(Department of Botany, University of Calcutta, India)

A Study on *Merulius similis* B. & BR. and the associated Bamboo-Rot

By

Sachindranath Banerjee and **Sankar Mukhopadhyay**

With 17 Figures and 4 Tables

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Introduction

In this country *Merulius similis* B. & BR., an interesting member of Hymenomycetes grows in association with bamboo clumps. At first it has been recorded to grow on an unidentified wood (BERKELEY and BROOME, 1875) but later its occurrence only at the bases of bamboo stumps has been reported by several workers (PETCH, 1910; BOSE, 1921; BANERJEE and BAKSHI, 1945). As such *Bambusa bambos* DRUCE can so far be assigned as its singular host species.

The distribution of the fungus also appears to be very limited. It has been reported from Portuguese Guinea, Malaya Peninsula, Ceylon and India suggesting its distribution only in the tropical countries. The highest altitude from which it has been collected is almost 3281′ above the sea level.

In Bengal, its large, soft, fleshy and resupinate to effuso-reflexed fruit bodies with ochreaceous hymenial surface are abundantly formed during the rainy seasons (July–September) but abruptly disappear at the end of the season. This short span of life of fructification seems peculiar when contrasted with several other commonly occurring persistent wood-rotting fungi. The high moisture-content of the fruit-body, its soft fleshy nature, its occurrence in damp situations, its absence in higher altitudinal areas, its sudden appearance at the onset of monsoon and eventual disappearance after a brief period of existence, seem to indicate its definite relationship with environmental conditions, particularly temperature and rainfall. The identical conditions of average

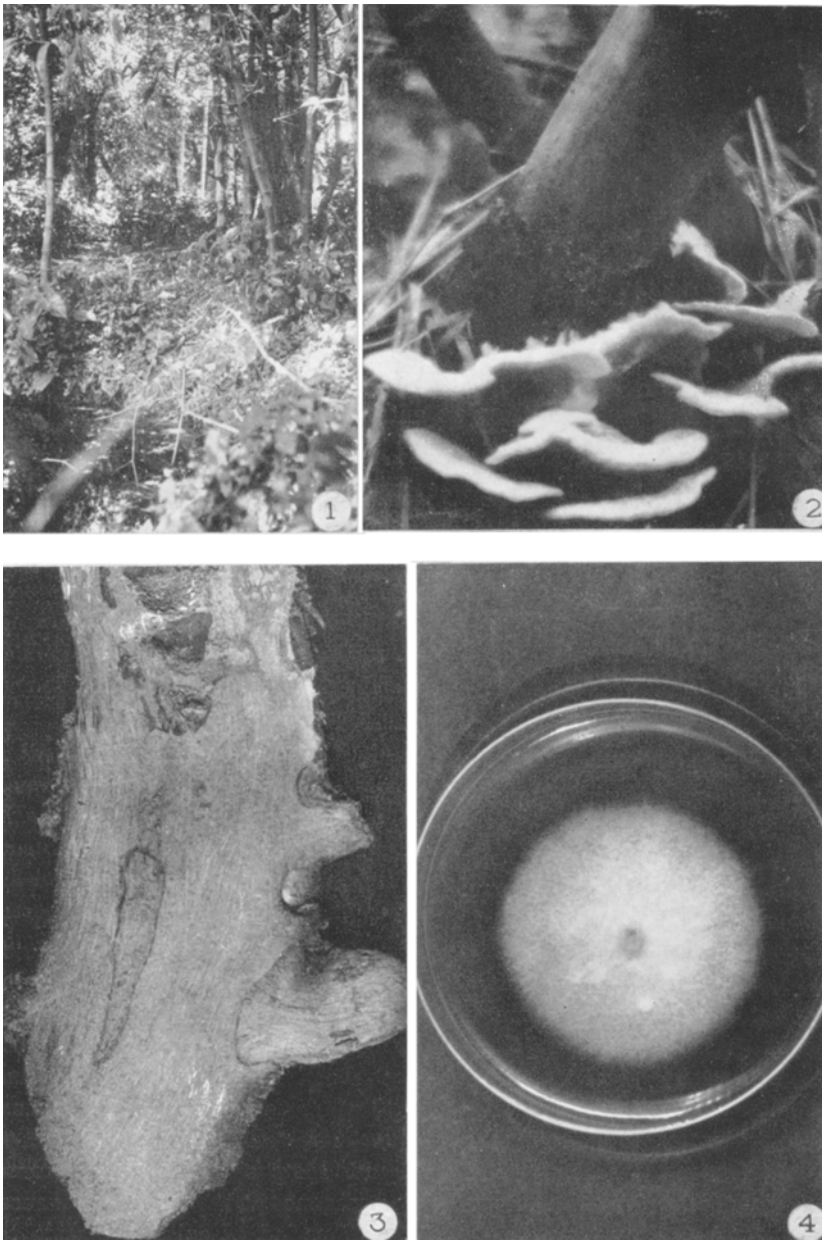

Fig. 1—4

temperature (70°–80° F) and rainfall (12″–16″) during the growing season in all the countries from which it has been reported, may be regarded as an added evidence for it.

Merulius similis has been first described by Berkeley and Broome, 1875. Petch, 1910, 1916 reports it as *Merulius eurocephalus* B. & Br. and considers *Polyporus (Merisimia) eurocephalus* B. & Br. as its synonym.

The genus *Merulius* (Hall) Fr. is generally included in the family Polyporaceae (Ainsworth and Bisby, 1954) but others (Rea, 1922; Bourdot and Galzin, 1927) in Meruliaceae. In a recent work, Harmsen, 1960, has discussed in detail the views so far held as to the generic conception of *Merulius*. He advocates for the retention of the name *Merulius* to the brown-spored group.

Though considerable amount of work has been done on the related members, particularly on *Merulius lacrymans*, yet informations on *Merulius similis* still remains very limited. Considering its complex association and economic importance of the host, the present investigation has been undertaken.

Source of Material

The material was collected from Calcutta and suburbs during the months of July to September. Its frequency of occurrence had been noted to be maximum during the month of August. In majority of cases the infected bamboo groves were located near the ponds and situated in such a way as to get the minimum amount of direct sun-light (Fig. 1).

The large, soft, fleshy, irregular or resupinate to bracket-shaped fructifications had been found on the exposed roots at the base of bamboo clumps (Fig. 2).

The stumps with fructification were collected and macroscopically examined. Blocks ($2'' \times 1'' \times {}^1/_2''$) were immediately prepared from their nodal, internodal and also from rhizomatous portions. Microscopic observations indicated the presence of a few fungal hyphae in the nodal and the internodal areas, while the sections from the rhizomes showed

Fig. 1. A typical bamboo grove beside a pond in cold and shady situation where *Merulius similis* B. & Br. grows profusely

Fig. 2. Imbricated clusters of fructification at the exposed root of a bamboo stump ($^1/_8$ nat. size)

Fig. 3. Longitudinal section of the infected rhizome of *Bambusa bambos* Druce, showing rotted areas due to the attack of *M. similis* ($^1/_8$ nat. size)

Fig. 4. Growth characteristics of secondary mycelium of *M. similis* on malt-agar on the 10th day after inoculation

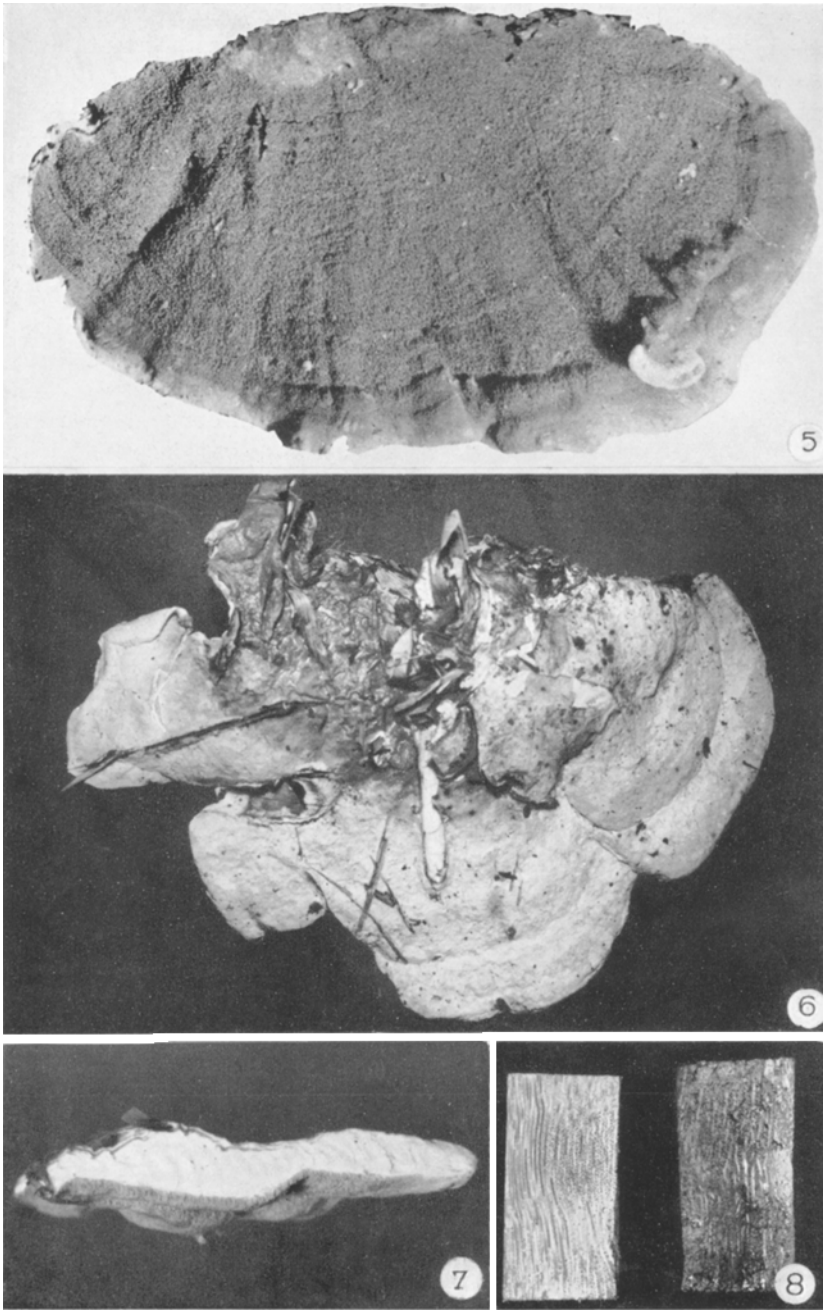

Fig. 5 -8

numerous, much branched septate hyphae with frequent clamp-connexions. Copious spore-discharge occurred from the freshly collected fructifications and in contrast to some other commonly found polypores, it did not take place from the dried specimens even after being sufficiently soaked in water.

The Sporophore

Fructification: Annual; usually sessile, sometimes resupinate or effuso-reflexed in part; when sessile attached to the substratum by a broad or a comparatively narrow base, in some cases the base partly encircling the substratum, dimidiate or reniform, single or in imbricate clusters, sometimes laterally confluent, soft, fleshy, breaking easily, on drying coriaceous and somewhat brittle, 5.5–30 cm across, 2.5–6 cm deep, and 0.5–1 cm in thickness. Margin thick, entire or wavy, whitish throughout or cream coloured at places when fresh, becoming involuted when dry, Caldera coloured with patches of Light Stone, Antique Gold on drying. Upper surface: Uneven, smooth, slightly sticky when fresh, somewhat wrinkled like dried leather when dry; azonate throughout, at first white, soon becoming Tawny, Yellow-Ochre or Chamois behind, but with an irregular whitish zone towards the margin, on drying colour changes to Chamois, with patches and streaks of Caldera colour (Fig. 6). Context: White, soft and fibrous, about 3–5 mm thick; an intermediate narrow, water-soaked zone, about 1 mm or less thick, present at the bases of pore tubes (Fig. 7). Hymenial surface: Poroid, soft, faintly zonate towards the margin, Yellow-Ochre, Negget Bronze Y, or Tan coloured, changing to Oak or Sudan Brown on drying; pore-mouths sub-circular or irregular, 1–3 mm across, in some cases intervening dissipiments break down; pore tubes short, dipping unequally into the substance of the context, about 2–3 mm long or reduced to shallow pits a little behind the broad sterile zone at the margin (Fig. 5). Basidia: Clavate, tetrasterigmatic and quadrisporous, dimension about 16–20 (28) μ × 3–3.5 (6) μ, with simple clamp-connexions at the base (Fig. 16). Basidiospores: Yellow, spherical to oval, wall very thick and darker than the central region, smooth; about 3.5–6 (7) μ across (Fig. 16).

Fig. 5. Fructification of *M. similis* showing hymenial surface with shallow pore-tubes and the sterile margin ($^1/_4$ nat. size)

Fig. 6. Fructification of *M. similis* showing the upper surface ($^1/_4$ nat. size)

Fig. 7. Vertical section through the fructification showing the context and the pore-tubes ($^1/_8$ nat. size)

Fig. 8. Test-block after 4 months' decay-resistance-tests in the laboratory showing an appreciable amount of shrinkage and the appearance of dark brown colouration; the control block does not show any such change

Isolation and Identification of the Causal Organism

In order to study the causal organism in culture, the mycelium from the rotted tissues of the suscept was isolated. The isolates were made from different areas of the rhizomes which bore large fructifications externally. Following the method described by BANERJEE (1955), small pieces of infected wood were cut carefully from the selected regions of each rhizome. The pieces were soaked in distilled water for a couple of hours and were surface sterilized by quickly dipping them in rectified spirit and subsequent flaming. One side of the exterior portion of such a block was then removed by a sterile scalpel in order to expose the internal tissue. A small bit of tissue thus exposed was then aseptically cut out and transferred to a sterile 2% malt-agar slant. In this way several transfers were made. These were then incubated under ordinary laboratory conditions of temperature (27°–30° C), humidity (68–98%) and diffused light of the laboratory, allowed to grow for about 3 weeks and then examined. Both macroscopic and microscopic characteristics of these cultures were found to be identical in all respects to the tissue-cultures of the fructifications already made available for the purpose of comparison.

Fungus in Culture

In order to study the cultural characteristics, the fungus was grown in Petri-dishes on malt-agar (malt-extract 2.5%, agar 2.5%, distilled water 100 ccm). The p_H of the medium, after preparation, was adjusted to 5.4. The plates were inoculated and incubated at 32° C in complete darkness. Observations were daily recorded upto 10th day and cultural characteristics together with the daily increment in diameter of the mat were noted.

The mycelium started its growth within 24 hours after inoculation, but no characteristics could be found till the 4th day when it appeared to be more profuse on the sides of the inoculum and exhibited plumose to felty habit on the medium the mycelium became sub-felty and at some places appeared to be chamois-like. Advancing zone was found to be raised and to some extent silky in texture. On the 6th day, the superficial mat was found to be felty to sub-felty throughout but at the periphery the mat appeared to be cottony to silky. On the 10th day, the mycelium including its zone of advance became raised, smooth, felty and white throughout, while on and around the inoculum it was densely felty (Fig. 4). The average daily increment in diameter of the mat was found to be 6.5 mm.

Fungus in Relation to the Host

External symptoms. The only visible symptom in the field is the appearance of fructifications. On cutting cross and longitudinal sections

of the infected rhizomes, distinct macroscopic characters of decay become apparent. The rot first appears at the periphery and gradually spreads towards the centre. With the advance of decay, the rhizome loses its toughness. It becomes soft, light, dry, somewhat fibrous and appears to be full of rot-pockets (Fig. 3). The colour changes from yellowish white to dark-brown. The decayed tissues on drying shrinks more than the sound ones. The diffusion of water in the former has also been found to take place at a much higher rate in comparison to the latter. When blocks of same size and shape are exposed to water under pressure for 1 hour, the average percentages of water diffused into sound and decayed tissues are found to be 75.72 and 106.71 respectively.

Microscopic characters of the rot. To study the microscopic characters of the rot, infected rhizomes at various stages of decay were fixed and preserved in Formal-Acetic-Alcohol (10 : 5 : 85 ccm 70% alcohol). For early stages of decay, samples were also obtained from test-blocks exposed to the fungal attack for a period of 4 months under controlled conditions. These were softened by boiling and subsequently treating in mixture of glycerine and methylated spirit. Transverse and longitudinal sections, 15–20 μ thick were cut. Of the various differential staining methods tried, the combination stains Safranine and Picro-aniline-blue (Cart-Wright, 1929) proved to be very suitable for detecting the hyphae within the tissues. Microscopic characters of the rot become very prominent in advanced stages of decay.

The distribution of hyphae is not uniform throughout but they are more common in the parenchyma. The hyphae, about 1.5–5 μ wide, are thin-walled, hyaline, sparsely branched and distantly septate with frequent simple clamp-connexions. They are full of granular protoplasmic contents and take up the blue stain deeply (Fig. 9). In the initial stage, the hyphae are not found within the vessels but in later stages they are found to accumulate in their lumina and extend both longitudinally and laterally (Figs. 12 and 15). In advanced stages of decay, the tracheids and xylem parenchyma are also attacked. Very little infection has been found to be taken up by the sclerenchyma cells. In the final stage of decay, due to dissolution of parenchyma cells, the bundles become somewhat isolated into groups. The wider vessels also become isolated due to the disintegration of the walls of xylem parenchyma. The compactness of the tissues is completely lost at this stage. In case of parenchyma, the hypha is found at first to penetrate through the simple pits (Figs. 10 and 13) and later also through the cell-walls directly at right angles to them forming prominent bore-holes. The diameter of the bore-holes is appreciably wider than the penetrating hyphae (Fig. 11). In case of vessels and tracheids, the hyphae penetrate chiefly through the pits but formation of bore-holes are also noticed (Fig. 14). At the final stage

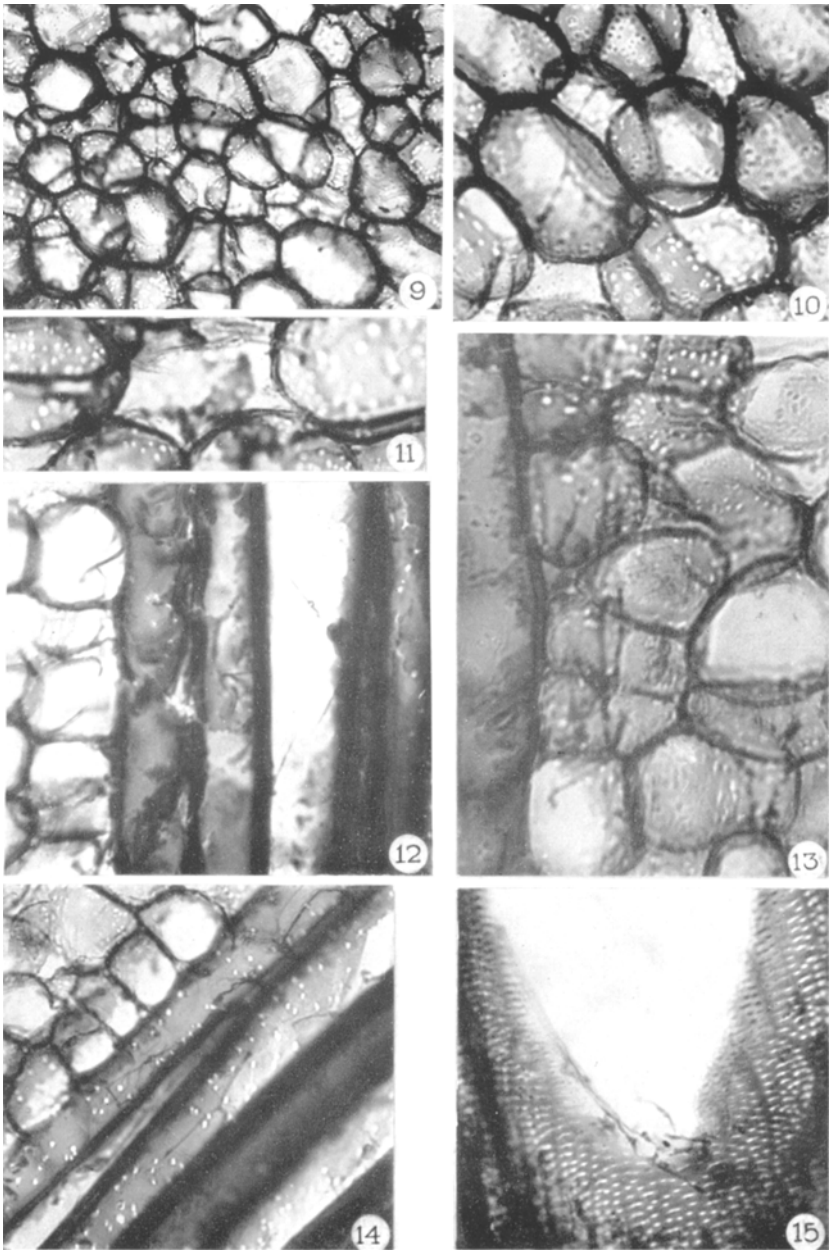

Fig. 9—15

of decay, the parenchyma cells become totally disorganized, xylem vessels become completely isolated and sclerenchyma cells appear as discrete segregated bundles showing very little change.

Decay-Resistance-Tests

The resistive capacity of the host tissue against the activity of the causal organism had been estimated by the quantitative measurement of decay under controlled conditions. Small samples of sterilized test-pieces of sound tissue were exposed to the attack of fungal mycelium under a set of conditions. The amount of decay was estimated in terms of final loss in dry weight of the test-pieces.

Three local varieties of bamboo ("Basni", "Valka" and "Talla"), had been selected for the study. Small rectangular blocks of uniform size ($2'' \times 1'' \times 1/2''$) were cut from sound internodes and dried at 60° C, to constant weight. The blocks were then soaked in distilled water till the average moisture of the individual block rose much above the fibre-saturation-point. The test-blocks were then sterilized in a moist atmosphere following Chidester (1937, 1939). Sterile 2.5% malt-agar slants in Kolle flasks were prepared and inoculated with actively growing mycelium. These were then incubated at 25° C in the diffused light of the laboratory. After 20 days the blocks were transferred to Kolle flasks, 4 in each, and exposed to the mycelium. These were kept as such for a period of 4 months. The average moisture-content of the blocks were recorded just before their exposure to the mycelium and were determined following the method suggested by Savory (1954). After the experimental period the blocks were taken out and the superficial mycelium on each was carefully removed. Each block was weighed immediately to record its moisture-content after 4 months' decay. The blocks were then examined to note the external manifestations of decay and dried at

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Fig. 9. Photomicrograph representing a portion of the longitudinal section of rhizome of bamboo showing the distribution of hyphae of *M. similis* through the parenchyma cells (× 105)

Fig. 10. Photomicrograph representing part of the longitudinal section of the rhizome showing the entry of the hyphae through the pits of the parenchyma cells (× 210)

Fig. 11. Photomicrograph representing a part of the longitudinal section of the rhizome showing the direct entry of the hyphae through the walls of the parenchyma cells (× 210)

Figs. 12 and 15. Photomicrographs representing parts of the longitudinal sections of rhizome showing hyphae within lumina of the vessel (× 105)

Fig. 14. Photomicrograph representing a portion of the longitudinal section of the rhizome showing the penetration of hyphae through the pits and bore-holes in the vessels and tracteids (× 105)

60° C to constant weight. The losses in weight of the test-blocks were finally determined. The results thus obtained are given in Table 1.

Table 1. Decay of test-blocks obtained from different varieties of bamboo due to 4 months' exposure to the attack of *Merulius similis*

Varieties	Basni	Valka	Talla
Number of replicates	12	12	12
Average loss (in %)	22.65	14.19	30.53

A comparative study of the losses in dry weight of different test-pieces clearly indicate their respective resistive capacities. The "Talla" variety appears to be perishable (FINDLAY, 1938) as the average loss in dry weight is above 30%. While the "Valka" and "Basni" may be classed as "non-resistant" as they suffer more than 10% loss in 4 months test.

The average moisture-content of different blocks before the experiment were about 80%. After the experimental period the blocks retained the moisture-content at about 60%. Though no control measure could be adopted to maintain the exact moisture-content of the blocks during the experimental period, yet the normal activity of the fungus did not suffer in any way, as the moisture-content after the experiment had been found to be much above the fibre-saturation point.

The results clearly indicate that *M. similis* attacks all the local varieties of bamboos under controlled conditions of the laboratory and a relationship may be noticed between the intensity of attack and the type of the blocks exposed.

Considering the occurrence of fruit-bodies only on the rhizomatous portion of bamboos, another set of similar experiments had been performed to determine the decay resistive capacities of fresh and partially decayed rhizomes. The blocks prepared from "Basni" variety only were exposed to the fungal mycelium and the amount of decay was determined following the procedure as adopted before. The results are given in the Table 2.

Table 2. Decay of test-blocks obtained from fresh and partially decayed rhizomes of bamboo due to 4 months' exposure to the attack of *M. similis*

Source of test-pieces	Fresh rhizome	Partially-dacayed rhizome
Number of replicates	12	12
Average loss (in %)	39.00	19.74

From the table it is evident that the fungus attacks fresh rhizomatous tissues more vigorously than the partially decayed ones. The fresh blocks after the experimental period exhibit pronounced macroscopical characters of the rot. The colour changes from yellowish white to dark-brown and an appreciable amount of shrinkage also takes place (Fig. 8).

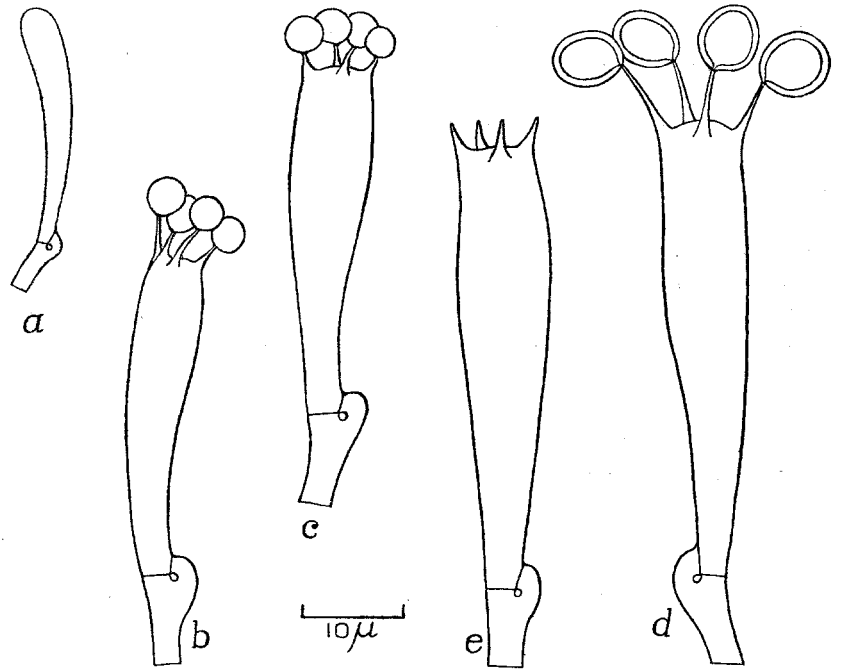

Fig. 16*a–e*. Different stages of basidial development and basidiospores

From the comparative study regarding the decay-resistance tests of internodes and rhizomes, it is evident that the decay in fresh rhizomes is far more greater than that in internodes of aerial parts. The loss in case of internodes of "Basni" variety is 22.65%, while in case of the rhizomes of the same variety is 39.00%.

Chemical Effects of Decay

Microchemical tests. Though microchemical tests do not always give positive proof of various chemical changes that take place during the process of decay but their importance can not be ignored owing to their utility in securing preliminary informations about such changes. Therefore, the microchemical tests for cellulose and lignin were performed in the present study, with fresh and partially decayed host tissues.

Following the usual practice, the sections were treated with phloroglucin-Hcl and Chlor-zinc-iodine to detect the presence of lignin and cellulose respectively and the intensity of colourations indicated the relative amount of them present in each case. Phloroglucin-Hcl turned the lignified walls red while chlor-zinc-iodine turned cellulosic walls blue. The results thus obtained had been verified by treating the sections with combination stains Gentian Violet and Bismarck brown to differentiate the lignified (violet staining) from non-lignified (brown staining) structures.

The intensity of the reaction for lignin had been apparently found to be greater in partially decayed tissues than that in sound ones while the tests for cellulose revealed its considerable loss with the progress of decay.

Chemical estimation. In order to estimate the effects of decay by chemical means, samples were taken from sound tissues and from those exposed to the fungal attack for a period of 4 months under controlled conditions and their average percentage content of cellulose and lignin were estimated. CROSS and BEVAN'S method (1903) was followed for cellulose estimation and lignin content was determined according to 72% sulphuric acid method of RITTER, SEBORG and MITCHEL (1932).

It was found that the average percentage of depletion of cellulose due to 4 months' exposure to fungal attack was 28.5%, while the lignin content appeared to increase apparently by 20.8% after the aforesaid period (Table 3).

Table 3. Relative percentage of cellulose and lignin content of sound tissues and of those after 4 months' exposure to the attack of *M. similis*

	Replicate	Average % in sound tissues	Average % in decayed tissues	% of apparent increase	% decrease
Cellulose	3	56.1	27.6		28.5
Lignin	3	25.3	46.1	28.8	0

Oxidase tests. It has been found from the study of the chemical effects of decay that the fungus concerned primarily consumes the cellulosic materials of the host tissues. Therefore, oxidase tests were performed in order to determine the nature of the organism. The method of BAVENDAMM (1928) was followed for this purpose.

The test consists of allowing the fungus to grow on 2.5% malt-agar medium each containing 0.5% gallic acid or tannic acid in Petri-dishes. The media after preparation was sterilized and plated in Petri-dishes (100 mm in diameter), each containing about 30 c.cm of the medium. These were then incubated with uniform agar-discs from young cultures and incubated at 30° C in darkness.

The test appeared to be a negative one as no dark brown zone around the inoculum was observed even after 72 hours. Thus, it can be stated that the fungus is a "brown-rot" one.

Toxicity tests. Three water-soluble chemicals Zinc chloride, Ascu A and Copper sulphate were selected to perform toxicity tests. The effectiveness of these preservative were evaluated in the loboratory by determining their toxic limits for the growth of the test-fungus on a nutritive agar medium containing graded concentrations of each of them. Media were prepared by adding required amounts of sterile 2% Zinc chloride solution to 2.5% malt-agar in Petri-dishes in such a way that the following concentrations were obtained: 0.02%, 0.04%, 0.06%, 0.08%, 0.1%, 0.12%, and 0.14%. Following the same procedures, media were also prepared with Ascu A and Copper sulphate solution. The media were plated, incubated and kept under ordinary laboratory conditions of temperature (32°–34° C), humidity (62%–87%) and in complete darkness.

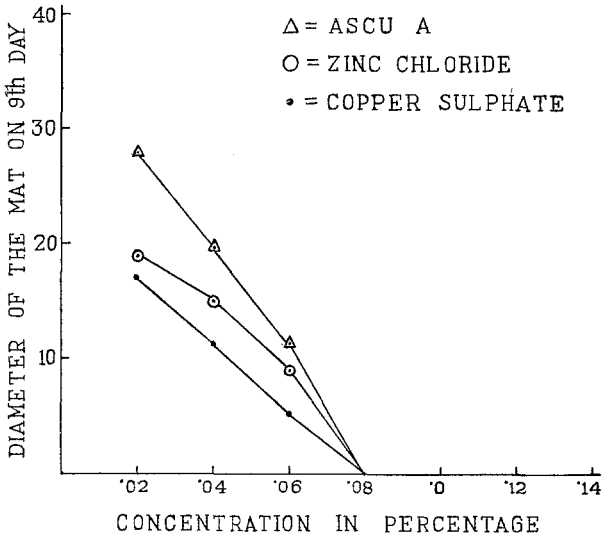

Fig. 17. Effect of concentration-variation of different preservatives on the growth of the secondary mycelium of *M. similis*

Results obtained on the 9th day after inoculation have been recorded in the Table 4 (Fig. 17).

Table 4. Effect of different preservatives on the growth of the secondary mycelium of *Merulius similis*

Grades in %	Average diameter of the mat on 9th day after inoculation (in mm)		
	Zinc chloride	Ascu A	Copper sulphate
0.02	17	27	19
0.04	12	20	15
0.06	8	11	9
0.08	0	0	0
Control	60	63	62

It is interesting to note that total inhibition of growth takes place at the concentration of 0.08%, for all the selected chemicals. It has also been observed that the fungus has the least tolerance for Zinc chloride and Copper sulphate is more toxic than Ascu A. But all the 3 chemicals appear to be very toxic in comparison with the controls.

Discussion

An investigation on *Merulius similis* in association with bamboo clumps and its effect on sterilized tissues of the host make it possible to discuss in a general way some of the salient features of the fungus. The dependence of the fungus on the environmental conditions, particularly temperature and rainfall, its behaviour and host-specificity reveal a fascinating but much complicated biological phenomenon. The highly limited span of life of the fructification, its appearance only during the most humid months of the rainy season and its high moisture-content are in striking contrast to other common wood-rotting Hymenomycetes. The fructification has been found to grow on the exposed roots at the base of the bamboos. The fructifications on the rhizome discharge their spores heavily on the soil just beneath this hyminial surface forming rusty areas. The soil around the bamboo clump always contains considerable amount of water and humus. In all probability, it is due to high moisture-content of the soil, a very little amount of spores is disseminated by the wind and as such they germinate in situ. Under favourable conditions they germinate and bring about infection.

The study on the effect of the fungus on sterilized tissues of the host by the decay-resistance tests in the laboratory for a period of 4 months indicate its capability to cause appreciable rot to the test blocks derived from the serial internodes of 3 local varieties of bamboo, namely, "Talla", "Basni" and "Valka" under controlled conditions. Though relative amounts of decay in these cases differ significantly, the "Talla" variety appears to some extent "perishable" to the attack, while both "Basni" and "Valka" are non-resistant. The blocks from healthy rhizomes also exhibit comparatively greater amount of losses than those obtained from partially decayed ones. It is further evident that the loss in case of fresh rhizomes is significantly greater than that of the aerial internodes.

The ramification of the fungal hyphae through the tissues of the host is somewhat striking. With the progress of decay the hyphae ramify chiefly through the parenchyma both intra and intercellularly. With the progress of decay the hyphae gradually spread through vessels, tracheids and to some extent the fibres. The penetration of the cell wall by the hyphae is first accomplished through the pits, but later

directly through the cell walls forming bore-holes. The preliminary informations regarding the comparative utilization of the chief chemical constituents of the tissue-elements have been obtained by performing micro-chemical tests of fresh and partially decayed tissues. The tests reveal considerable decrease in the amount of cellulosic materials only. The increased intensity of reaction of lignin indicates that the fungus does not consume an appreciable amount of that substance. Estimation of lignin and cellulose also serves a corroborative evidence for the data obtained in micro-chemical studies. The oxidase tests with gallic acid and tannic acid appear to be negative. Thus, from the integrated results it can be concluded that the fungus is a "brown-rot" one.

Summary

1. An investigation has been undertaken on *Merulius similis* B. & Br. and the associated bamboo-rots frequently found in Calcutta and suburbs. The fungus, so far reported, has been found to be host-specific, and *Bambusa bambos* Druce may be assigned as the singular host species for it.

2. Its occurrence has been reported only from a few tropical countries where the temperature is neither too low nor too high (70°–80° F) and the average rain-fall is moderate (12″–16″) during the growing season.

3. The mycelium has been found only in the tissues of the rhizomatous portion of the infected stumps and it has been isolated from those regions. The cultures reveal identical characteristics with those obtained from the tissue of the fruit-bodies.

4. Macroscopically the decayed tissues appear somewhat dark brown and with minute rot-pockets. In the advanced stage of decay the tissues become soft, light, dry, and somewhat fibrous.

5. Microscopic examinations of the rotted tissues reveal that the parenchyma cells are first attacked by thin-walled hyaline hyphae with frequent clamp-connexions and their entry is mainly through pits. In the advanced stage, the mycelium can be detected within the vessels and tracheids and to some extent within the fibres. Finally, the walls of the parenchyma cells dissolve and due to this dissolution by xylem parenchyma and vessels become separated.

6. Decay-resistance-tests in the laboratory with internodal tissues of 3 local varieties of bamboo ("Talla", "Valka" and "Basni") and also with fresh and partially decayed rhizomatous portions of one variety ("Basni") indicate 22.65%, 14.19%, 30.53%, 39.00%, and 19.74% average losses in dry weights respectively.

7. Micro-chemical studies show the chemical changes that take place in the host tissues during the progress of decay. The results indicate that only cellulosic materials are consumed. Estimations of cellulose

and lignin show considerable depletion of the former only, as such apparent increase in the percentage content of the latter has been noted in decayed tissues.

8. Oxidase tests with tannic acid and gallic acid have been performed but yield negative results.

9. Toxicity tests have been performed with Zinc chloride, Ascu A and Copper sulphate following the standard agar method. It has been observed that the mycelium possesses least tolerance for these compounds and Zinc chloride is most toxic in comparison to the other two.

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