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INVESTIGATIONS ON THREE STRAINS OF *FOMES DURISSIMUS* LLOYD ASSOCIATED WITH DECAY OF ECONOMIC TREES

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The present investigation has been undertaken to study the biology of three strains of *Fomes durissimus* Lloyd, growing luxuriantly on branches and trunks of living trees of *Swietenia mahogani, Casuarina equisetifolia* and *Mimusops elengi* during the months of July to October in Burdwan University campus, West Bengal, India. Besides these three host-species it attacks a number of timber yielding trees and decaying logs. The distribution of the fungus appears to be very limited.

Decay-resistance tests reveal that the three strains of the test-fungus can cause considerable amount of decay of the host-wood. Sapwood of *S. mahogani* appears 'non-resistant' and the sapwood of *C. equisetifolia* and *M. elengi* may be classed under 'moderately resistant' while the heartwoods in all the three hosts are 'resistant'. The gross character of the rots reveals that the decay causes bleaching of colour of both sapwood and heartwood. The wood ultimately becomes brashy with age.

Oxidase tests with 0.5% gallic or tannic acid reveal that all the three strains are 'positive reactor' producing brown rings around the inocula. Thus, the aforesaid strains of the test-fungus are of 'white-rot' types. A parallel test with 0.007% Gentian violet has also been performed in conformity with the previous tests.

Toxicity tests following American agar method and European wood-block-method have been performed with five different preservatives, *viz.*, borax, zinc chloride, sodium arsenate, creosote and 'Ascu A'. It is obverved that 'Ascu A' is most effective of all the five preservative used since mycelia of the three strains of the test-fungus have least tolerance for it.

INTRODUCTION

Fomes durissimus Lloyd, a very common member of Polyporaceæ in India, attacks a number of economic trees and timbers during the rainy season extending from July to October every year. Moist and somewhat shady place with temperature between 28 – 32°C seems to be favourable for the luxuriant formation of its basidiocarp.

Lloyd (1920) first described the fungus from the African collection of John Gossweiler. He referred a Madagascar collection to *Fomes pseudosenex* which shows close similarity with *F. durissimus*. He further stated that Berkeley misreferred the fungus to *F. rhabarbarinus* and one Cuban collection to *F. calcitratus* both of which differ under the microscope in having setæ. Murrill also misreferred the fungus to *F. fastuosus*. According to Lloyd., *F. carryophylli* is so close to *F. durissimus* that it is difficult to show the difference.

The distribution of the fungus appears to be limited to tropics. Lloyd reports it as a common fungus in both Eastern and American tropics. It has also been reported from Africa. In India, it has been reported from Lakra Hills in Assam (Bose, 1937) and from Calcutta on a large number of economic timbers (Banerjee, 1947). An anonymous worker (1950) and Bakshi (1971) reported the fungus to grow all over the plains commonly as saprophytes but also as parasite on hardwoods and living economic trees. Considering the fairly wide distribution of the species in India, the economic importance of the hosts and the very limited informations available on the nature of decay caused by the fungus, present investigation has been undertaken.

Materials and Methods

The fructifications of *F. durissimus* were collected during the months of July to October while growing luxuriantly on standing trees of *Swietenia mahogani* (strain I), *Casuarina equisetifolia* (strain II), *Mimusops elengi* (strain III) in the Burdwan University Campus, West Bengal, India (Plates I-III, Figs. 1, 7 and 13). The causal organism from different hosts were isolated in culture. For confirmation of species, the material was compared with the huge collections in the herbaria of Dr. S. R. Bose and of Dr. S. N. Banerjee of Calcutta University, particularly the authentic materials identified by Dr. E. M. Wakefield, formerly of Royal Botanical Garden, Kew. It was found to be identical in all respect with the authentic collection of *F. durissimus*. On critical examinations, however, it was found that the organism from three hosts differed to some extent in structure as well as in the cultural behaviour and were represented by strain I, II and III.

The Basidiocarp

Fructification : Perennial ; always sessile, attached to the substratum by broad base ; solitary or several growing together in imbricate manner with laterally confluent margins forming larger fructifications ; demediate ; applanate ; particularly distinct in younger specimens ; heavy, hard and woody ; 2.3 – 12.0 cm across, 1.5 – 8.0 cm deep and 0.5 – 3.0 cm in thickness near the base. Margin

thick entire, yellowish brown in colour, in larger specimens uneven. **Upper surface :** Crustose, rugulose, sometimes cracky ; blackish brown to almost black in colour, younger fructifications and current year's growths in older specimens somewhat tomentose ; concentrically zoned, having various shades of brown colouration (Plates I-III, Figs. 3, 9 and 15). **Context :** Indistinctly stratified, corky-woody ; Argus brown in colour ; 0.5 – 2.0 cm thick, sterile tissues between pore tube layers 1.0 – 1.5 mm thick. **Hymenial surface :** Poroid, usually smooth, azonate, somewhat darkbrown with a greenish brown shinning lustre in fresh specimens, becoming darker when dry. Pore mouths uniform throughout, minute, 5.6 – 11.6μ in diameter ; circular or somewhat angular ; a narrow sterile zone present just behind the margin. Dissepiments 2.8 – 15.4μ thick, concolourous with context (Plates I-III, Figs 4, 10 and 16). **Basidia :** Clavate ; tetrasterigmatic and quadrisporous ; dimension 7.25—9.0μ × 3.75—5.0μ (I), 6.25 – 7 5μ × 3.12—5.0μ (II) and 2.7—5 25μ × 1 25 – 3.0μ (III). **Basidiospores :** Subglobose, slightly thick walled, 1.5 – 5.9μ × 1.25—5.6μ (I), 0.75—5.8μ × 0.62—5.0μ (II) and 0.65—2.5μ × 0.5 – 2.0μ (III) in diameter (Plates I-III, Figs. 2, 8 and 14). The hyphal composition of the three strains consisting of one type of thin walled and two types of thick walled hyphæ resembling generative, skeletal and binding type. Slight variations in diameter of hyphæ have, however, been observed in them.

Natural Resistance of the Host Wood

Much interest has developed in the study of wood decay by wood-destroying fungi since the magnitude of loss is known to be directly correlated with the durability of the host-wood in offering natural resistance (Scheffer *et al*, 1966). In order to study the relative resistance offered by *S. mahogani, C. equisetifolia* and *M. elengi* against decay by three strains of the test-fungus, the method recommended by Banerjee (1955) was mainly followed. Rectangular wood-blocks (2″ × 1″ × ½″) were cut from sound heart and sapwood of three hosts and dried to constant weight. The blocks were rapidly soaked in distilled water under reduced atmospheric pressure to avoid leaching until the moisture content was raised above "fibre-saturation-point '. The test-blocks were then sterilized, exposed to the attack of actively growing mycelia of the test-fungus on malt agar medium in Kolle flasks and incubated at 30°C in complete darkness for four and eight months. Sterilized distiiled water was poured to the reservoir at the neck of the Kolle flasks at intervals during the period of incubation. At the end of experimental periods, the superficial mycelia over the test-blocks were carefully removed, the physical changes due to decay were noted and the moisture contents were recorded. The blocks were then dried to constant weight at 60°C and the losses in dry weights due to decay after four and eight months were calculated.

The results are given in Table 1.

Table 1. *Comparative losses in dry weight (%) of sapwood and heartwood blocks of* S. mahogani, C. equisetifolia *and* M. elengi *to the attack of* F. durissimus *after 4 and 8 months' test*

Host-species	Period	Loss in dry wt.*			
		Sapwood		Heartwood	
		Mean (%)	Range (%)	Mean (%)	Range (%)
S. mahogani	4 months	10.3	5.4—14.2	1.3	0.9—1.9
C. equisetifolia	,,	9.3	5.0—12.1	1.2	0.7—1.7
M. elengi	,,	6.8	4.7—11.3	1.6	1.1—2.3
S. mahogani	8 months	21.3	14.9—33.3	3.8	3.0—5.0
C. equisetifolia	,,	12.6	10.0—15.0	2.3	2.0—2.6
M. elengi	,,	13.3	11.8—14.4	3.2	1.2—4.4

* Average of 10 replicates.

Decay caused by three strains of *F. durissimus* to their respective hosts indicates that loss in heartwood is much less than that of the sapwood in all cases. The difference indicates the variations in natural resistance offered by the sap-and heartwood of different hosts. This result is valid so long as the size of the wood-blocks remains constant. The sapwood of *S. mahogani* thus appears "non-resistant" (Findlay, 1938), as it suffers more than 10% loss while that of *C. equisetifolia* and *M. elengi* are "moderately resistant" showing less than 6% loss in four months' test while heartwood of all the species appear to be "resistant". After eight months, loss appears to be more than double over the values obtained in four months. The moisture content of the test-blocks during the experimental period remained much above the fibre-saturation-point and hence the normal activity of the fungus did not suffer.

The physical characters of sapwood of *S. mahogani* and *C. equisetifolia* due to decay showed considerable changes from darkbrown colour to irregular bleached somewhat fibrous areas of lighter shades with rusty coloured patches at places (Plates I-II, Figs. 6 and 12). In *M elengi* the blocks showed similar bleached fibrous but less prominent areas (Plate III, Fig. 18). Heartwood blocks of all the host-species also showed discoloured areas of somewhat fibrous texture.

Chemical Effects of Decay

The nature of decay of the three host-wood was studied from biochemical standpoint by the quantitative analysis of lignin and cellulose using fine powder (40-mesh) of sound and decayed wood. Quantitative estimation of lignin was

made following Sæman *et al* (1954) and cellulose following essentially the Tappi standard (1954) and Cowling (1961).

The results are recorded in the Table 2.

Table 2. *Percentage of lignin and cellulose in sound and partially decayed sap-and heartwood of* S. mahogani, C. equisetifolia *and* M. elengi

Nature of wood and the strain of *F. durissimus*	Lignin (%)	Cellulose (%)
S. mahogani sapwood	31.5	69.0
S. mahogani sapwood+strain I	21.5	66.0
S. mahogani heartwood	37.3	64.0
S. mahogani heartwood+strain I	35.3	62.0
C. equisetifolia sapwood	39.5	66.0
C. equisetifolia sapwood+strain II	33.4	64.0
C. equisetifolia heartwood	44.1	62.0
C. equisetifolia heartwood+strain II	42.7	61.0
M. elengi sapwood	35.5	66.0
M. elengi sapwood+strain III	27.5	63.0
M. elengi heartwood	37.8	59.5
M. elengi heartwood+strain III	36.0	58.0

The results show that the percentages of lignin in decayed wood of the three hosts are much lower than those in sound wood while the percentage of cellulose decreases slightly during the process of decay. Thus the utilization of lignin primarily with simultaneous destruction of cellulose to some extent proves the white-rot nature of the fungus.

To establish the strain variations and host-specificity of the strains, cross inoculation experiments were made, the results of which clearly indicated the existence of variations in strains which were not host-specific (Santra, 1974).

Oxidase Test

On the basis of chemical decomposition, white-rot fungus producing extracellular phenoloxidizing enzymes (Nobles, 1958), decomposes lignin primarily, while brown-rot type destroys cellulose by hydrolyzing enzymes leaving the lignin almost undigested. To determine the nature of enzyme produced by the test-fungus, method recommended by Bavendamm (1928) was followed. The mycelia of test-fungus was grown on malt agar media separately containing 0.5% gallic or tannic acid in Petridishes. In all cases, after 24 hours, dark brown ring appeared around the inoculum due to reaction of oxidase secreted by the fungus with both gallic and tannic acid corroborating the white-rot nature

of the fungus. Considerable variation in intensities of colour reactions was, however, observed in the three strains. This was further confirmed by a parallel test following Preston and McLennan (1948) by allowing the mycelia to grow on 2.5% malt-agar media containing 0.007% Gentian violet. The violet colour of the medium was bleached slowly but completely in about a month. Complete decolourization of the dye occurs only when the oxygen concentration is comparatively higher and in presence of an extracellular enzyme system by white-rot fungi (Preston and McLennan, 1948).

Toxicity Test

With a view to protect wood from deterioration by the test-fungus, toxicity tests using several preservatives were performed. Property of toxicity, which is used in the determination of effectiveness of the preservatives was measured with a view to find out the inhibition point. This was done in the laboratory by undertaking both the agar method using culture media containing different concentrations of the preservatives (Hunt and Garratt, 1953 ; Carr, 1955 ; Cowling, 1957 ; Da Costa and Kerruish, 1964 ; Young, 1961) and the wood-block method exposing small wood-blocks treated with different concentrations of the preservative to the action of the mycelium of the test-fungus growing vigorously on nutrient agar media in Kolle flasks. One oil-borne preservative, Creosote and four water soluble preservatives, 'Ascu A' (a standard wood preservative in market), Zinc Chloride, Borax and Sodium arsenate were selected. Required amounts of the preservatives were added to sterilized 2.5% malt agar media to obtain a graded concentration 0.03%, 0.06%, 0.09%, 0.12%, 0.15%, 0.18% and 0.21% of each preservative. In case of 'Ascu A' the concentration grades 0.005%, 0.01%, 0.02%, 0.03% and 0.04% were selected. The media with such graded concentrations of each chemical were plated, inoculated at the centre separately with uniform discs of mycelia of the three strains of *F. durissimus,* incubated at 30°C in darkness. Three replicates were made for each concentration and for each strain of the test-fungus.

The results have been recorded in the Table 3.

Table 3. *Data showing inhibition point of growth of mycelia of three strains of* F. durissimus *after 20 days*

Preservatives	Concentration in %		
	Strain I	Strain II	Strain III
Zinc Chloride	0.12	0.12	0.12
Borax	0.12	0.12	0.12
Sodium arsenate	0.15	0.15	0.15
Creosote	0.12	0.12	0.12
'Ascu A'	0.02	0.02	0.02

From Table 3, it is clear that the inhibition point for all the strains of the test-fungus lies at 0.15% for sodium arsenate, 0.12% for borax, zinc chloride and creosote and 0.02% for 'Ascu A'. Thus 'Ascu A' proves to be the most effective preservative among the substances tested and seems to be effective even at a very low concentration against all the three strains.

For wood-block method, inhibition concentration and one higher and one lower of each preservative were selected. Dried wood-blocks of only sapwood of the three host-species which proved to be more susceptible than heartwood were impregnated in different concentrations of the preservatives. The quantity of the preservative retained in the wood in terms of grams per cubic inch were noted. The blocks were sterilized and exposed to the attack of mycelia of test-fungus in Kolle flasks for about 4 months at 30°C in darkness (Plates I-III, Figs. 5, 11 and 17). At the end of experimental period the final dry weights of the test-blocks were taken and the loss in weight due to fungal action was finally calculated.

The results are recorded in Table 4.

Table 4. *Toxicity test with sapwood of* S. mahogani, C. equisetifolia *and* M. elengi *treated with different preservatives and exposed to mycelia of three strains of* F. durissimus *for a period of 4 months at 30°C.*

Host wood and strain of *F. durissimus*	Preservative	Concentration (%)	Absorption (gm/inch^3)	*Loss (%)	Condition of the test block
S. mahogani+strain I	Zinc Chloride	0.09	0.007	5.8	+
		0.12	0.009	3.1	+
		0.15	0.012	2.6	++
C. equisetifolia+strain II	-do-	0.09	0.007	5.0	+
		0.12	0.009	3.0	+
		0.15	0.012	1.5	++
M. elengi+strain III	-do-	0.09	0.008	3.5	+
		0.12	0.010	2.3	++
		0.15	0.012	1.9	++
S. mahogani+strain I	Borax	0.09	0.006	4.3	+
		0.12	0.009	2.3	++
		0.15	0.012	1.3	++
C. equisetifolia+strain II	-do-	0.09	0.007	4.9	+
		0.12	0.008	2.0	++
		0.15	0.011	1.2	++
M. elengi+strain III	-do-	0.09	0.007	3.2	+
		0.12	0.011	2.0	++
		0.15	0.012	1.1	++

Contd...

Table 4 contd...

Host wood and strain of *F. durissimus*	Preservative	Concentration (%)	Absorption (gm/inch³)	*Loss (%)	Condition of the test block
S. mahogani+strain I	Sodium arsenate	0.12	0.011	5.7	+
		0.15	0.015	3.3	+
		0.18	0.018	2.2	++
C. equisetifolia+strain II	-do-	0.12	0.010	5.6	+
		0.15	0.012	3.2	+
		0.18	0.014	2.1	++
M. elengi+strain III	-do-	0.12	0.010	3.4	+
		0.15	0.015	2.4	++
		0.18	0.018	1.8	++
S. mahogani+strain I	Creosote	0.09	0.007	3.4	+
		0.12	0.008	2.4	++
		0.15	0.010	1.8	++
C. equisetifolia+strain II	-do-	0.09	0.007	5.7	+
		0.12	0.010	3.2	+
		0.15	0.011	2.4	++
M. elengi+strain III	-do-	0.09	0.008	4.5	+
		0.12	0.012	2.4	++
		0.15	0.014	1.5	++
S. mahogani+strain I	'Ascu A'	0.01	0.001	2.53	++
		0.02	0.002	1.93	++
		0.03	0.003	1.37	++
C. equisetifolia+strain II	-do-	0.01	0.001	2.56	++
		0.02	0.002	1.90	++
		0.03	0.003	1.20	++
M. elengi+strain II	-do-	0.01	0.001	2.55	++
		0.02	0.002	1.69	++
		0.03	0 003	1.16	++

* Average of three replicates

+= Partially decayed ; ++= Almost sound.

From table 4, it is evident that the fungal activity is not totally inhibited even at the concentration showing inhibition in the agar-method. It is also evident that 'Ascu A' is most effective of the five preservatives used, since the mycelia of the three strains of *F. durissimus* have least tolerance for it. Further, the sapwood of *S. mahogani* which was found to be non-resistant and those of *C. equisetifolia* and *M. elengi* which were 'moderately resistant' in decay-resistance-tests, became almost resistant to the attack of mycelia when approximately 0.001 gm of this toxic substance is present per cubic inch of the wood.

Discussion

Fomes durissimus Lloyd is a member of the family Polyporaceæ which attacks a large number of timber yielding trees in India thereby lowering the quality and market value. The fungus is also very common during rainy season in the University campus on trees of *S. mahogani, C. equisetifolia* and *M. elengi* which induces the present investigators to deal in general way some of the aspects of its life-processes and its relationship with the hosts.

The fungus growing on the three hosts shows difference among themselves in detailed structures as well as in their cultural behaviour, thus exhibiting the existance of strain variations. The nature and extent of decay of the three host-species have been found to depend much on the nature of the wood and the strain of the fungus as revealed by 'decay-resistance-tests'. The sapwood of *S. mahogani* appears to be "non-resistant" as it suffers 10.3% loss in dry weight. Sapwood of *C. equisetifolia* and *M. elengi* is "moderately resistant" as it suffers 9.3% and 6.8% losses respectively. The heartwoods in all the host-species are "resistant" showing losses below 4%. This amount of losses due to decay is valid, so long as the particular size of the wood-blocks taken remains constant with moisture content far above the "fibre-saturation-point". Since the overall size of the test-blocks has considerable effect on the rate of decay (Findlay, 1953). Quantitative estimation of cellulose and lignin of the three host-species reveals that the loss in dry weight of wood is due to considerable loss of lignin with some cellulosic materials taking place during the process of decay. The destruction of mainly lignin by all the three strains of *F. durissimus* is essentially a process of oxidation by phenoloxidase characteristically produced by 'white-rot' fungus. The positive reactions exhibited by the three strains with gallic or tannic acid confirm the said nature of the fungus.

By using proper concentrations of wood preservatives, the woods of the host-species have been found to be protected from the strains of *F. durissimus* under a set of controlled conditions in the laboratory. Of the five preservatives used, "Ascu A" is most effective since all the strains show least tolerance for it. Borax, zinc chloride, sodium arsenate and creosote almost inhibit the fungal activity to some extent.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. Fructifications of *Fomes durissimus* Lloyd strain I growing on standing tree of *Swietenia mahogani* (Reduced).

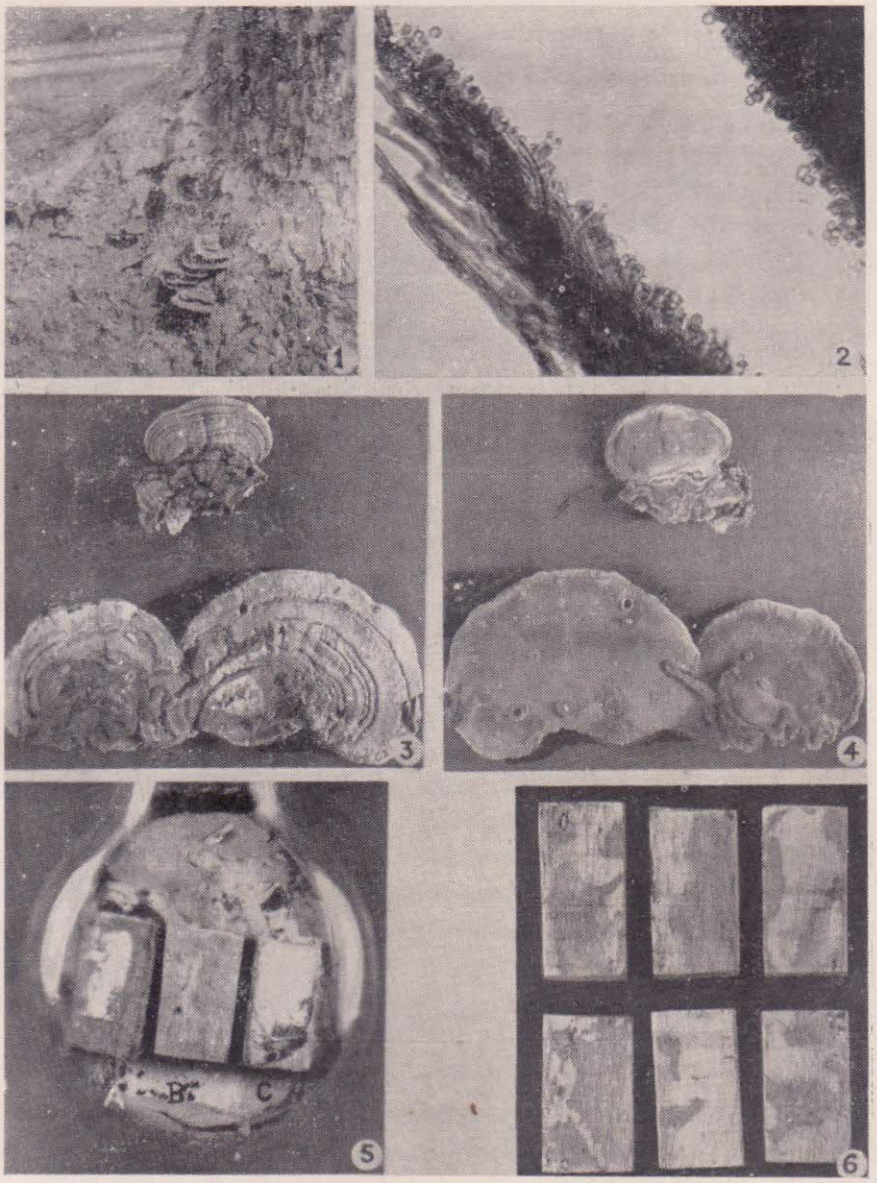
Fig. 2. Part of a section through hymenium of *F. durissimus* strain I showing pore tube, basidia and basidiospores (×5500 approx.).

Fig. 3. Fructifications of *F. durissimus* strain I showing concentrically zoned upper surface.

Fig. 4. Fructification of *F. durissimus* strain I showing poroid hymenial surface.

Fig. 5. Toxicity test in Kolle flask with wood-blocks of *S. mahogani* treated in different concentrations of 'Ascu A' and subjected to the attack of *F durissimus* strain I for four months ; A, treated with 0.02% 'Ascu A', B, treated with 0.03% 'Ascu A' and C, untreated.

Fig. 6. Decayed sapwood (upper row) and heartwood (lower row) blocks of *S. mahogani* showing discolouration at places after eight months' exposure to the strain I of *F. durissimus*.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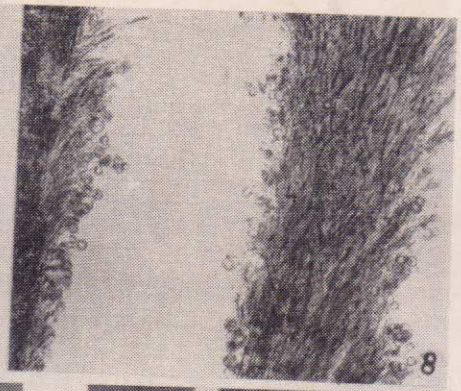
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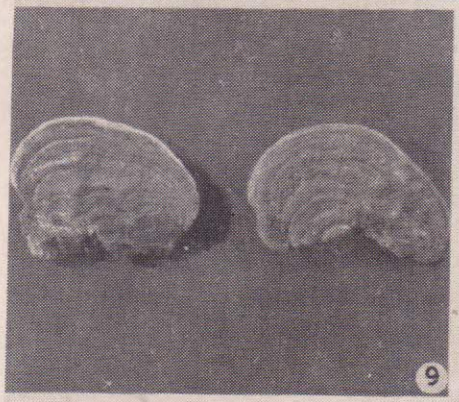
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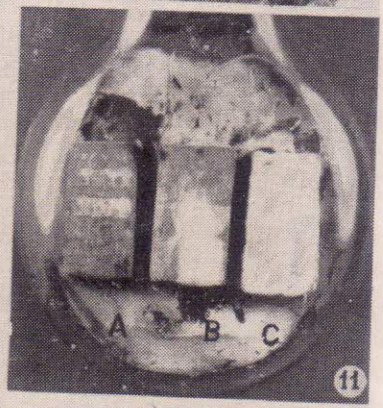
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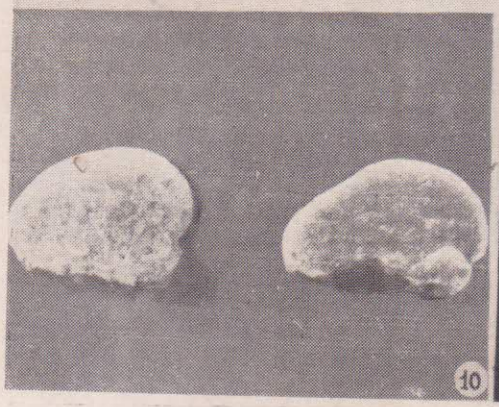
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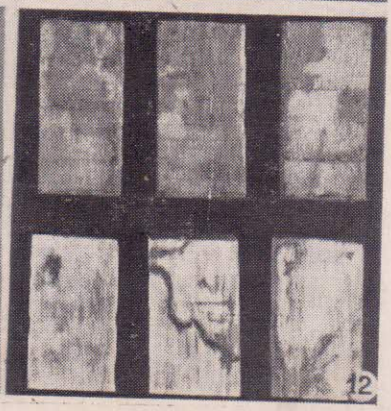
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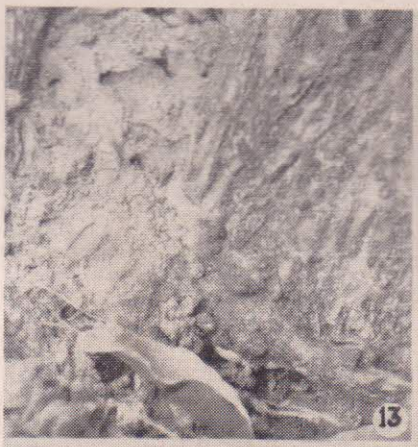
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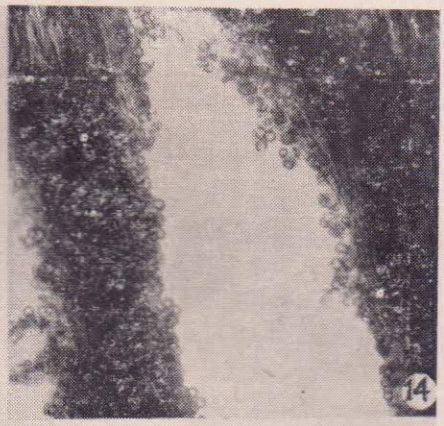
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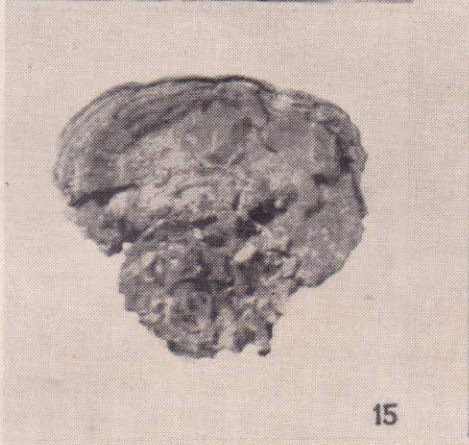
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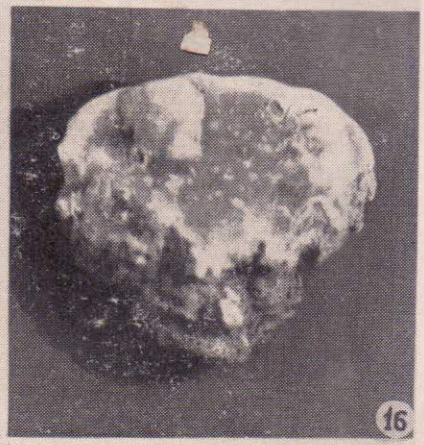
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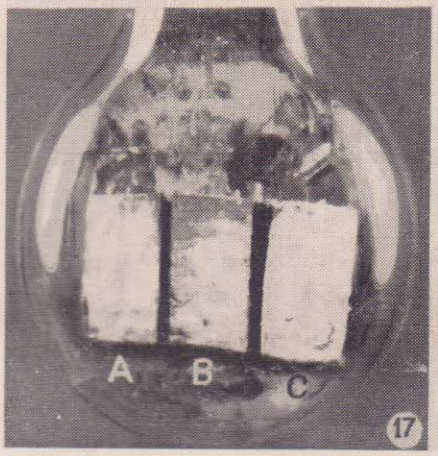
A
B
C
17

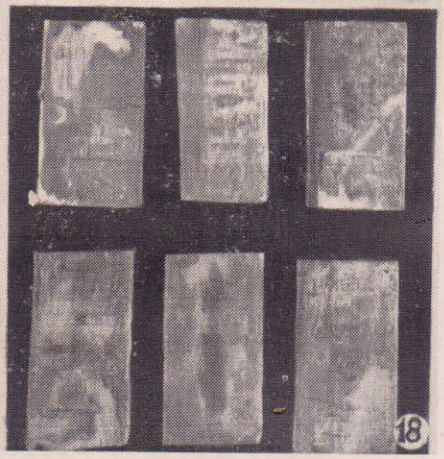
18

Plate II

Fig. 7. Fructifications of *F. durissimus* strain II growing on standing tree of *Casuarina equisetifolia* (Reduced).
Fig. 8. Part of a section through hymenium of *F. durissimus* strain II showing pore tube, basidia and basidiospores (×5500 approx.).
Fig. 9. Fructifications of *F. durissimus* strain II showing concentrically zoned upper surface.
Fig. 10. Fructifications of *F. durissimus* strain II showing poroid hymenial surface.
Fig. 11. Toxicity test in Kolle flask with wood-blocks of C *equisetifolia* treated in different concentrations of 'Ascu A' and subjected the attack of *F. durissimus* strain II for four months ; A treated with 0.02% 'Ascu A', B, treated with 0.03% 'Ascu A' and C, untreated.
Fig. 12. Decayed sapwood (upper row) and heartwood (lower row) blocks of *C. equisetifolia* showing discolouration at places after eight months' exposure to the strain II of *F. durissimus*.

Plate III

Fig. 13. Fructifications of *F. durissimus* strain III growing on standing tree of *Mimusops elengi* (Reduced).
Fig. 14. Part of a section through hymenium of *F. durissimus* strain III showing pore tube, basidia and basidiospores (×5500 approx.).
Fig. 15. Fructification of *F. durissimus* strain III showing zoned upper surface.
Fig. 16. Fructification of *F. durissimus* strain III showing poroid hymenial surface.
Fig. 17. Toxicity test in Kolle flask with wood-blocks of *M. elengi* treated in different concentrations of 'Ascu A' and subjected to the attack of *F. durissimus* strain III for four months ; A treated with 0.02% 'Ascu A', B, treated with 0.03% 'Ascu A' and C, untreated.
Fig. 18. Decayed sapwood (upper row) and heartwood (lower row) blocks of *M. elengi* showing discolouration at places after eight months' exposure to the strain III of *F. durissimus*.