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LENZITES REPANDA (MONT.) FR. IN RELATION TO TIMBER DECAY OF *MANGIFERA INDICA* LINN.

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(With 3 Plates)

Lenzites repanda, a common wood-rotting polypore, has been found to grow in abundance on logs and lumbers of *Mangifera indica* during the rainy season. An investigation on certain aspects of its activities on mango wood have been discussed in the present paper.

The isolates obtained from the infected logs and lumbers of mango resemble in detail with the secondary mycelium obtained by random pairing of the primary mycelia grown from single basidiospores.

The externally visible symptoms of the rot have been carefully noted. The rot pockets, distributed from the periphery to the centre, are highly bleached and delimited by blackened zone-lines.

The microscopic changes in the wood due to decay show the presence of abundant mycelium in vessels and wood parenchyma, thining of the walls of different wood components, hyaline, thin-walled hyphæ with clamp-connexions mainly running through the simple and bordered pits and often penetrating the cell-walls forming bore holes.

The observations with standard microchemical reagents have shown that the wood in very advanced stage of decay responds clearly to the test for the detection of cellulose and indicates that the wood is more or less delignified The chemical analysis of the wood further shows that in the early stage of decay, the primary mycelium utilizes more lignin than cellulosic materials whereas, the secondary mycelium utilizes both lignin and cellulosic materials simultaneously. In advanced stage of decay the lignin-content is depleted to the maximum and the cellulosic materials are left in free form.

Decay resistance test under laboratory conditions shows that the wood has been found to be 'non-resistant' to decay when attacked by both the types of mycelia. The primary mycelium is more destructive than the secondary one. They produce identical decay characteristics which are similar to those found in the logs collected from the timber yards.

Positive oxidase reactions produced separately by both primary and the secondary mycelia on different standard media place the fungus in the 'white rot' group.

The toxicity tests carried out with some common water soluble preservatives and creosote against the fungus in question have clearly shown that creosote is very effective for wood preservation.

Introduction

In India, investigations on fungi causing decay of important timbers due to higher Basidiomycetes, have been carried out mainly by Bose (1930), Banerjee and his co-workers (1945, 1947, 1954a, 1954b, 1955, 1956, 1957, 1959, 1960, 1962), Bagchee *et al* (1951, 1953, 1954, 1955, 1960), Bakshi and his associates (1957, 1961), and a few others. The Indian wood-rotting fungi are, however, legion and in comparison to the immensity of the task ahead, these workers have touched only certain aspects of the problem.

The present investigation is a further addition to this line of research and it is one of the series of investigations on higher fungi that are being carried out in the Department of Botany, Calcutta University during the last three decades. The rot selected for the purpose is caused by *Lenzites repanda* (Mont) Fr. in Mango timber (*Mangifera indica* Linn.). From available literature it appears that practically no detailed work has so far been done on *L. repanda* in relation to decay of this timber.

The importance and the structure of the wood may be incidentally noted. This timber is used for the purpose of making heavy furnitures, floor and ceiling boards, window frames, packing boxes, ply wood, oar blades, dug-outs and boats etc. The major of using this timber in exposed condition is that it is rot resistant to decay caused by higher fungi. The durability of the timber is further affected due to its internal structure. The wood is not differentiated into heart wood and sapwood. The presence of abundant wood parenchyma and unevenly distributed large vessels with window like pits leading to ray parenchyma fecilitate rapid distribution of the fungus within the tissues. In the adjacent vessels the pits however, are angular.

The present investigation consists of several lines of inquiry. In order to obtain a clear knowledge regarding the interactions existing between the host-wood and the causal organism, the symptoms of decay, its microscopic studies including penetration and distribution of fungal mycelia within the host-tissues, and the changes brought about by them in the chemical constituents of the wood have been studied. In addition to these, the role of the natural resistance of healthy wood to decay, the nature of the rot produced, and the toxic reactions of different wood-preservatives against primary mycelium and secondary mycelium of the fungus have also been ascertained on a comparative basis.

Materials for Study

The materials for this investigation have been collected from timber-yards of Calcutta in 1964. The fructifications of *L. repanda* have been found to grow

luxuriently on heavily soaked logs and lumbers of *M. indica*, espicially during the months of July and August. Selected short sections (2 ft. long) of the lumber with well developed fructifications (Plate 1, Figs. 3 & 4) have been collected and later transeverse, median and tangential longitudinal sections have been cut to note the internal symptoms in detail. The fructifications have been revived readily after soaking with distilled water. Following Banerjee (1956) viable basidiospores have been obtained from small pieces of these basidiocarps and several monosporous cultures of primary mycelia have been made following the technique suggested by Smith (1954).

As *L. repanda* has been ascertained to be a heterothallic species (Ghosh, 1971) typical secondary mycelia have been obtained by making a series of mating experiments with several primary mycelia of monosporous origin in culture tubes. By random pairing between these mycelia in some cases typical secondary mycelia with clamp connexions have been obtained. Both the types of mycelia have been subcultured on 2.5% malt agar slants and preserved for further investigation.

Fungus in Relation to Timber Decay

(a) Isolation and Identification of the Causal Organism :

Isolation of the causal organism from different portions of infected mango wood has been made following Long and Harsch (1918) modified by Fritz (1923) and by Banerjee (1955). Several transfers have been done and incubated at 30°C in darkness for about a week. On microscopic examination in all cases mycelia have developed and these have been found to be identical in microscopic details with the stock cultures of typical secondary mycelium of *L. repanda*. Furthermore, all the stock cultures and the isolates when transferred to sterilized wood blocks of mango have produced the basidiocarps of *L repanda* under a set of controlled conditions and this has readily helped in the identification of the isolates.

(b) Gross character of the rot :

The visible external symptoms of the decayed mango wood due to attack by *L. repanda* have been carefully studied under field conditions. The discoloured lumbers in the timber-yards have been found to be heavily damaged bearing abundant fructifications of *L. repanda* on their surface and at their cross-section-ends (Plate I, Fig. 1). At these ends of the wood several groups of isolated rot pockets at various stage of decay are distinctly visible extending from the periphery to the centre. Irregular decayed regions often coalesce and form larger rotted areas. These are usually delimited by blackened zone-lines (Plate I, Fig. 5). At the periphery, the pockets are filled with short dusty fibres. These are separated

here and there with compact areas. On testing the decay with the pointed end of a knife it has been found that the texture of wood is soft and it is practically impossible to get long splinters, as the fibres break off short over the top of the knife. This shows that they have become 'brash'. In the advanced stage of decay the rotted wood ultimately becomes spongy and the identity of pockets is completely lost.

In early stage of decay the wood appears rather slightly bleached in contrast to the colour of the normal wood. The colour of the decayed patches is Buff to Yellow-buff, usually towards the centre, but sometimes towards the periphery the colour of the rots includes various shades of brown. In the advanced stage the decayed wood gives mushroomy smell due to the presence of appreciable amount of mycelium and decomposed materials.

Median longitudinal section of the lumber shows similar characteristics. It is more decayed at the periphery, lesser at the centre. The pockets are somewhat elongated, narrow, running parallel to the grain and separated by apparently sound wood. In the pockets bleached soft fibrous mass of decayed wood is evident. Brown to black zone-lines may sometimes be seen separating the decayed areas.

Freshly cut sections of the lumber when moistened in the laboratory for a few days, mycelial growth begins to appear in less decayed areas in the form of white mycelial sheet (Plate 1, Fig. 7) extending radially in a fan shaped manner (Plate 1, Fig. 6). At the margin of the matted sheet in some areas short white mycelial strands make their appearance (Plate 1, Fig. 7) while the older central region becomes some what Yellowish buff in colour.

In extreme case of decay, the rotted areas breakdown completely forming cavities being filled with masses of broken fibres and rendering the wood useless (Plate 1, Fig. 2).

(c) Microscopic details of the rot :

In order to study the changes brought about in the infected host-tissues anatomical characteristics of both sound and decayed woods have been microscopically examined and compared. It has been found that the infected wood brought into the laboratory is already in an advanced stage of decay. As such, the determination of early stages of decay including the mode of infection, distribution of the mycelium within the host-tissues, and the effect of hyphæ on the wood elements has been found to be difficult to ascertain. The age of the decay of the collected timber cannot, therefore be determined due to the non-availability of necessary data. This sample has thus been selected for studying the later stages of decay only. Small wood-pieces from different portions of the lumber showing different stages of infection have been sectioned. The internal mycelium has been found to be in a better condition for observation than that in a highly decayed sample which has become 'brash' and found unsuitable for sectioning.

The materials for studying the early stages of decay have been obtained by following the method suggested by Banerjee and Sinha (1954). Sound, rectangular (5×2.5×1.2 cm) wood-blocks have been subjected to decay after proper soaking and sterilization Blocks exposed aseptically to the attack of both the primary and the secondary mycelia of *L repanda* grown separately on 2.5% *malt-agar* in Kolle-flasks for a period of four months. These have been taken out in batches, one set at the end of each month in order to observe gradual progress of decay during early stages of infection. These test-samples as well as samples from the collected lumber have been fixed and preserved in Formal-Acetic-alcohol (70% Ethanol 85 ml, acetic acid 5 ml and Formaldehyde 10 ml) for future use.

Before sectioning the wood pieces have been softened in gently boiling water until they have become completely water logged and sunk. Both microtome sections (15 – 20μ thick) and free-hand sections of these materials have been cut. The sections are transverse, radial longitudinal, and tangential longitudinal. Free-hand sections have been found to be sufficiently thin and most suitable for detailed examinations in rendering the internal mycelium visible without much distortion. These sections have been differentially stained following the methods described by Cartwright (1929) and that later modified by Proctor (1941). This combination Safranine Piero Aniline blue has been found to be very satisfactory for detecting the hyphal elements within the woody tissues. The lignified cell-walls of the wood elements have shown various shades of red and the fungal hyphæ appeared in deep blue colour.

In early stages of decay no appreciable thining of cell-walls is evident. The fungal hyphæ are mainly found within the vessels and wood parenchyma (Plate II, Fig. 8). The abundance of parenchyma within the wood and large vessels having window-like pits leading the ray parenchyma help the fungus for its easy distribution. All the elements are, however, not equally invaded. Only a few ray cells are found to be infected with fungal hyphæ. At certain places they also invade the wood fibres lying in between the distantly placed vessels (Plate II, Fig. 9), and ray cells. The invaded vessels show the presence of large number of tylosis with hyphæ penetrating them (Plate III Fig. 15). The hyphæ are thin-walled, hyaline, freely branched, distantly septate and with frequent clamp-connexions. They vary in thickness (.85 – 5.1μ wide) and are filled with granular protoplasmic contents. The hyphæ with broader diameter are common within the vessels as well as in the wood parenchyma. The cell to cell distribution of hyphæ are both transverse and longitudinal (Plate III, Figs. 16, 18). The types of hyphal penetrations, as demonstrated by Hartig 1878, 1882, 1894) are present. In one of them, penetration takes place through bore-holes without much reduction in size of the hyphæ. A short narrow branch with more or less acute tip arises from an actively growing hyphæ. It penetrates the wall through the simple pits of fibres and parenchyma cells (Plate III, Figs. 16, 18) and through bordered pits of the

vessels (Plate II, Figs. 11, 13). It bores directly the lignified walls of all these elements and also the overhanging borders of the broad bordered pits. In case of the hyphæ with larger diameter, on the otherhand, the apices become narrow and attenuated and pass through the formed bore holes in the wall till the penetration is complete. On emergence they regain their former width, while the portions within the bore-holes remain extremely thin. In both cases, the bore-holes are as large as or larger than the diameter of the penetrating hyphæ. The uniform smooth contour of the bore-holes support Proctor's view of cell-wall penetration by chemical dissolution of the wall due to enzymes secreted by the invading hyphæ forming the passages Within the vessels, most of the hyphæ run lengthwise and often send out branches at right angles to the paratracheal parenchyma cells. In longitudinal sections a few hyphæ are seen to penetrate the fibres transversely but others run longitudinally through them forming short branches at intervals and those in their turn eventually penetrate the wall. In radial longitudinal sections, the freely branched hyphæ are seen mainly to run transversely through the ray cells (Plate III, Fig. 18).

With the progress decay (after 4 monthr), the mycelium becomes plentiful and the vessels are sometimes choaked with weft of hyphæ (Plate II, Figs. 8, 12). It also produces a heavy network within the parenchyma cells (Plate III, Figs. 14, 18). Ray cells and fibres are the comparatively least infected tissue-elements even at this stage. The tyloses disappear. The brown gummy materials (Plate III, Fig. 17) and the crystals within the ray cells are partially depleted. The continued corrosion of the bore-holes on opposite sides of the cell wall may produce an hour-glass shaped opening later on and in the advanced stage it becomes several times larger in diameter than the passing hyphæ and cylindrical in form. As a consequence the portion of the hyphæ within the enlarged bore-hole widen Considerable thinning of cell walls of all the elements takes place (Plate II, Fig. 10). The bore-holes ($1.7 - 10.2\mu$ in diameter) are numerous at this stage (Plate III, Figs. 19, 20 . The eye-let shaped orifics of the bordered pits enlarge by dissolution of the borders, but their shapes do not alter ($10.2 - 3.4 \times 3.4\mu$ and $15.4 - 3.4 \times 5.1 - 1.65\mu$), whereas other bore-holes remain almost circular to oval in form. Sometimes, adjacent bore-holes coalesce and a larger hole of irregular outline is produced in the wall.

In advanced stages the parenchyma cells gradually separate from one another by separations of middle lamella and produce large gaps in the sections. Even the fibres are thined out to such an extent that their lignified walls are nearly used up by the fungus. They also separate from one another. Innumerable bore-holes in all the tissue elements make it difficult to keep their original shape. However all the tissues are not decayed simultaneously. The most decayed parts fit loosely to their former positions within the less decayed portions Occasionally, within the wood the mycelia produce chanels of certain length ($47.6 \times 5.1\mu$) along their course and by dissolution of contiguous wood elements.

(d) **Chemical effect of Decay :**

(i) *Microchemical tests :*

The preliminary information regarding the chemical changes in wood components that are associated with the progress of decay are usually gathered with the help of simple microchemical methods. These components remain in a highly complex form in the secondary walls and require specific reagents to separate them. In many instances, therefore, the absence of any component cannot be definitely proved in the absence of typical colour reaction.

These methods are employed on the sections of sound wood, partially decayed wood and the wood in an advanced stage of decay. Thin sections of these materials have been treated with Phlorogluein-HCl (Johansen, 1940) and chlor-zinc-iodine (Rawlins and Takashi, 1952) for the detection of lignin and cellulosic materials respectively. The results have further been verified by Maul's treatment (Baxter, 1925) and Iodine Potassiumiodide and sulphuric acid (Whaley, Mericle and Heimsch, 1952).

The observations noted from the tests for lignin and cellulose are give in Tables 1 and 2.

Chlor-zinc iodine and Iodine Potassium iodide and sulphuric acid have failed to produce colour reaction in all the specimens except in case 'brash' wood, when it has been teased and properly treated.

In addition to the above tests, the combination stain safranine and fast-green (Anonymous, 1964) has also been used to differentiate the lignified and non-lignified structures. (Table 3).

From the results obtained by the specific tests for lignin, it can be stated that both the primary and the secondary walls of different wood elements give positive indications of gradual delignification. The intensity of colour reactions produced by the reagents in sound wood diminishes with the progress of decay, which clearly indicates gradual depletion of lignin. In extreme cases of decay the lignified walls of infected wood become colourless indicating complete delignification. The absence of colour reaction in the reagents used for the detection of cellulose, strongly suggests the posibility of masking of cellulosic materials by lignin in the secondary walls. Lignin may remain as a chemical complex with cellulosic substances. The positive reactions obtained in case of 'brash' wood again confirm complete delignification of tissues due to fungal decay which frees cellulose from chemically bound conditions.

The results of combination stains, safranine and fast green, also closely correspond to the above findings and confirm cellulose depletion by exhibiting paler shades of green colour.

Table 1. *Results of staining reactions with Phloroglucin HCl for lignin in sound and decayed wood of* M. indica.

	Sound wood	Partially decayed wood	Advanced decay
Vessels	Primary walls deep red. Secondary walls light red. Tyloses unstained.	Primary walls red. Secondary walls pink to light violet, not uniformly stained.	Primary walls pale red at places. Secondary walls pale pink to colourless, not uniformly stained. Bore-holes bigger opening colourless.
Wood Parenchyma	Primary walls red. Secondary walls high red to light violet.	Primary walls red. Secondary walls light violet to pale pink. Not uniform through out. Opening of the bore-holes colourless.	Primary walls pink to violet at corners, secondary walls pink, pale pink to colourless, not uniform, some cells distorted. Bore-holes colourless.
Wood fibres	Primary walls deep red. Secondary walls light red to violet, except the lining layer, which is nearly as red as primary walls.	Primary walls deep red. Secondary walls pale violet. The layer lining the lumen stained red, but not uniform through out, rarely distorted at places.	Primary walls red to deep red at corners, other parts pink, secondary walls almost colourless. The layer lining the lumen not continuously stained.
Ray cells	Primary walls red, secondary walls light red.	Primary walls red to light red, secondary walls light red to pink not uniform. Badly infected ones are colourless.	Primary walls light red to pink, secondary walls pale pink to colourless, not uniform, some are distorted.

Table 2. *Results of staining reactions with Maul's-treatment ($KMnO_4$-HCl-NH_4OH) for lignin in sound and decayed wood of* M. indica.

	Normal wood	Partially decayed wood	Advanced decay
Vessels	Primary walls red. Secondary walls light red to pink.	Primary walls red to pink. Secondary walls pale pink to colourless at places.	Primary walls pale pink. Secondary walls almost colourless towards the lumen.
Wood parenchyma	Primary walls red. Secondary walls pale red, red along the lumen.	Primary walls pale red to pink, secondary walls pale pink to colourless at places.	Primary walls pale pink. Secondary walls pale pink to colourless in patches. Distorted cells colourless.
Wood fibres	Primary walls bright red. Secondary walls pink at the middle. Red along the lumen.	Primary walls red, secondary walls pink to light red towards the lumen, not uniformly stained.	Primary walls red at corners. Secondary walls pale pink. Pink patches towards the lumen.
Ray cells	Primary walls red. Secondary walls pale pink. Red towards the lumen.	Primary walls pale pink. Secondary walls pale orange in patches.	Primary walls pale pink. Secondary walls pale orange to colourless in patches. Distorted cells colourless.

Following Ritter (1925) sections of the same materials have also been treated with 72% sulphuric acid. The cells of normal and partially decayed wood swell up and break apart leaving mixture of distorted vessels, fibres and fragments of tissues, whereas, in case of wood in advanced stage of decay some unidentified particles are only visible.

Though this test is not conclusive in itself, it may be concluded, that at least in sulphuric acid test positive cellulosic materials are present not only in the partially decayed wood but also in its advanced stage of decay.

Since the microchemical tests have shown progressive loss of lignin and some amount of cellulose, it can be concluded that *L. repanda* is primarily a lignin destroyer.

(ii) *Chemical studies*

The decomposition of wood by fungi is an enzyme mediated chemical process. The decay produced by wood-rotting fungi has been divided into two groups viz., 'white rots' and 'brown rots', on the basis of the wood components they effectively decompose. Analysing the woods decayed by pure cultures of various wood-rotting fungi, Bray and Andrews (1924), Wiertelax (1932), Campbell (1932, 1952), Findlay (1940), Lindeberg (1946), Faræus, Nilsson and Nilsson (1949), Leopold (1951), Mizumot (1955), Cowling (1957), Grohn and Deters (1959), Schanel (1967) and Seifert (1967) have conclusively proved that 'white rot' producing fungi consume certain amount of cellulose along with lignin and this activity varies with the virulence of the species. On the other hand, 'brown rot' fungi utilize mainly cellulosic materials and the lignin content remains more or less constant. The 'white rot' fungi have further been subdivided by Campbell (1930, 1932) into three groups, according to the sequence of attack on the wood components at the initial stage of decay.

In order to obtain accurate information regarding the total changes brought about in the wood components and to find out the possibility of the existence of any difference in the activities of the primary and the secondary mycelia of *L. repanda*, several sound wood blocks of *M. indica* were separately decayed under laboratory conditions. After exposoure to fungal action the blocks were taken out in batches at the end of first, second, third and fourth month from each set. These, along with the sound wood and the naturally decayed wood cellected from the timber-yard were analysed following the methods recommended by Cowling (1960).

The individual sample was ground into small particles to pass through 40 mesh standard seive for better penetration of reagents and stored in screw capped bottles after thorough shaking.

(a) **Moisture-content :**

The moisture content of each sample was first determined before proceeding to any analytical process. 500 mg. of each type of wood meal in replicates of three were weighed in weighing bottles and placed in a vacuum desiccator over magnesium perchlorate. Their tops were removed and the pressure within the desiccator was reduced to 3 mm of mercury. After 24 hours, the pressure was released through a tube containing magnesium perchlorate. Each bottle with its respective top quickly fitted was removed from the desiccator and weighed. The process was repeated till they attained constant weight. The moisture-free-weight was subtracted from the original weight of the sample and the difference represents moisture content.

The results are expressed as mean percentage as shown in Table 4.

(b) **Hot and cold water solubility :**

500 mg. of each type of wood meal in replicates of three were weighed separately in 250 ml. and 500 ml beakers and each was covered with 25 ml of distilled water. The 250 ml. beakers were kept in room temperature (30°C±2°C) for 24 hours while the 500 ml. beakers were kept over a boiling water bath for 3 hours with occasional stirring. After each extraction period the samples were filtered through crucibles (1 G_3), washed 10 times with 15 ml. of hot or cold distilled water accordingly with suction. Each sample was then dried at 60°C for 24 hours and over magnesium-perchlorate to constant weight. The extractive free weight when subtracted from the moisture free weight of the sample gave the hot or cold water solubility in question.

The results are expressed in mean percentage as shown in Table 4.

(c) **Alkali solubility :**

25 ml. of 1% sodium hydroxide solution was used for every 500 mg. of each material contained in 500 ml. beaker. All the samples were treated for 1 hour on boiling water bath with frequent stirring. These were filtered through crucible (1 G_3), washed with hot distilled water followed by neutralization with 15 ml. of 10% Acetic acid (v/v) These were again washed several times with 15 ml. of hot distilled water with suction till they became acid free. Each sample was dried to constant weight.

The results have been determined and expressed as before in Table 4.

(d **Total extractive :**

500 mg. of weighed sample of each type was packed in No. 1 Whatman filter paper and extracted successively in a Soxhlet apparatus with 1 : 2 (v/v) of 95% ethanol and benzene and with ethanol alone. In each case the extraction was

Table 3. *Results of staining reactions with Safranine and Fast green for lignin and cellulosic materials in sound and decayed wood of* M. indica.

	Sound wood	Partially decayed wood	Advanced decay
Vessels	Primary wall deep red. Secondary walls light red to violet.	Primary walls deep red. Secondary walls light red to green in patches.	Primary walls light red to green. Secondary walls light green, not uniformly stained.
Wood paranchyma	Primary walls deep red. Secondary walls light red to violet but partly greenish.	Primary walls light red. Secondary walls light green.	Primary walls light red to pale green. Secondary walls almost absent or light green to faintly green, not uniform, distorted.
Wood fibres	Primary walls deep red. Secondary walls light red to violet.	Primary walls deep red. Secondary walls light red to green in patches.	Primary walls deep red at corners but pale pink to green in patches. Secondary walls green or light red.
Ray cells	Primary walls deep red. Secondary walls light red to violet but partly green.	Primary walls light red. Secondary walls pale pink to pale green not uniform.	Primary walls light red to pale green. Secondary walls faintly green to light green. Distorted cells green.

Table 4. *Data (mean) showing percentage of different wood components in sound wood, partially decayed wood and wood showing advanced stage of decay in* M. indica *by* L. repanda.

	Sound wood	Partially decayed wood								Wood in an advanced stage of decay
		1 month		2 months		3 moths		4 months		
		Primary mycelium	Secondary mycelium	Primary mycelium	Secondary mycelium	Primary mycelium	Secondary mycelium	Primary mycelium	Secondary mycelium	
Moisture-content	6.26	6.36	4.64	7.12	4.24	7.34	7.46	7.4	7.54	7.02
Cold water solubility	8.27	7.73	11.40	3.10	9.12	5.80	5.64	3.11	6.49	8.49
Hot water solubility	11.5	7.54	10.08	5.6	8.3	8.72	7.96	5.66	6.04	8.66
Alkali solubility	22.63	22.55	23.11	16.34	24.31	20.44	20.00	14.60	16.52	31.87
Total extractive	13.28	7.01	8.23	6.90	7.21	9.26	9.13	9.62	8.87	9.56
(i) Alcohol : Benzene	9.98	5.59	5.85	5.16	5.57	5.97	5.55	5.81	5.68	3.61
(ii) Ethyl alcohol	1.92	0.81	0.90	1.03	0.75	0.73	0.82	1.03	1.55	0.96
(iii) Hot water	1.38	0.61	1.84	0.71	0.89	2.56	2.76	2.78	1.64	5.44
Holocellulose content	50.0	57.41	52.6	60.33	51.53	55.25	49.21	56.56	56.56	72.70
(i) Alpha-cellulose	36.54	40.95	38.84	48.02	38.55	37.69	36.99	39.65	36.37	42.81
(ii) Beta-cellulose	3.25	5.94	8.12	5.69	4.62	5.63	5.63	7.14	4.70	20.53
(iii) Gamma-cellulose	10.19	10.50	5.63	6.60	8.35	11.91	6.75	9.76	16.01	9.34
Lignin-content	35.1	32.7	28.94	25.73	29.66	31.62	30.79	30.1	29.74	8.8

continued for 8 hours and the Soxhlet siphoned 3 times per hour. Each extract was evaporated on a waterbath separately and dried to constant weight. Finally the sample was extracted with 400 ml. of distilled water on a hot waterbath for 3 hours. It was then filtered through crucible (1 G_3), washed 5 times with 15 ml. of hot distilled water with suction and dried to constant weight.

The results are expressed in mean percentage in Table 4.

(e) **Holocellulose :**

After determining the moisture-content 200 mg. of each total extractive free sample was weighed in crucible (1 G_3) and fitted to a chlorination apparatus. It was surrounded by ice-water and chlorinated for 30 minutes with moderate suction. At the end of each chlorination period. the ice-water was removed and the material was washed with ethanol for 1 minute. The material was then treated twice with 3% (v/v) hot (75° – 80°C) ethanolic monoethanol amine for two minutes each, washed two times with ethanol and twice with ice-cold distilled water with suction. This process was repeated till monoethanol amine produced no colour reaction. Finally, the washing was done twice with 15 ml. of ethyl ether, kept in air to evaporate the ether and dried to constant weight over magnesium-perchlorate.

The results are expressed in mean percentage as shown in Table 4.

Alpha-, Beta-, and Gamma cellulose :

The moisture content of holocellulose thus obtained was determined. 200 mg. of holocellulose thus obtained was treated twice with 2.8 ml. of 17.5% sodium hydroxide (5.23 N) at an interval of 5 minutes at 20°C, and kept for 45 minutes. Then 5 ml. of distilled water at same temperature was added to it and the whole thing was poured through crucible (1 G_3) with suction. It was washed twenty times with 1.5 ml. of distilled water, then treated with 2.6 ml. of 10% acetic acid for 5 minutes and again washed 8 times with distilled water, till it became acid free. The filtrate was stored in a beaker for the determination of beta and gamma fractions of holocellulose. The final washing of the alpha-cellulose fraction was performed twice with ethanol and thrice with ethyl ether. The ether was evaporated in air and the sample was dried to constant weight over magnesium perchlorate.

3.2 ml. of acetic acid was added to the previously stored filtrate. The precipitate obtained was filtered through crucible (1 G_5), washed twice with distilled water by suction and dried to constant weight over magnesium-perchlorate.

The results have been tabulated as mean percentage in Table 4.

(f) Lignin :

100 mg. of each sample was treated with ice-cold 72% sulphuric acid (v/v) in test-tube (in replicates of three) kept in ice bath and macerated to the sides of the test-tube with a glass rod. After 2 minutes, the tubes were removed and placed in a water bath fastened to a shaker at room temperature (30°±1°C) for an hour. During this period the samples were stirred and macerated at every 15 minutes interval. Finally, the content of each tube was transferred in a beaker with 28 ml. of distilled water covered with watch glass and autoclaved at 15 lbs pressure for 55 minutes. On cooling, the content was filtered through crucible ($1^{'}G_3$), washed with 10 ml. of distilled water with suction till the sample became acid free and dried to constant weight over magnesium-perchlorate.

The result has been expressed as mean percentage in Table 4.

When a fungus utilizes all the constituents of wood it is nutural that the analyses may not reveal a gross change in the relative proportions of the component. In this investigation, however, a marked difference in the activity of *L. repanda* has been observed with regard to the primary and the secondary mycelia at the initial stages of decay.

Fractions of holocellulose was determined following the equation :

$$\text{Yied} = \frac{\text{wt. of } \alpha \text{ or } \beta \text{ cellulose obtained}}{\text{wt. of holocellulose taken}} \times \% \text{ holocellulose}$$

The hot water solubility of the wood samples partially decayed by the primary and the secondary mycelia never exceeds that of the sound wood, and gradually decreases with the progress of decay. On the otherhand, the cold water solubility of the sample decayed by the secondary mycelium is higher than that of the sound wood in the first two months and then it suddenly diminishes. The 1% alkali soluble materials of samples decayed by the primary mycelium never exceed the solubility of the sound wood, but that of the secondary mycelium increases in the first two months and then decreases On the whole, the solubility bears no definite relation with the primary and the secondary mycelia, but in most cases the secondary mycelium shows greater solubil ty than that of the primary mycelium in the first two months of decay and then nearly comes to the same level in the next two months.

The highest yield of the alcohol-benzene (1 : 2) and alcohol extracted materials has been obtained from sound wood. This yield decreases considerably in the first two months of decay but slightly increases than that in the fourth month. The water soluble fraction, however, increases with the progress of decay.

It appears that the cellulose-content of the samples bear no definite relation with the progress of decay. In most cases of the decay caused by the primary mycelium, the cellulose-content is higher than that of the sound wood, but it comes to the same level in the fourth month with that of the samples decayed by

the secondary mycelium. It clearly indicates greater lignin utilizing capacity of the primary mycelium than that by the secondary mycelium. The results obtained for lignin-content also correspond with this finding and confirms that the secondary mycelium attacks both lignin and cellulose at the same time.

The sample decayed in nature shows highest alkali solubility and increased β-cellulose content than that of the sound wood and partially decayed wood thus confirming cellulose consumption during decay. According to Campbell (1930, 1932) and others it is roughly proportional to cellulose depletion. The relative proportion of cellulose and lignin content in decayed wood further proves greater amount of lignin utilization at the advanced stage of decay.

The total amount of lignin, cellulose and the materials of total extractions shows that in case of samples decayed by the primary mycelium it comes near about 93 – 97%, whereas, in case of secondary mycelium, it is 88 – 95% and in case of sound wood it is 99.38%. The differences obtained between these results are possibly due to the production of some intermediate products in higher quantity in case of secondary mycelium, than by the primary mycelium. It can be otherwise explained as the secondary mycelium decomposes more wood substances than it can possibly utilize.

From the above findings, it can be concluded that the two types of mycelia according to their preference on wood components, fall under two different groups proposed by Campbell (1930 – 1932). The primary mycelium may be included under the group utilizing lignin at the initial stages of decay, and the secondary mycelium under another group, attacking both lignin and cellulose simultaneously.

(e) **Decay-resistance :**

In nature, extensive destruction of timber is mainly caused by wood-rotting fungi. The durability of a timber is determined by its natural resistance to this invasion. The study of the correlation between the role of natural resistance in timbers and the destructive capacity of the causal fungi require quantitative estimation under controlled conditions. The underlying principle of the techniques followed by various authors is to expose sterilized test-pieces of sound wood of the species selected for the investigation to the attack of pure cultures of the fungus in question for a specific period and the amount of decay caused by it is estimated in terms of percentage loss in their final dry weights. These investigations are often conducted with the natural isolates from the decayed wood. In most cases the isolates are of the secondary type showing clamp-connexions. A comparative study on the respective behaviour of the primary and the secondary mycelia appears to be meagre. Kaufert (1936), Verrall (1937) and others have mentioned that the primary mycelia are less vigorous than the secondary ones causing the decay, where as Robak (1942), Aoshima (1954), Da, costa and Kerruish (1965) and

Amburgey (1967) have stated that the primary mycelium of a given species shows greater decaying capacity than the secondary one.

For the determination of actual natural resistance to decay of the sound timber of *M. indica* against *L. repanda* the method adopted by Banerjee and Sinha (1954) has been essentially followed and comparison between the relative wood-destroying abilities of the primary and the secondary mycelia under consideration has been made. Several rectangular blocks (5 × 2.5 × 1.2 cm.) of sound wood of *M. indica* of known dry weights have been completely soaked in distilled water under reduced pressure, sterilized and transferred aseptically to a number of Kolle flasks, four in each, to primary and secondary mycelia of *L. repanda* growing separately on sterilized 2% *malt agar* slants. These have been incubated at 32°C in complete darkness for a period of four months. For the determination of the moisture-content of the test-blocks another set of blocks has been removed after sterilization and immediately weighed. The original dry weights of these blocks when subtracted from the weights of the sterilized blocks, the moisture-content of each block has been obtained. The mean moisture-content of the test-blocks thus obtained has been found to be 108% which is far above the fibre-saturation-point. A set of control has also been kept in which the medium of the Kolle flasks has not been inoculated with any type of the mycelium.

At the end of the test period the blocks have been taken out of the flasks. The superficial mycelium of each test-block has been carefully removed and immediately weighed for the determination of moisture-content at this stage. The blocks have been examined for the amount of softening they have undergone and the external manifestations of decay, and lastly dried in an oven at 60°C to constant weights. Their final dry weights have given the loss in weights of the test-blocks due to fungal decay.

The results thus obtained are given in Table 5.

From the respective values of the losses in weights of sound wood-blocks, it can be easily concluded that the decay caused by primary mycelium is greater than that of the secondary mycelium. The mean weight loss during the test period is 19.49 ± 0.22 in case of primary mycelium and 13.32 ± 0.15 in case of secondary mycelium. The percentage of weight losses for the sound wood has been compared and the value of t is found to be 11.75 for 23 degree of freedom, which is highly significant. It further confirms the presence of great difference in the activity of the two types of mycelia.

Following Findlay (1938) the wood of *M. indica* exposed to the attack of primary and secondary mycelia of *L. repanda* has been found to be 'non-resistant', because in both the cases they have caused average weight loss more than 10%. The blocks kept as control show no appreciable loss in weights.

Table 5. *t-test of comparison of loss in dry weight (%) of sound wood blocks of* M. indica *exposed to the attack of the primary and the secondary mycelia of* L. repanda.

Serial Number	Loss (%) due to primary mycelium	Loss (%) due to secondary mycelium	Difference
1	39.63	27.76	11.87
2	31.01	21.74	9.27
3	27.39	16.97	10.42
4	26.84	15.36	11.48
5	19.16	15.00	4.16
6	18.97	13.55	5.42
7	12.94	12.77	0.17
8	11.85	8.53	3.32
9	11.74	6.90	4.84
10	11.59	5.22	6.37
11	10.05	4.76	5.29
12	12.70	11.37	1.33
	S.E. = ±1.111	t = 11.57	

It has been stated the initial moisture-content of the wood blocks before the experiment has been on the average about 108%. After the experimental period, the blocks contained 153% moisture on the average, which indicates that the fungus has not suffered any desiccation during the long course of this experiment as no control measure has been adopted to maintain the moisture-content inside the Kolle flasks.

The decay of the test-blocks is externally manifested by their heavily damaged areas, which appear rough, loose fibrous in texture, and crumble into 'brashy' needles of wood elements under rubbing pressure. They have also become light, soft and somewhat porous. The blocks exposed to the primary mycelium have shown considerable bleaching in comparison to that the sound wood block. In most cases, the entire surface in contact with the fungal mat, and in extreme cases, the entire block has been bleached. On the other hand, the blocks exposed to the secondary mycelium show variable extent of bleached areas, which in turn, have been marked by black zone-lines.

(f) **Oxidase tests :**

The wood-rotting fungi consume complex wood substances by decomposing them into simpler products through extracellular enzymatic activities. The rots due to this decomposition are of two main types, viz., 'brown rots' and 'white rots'. In the former the cellulose and its associated pentosans are decomposed leaving the lignin components unaffected while in the latter all the components are attacked including lignin. On this basis the decay types are classified by Falk and Haag (1927), Wehmer (1927), and Bavendamm (1928), who designated the two

types of rots as 'destruction rot' and 'corrosion rot'. The former covers the 'brown rot' and the latter 'white rot'. According to Wehmer (1927) the degradation of cellulose is a hydrolytic process. The oxidizing enzyme system usually produced by 'white rot' fungi is totally absent in 'brown rot' types. The simple laboratory test followed by Bavendamm (1928) to distinguish 'white rot' fungi from 'brown rot' types is based on colour reaction produced by oxidizing enzymes in presence of phenolic compounds like tannic acid or gallic acid. A few other tests by using certain substances, like gum-guaiacum (Cartwright and Findlay, 1943 ; Boidin, 1951 ; Nobles, 1958) ; gentian violet (Preston and McLennan, 1948) etc., have also been reported.

The nature of the rot caused by *L. repanda* has been determined on the basis of the recommended oxidase tests of Bavendamm (1928), Preston and McLennan (1948), and Nobles (1948) with both the primary and the secondary mycelia. The results of Bavendamm's tests (0.5% tannic or gallic acid in 2 5% *malt agar*) have been summarized in Table 6.

In Preston & McLennan's test (0.007% gentian violet in 2.5% *malt agar*) bleaching of the colour of the medium has been started within 15 days from periphery in case of the secondary mycelium but uniformly throughout in case of the primary mycelium. Complete bleaching of the medium has not, however, been acomplished in any case even after 3 months.

When gum-guaiacum solution (0.5 g. dissolved in 30 ml. of 95% alcohol) has been added to the advancing zones of both primary and secondary mycelia characteristic dark blue colouration has been appeared within 9 minutes and 15 minutes respectively.

From the above observations it can be definitely stated that *L. repanda* is a 'white rot' producing fungus. Both the primary and the secondary mycelia have positively reacted with all the test substances used but the intensity of reactions varied with respect to the specific substance.

Table 6. *Results of oxidase tests with primary and secondary mycelia of* L. repanda.

Types of mycelium	0.5% Gallic acid	0.5% Tannic acid
Primary mycelium	Strong (++++) ; diffusion zone dark brown, opaque, diameter 34—42 mm. forming a wide 'Corona' beyond the slight superficial growth around the inoculum.	Moderately strong (+++) ; diffusion zone light to dark brown, comparatively narrow, diameter 28—34 mm., slight growth above the inoculum.
Secondary mycelium	Moderately strong (+++) ; superficial growth 8 mm. in diameter ; diffusion zone light to dark brown, comparatively narrow, 15—16 mm. in diameter.	Strong(++++); slight superficial growth and or no growth above the inoculum ; diffusion zone dark brown, opaque, 18—20 mm. in diameter.

(g) **Toxicity test :**

Most timbers need protective measures to make them durable against the organisms capable of destroying them. Fungal destruction of wood is by far the most serious one and of most wide-spread occurrence. A great variety of substances and their combination mixtures have been suggested by various workers as wood preservatives for a long time. There is no universally ideal preservative for all kinds of timbers. Even the causal organisms vary greatly in their susceptibility to different toxicants.

In the present investigation toxicity tests with the primary and the secondary mycelia of *L. repanda* have been performed by following both the 'agar plate' and the 'wood-block' methods (Cartwright and Findlay, 1946). Of the recommended preservatives three water soluble compounds (Zinc chloride, AsCu A, and Borax) and a preservatives oil (Creosote) have been selected for the purpose.

Agar-method : 2% stock solution of each water soluble chemical has been prepared and all the under mentioned graded tests have been performed with it. For each concentration grade the required amount of stock solution has been pippetted into separate flasks and mixed hot with 2% *malt agar* after sterilization. The concentration grades prepared for each chemical have been 0.02%, 0.04%, 0.06%, 0.08%, 0.10%, 0.12%, 0.14%, 0.16% 0.18%, and 0.20%. The media thus prepared have been poured in sterile Petri-dishes and separately inoculated with agar discs (6 mm. diameter) cut out from the advancing zone of 7-days-old cultures of the primary and the secondary mycelia and incubated at 30 – 32°C.

The same graded concentrations of creosote have been prepared by blending method following Marsden (1954). For each concentration, 2% sterilized *malt agar* has been blended hot in a blender with required amount of creosote directly pippetted in it. The media have been quickly poured in sterile Petri-dishes. Those have been inoculated and incubated as before. A set without the toxicants has also been kept as control under similar conditions for each test chemical.

After 7 days of incubation linear growth of the mycelial mat on each chemical has been recorded for the concentrations upto the inhibition point and represented in Table 7.

Wood-block method : The respective concentrations obtained at the inhibition points by agar-method and a few grades above them have been employed for each preservatives in this method. Sound woods (5 × 2.5 × 1.5 cm.) of *M. indica* dried to constant weights have been impregnated with graded concentration of the preservatives following Cartwright and Findlay (1946) in a flask and subjected under reduced pressure till they have sank down. Each block has been immediately weighed separately. Final retention of a preservatives in

each wood-block has been calculated from the amount of solvent absorbed in a measured area and the concentration of the solution (Kg/m³) used. The blocks have been sterilized in test-tubes with a little solution of higher concentration at the bottom, 3 in each, and then exposed to the activities of primary and secondary mycelia in Kolle-flasks (Vide for detail section e). All the flasks have been incubated in darkness for 4 months at 30° – 32°C.

Table 7. *Data showing the effect of different water soluble preservatives on growth of both the primary and secondary mycelia of* L. repanda *after 7 days.*

Conc. of Preservative %	Growth in diameter (mean) of the mycelium (in mm.)							
	Zinc chloride		Ascu A		Borax		Creosote	
	Pri. Myce.	Sec. Myce.	Pri. Myce.	Sec. Myce.	Pri. Myce.	Sec. Myce.	Pri. Myce.	Sec. Myce.
0.02	16	8	6.6	10	36	39	6.7	6.8
0.04	—	—	—	—	30	25	—	—
0.06	—	—	—	—	23	19	—	—
0.08	—	—	—	—	11	11	—	—
0.10	—	—	—	—	6	6	—	—
0.12	—	—	—	—	—	—	—	—
0.14	—	—	—	—	—	—	—	—
0.16	—	—	—	—	—	—	—	—
0.18	—	—	—	—	—	—	—	—
0.20	—	—	—	—	—	—	—	—
Control	28	34	30	35	29	35	28	33

In case of Creosote, Petroleum ether has been used as solvent to prepare the graded concentration for the impregnation of the test-blocks. Following impregnation and after complete evaporation of the solvent in air the treated blocks have been used without sterilization.

The weight losses (%) of the treated blocks at different concentrations of the preservatives after experimental period have been determined from the original dry weights and expressed in Tables 8 – 15.

Table 8. *Toxicity test with sound wood-blocks of* M. indica *treated with 'Ascu A' and exposed to the primary mycelium of* L. repanda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (g)	Loss in dry-weight (g)	Loss (%)	Average loss (%)
0.22	11.600	1.66	10.960	0.640	5.51	5.56
	10.330		9.700	0.630	6.09	
	9.640		9.150	0.490	5.08	
0.24	9.440	1.91	9.000	0.440	4.66	5.07
	10.400		9.790	0.610	5.86	
	9.350		8.910	0.440	4.70	
0.26	10.280	2.13	9.610	0.670	6.51	4.86
	10.550		10.160	0.390	3.79	
	10.500		10.050	0.450	4.28	

Table 9. *Toxicity test with sound wood-blocks of* M. indica *treated with 'Ascu A' and exposed to the secondary mycelium of* L. reganda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (g)	Loss in dry-weight (g)	Loss (%)	Average loss (%
	10.500		10.060	0.440	4.19	
0.22	11.150	1.66	10.960	0.190	1.70	3.27
	9.930		9.540	0.390	3.92	
	10.650		10.390	0.260	2.44	
0.24	10.6 0	1.91	10.280	0.400	3.74	2.95
	10.860		10.570	0.290	2.67	
	9.430		9.150	0.280	2.97	
0.26	10.200	2.13	9.900	0.300	2.94	2.25
	10.500		10.460	0.090	0.85	

Table 10. *Toxicity test with sound wood-blocks of* M. indica *treated with Zinc chloride and exposed to the primary mycelium of* L. reganda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (g)	Loss in dry weight (g)	Loss (%)	Average loss (%)
	10.040		8.940	1.100	10.95	
0.18	10.380	1.41	9.770	0.610	5.87	6.83
	8.990		8.660	0.330	3.67	
	10.130		9.640	0.490	4.83	
0.20	9.750	1.52	9.160	0.590	6.05	6.36
	9.490		8.710	0.780	8.21	
	10.010		9.510	0.500	4.99	
0.22	10.850	1.7	10.170	0.680	6.26	5.15
	10.200		9.770	0.430	4.21	

The tables show that the inhibition point obtained in agar method for each preservative bear little or no correlation with that obtained in wood-block method. The concentrations of the preservatives used for the wood-block method are

Table 11. *Toxicity test with sound wood-blocks of* M. indica *treated with Zinc chloride and exposed to the secondary mycelium of* L. repanda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (g)	Loss in dry weight (g)	Loss (%)	Average loss (%)
0.18	11.250	1.41	10.320	0.930	8.26	6.72
	9.770		9.210	0.560	5.73	
	10.500		9.850	0.650	6.19	
0.20	9.830	1.52	9.250	0.580	5.90	5.67
	10.540		10.030	0.510	4.93	
	9.680		9.080	0.600	6.19	
0.22	9.800	1.7	9.330	0.470	4.79	5.26
	10.350		9.730	0.620	5.99	
	10.390		9.870	0.520	5.00	

Table 12. *Toxicity test with sound wood-blocks of* M. indica *treated with Borax and exposed to the primary mycelium of* L. repanda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (g)	Loss in dry weight (g)	Loss (%)	Average loss (%)
0.18	9.170	1.44	8.550	0.620	6.75	6.84
	10.020		9.420	0.600	5.98	
	9.350		8.620	0.730	7.80	
0.20	10.070	1.55	9.550	0.520	5.16	5.06
	8.940		8.490	0.450	5.03	
	11.000		10.450	0.550	5.00	
0.22	9.950	1.66	9.540	0.410	4.12	4.82
	10.100		9.540	0.560	5.54	
	10.190		9.700	0.490	4.80	

approximately two to five times greater than those found in the agar method. Even then complete inhibition of fungal growth has not been observed. The finding confirms the conclusion of Cartwright and Findlay (1942), Leutritz (1946),

Table 13. *Toxicity test with sound wood blocks of* M. indica *treated with Borax and exposed to the secondary mycelium of* L. repanda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (g)	Loss in dry weight (g)	Loss (%)	Average loss (%)
0.20	10.200	1.55	9.800	0.400	3.93	4.70
	11.290		10.620	0.670	5.93	
	10.310		9.870	0.440	4.26	
0.22	9.440	1.66	9.080	0.360	3.81	3.53
	9 600		9.220	0.380	3.95	
	9.840		9.560	0.280	2.84	
0.24	9.500	2.14	9.300	0.200	0.2'0	2.32
	10.630		10.520	0.110	1.03	
	9.910		9.530	0.380	3.83	

Table 14. *Toxicity test with sound wood blocks of* M. indica *treated with Creosote and exposed to the primary mycelium of* L. repanda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (g)	Loss in dry weight (g)	Loss (%)	Average loss (%)
0.08	10.920	0.24	8.350	2.570	23.54	20.43
	10.550		8.690	1.860	17.44	
	11.132		8.920	2.150	19.31	
0.10	10.940	0.32	9.460	1.480	13.52	9.7
	10.490		9.390	1.100	10.48	
	9.990		9.480	0.510	5.1	
0.12	10.180	0.49	9.510	0.670	6.48	3.72
	10.550		10.430	0.120	1.13	
	10.130		9.770	0.360	3.55	

Torgeson (1967) and others regarding poor correlation between results obtained by following the two methods. The primary mycelium of *L. repanda* show greater tolerance of preservatives than the secondary one under similar conditions.

Table 15. *Toxicity test with sound wood-blocks of* M. indica *treated with Creosote and exposed to the secondary mycelium of* L. repanda *for a period of 4 months at 30° – 32°C.*

Concentration of treating solution (%)	Initial oven-dry weight (g)	Absorption Kg./m³	Final dry-weight (%)	Loss in dry weight (g)	Loss (%)	Average loss (%)
	9.570		7.720	1.850	19.33	
0.08	10.170	0.24	8.862	1.308	12.05	14.05
	10.240		9.220	1.020	9.96	
	9.390		9.310	0.080	0.851	
0.10	10.080	0.32	9.560	0.520	5.158	2.49
	10.170		10.020	0.150	1.484	
	10.020		9.500	0.320	3.239	
0.12	9.950	0.49	9.950	—	—	1.079
	10.080		10.080	—	—	

The minimum decay of wood-blocks have been caused by the primary and the secondary mycelia in *L. repanda* at the concentration of 0.26% of Ascu A, 0.22% of Borax and Zinc-chloride and 0.12% of Creosote. The minimum weight losses in lowest concentration has been obtained in creosote treatment. It indicates that further trials with the wood-blocks treated with higher concentrations of creosote will completely inhibit the decomposition of wood by both the primary and the secondary mycelia of *L. repanda.* Hence Creosote can be safely recommended as an effective fungicide for the fungus in question.

Discussion

The frequent occurrence of *Lenzites repanda* on one of the useful timbers, viz., *Mangifera indica,* and the extensive destruction of the wood caused by it appear as a question of great concern with regard to timber economy in this country. It will, therefore, be worthwhile to discuss in a general way some of the aspects of this fungus in relation to timber-decay.

The logs of *M. indica,* so common in the timber-yards with large fructifications of *L repanda,* have been found to be heavily attacked by the fungus often making them practically useless. The cross-section-ends of the log or the lumbers show several rot pockets at various stages of decay extending from the periphery right upto the centre. The tissues of the rotted areas in the advanced stage become loose, fibrous and highly bleached. In extreme cases, they break down completely forming cavities with broken masses. At a comparatively early stage the decayed areas are often delimited by dark zone-lines from the apparently sound wood.

Certain anatomical features and properties of the host-wood quite possibly help in causing the infection. The porous wood when completely soaked retains water more than its own dry weight. The mycelium of the fungus easily passes through its big conspicuous vessels and their large "windo-like" bordered pits (Pearson and Brown, 1932) without least resistance. The microscopic examinations of the infected wood fully confirms this statement, as abundant fungal hyphæ are present in the vessels and the wood-parenchyma. The hyphæ, though chiefly located in the vessels and the wood-parenchyma, eventually invade the fibres lying between them. The cell to cell penetration by the hyphæ takes place mainly through the simple pits of wood parenchyma and wood fibres and bordered pits of the vessels. In an advanced stage of decay they also directly penetrate the cell-walls by enzymatic dissolution forming bore-holes whose even contours confirm Proctor's (1941) theory of cell-wall penetration.

Both the primary and secondary mycelia of *L. repanda* are capable of producing decay with identical characteristics on sound wood-blocks after being exposed to them separately for a limited period. The primary mycelium shows significantly greater (t=11.57 for 23 degrees of freedom) decaying eapacity than that of the secondary one. As the dissemination of the fungus takes place by the basidiospores, it is obvious that the primary infection of the wood and the entry of the fungus within the host tissue take place in the primary condition. If per chance the wood is infected by basidiospores of opposite sex the resultant primary mycelia of compatible strains eventually unite within the wood elements and initiate the secondary stage of development of the fungus.

This secondary condition of the mycelium has only been found in any infected wood in which the decay has progressed to a considerable extent. It is this secondary mycelium that produces the fructifications of the fungus esternally under favourable conditions for their development.

From the mode of distribution of rot pockets within the wood it is clear that the infection spreads from the periphery of the logs towards the centre. The non-living wood-elements are non-resistant to decay. The fungus causes 19.49% and 13.32% decay in 4 months test when sound wood-blocks of *M. indica* are exposed separately to cultures of primary and secondary mycelia of *L. repanda* respectively.

The available records do not provide any information regarding the activities of *L. repanda* on living mango trees. As the fungus is a wood inhabiting one, it evidently utilizes the wood components saprophytically to obtain its nourishment. The preliminary qualitative tests on the chemical effects of decay in the host wood have helped to reveal specifically the degradation of lignin by the fungus. Chemical analyses of the decayed wood, under laboratory conditions and in nature, present a clear picture of the biological decomposition of the wood. The fungus is primarily a lignin destroyer. The cellulosic components are also

decomposed along with it. At least at the initial stages of decay, the primary mycelium utilizes more lignin than the cellulosic materials, whereas the secondary mycelium utilizes both the lignin and the cellulosic materials simultaneously. These differences are more or less minimized at a later stage of decay.

The degradation of lignin being an oxidative phenomenon, it is obvious that the fungus should possess an oxidizing system. The simple standard oxidase tests on medium containing either gentian violet or gallic acid or tannic acid in addition to gum-guaiacum test confirm the production of extracellular oxidases by the fungus As such, the rot produced by the fungus can easily be classed under the 'white rot' group. The more pronounced oxidase reactions produced by the primary mycelium than those by the secondary mycelium show its greater oxidizing capacity. It explains, on the other hand, the result of chemical analysis which shows that the primary mycelium is capable of degrading more lignin, which is an oxidative process, than that by the secondary mycelium.

It can be suggested that the decay caused by the fungus can effectively be prevented by using creosote as the suitable preservative. It has been found to be highly toxic to this fungus at lowest concentration than other common water soluble wood-preservatives. For temporary preservation of wood, therefore, painting the external surface with creosote is advisable. But impregnation of timbers with creosote in a pressure plant is essential to use the timber in exposed condition for a long period.

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EXPLANATIONS OF PLATES

PLATE I

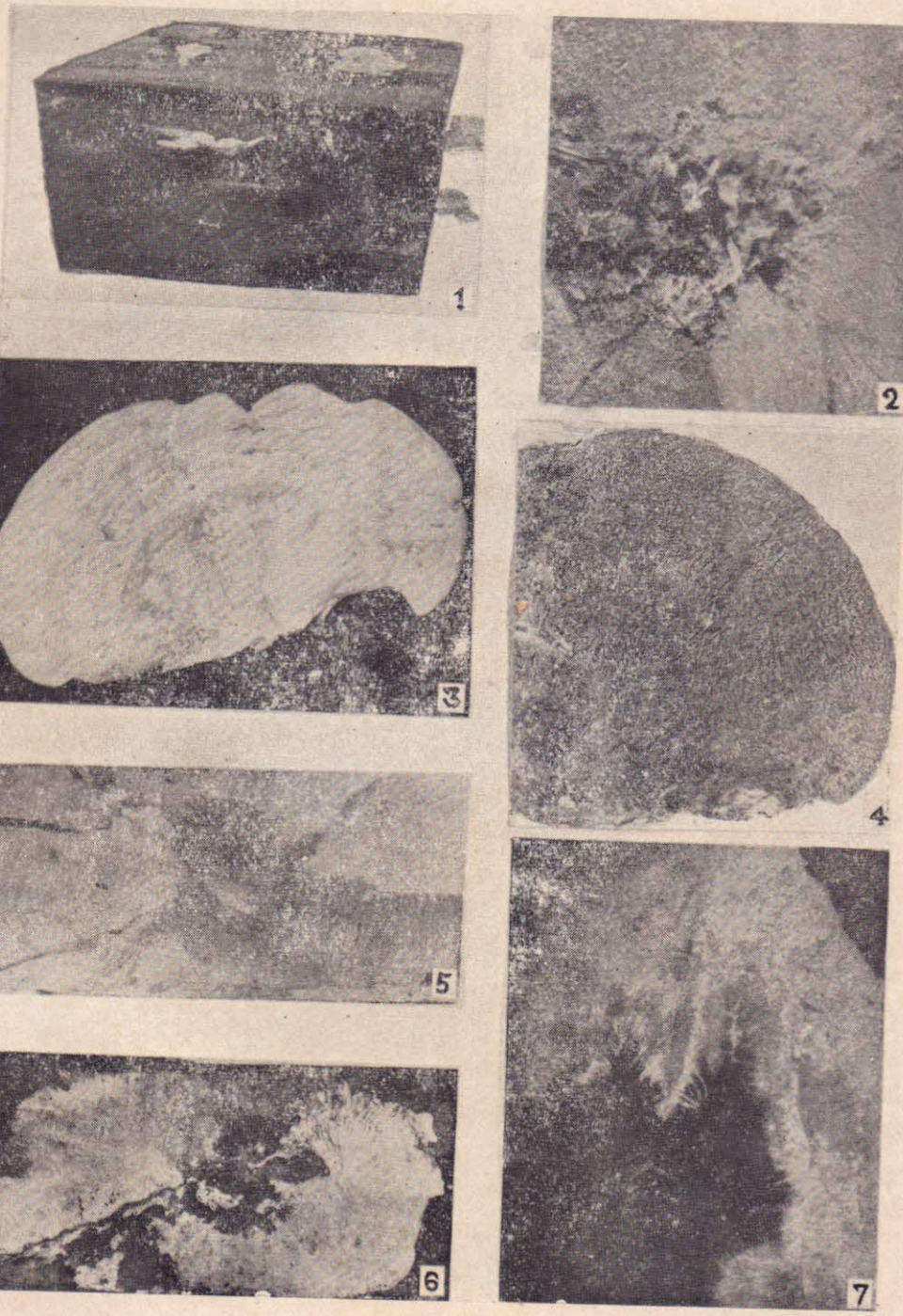
Fig. 1. Fructifications of *L. repanda* on lumber of *M. indica* (7/8 reduced).
Fig. 2. Part of a cross section of the infected lumber of *M. indica* in an advanced stage of decay showing cavity filled with masses of broken wood elements and conspicuous shrinkage cracks (½ reduced).
Fig. 3. Upper surface of the fructification of *L. repanda* showing broad zonations and irregular pustules (¼ reduced).
Fig. 4. A part of hymenial surface of the fractification of *L. repanda* showing lamellæ (½ reduced).
Fig. 5. Transverse section of the infected lumber of *M. indica* showing irregular bleached areas at various stages of decay delimited by blackened zone lines (½ reduced).
Fig. 6. The mycelial mat expands radially in a fan shaped manner (½ reduced).
Fig. 7. Short mycelial strands at the margins of the matted sheet (½ reduced).

PLATE II

Fig. 8. Photomicrograph of a part of a transverse section through partly decayed wood of *M. indica* showing masses of hyphæ within the vessel and ray cells (×360).
Fig. 9. Photomicrograph of a part of t. s. through decayed wood of *M. indica* showing distribution of hyphæ within the ray cells and wood fibres (×500).
Fig. 10. Photomicrograph of a part of a transverse section through decayed wood of *M. indica*, showing thinning of the vessel walls and of other wood elements (×500).
Fig. 11. Photomicrograph of a part of a vessel wall of the infected wood of *M. indica* showing penetration of hyphæ through large bordered pits and also directly through the walls forming bore-holes (×900).
Fig. 12. Photomicrograph of a part of a radial longitudinal section of the decayed wood of *M. indica* showing abundant hyphæ within a vessel and wood parenchyma.
Fig. 13. Photomicrograph of a part of a vessel wall of the infected wood of *M. indica* showing penetration of hyphæ through hexagonal bordered pits and also directly through the borders forming bore-holes (×900).

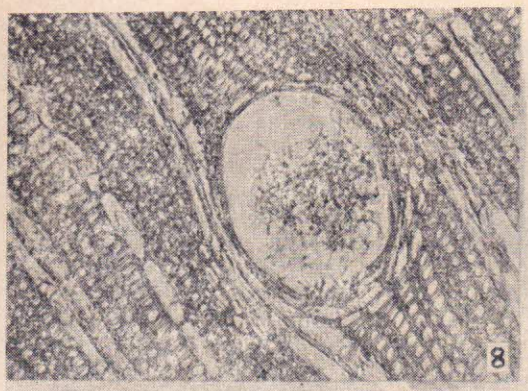
PLATE III

Fig. 14. Photomicrograph of a part of a tangential longitudinal section of the decayed wood of *M. indica* showing distribution of hyphæ within the vessels and wood parenchyma (×400).
Fig. 15. Photomicrograph of a part of a radial longitudinal section of the decayed wood of *M. indica* showing presence of hyphæ within the tyloses (×700).
Fig. 16. Photomicrograph of a part of a tangential longitudinal section of the infected wood of *M. indica* showing penetration and distribution of hyphæ within the wood-fibres and ray cells (×900).
Fig. 17. Photomicrograph of a part of a transverse section through the infected wood of *M. indica* in early stage of decay showing deposition of gummy materials (×700).
Fig. 18. Photomicrograph of a part of wood parenchyma of decayed wood of *M. indica* showing distribution of hyphae (×900).
Fig. 19&20. Photomicrographs of parts of the wood fibres and wood parenchyma of advanced decayed wood showing numerous bore holes of different sizes (×900).



On *Lenzites repanda* (Mont.) Fr.

P. Ghosh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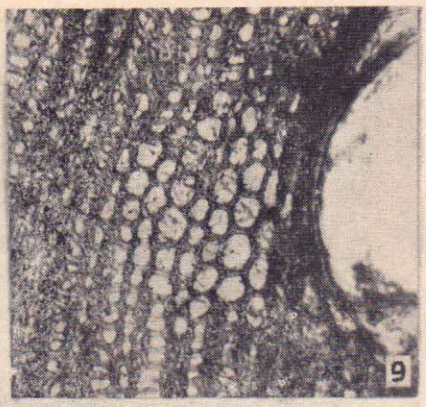
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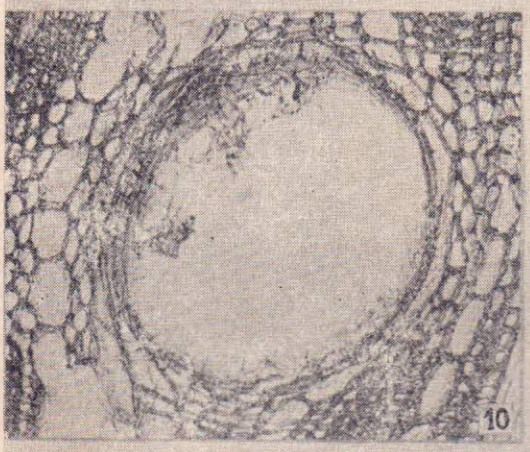
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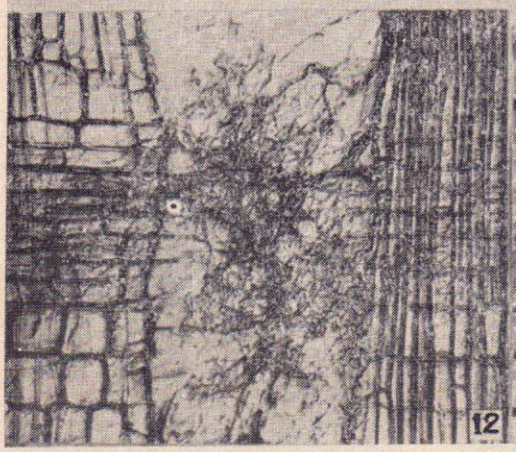
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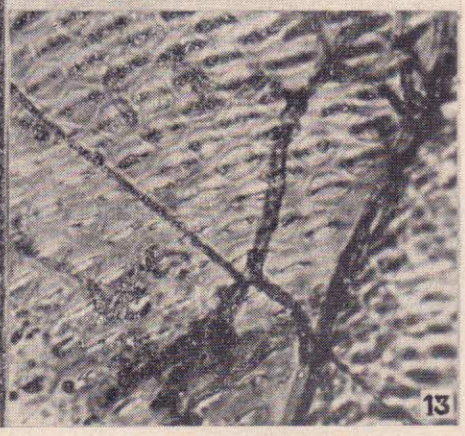
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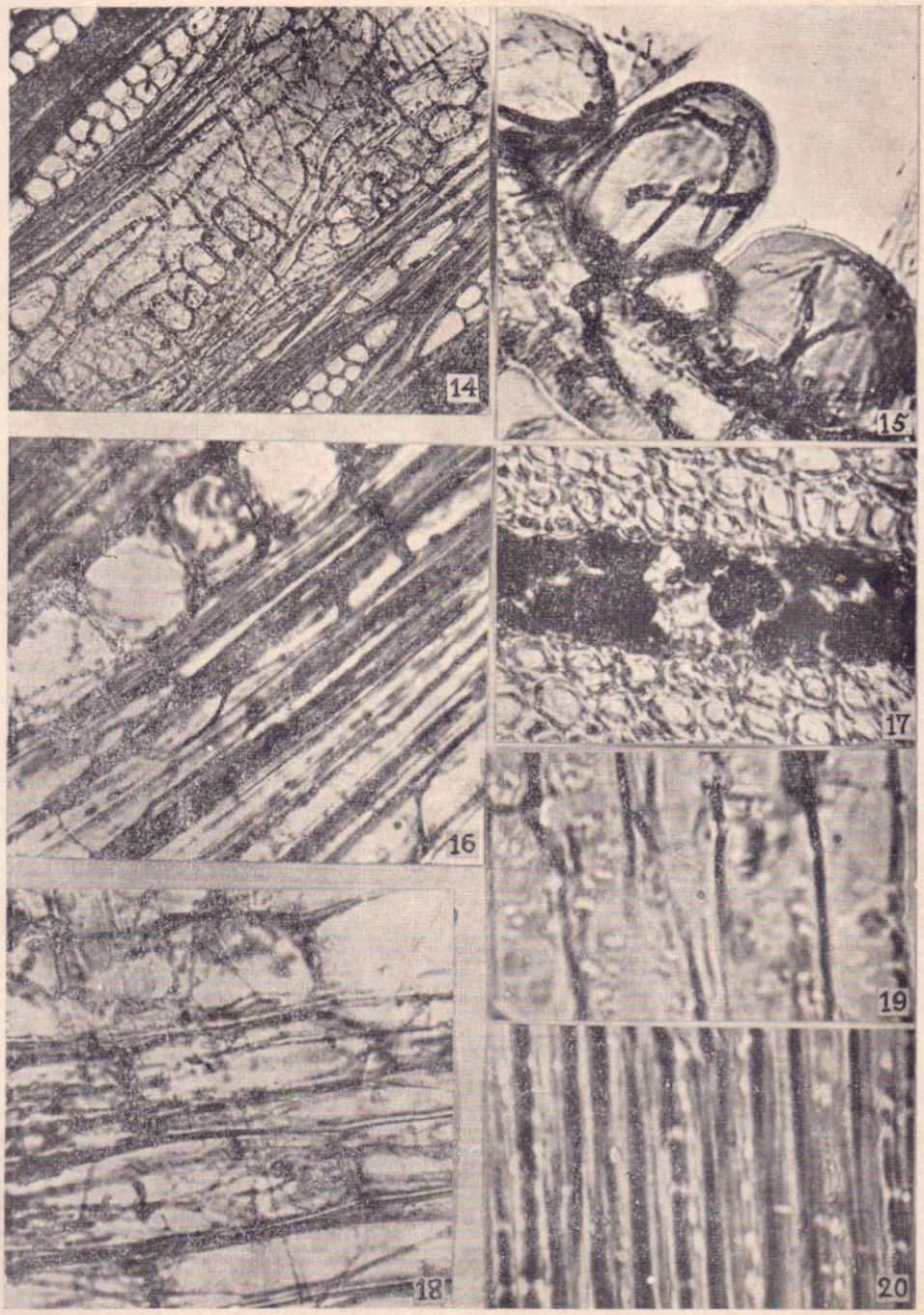

On *Lenzites repanda* (Mont.) Fr. P. Ghosh