28	28
	0.75	68	64	60	60
	control	0	0	0	0

c) Effect of dipping time on the control of fungal growth over the wood blocks

In the wood blocks dipped in 0.5% sodium azide for 30 minutes, 100% inhibition was noted at the end of the fourth week whereas in 10 and 20 minutes dipping, inhibition was 60 and 92% respectively (Table 11). Two treatments, such as dipping in 0.5% concentration of sodium azide for 20 or 30 minutes were found significantly different from all other treatments. When the dipping time was increased, the percentage of inhibition of fungal growth also increased. This increase in inhibition of growth may be due to the diffusion of more chemical solution during long dipping periods.

The use of sodium azide as a preservative chemical against mould and sapstain fungi has not been reported earlier. However, it has been used effectively to control a few fungal diseases. Row et al., (1974) reported that Cylindrocladium black rot of peanuts was effectively controlled by applying sodium azide in the soil. Similarly 10 ppm solution of potassium azide inhibited the germination of microsclerotia of *Cylindrocladium floridanum* (Weaver, 1971). Sodium azide was found to be effective in controlling the nursery diseases of eucalypts (Sharma and Mohanan, 1991). They have found that in laboratory evaluation, sodium azide (0.05 and 0.1%) was 100% effective against *Cylindrocladium quinqueseptatum, C. ilicicola, C. floridanum, C. parvum* and *C. camelliae.* All these reports indicate that sodium azide is an effective chemical for controlling fungal growth. However, only after studying the toxicity and testing the efficacy in the field, it can be recommended as a preservative against stain and mould fungi.

Table 11 Percent control of fungal growth on wood blocks dipped in sodium azide solution for 10, 20 and 30 minutes.

Dipping time (minutes)	Concentration %	Period of incubation (weeks) 1st	2nd	3rd	4th
10	0.1	100	100	80	40
	0.25	100	100	100	48
	0.5	100	100	100	60
	control	0	0	0	0
20	0.1	100	100	70	60
	0.25	100	80	80	80
	0.5	100	100	100	92
	control	0	0	0	0
30	0.1	100	100	72	60
	0.25	100	100	100	80
	0.5	100	100	100	100
	control	0	0	0	0

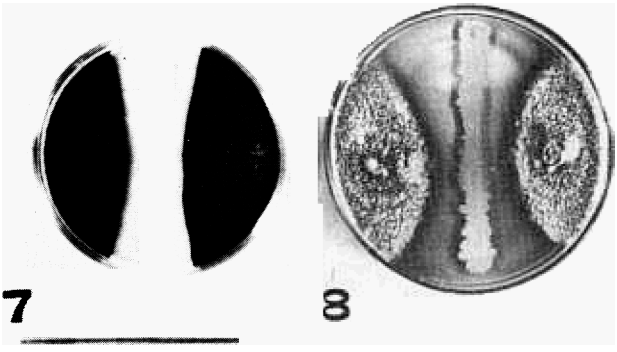
Biological control

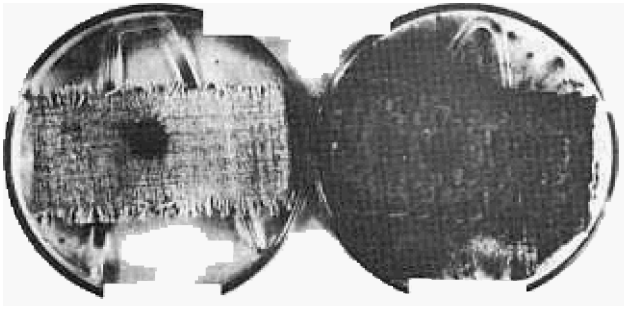
(a) Testing of the bacterium on culture medium

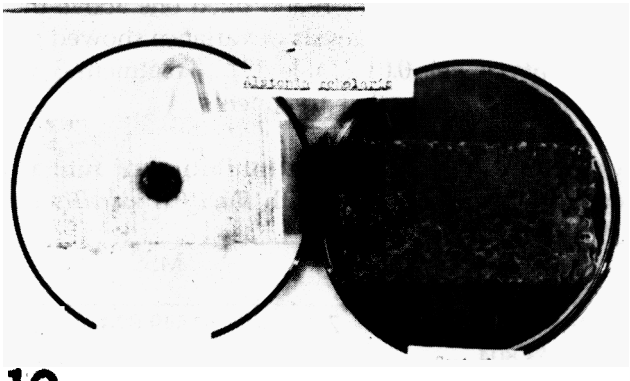
The bacterium, *Bucilliis subtilis* inhibited the growth of all fungi tested except *Pythium* sp. (Table 12). The maximum inhibitory zone was noted in the case of B. *theobromae* (Figs. 7, 8) followed by *Memnoniella echinata, Scytalidium lignicola, Trichoderttia viride, Acremonium recefei, Aspergillus niger, A. flavus* and *Cerutocystisfimbriata* Among the pathogens tested, *Pestulotiopsis* sp., the common foliar pathogen had shown the inhibitory zone upto 17 mm.

Testing of the bacterium on wood blocks

The wood blocks of three timber species, *Hevea brasiliensis, Ailanthus triphysA* and *Alstonia scholaris* treated with the suspension of *B. subtilis* were found to be devoid of any fungal growth (Figs. 9,l0). On the control blocks the fungus grew well and covered the entire surface of wood and also resulted in internal stain.







Figs. 7 - 10. 7. Inhibition of the growth of *B. theobromae* by *Bacillus subtilis*.
8. Inhibition of the growth of *Fusarium solani* by *B. subtilis*.
9. Inhibition of the growth of *B. theobromae* on rubber wood block by *B. subtilis*.
10. Inhibition of the growth of *B. theobromae* on *Ailanthus triphysa* wood block by *B. theobromae*.

Table 12 Inhibitory zone (in mm) produced by antagonistic *Bacillus subtilis* against various fungi.

No.	Fungi tested	Clear zone
1	*Aspergillus flavus*	6
2	*A. niger*	7
3	*Trichoderma viride*	9
4	*Meninoniella echinata*	13
5	*Botryodiplodia theobromae*	18
6	*Acremoniuni recefei*	9
7	*Scytalidium lignicola*	11
8	*Ceratocystis fim briata*	5
9	*Fusarium solani*	14
10	*Corticium salnionicolor*	14
11	*Sclerotium rolfsii*	9
12	*Pestalotiopsis sp.*	17
13	*Rhizoctonia solani*	14
14	*Collectotrichum gloeosporioides*	10
15	*Pythium sp.*	00

(c) Field testing of the bacterium

The maximum control of fungal growth was recorded on rubber wood planks sprayed with bacterial suspension and piled one above the other without leaving any space in between. The analysis of variance showed significant difference between treatments at p = 0.01 (Table 13). Treatment 3 was found to be significant when compared to all other treatments.

Table 13 Analysis of variance of data on inhibition of fungal growth on rubber wood planks treated with *Bacillus subtilis* suspension.

Sources	SS	DF	MSS	F
Treatments	639443.180	7	91349.026	8.4221**
Replication	28163.804	3	9387.935	0.8655(ns)
Residual	227772.598	21	10846.314	
Total	895379.582	31		

** Significant at p = 0.01 ns = non-significant-

Biological control of plant pathogens, free from hazards, is a difficult but important necessity. The antagonistic potential of certain bacteria can be quite effective and if well regulated, provide an industrially useful method for wood protection. Among the various bacterial antagonists tested *Bacillus* subtilis has been reported to control several plant pathogens (Dunleavy; 1955; Aldrich and Baker, 1970; Broadbent et al., 1971; Swinburne and Brown, 1976; Singh and Deverall, 1984). Results of this study are in agreement with earlier reports on the efficacy of *Bacillus subtilis* in controlling sapstain and mould on various timber species. Laboratory studies were successfully carried out to evaluate the efficacy of bacteria as a biological control agent against 5 brown rot and 3 white rot fungi (Benko and Highley, 1990). Tests were also carried out in Australia to screen bacteria effective against fungi causing blue stain (Benko, 1988). *Bacillus subtilis* strain 186 inhibited the growth of three sapwood inhabiting fungi on agar and prevented colonisation of spruce blocks placed on agar plates (Bernier et al., 1986). More detailed studies with the same strain of bacterium revealed that it can be used as a potential biological control agent for sapstain and mould growth on unseasoned timber (Seifert et al., 1987). All these reports indicate that *B. subtilis* can be used in the biological control of sapstain. However, large scale pilot trials are necessary to work out the economic feasibility of this method.

CONCLUSIONS

1. *Botryodiplodia theobromae* is the predominant sapstain fungus of economically important timbers of Kerala such as *Hevea brasiliensis* (Rubber), *Ailanthus triphysa* (Matty), *Bombax ceiba* (Elavu), *Alstonia scholaris* (Ezhilam-pala), *Mangifera indica* (Mango), *Anacardium occidentale* (Cashew), *Macaranga peltata* (Vatta) and *Erythrina stricta* (Murukku).

2. Sapstain caused by *B. theobromae* effects weight loss in timbers. Weight loss in rubber wood after a period of four months was 12.2%.

3. If the wood moisture content is reduced to <24%, timbers can be protected from sapstain to a large extent.

4 Among the various fungicides tested, sodium azide which was found effective in controlling stain and mould fungi in the laboratory, appears to be a promising chemical worthy of testing in the field to tap its industrial potentials.

5. A bacterium, *Bacillus subtilis* isolated from rubber wood was found to be antagonistic against several stain and mould fungi. The efficacy of this bacterium was tested in the field and proved to be effective in controlling sapstain upto a level of 80% in green timber.

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