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STUDY OF FUNGI DISEASES ON *TECTONA GRANDIS*L.F.

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ABSTRACT:

Fungi that degrade wood produce extra cellular enzymes that break down the woody cell wall. Growth characteristics of the microorganisms in wood and the type of degradative system produced results in different decay patterns being produced (Blanchette, 1998). During the study on plant pathological effects on teak wood find out three types main diseases on teak.

KEY WORD: *Pathology, Fungi, Teak, Wood.*

INTRODUCTION:

Wood deterioration is an essential process in the environment that recycles complex organic matter and is an integral component of life. These processes, however, also destroy historic wood that has been used as shelter, utility and art resulting in a loss of valuable cultural properties from archaeological sites. Woods with natural resistance to microbial degradation were often used in ancient times for an application where wood was in contact with the ground, for shipbuilding and for other uses (Meiggs, 1982; Blanchette, 2000). These extractive-rich woods helped to preserve the wood and resist microbial attack but even the most resistant woods are not immune from decomposition. Wood that persists for long periods of time is usually protected by an environment that limits microbial activity. These special conditions may allow wood to survive centuries or even thousands of years but even in the most extreme environments some physical and chemical modification of wood from bio-deterioration takes place. What type of deterioration occurs and how these processes impact the wood are important questions that need consideration if wooden cultural properties are to be studied and properly pre-served. Since there are relatively few wooden objects surviving from past civilizations, they are extremely valuable resources that deserve careful attention. It is essential to improve our understanding of the microbes and processes that affect archaeological woods and to increase our knowledge of structural and chemical changes that occur in wood from degradation. This review provides information about bio-deterioration mechanisms affecting wood and describes a wide variety of examples with deterioration found in archaeological wood from different environments.

Study area:

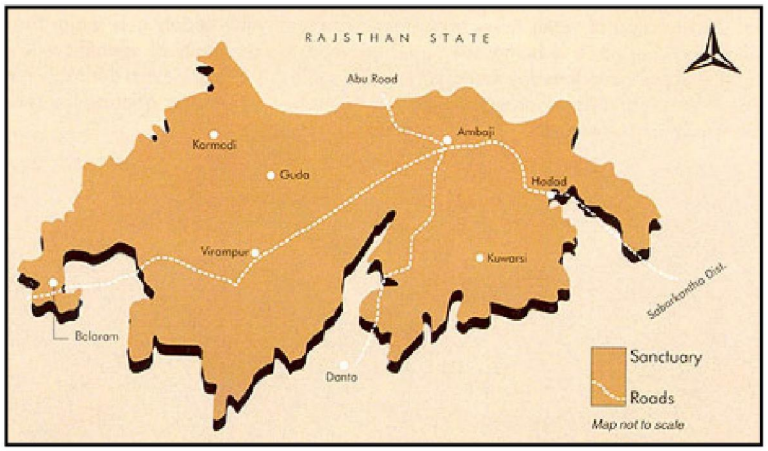

Figure 1: Map of study area

Wood samples of *Tectona grandis* L.F. infected with fungi were collected from trees growing in Balaram Ambaji Wild Life Sanctuary.

The sanctuary is lying between 24° 10' to 24° 30' N latitude and 72° 20' to 73° 00' E longitude, this picturesque 542.08 sq. km. tract of forest in Banaskantha district was declared as a Sanctuary by Government of Gujarat vide its notification No. GVN/27/WLP/1088/850-V2 dated 7th Aug. 1989 for the purpose of protection, propagation and conservation of the wild life and its environment. The sanctuary covers an area of 542.08km 2 in the Banaskantha District of Gujarat. The sanctuary gets its name from 2 historic temples, one to Lord Balaram and the other to Mata Ambaji, located at opposite corners of the Sanctuary.

MATERIAL AND METHODS:

1. Sample Fixation:

Samples disk measuring about 60X60X60 mm. Samples were fixed immediately in Formaldehyde Acetic Acid (Berlyn and Miksche, 1976) while some of the unfixed samples were packed in sterile polyethylene bags.

2. Fungal Isolation and Identification:

After coming back from the field the unfixed samples were inoculated in PDA (PDA: 20 g potato, 20 g of dextrose, 18 g agar and 1000 ml distilled water) and malt extract media contain Petri plates for isolation and identification of the fungi. After two weeks in Petri plates which are containing malt agar medium prior to inoculating the wood blocks all the strains were growing separately. The strains were spread on malt agar medium in order to obtain spores and mycelium production for pathogenicity test, and freeze drying and preservation.

For isolation of fungal pathogen, small pieces of wood blocks were surface sterilized with 95% ethanol for 10-15 seconds followed by 1% sodium hypochlorite for 45-50s. These wood pieces were then aseptically inoculated on PDA medium. Inoculated blocks were incubated in an environmental incubator at 28˚c for complete growth. Pure culture was obtained after successive transfer on PDA medium and preserved at 8˚C. Both the fungi were isolated from the trees growing in the study area. Pure cultures

were established by routine methods and were identified Agarkar institute of Pune and Plant Pathology division, Forest research institute Dehradun.

For decay test, oven dried cubic blocks (2X2X2cm) were exploited. Before weighing some of the blocks were marked to take final weight each incubation period. After weighing these blocks were soaked in water for 24 hrs and then autoclaved for 30 min at 120˚. After cooling, these blocks were surface sterilized with 70% alcohol and inoculated with pure cultures of infected strains. These samples were incubated for 30, 60, 90,120, 150 etc…days at 27 ˚C and 70% relative humidity. After each incubation period the wood blocks were removed from the Petri plates, cleaned to remove mycelia. The marked blocks were weighed to determine final volume while rests of the blocks were fixed in FAA. The % of weight loss due to degradation was then calculated whereas uninoculated blocks were treated as control samples. After 12 hrs of fixation in FAA, samples were transferred to 70% alcohol for histological study.

3. Pathogenicity tests

4. Microtomy and staining

After 12 hrs of fixation in FAA (Formaldehyde, Acetic Acid. Ethyl alcohol (70%); 10:5:85, v/v), samples were cut in to smaller pieces and transferred in 70% alcohol. Samples were processed by both sliding and rotary microtome. Transverse, Radial and Longitudinal sections of 12-15 µm thickness were directly cut on the sliding microtome and stained with Safranine and Astra blue combination (Srebotnik and Messner, 1994). For rotary microtome, suitably trimmed (5 mm^2) samples were dehydrated with tertiary butanol series (30%, 50%, 70%, 90% followed by 3X1005 pure TBA) and processed by routine method of paraffin embedding. After dehydration in ethanol-xylene series the sections were mounted in DPX. Some of the sections were also treated with potassium Iodide, Coommasie Brilliant Blue (CBB), Sudan Black B and ferric chloride for the localization of starch, proteins, lipids and tannins respectively (Krishnamurthy, 1999). Important results were micro-photographed with Leica DM 2000 trinocular research microscope.

RESULT:

Decay by *Trichoderma harzianum*:

After 30 days of fungal inoculation, there was no appreciable weight loss of wood block (Table 1), but fungal mycelia invaded all the cell types of the secondary xylem. Initially fungal mycelia began to grow on wood block and ultimately covered the whole block within 15 days. In the beginning mycelia invasion was observed through the vessel lumen (Figure 1A). From the vessels, hyphae traversed into the neighboring rays (Figure 1B) and gradually extend in all direction including xylem fibres and adjacent axial parenchyma cells (Figure 1C, D). At this stage no visual damage was observed in the cell walls.

Fungal mycelia moved from one cell to the next through the pits present on their walls (Figure 1E). Presence of fungal mycelia in all the cell types of xylem adjacent to vessel elements was a common feature in all the samples studied.

Samples exposed to fungi for 60 days showed sign of selective delignification, though the signs were not that distinct in all the cell types, but can be easily observed in fibres. In transverse view, many of them showed concentric delignification starting from middle lamellae towards lumen. As a result the secondary wall adjacent to the middle lamellae stained blue with astra blue instead of red by safranine. This feature was initially observed in the fibres adjacent to the rays, axial parenchyma cells and vessel elements. Thereafter, it gradually invaded all the cell types.

After 90 days of inoculation, all the cell types of the secondary xylem were invaded by the fungal mycelia. The middle lamellae that were stained blue began to lose the integrity and individual cells became separated from each other (Figure 2A). As the degradation progressed further, complete separation of fibres was a common feature and it was observed in most of the sections (Figure 2B). Due to delignification, pits of the ray and axial parenchyma cells became more pronounced and became larger in size and irregular in shape. At the same time, formation of several bore holes on the lateral walls of the rays was a common feature (Figure 2C). Compared to axial elements, ray cells were more affected showing advanced thinning of the cell walls that stained blue colored with astra blue part of the cell wall in which lignified part was relatively unaffected stained red with safranine.

Wood blocks exposed to fungi for 120 days showed similar pattern of delignification but the effects were more pronounced. At this stage, walls of ray cell showed larger erosion troughs (Figure 2D) and the bore holes on the walls were more distinct and became irregular in shape (Figure 2E). With the advancement of decay, many of the ray cells were either partially or completely disintegrated (Figure 2F). Vessel elements began to deform (Figure 3A) due to loss of rigidity while fibres appeared almost completely separated from each other (Figure 2D). In longitudinal sections xylem fibres were seen completely separated while ray cells not only got separated from each other but also they became completely disintegrated due to the loss of rigidity (Figure 3B). At this stage xylem cells showed characteristic of simultaneous rot i.e. cell-wall thinning, the walls were completely bleached and showed erosion trough across the cell walls. These troughs were irregular in shape and size (Figure 3C). In some instances, the erosion reached the middle lamella completely removing the cell wall in a localized area (Figure 3C). However, cell wall thinning is not much distinct in the axial elements as compared to ray cells (Figure 2F).

Decay by *Chrysosporium asperatum*: As seen in case of *Trichoderma harzianum*, *C. asperatum* also showed both selective and simultaneous decay. In the initial stage (60 days) wood blocks inoculated with *C. asperatum* showed selective delignification by defibration due to dissolution of middle lamella

whereas erosion troughs and bore holes were also observed occasionally. Fungal hyphae were most abundant within the lumina of vessels, xylem rays and axial parenchyma cells. Similar to former strain, selective delignification by *Chrysosporium* resulted in the degradation of middle lamella which eventually led to separation of individual cells. Up to 60 days, degradation pattern by *C. asperatum* remained more or less similar to *T. harzianum*. After 90 days of inoculation, considerable variations were observed in the samples investigated. The fibre walls were completely interrupted due to extensive delignification and showed advanced erosion troughs from the lumen surface to middle lamella (Figure 3D). Cell walls were comprehensively degraded and showed larger bore holes and erosion troughs (Figure 3E, F). In longitudinal view, obliquely arranged erosion troughs run parallel with the cellulose micro fibrils on the fibre walls, which showed blue staining with astra blue (Figure 4A). Most of the time these troughs were merged to form tunnels of indefinite length and shape. They may be oval, circular, irregular or fusiform shaped.

As compared to wider vessels, narrow vessels present in groups (i.e. radial or diagonal multiples) were not much affected by the fungal invasion, except they were separated from each other due to crumbling of middle lamella (Figure 4B). On the other hand larger and solitary vessels within the same section showed extensive crumbling of the vessel walls (Figure 4C). These walls were totally degenerated and eventually resulted into complete collapse of the vessel element due to loss of rigidity (Figure 4C). As shown in Figure 4D, in advance stage of decay almost all the fibres were separated from each other and the walls were crumbled due to lose of rigidity and integrity (Figure 4D). At several places fibre walls were interrupted due to advance erosion troughs (Figure 4D).

DISCUSSION:

Lignin is a highly branched polymer of phenylpropanoid compounds, and is an important component of secondary wall of the plant (particularly secondary xylem). After cellulose, lignin is the second most abundant organic compound in plants, representing approximately 30% of the organic carbon in the biosphere (Boerjan *et al*., 2003). The functional significance of lignin is associated mainly with the mechanical support allowing plants to stand erect and as a defense against pests and microorganisms (Boudet, 2000; Lagaert *et al.,* 2009). Despite the importance of lignin in plant as a defense against microorganisms, it is most often observed that secondary xylem is invaded by the microbes such as bacteria and fungi. Now a day, white rot fungi are of particular interest because they are one of the few groups of microorganisms that can selectively degrade lignin without appreciable losses of cellulose (Otjen and Blanchette, 1985). Thus, white rot Basidiomycetes are not only extremely attractive for use in biological pulping processes but are also equally imperative in bioremediation of textile dyes, poly aromatic hydrocarbons and xenobiotic compounds.

On the basis of decay pattern, three general types of decay are recognized (Liese, 1970; Nilsson, 1988; Blanchette, 1991; Zabel and Morrell, 1992; Eaton and Hale, 1993). In white rot, all cell wall components (i.e. lignin, cellulose and hemi cellulose) are either degraded simultaneously (Blanchette and Reid, 1986) or they may be degraded preferentially, especially in the early stages (Otjen and Blanchette, 1985). There are reports that some white rot fungi are capable to cause both types of decay in the same wood or in different wood species (Blanchette, 1984a, b, 1991). In the present investigation, *Trichoderma harzianum* showed selective delignification pattern and the most remarkable effect is defibration by dissolution of the middle lamella in *Tectona*. Formation of erosion trough on the cell wall from the lumen surface is a prominent anatomical feature in both the samples exposed to fungi for 120 days. In *Tectona grandis* (teak), pattern of delignification differed from the previous species. In teak, *Chrysosporium asperatum*, though belongs to Ascomycetes it shares the characteristics of both white rot and soft rot type of wood decay. Initially *C. asperatum* showed selective delignification by separation of individual xylem elements due to dissolution of middle lamella and later on it showed formation cavities characteristic to soft rot, particularly type-1. Soft rot is characterized by the formation of cavities ("L" bending or "T" branching and hyphal tunnelling) around hyphae growing in the secondary cell walls of wood (Nilsson 1974; Blanchette, 2000). Formation of these cavities is further classified into two types. In type 1, cavities are formed in the secondary wall whereas in type 2, progressive erosion of secondary walls occur but middle lamella is not degraded in contrast to cell wall erosion by white rot fungi but may be modified in advanced stages (Nilsson *et al.*, 1989; Blanchette, 2000). In addition to cavity formation within the cell wall, development of discrete notches of cell wall erosion by hyphae lying within the lamina is also observed frequently in wood degraded by soft rot fungi (Schwarze and Fink, 1998).

Available literature indicates that both the forms of white rot may be caused by one fungus in different portions of the same wood or in different wood pieces (Blanchette, 1984a, b, 1991). In the present study, light microscopy showed that *Chrysosporium asperatum* and *Trichoderma harzianum* caused different patterns of decay in *Tectona*. This may be evidenced by the presence of distinctive anatomical features and by the staining technique. Initially, both the strains produced a selective delignification of the tissue, manifested by cell separation. Separation of xylem cells owing to the dissolution of middle lamella is considered to be the best indicator of the selective type of decay (Anagnost, 1998; Luna *et al.,* 2004). The staining technique contributed also to separate the selective delignification from the simultaneous decay, as proposed by (Srebotnik and Messner, 1994). Delignified tissue of wood stained blue with astra blue due to absence of lignin while portion of relatively unaffected cell wall stained red with safranine. Our earlier study also demonstrated similar feature to distinguish the delignified xylem cells in *Ailanthus* (Koyani *et al*., 2010).

In the advanced stage of decay, other signs of degradation such as formation of bore holes in the fibres and erosion channels in *Tectona* were also detected on the cell wall of xylem fibres and ray cells. Formation of bore holes, erosion troughs and erosion channels are considered to be the characteristic features of simultaneous rot (Liese, 1970; Rayner and Boddy, 1988; Eriksson *et al*., 1990; Schwarz and Fink, 1998). Our results are in agreement with the earlier reports on selective delignification and simultaneous rot produced by both the fungal strain (Otjen and Blanchette, 1985; Anagnost, 1998; Luna *et al.,* 2004; Koyani *et al*., 2010). Wood samples inoculated with *Trichoderma harzianum* initially showed selective delignification but later on it showed formation of erosion troughs in samples after 120 days of incubation period. On the other hand, *Chrysosporium asperatum* showed such erosion troughs within 90 days after the inoculation. Cell wall thinning is another important feature that was observed in the present study.

Degradation ability of the secondary xylem is considered to be associated with the lignin composition of individual cell type. The libriform fibres and xylem ray parenchyma reported to have relatively high syringyl monomer content (Nakano and Meshitsuka, 1978; Iiyama and Pant, 1998) and it shows peak UV-absorbance at short wave length (Fergus and Goring, 1970a, b). In contrast, fibre-tracheids appear to have high guaiacyl monomer content and show their peak UV-absorbance at longer wave length. Compared to xylem fibres, vessels are found to be more resistant to decay caused by both the strains of fungus in the wood blocks of both the timber species tested. Among the vessels also, narrow vessels were relatively more resistant to fungal attack as compared to wider ones. Wood samples inoculated with both fungi showed that there was no appreciable change in the cell wall of narrow vessels except their separation. On the contrary, wider vessels collapsed completely and became deformed in the samples inoculated with both the strains. In hardwoods, vessel walls are considered to be resistant to degradation by white rot Basidiomycetes as has been described in details (Blanchette *et al*., 1987, 1988). Similar observations are also reported in our earlier study on naturally infected *Ailanthus excelsa* by *Inonotus hispidus*. The persistence of lignin rich vessel elements in the wood inoculated with *Trichoderma harzianum* and *Chrysosporium asperatum* may be due to high percentage of guaiacyl monomer content as reported in earlier investigations (Blanchette *et al*., 1987, 1988; Schwarze *et al.,* 2000; Koyani *et al.*, 2010).

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Table 1: Average per cent weight loss during each incubation period (60, 90 and 120 days)

Decay fungi	% Weight loss (60 days)	% Weight loss (90 days)	% Weight loss (120 days)
Trichoderma harzianum	23.11 (± 7.32)	35.73 (± 6.88)	43.39 (± 7.76)
Chrysosporium asperatum	29.87 (± 5.13)	37.08 (± 7.82)	46.73 (± 8.64)

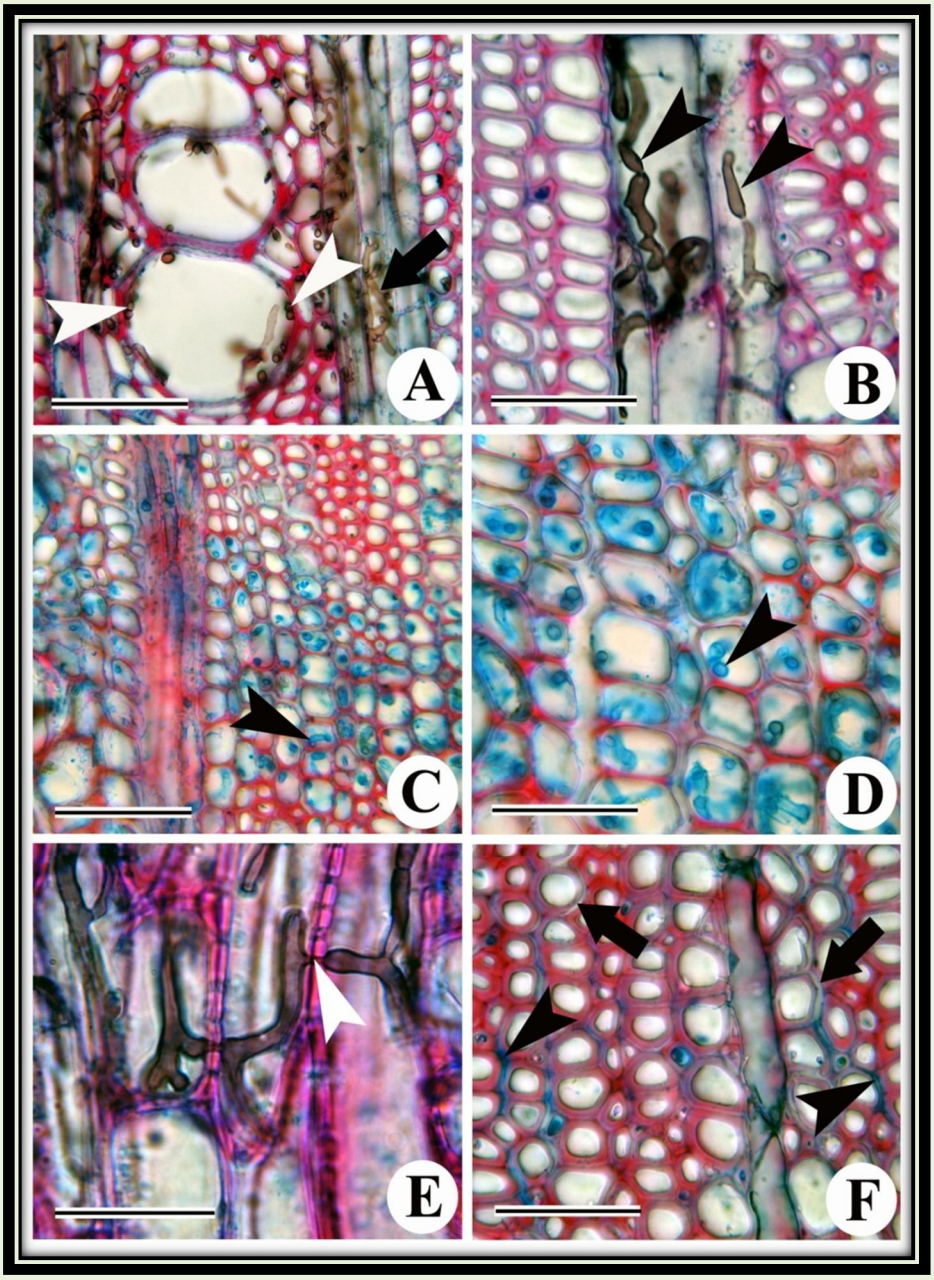

Figure 1: Transverse (A–D & F) and tangential longitudinal (E) view of secondary xylem of ***Tectona grandis*** showing features of decay by ***Tricoderma harzianum***.

A: Fungal hyphae (arrow) passing through vessel lumen, vessel associated parenchyma and ray cells (arrow). Note that all cell types of secondary xylem showing fungal invasion.

B: Fungal hyphae passing through the xylem ray (arrowheads).

C: Invasion of fungal mycelia into axial parenchyma cells (arrowhead).

D: Enlarged view of axial parenchyma cells showing fungal mycelia cut in transverse view (arrowhead).
E: Movement of fungal mycelium from one of the ray cell into neighbouring cell through the pit present on the lateral wall (arrowhead)
F: Initiation of degradation at the cell corners and along the middle lamella of fibre walls (arrowheads). Note the separation of fibres from the middle lamella (arrow).

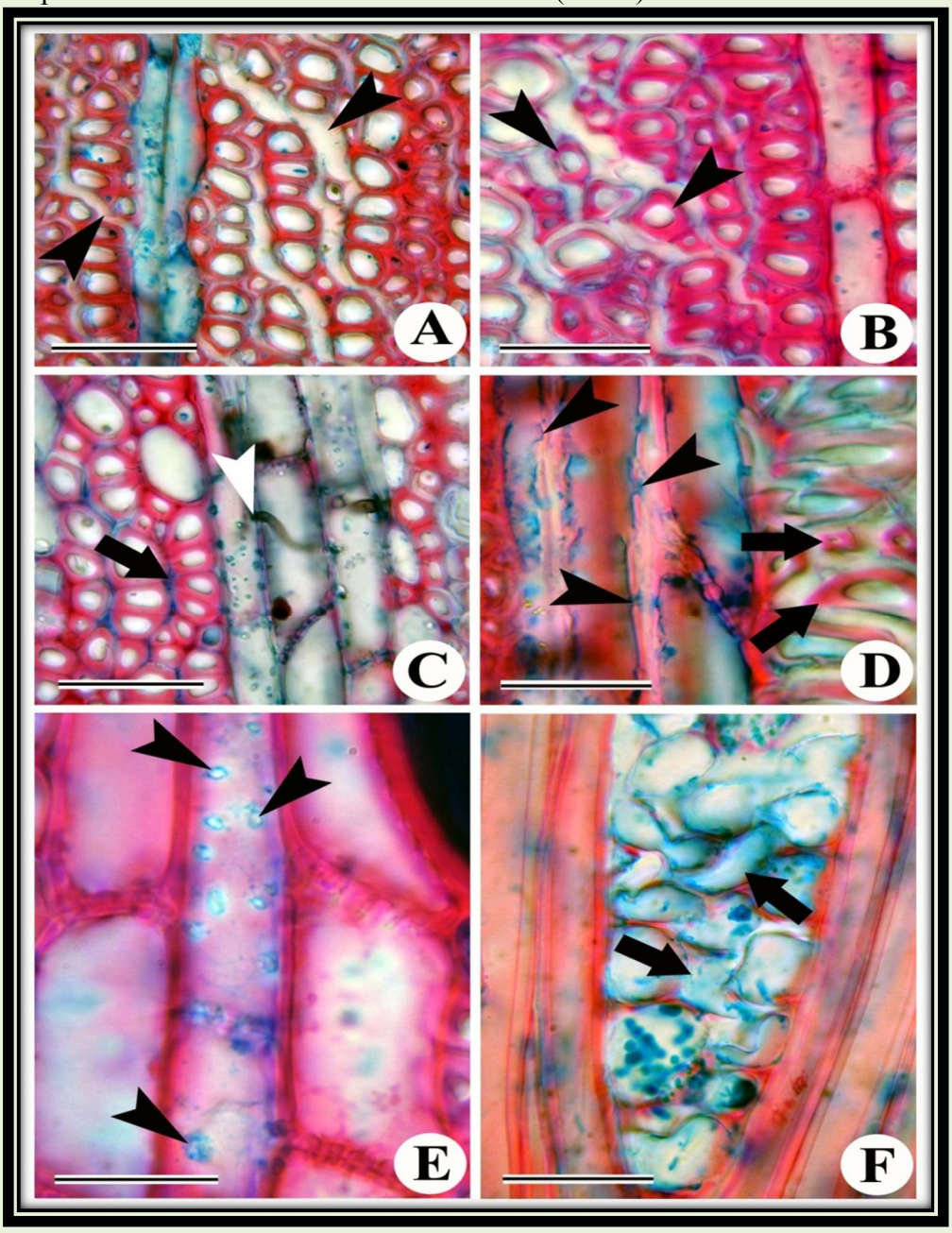

Figure 2: Transverse (A–E) and tangential longitudinal (F) view of secondary xylem of ***Tectona grandis*** *showing* features of decay by ***Tricoderma harzianum*.**

A: Degradation of middle lamella resulted in the separation of xylem fibres from each other (arrowheads).
B: Completely separated xylem fibres (arrowheads) in advanced stage. Note the distinct separating lines passing through the xylem fibres.
C: Xylem rays showing larger bore holes (dotted structure) on the walls. Note the fungal mycelium passing through one of the bore hole (arrowhead). Arrowhead indicates initiation of degradation at the cell corners and along the middle lamella of fibre walls (arrow).

D: Ray cell wall damaged by fungal attack. Note the eroded wall showing erosion troughs (arrowheads). Arrows indicate completely separated fibres.
E: Ray cell wall showing large erosion holes on the wall (arrowheads).
F: Complete collapse of ray cells in advance stage of decay (arrows).

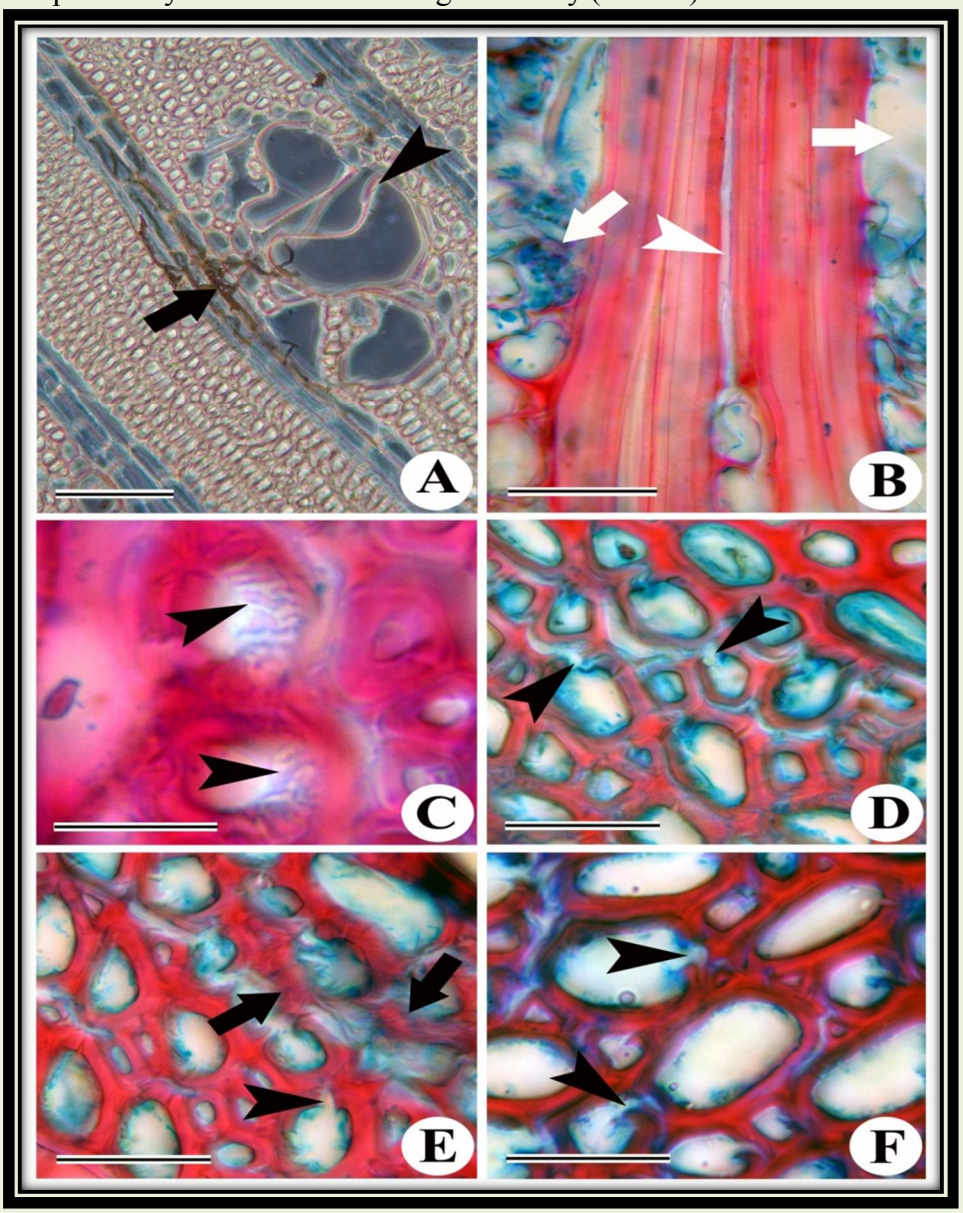

Figure 3: Transverse (A, C–E) and tangential longitudinal (B) view of secondary xylem of ***Tectona grandis*** showing features of decay by ***Tricoderma harzianum*** (A–C) and ***Chrysosporium asperatum*** (D–F).
A: Phase contrast micrograph showing deformed vessel wall (arrowhead) whereas arrow indicates fungal mycelia in the rays, vessel and vessel associated cells.
B: Completely separated xylem fibres (arrowhead). Arrows indicates fully disintegrated ray cells.
C: Fibres showing erosion trough across the cell walls (arrowheads). Note that erosion troughs are irregular in shape and sized and continue across the lumen to middle lamella.
D: Completely separation of xylem fibres showing interrupted walls with erosion troughs (arrowheads).
E: Fibre wall showing erosion trough (arrowhead). Note the distorted fibre wall showing loss of integrity and rigidity (arrows).

F: Fibre walls showing erosion trough (arrowheads).

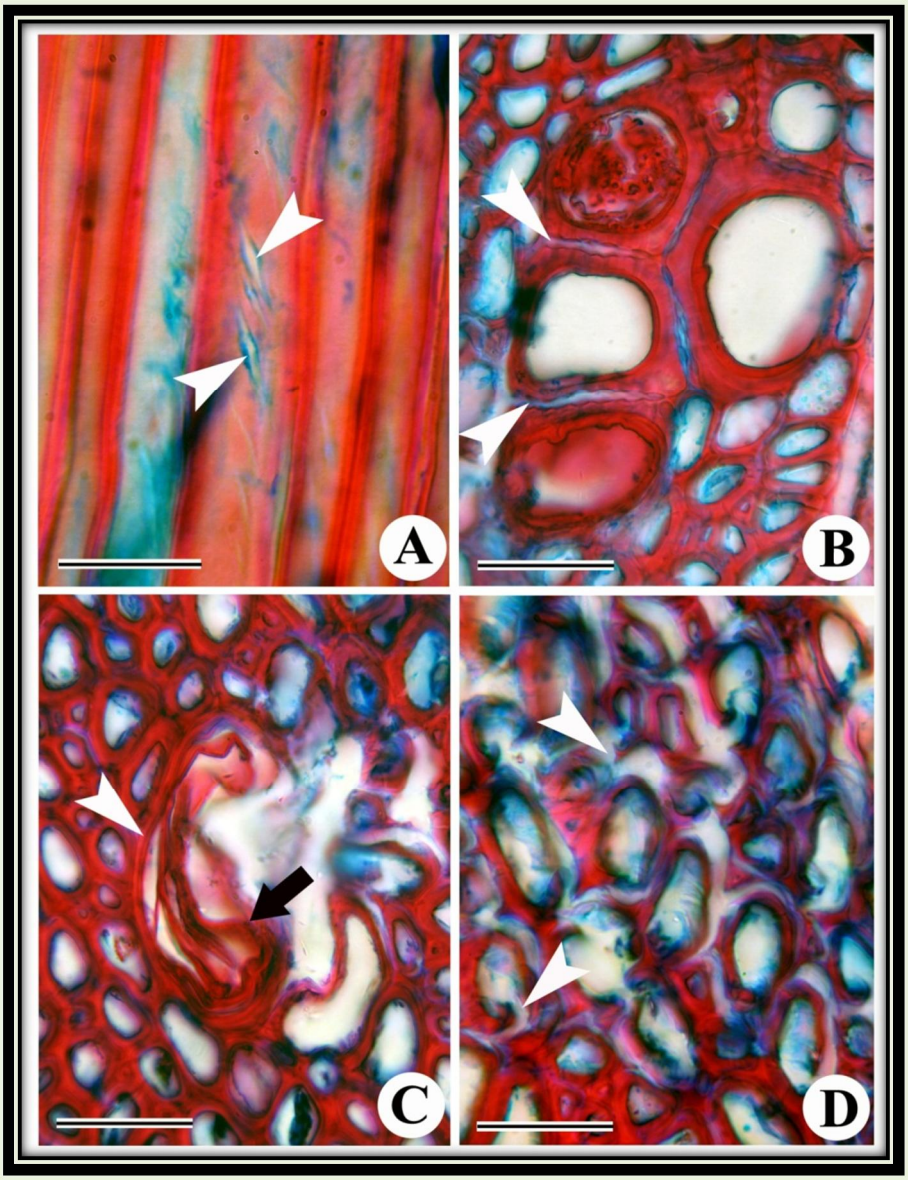

Figure 4: Tangential longitudinal (A) and transverse (B–D) view of secondary xylem of ***Tectona grandis*** showing features of decay by ***Chrysosporium asperatum*.**

A: Fibre wall showing obliquely arranged erosion trough with varying dimensions (arrowheads).

B: Dissolution of middle lamella and separation of small vessels (arrowheads) while wall remains intact.

C: Deformed vessel element showing loss of rigidity and complete collapse of vessel wall (arrow). Note the other side of the vessel element (arrowhead).

D: Advance stage of decay showing loss of cell wall rigidity and interrupted xylem fibres. Arrowhead indicates breaking of the fibre wall at erosion troughs.