DISCUSSION

Efficient utilization of various timber species in India is becoming more important as the supply of qualitative wood diminishes. Hence, more judicious use of these resources requires adequate knowledge of various timber species that are available. Natural resistance or susceptibility of different wood species to deterioration by various fungi, insects and borers are also of common occurrence in the field. Since very little is known so far about natural decay resistance of Sissoo wood, it was considered worthwhile to undertake this investigation with a few specific objectives. These are (1) to determine the natural decay resistance of Sissoo wood to three common wood-rotting polypores; (2) to study the interaction effect of three wood-rotting fungi on decay resistance of wood; (3) to ascertain the role of wood extractives in decay resistance; (4) to isolate the antifungal substance (active principle) from wood extractive, if possible and also (5) to select the best wood preservative for controlling fungal decay of Sissoo wood.

Although a large number of organisms grow on wood under natural conditions only a few of them are capable of breaking down the wood substances. Usually lignocellulosic materials of wood confer resistance to degradation by microorganisms than other types of materials present in the wood (Scheffer and Cowling, 1966). The decomposition of lignin concurrently with plant carbohydrates in wood by white-rot fungi was reported by Campbell as early as in 1952. However, the white-rot fungi differ considerably in the relative rates at which they attack lignin and carbohydrates (Higuchi et al., 1955). The decay of Sissoo wood caused by F. sanguineus, C. hirsutus and F. flavus in the natural forest was considerable but it was not possible to estimate the extent of damage caused by these organisms accurately. Besides, the age of infection of wood was also not
known. Hence, decay resistance of Sissoo wood to three fungi was tested in the laboratory under identical conditions. The results of decay resistance test reveal that sapwood of Sissoo is perishable (weight loss >30%) to all three test fungi irrespective of rot isolates and polysporous mycelia while the heartwood is "resistant" to all but moderately resistant to polysporous mycelia of P. sanguineus. The percentage weight loss was slightly higher than 5%. According to Findlay (1938) weight loss up to 5% is considered as "resistant" and 6-10% is "moderately resistant". Scheffer and Cowling (1966) stated that ASTM (American Society for Testing and Materials) standard soil block test of natural decay resistance has proved highly satisfactory for laboratory evaluations. By this method weight losses of 0-10%, 11-24%, 25-44% and 45% or even greater are considered as "very high", "high", "moderate" and "slight" decay resistance. In accordance the ASTM standard, sapwood of Sissoo is slightly resistant to all test fungi except to rot isolate of F. flavus. To rot isolate, the wood is "moderately resistant". Heartwood, on the other hand, showed "very high" resistance to all test fungi.

Puri and Khan (1968) tested the natural resistance of Sissoo wood to Polyporus [Coriolus] hirsutus, Polystictus [Pycnoporus] sanguineus and Irpex [Flavodon] flavus and concluded that the heartwood was highly resistant to all the test fungi. Present results also confirm the findings of Puri and Khan. The greater decaying capacity of polysporous mycelia than their corresponding rot isolates is not unusual because Chakravorty (1980) also observed differential decaying activities of polysporous mycelia and rot isolates of 3 polypores when tested on sapwood of Artocarpus chaplasha. It is also interesting to note that both polysporous mycelia and rot isolates of test fungi caused more or less similar decay of heartwood of Sissoo although extent of decay was negligible. Significant inhibition of growth of fungi was probably due to the presence of toxic substances in the heartwood of Sissoo.
Similar results were reported earlier by a number of workers (Banerjee and Sinha, 1954; Banerjee and Purkayastha, 1966) who studied the decay resistance of heartwood of different timber species against different types of fungal cultures (i.e., polysporous, monosporous and rot isolates). Artificially decayed wood (after 16 weeks' exposure to fungi) blocks of Sissoo appeared to be partially bleached. This bleaching was perhaps due to destruction of pigmented materials by fungi during the process of decay (Scheffer, 1936). Among the three fungi, *F. flavus* and *C. hirsutus* caused spongy rot while *P. sanguineus* exhibited stringy rot. Microscopic examination of thin sections of artificially decayed wood revealed the presence of bore holes on the cell walls. It suggests that penetrating hyphae certainly secrete cell wall dissolving enzymes. Proctor (1941) expressed similar views with regard to the formation of bore holes. He investigated the mode of penetration of cell walls in four different types of wood by six species of fungi. Apart from bore holes, rupturing and thinning of cell walls, presence of mycelial web in the lumina of vessel and fragmentation of vessel walls were of common occurrence in the wood during advanced stages of decay. Microscopic findings supported that *F. flavus* and *P. sanguineus* caused minimum and maximum decay of wood elements, respectively.

Scanning Electron Microscopic (SEM) examination of sections of artificially decayed Sissoowood also substantiates the earlier findings under the light microscope. In addition, SEM studies also reveal certain new and interesting facts. These are (1) swelling of cell walls in some places; (2) fragmentation of cell walls; (3) web-like hyphal network in vessels and (4) clear bore holes in the walls. Sachs et al. (1989) examined the Aspen chips under Scanning Electron Microscope after 3 weeks' exposure to *Phanerochaete*
chrysosporium (strain BKM-F-1767). They also observed a strong web-like network over the chip exterior surfaces and fungal bore holes in the wood cell walls. The resistance of heartwood vessels to degradation by white-rot basidiomycetes was studied by Blanchette et al. (1988). Wood blocks of Acer saccharum were inoculated with Dichomitus squalens and incubated for 12 weeks. The sections of decayed wood (white stringy rot) along with transverse and radial sections of sound wood were examined under Scanning Electron Microscope. Complete degradation of fibres, selective attack on ray parenchyma and voids among the wood elements were noted. Anatomical and chemical changes in birch wood attacked by white rot fungi were also reported by Ozolinya et al. (1987). In the present investigation the sections of decayed Sissoowood were examined under Scanning Electron Microscope. Scanning Electron micrographs showed that Coriolus hirsutus removed the secondary cell wall from the lumen outwards whereas P. sanguineus macerated the fibres while causing the cell wall to swell into the lumen. All the changes indicate that P. sanguineus is most destructive.

Microchemical test (Phloroglucin-Hcl and Chlor-zinc-iodine) revealed that lignin was primarily destroyed by all three test fungi. It suggests that all of them are white-rot causing organisms. It is necessary to mention that among the three fungi tested, F. flavus showed weak oxidase reaction after 72 hours of incubation. Banerjee and Purkayastha (1966) also recorded weak oxidase reaction for F. flavus. Abe (1989) tested 3 fungi, namely, Coriolus versicolor, Lenzites betulina and Inonotus xeranticus. All of them exhibited positive oxidase reaction and hence placed under white rot group.

Secretion of extra-cellular phenol oxidases by C. versicolor in wood meal medium was detected by Morohoshi et al. (1989). Decay of wood meal proceeded vigorously at the
beginning but the pH of the medium varied between 4.0 to 4.3 only. Kirk (1971) concluded that (a) phenol oxidases theoretically could bring about some oxidation and degradation of lignin. But the question is whether or not they play a significant role is not clear. (b) Phenol oxidases could be only a part of the enzyme complex that catalyse the complete decomposition of lignin. Its indirect role in detoxifying the toxic phenolic compounds that may be released from lignin during its decomposition has been suggested earlier by Gadd (1957) and Rösch (1967). Several earlier studies on the enzymes of lignin decomposition focussed on phenol oxidizing enzymes such as laccase and peroxidase produced by white-rot fungi. It is unlikely, however, that this type of activity is important in structural decomposition (Kirk, 1984), because the enzymes may have some other role in lignin decomposition (Ander and Eriksson, 1978). Since the lignin polymer is attacked by extra-cellular non-specific oxidizing agents, it is possible that the enzyme may not be involved directly, just as cellulose apparently is non-enzymatically oxidized by brown-rot fungi (Kirk and Cowling, 1984).

It appears from the results of present study that P. sanguineus is more lignin destroyer than the other two fungi. Therefore, it was considered worthwhile to extract and estimate both lignin and cellulose contents of wood decayed separately by P. sanguineus, C. hirsutus and F. flavus. As it was not possible to determine the age of fungal infection of wood in the field, sound wood blocks of Sissoo were artificially inoculated \textit{in vitro}. Sound wood blocks were exposed to test fungi for 16 weeks and subsequently both lignin and cellulose were extracted with a view to determine the extent of damage of lignin and cellulose caused by the test fungi. Results of the present study reveal that P.
sanguineus and F. flavus caused maximum and minimum degradation of lignin, respectively, under identical conditions. This finding also supports the results of oxidase test, microchemical test, light and electron microscopic observations and decay resistance test. There is no reason to suspect about preferential utilization of lignin by different white-rot causing fungi. It is known that the white-rot fungi vary considerably in their relative rates of attacking lignin and carbohydrate in a woody tissue (Higuchi et al., 1955; Kawase, 1962). Some removed lignin much more rapidly than the carbohydrate. Cowling (1961) demonstrated that Polyporus versicolor can destroy over 95% of the lignin in sweet gum wood. Reviewing the reports of several workers, Kirk (1971) concluded that under favourable conditions white-rot fungi can totally destroy the lignin content in wood. In the present case, P. sanguineus caused maximum (41.89%) loss of lignin under test conditions. On the contrary, loss of cellulose was not significant because all the test fungi were white-rot causing organisms. Lignin is degraded by ligninase.

The amount of wood decay caused by P. sanguineus, C. hirsutus and F. flavus was evaluated in the laboratory. But under natural conditions, wood provides an excellent venue for the community interactions. The fungi represent biologically a diverse group of microorganisms. Although different types of fungi often grow on a wood substrate under similar natural habitats their effects may be quite different. Interactions and competitions among these organisms may be important in determining their distribution, growth patterns and ecological roles within wood tissues (Rayner and Todd, 1979). Several workers (Purkayastha and Chaudhuri, 1980; Mallet and Hiratsuka, 1986) have studied the interaction effects of fungi on wood earlier and concluded that antagonism exists among some of the wood-rotting fungi. Because of the the antagonism they jointly cause less wood decay. This created an interest and
hence wood inhabiting hyphomycetes were used as biological control agents against wood-destroying fungi Basidiomycetes (Ricard and Bollen, 1968; Bruce and King, 1983). Cease et al. (1989) reported that *Scytabladium* isolates were an effective pretreatment against wood degrading Basidiomycetes in the laboratory test. These results were similar to those reported previously by Ricard and Bollen (1968) and Lundberg and Unestan (1980). They also reported that *Scytabladium* species inhibited wood decay.

A scheme proposed for fungal interactions by Cooke and Rayner (1984) is cited here. This may give an idea about the interaction pattern and its probable impact on wood decay.

Fungal interactions

![Diagram of fungal interactions]

Proposed Scheme of fungal interactions

[After Cooke and Rayner (1984)]

It is simple and precise and yet covers a wide range of interaction types known to occur in nature. In the present investigation, the test fungi were grown in all possible combinations in solid as well as in liquid media. Results reveal that maximum inhibition of growth occurred when both P.
sanguineus and F. flavus were grown in liquid culture. These two fungi together significantly inhibited decay of Sissoo wood. According to Cooke and Rayner (1984) this interaction (P. sanguineus x F. flavus) may be classified under "competitive" type (detrimental to both). Similar competitive interaction was also observed in case of P. sanguineus and C. hirsutus (PxC). Both the organisms significantly inhibited decay of wood. When Sissoo wood was exposed to C. hirsutus and F. flavus (CxF), the interaction was more or less of the neutralistic type because it is beneficial to one (F. flavus) but neither beneficial or detrimental to the other (C. hirsutus) in view of % loss in weight caused by C. hirsutus alone.

The results of interaction studies suggest that combination of fungi particularly (PxF) or (PxC) may cause significant reduction in wood decay in the field.

Antagonistic organisms could be exploited for controlling wood decay to some extent. Hulme and Shields (1972, 73) reported that Trichoderma viride inhibited wood decay caused by Polystictus versicolor, P. hirsutus, P. adustus and 2 Peniophora spp. This was attributed to the depletion of the more accessible nutrients by the primary saprophytes, thus delaying the rapid colonization by the secondary fungi. However, this explanation is not applicable in the present case since the wood was inoculated simultaneously by two fungi under identical conditions. It is not unreasonable to speculate that the capacity to utilise nutrients by one of the interacting fungi could be higher than that of the other species. The organisms with the lower utilisation capacity cannot colonize the wood as rapidly as the other species. The results of the present study show that the percentage increase in growth (in relation to control) of C. hirsutus was highest among the three fungi even in 1%
aspargine. It suggests that better utilisation of nitrogen is not a factor in wood decay. *C. hirsutus* utilized more nitrogen but it was less destructive than *P. sanguineus*. Therefore, it is not improbable that some other factor(s) inherent in the wood itself could preferentially inhibit the mycelial growth of invading fungi.

Rudman (1965) studied the antifungal activities of a number of wood extractives and related compounds in wood substrates. Some of the detected compounds such as genistein, feruginol, cinnapic acid were effective in controlling *Lentinus lepideus* but not *Coniophora olivacea*. Pinosylvin-mono methyl ether was effective in controlling *C. olivacea* but not *L. lepideus*. The decay resistance of heartwood of *Callistris collumaris* to *Coniophora olivacea*, *Fomes setuletis*, *Trametes liheino-giva* and *Hypoloma faciculai* is associated with the amount of petroleum ether solubles present in the heartwood of the test species.

Five phenolic compounds were detected in ether extractive of heartwood of *Artocarpus chaplasha* of which one was fungitoxic. This was confirmed by TLC bioassay test. However, the response of the test fungi (*P. sanguineus*, *C. hirsutus*, and *Trametes cingulata*) varied significantly. The active principle could not be identified (Chakraborty, 1980).

In 1974, Hart and Hills isolated some stilbenes and polyphenols from *Eucalyptus sideroxylon* which were highly inhibitory to growth of some wood-rotting polypores. Heartwood of *E. sideroxylon* was not decayed *in vitro* by *Polyporus versicolor* and *Poria monticola*. When methanol extract of heartwood was diluted thousand times in 3% malt extract, it inhibited the growth of *P. monticola* and not of *P. versicolor*. It was demonstrated that heartwood blocks retained its decay
resistance capacity even after extraction with methanol. The ether solubles (rich in stilbenes) and water solubles (rich in Ellagitanins) also inhibited the growth of both the fungi on 3% malt extract.

In the present study both ethanol and diethyl ether extractives inhibited the growth of all three test fungi. Reduction in decay resistance of wood was noted when the wood was free from ethanol or ether solubles. The antifungal substance isolated from ethanol extractives of heartwood by TLC, inhibited the growth of the test fungi. This substance had shown UV absorption peak at 258 nm. The differential response (F. flavus most and P. sanguineus least sensitive) of test fungi to this substance indicates that this could be a factor (not necessarily a sole factor) for the differential resistance of wood. It is worthwhile to mention here that P. sanguineus and F. flavus caused maximum and minimum decay of sapwood of Sissoo, respectively. It appears from the above statements that the isolated antifungal compound may be involved in the decay resistance of Sissoo wood. It would be more rewarding if this compound is properly identified in future and its exact role in decay resistance of Sissoo wood is ascertained. A number of coumarins were reported to be toxic to wood-rotting fungi such as Lentinus lepideus and Lentinus trabea but the stilbenes tested were not markedly toxic (Rudman, 1963). He concluded that petrostilbenes were not the sole or even the major factor conferring decay resistance in Pterocarpus spp. from which the compound was extracted by King et al. (1953).

According to Ingham (1972) the role of stilbenes in resistance of plant remains yet to be explained.

The toxicity of wood phenolics to several wood-rotting fungi was previously reported by many workers.
There is also evidence that the progressive removal of extractive from heartwood of Teak and Sal with various solvents reduced the resistance of timber to *Polyporus versicolor*, *P. sanguineus*, *P. hirsutus* and *P. meliae* (Puri, 1967).

In 1979, Chaudhuri and Purkayastha detected three phenolic compounds, namely, catechol, phloroglucinol and gallic acid in the wood extractives of *Pterocarpus marsupium*. None of them could be accounted for the differential resistance of *Pterocarpus* wood to their test fungi. Among the three identified compounds, catechol was most fungitoxic.

Hart and Shrimpton (1979) stated that decay resistance is a multifunctional phenomenon and therefore it is not possible to correlate the resistance of wood with a single substance.

Variation in decay resistance of heartwood of *Cunninghamia lanceolata* was noted after sequential extractions with different solvents by Wang et al. (1989). Samples from trees of *C. lanceolata*, *C. lanceolata* var. *konishii* [*C. konishii*] and *C. lanceolata* f. *daiten-u* (age 13-14 year) were treated with n-hexane, ether, dichloromethane, acetone, methanol and water and exposed to the white-rot fungus *Coriolus versicolor* and the brown rot *Laetiporus sulphureus*. Results indicated that the antifungal components were mainly in the hexane and acetone soluble fractions. The ether extracts of *C. lanceolata* f. *daiten-u* and the aqueous extract of *C. konishii* contained decay resistant components. *C. lanceolata*, however, showed an unusually low decay rate. Therefore, it is not unusual to find resistant factor in both ethanol and ether extractives of *Sissoo* wood.

In the present investigation the isolated antifungal
substance could not be identified due to some technical reasons and hence it was not possible to continue the work further in that direction. Eventually, it was decided to test the efficacy of some known wood preservatives which could be used for protection of wood against fungal attack. Although a large number of chemicals have been recommended by different workers for controlling wood decay only a few of the formulations have been found to be very effective.

It is well known that agar block tests for the toxicity of wood extractives or timber preservatives are simple and rapid but have two disadvantages: (1) antifungal activity in agar is a poor index of the activity in wood and (2) test involving agar in which a directly assimilable carbon source is available, cannot show the effect. A compound may have a role in limiting decay by inhibiting cellulolytic or lignolytic enzymes of the fungi. In view of the disadvantages of agar plate test it was considered desirable to use wood blocks and billets for testing the efficacy of wood preservatives.

Five water soluble (sodium penta chlorophenate, zinc chloride, arsenic trioxide, Ascu-A and Ascu-B) and two water repellent preservatives, namely, PS-2 and creosote were tested. Among the 5 water-borne wood preservatives Ascu-A (CCA) was most effective in controlling decay. It contains copper, arsenic and chromium salts in definite proportions (Hunt and Garratt, 1938). In the United States this preservative is known as "Green salt" and "Erdalitch". It is extensively used for wood treatment both in vivo and in vitro.

Results show that 2% Ascu-A gave maximum protection (about 81% reduction in decay) against *F. flavus* and minimum (69.29%) against *P. sanguineus* although the amount of retention of Ascu-A was less than that of Ascu-B. Variable
performance of wood preservatives may also be explained to some extent on the basis of microdistribution theory which suggests that some preservatives are distributed poorly in the wood, both at the macroscopic level and microscopically within cell walls (Nicholas and Preston, 1984). One fungitoxic element of the preservatives does not penetrate into the bulk of the fibre walls during treatment although the overall preservative loading is adequate for the protection of the wood. Most of the preservative remains close to the original penetration pathways in the vessels and rays (Nicholas and Preston, 1984).

Da Costa and Osborne (1968) stated that wood destroying fungi excrete or secrete large amount of organic substances which could react with the preservative and render it more soluble and therefore more readily removed by leaching. As a result of leaching, the toxicity of the preservatives reduced considerably. The differences in decaying activities of fungi are not unusual because it is likely that any metabolite from the fungus could affect the preservatives applied to the wood or a fungus may be either highly or least tolerant to the preservative. Da Costa and Kerruish (1967) reported that Poria monticola was highly tolerant to copper chromate but rather sensitive to the copper chrome arsenate preservatives. Creosote was much more effective than two water soluble wood preservatives, namely, Ascu-A and Ascu-B. About 90% reduction in decay was recorded when wood blocks were treated with creosote for one hour and then exposed separately to 3 test fungi for 16 weeks. The greater efficacy of creosote than that of PS-2 was probably due to greater amount of retention of creosote by the wood substrate and also greater toxicity of the same. Singh et al. (1989) studied the durability of creosote treated Malaysian timber. The observation made for more than 50 years at two sites at the Forest Research Institute, Malaysia on the durability of 96 species of wood.
treated only with creosote. The data were tabulated for each species on average creosote absorption, service life at lower and upper ground, average life and time of destruction of first and last stakes. In most cases increased absorption of creosote gave an increased service life, although these data are not available for all species. Timbers with absorption \( \geq 320 \text{ kg/m}^3 \) had very low failure rates. The timbers were classified into 5 classes on the basis of their durability. These are as follows: Class I (perishable) of service life 0.5 year to Class V (very durable) of service life \( \geq 25 \) years. Usually the effectiveness of preservatives depends upon several factors such as method of application, nature of timber substrate, macro- and microdistribution of preservative in wood, retention capacity of the wood and the environmental conditions. Variations in timber substrates greatly affect the performance of water borne preservatives. The effect is probably due to variation in fixation mechanisms and the type of toxic compound(s) found in the wood (Da Costa and Osborne, 1968).

Explanations given by previous workers for variable performance of wood preservatives have been discussed. The present work has confirmed and extended some of the findings of previous investigators. In addition, this study reveals certain new facts of fundamental importance. Finally, it can be concluded that (a) among the three test fungi, \textit{P. sanguineus} causes maximum decay of wood of \textit{D. sissoo}. (b) All the test fungi utilized significantly more lignin than cellulose but \textit{P. sanguineus} degraded lignin most efficiently than other two fungi. (c) Electron microscopic observation also confirms that \textit{P. sanguineus} and \textit{F. flavus} are most and least destructive respectively. (d) Sapwood is perishable to all the test organisms but differential susceptibility of sapwood to fungi may be due to several reasons. (e) It is significant to note that interaction of \textit{P. sanguineus} and \textit{F. flavus} or \textit{P. sanguineus} and \textit{C. hirsutus} caused considerable
reduction in wood decay. (f) No correlation was observed between decaying capacity and nitrogen utilizing ability of the organism. (g) An antifungal substance was detected in the ethanol extractive of heartwood of Sissoobut no valid comment can be made on the cause of durability of Sissoowood until the isolated antifungal compound is properly identified and tested on wood. Mechanism of decay resistance of different types of wood still remains a major problem to Forest Pathologists. (h) The efficacy of a number of wood preservatives has been tested but Ascu-A and creosote are undoubtedly the best preservatives and hence these may be recommended for use in the field.