The Role of Fungi in the Origin of Oleoresin Deposits (Agaru) in the wood of *Aquilaria Agallocha* Roxb.

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A brief review is given of investigations into the cause of agaru, a valuable aromatic oleoresinous deposit found in the stems of *Aquilaria agallocha* in Bangladesh, East India and other parts of South East Asia.

In previous investigations various fungi have been identified in association with agaru deposits but their casusal role in this context has not been fully established.

The present investigation includes microscopic examinations and identification of fungal isolates from four samples of agaru collected in the Sylhet region of Bangladesh. From this evidence it is concluded that it is unlikely that there is a specific fungal cause for agaru. Suggestions are made for further research.

বাংলাদেশ, পূর্বভারত ও দক্ষিণ পূর্ব এশিয়া অঞ্চলে এক্যুলারিয়া এ্যাগালোচা গাছের কাণ্ডে এক প্রকার মূল্যবান ওলিও রেজিনস, আগারুর গঠন সম্পর্কিত নিরীক্ষা সমূহের একটি সংক্ষিপ্ত পর্য্যালোচনা এই প্রবন্ধে উপস্থাপন করা হইয়াছে।

পূর্ববর্তী গবেষণাসমূহে আগারু সংলগ্ন বিবিধ প্রকার ছত্রকের পরিচিতি বর্ণনা করা হইয়াছে, কিন্তু এই প্রসংগে ঐ সমস্ত ছত্রকের ভূমিকা পুরাপুরি প্রতিষ্ঠিত করা হয় নাই।

এই প্রবন্ধে বাংলাদেশের সিলেট অঞ্চল হইতে সংগৃহিত আগারুর চারিটি নমুনার আনুবীক্ষণিক পরীক্ষা ও ঐ সমস্ত নমুনা হইতে পৃথককৃত ছত্রক সমূহের পরিচিতি বর্ণনা করা হইয়াছে। এই পরীক্ষার প্রেক্ষিতে এই সিদ্ধান্তে আসা গিয়াছে যে কোন বিশেষ ধরণের ছত্রকের কারণে আগারু গঠন হয় না। এই বিষয়ে আরও গবেষণা সম্পর্কে ইঙ্গিত দেওয়া হইয়াছে।

INTRODUCTION

The genus *Aquilaria* Lam. (Thymeliaceae) comprises eight tree species which are distributed throughout India, China, South East Asia and the East Indies (Willis, 1955).

Aquilaria agallocha Roxb., economically the most important of these, occurs in parts of Bangladesh, Bhutan, Burma, Assam and Eastern India where it provides useful light coloured timber and its bark has been used previously to prepare a kind of paper (Gamble, 1922 ; Burkhill, 1966). However, it is valued mainly for the dark brown to black oleoresinous deposits that are found in about 10% of the naturally occurring trees of *A. agallocha* and other species of the genus. This product, variously

known as agaru, eaglewood, aloewood or calamnac, has been collected from the forests of India, Burma, Malaysia, Indonesia, Cambodia and Annam for many centuries and has formed a valuable article of trade (Burkhill 1966).

Agaru occurs in irregular patches and streaks in the steam wood of trees over 25 years of age, increasing in quantity and quality with age so that the best yields are found in trees which are 50-60 years old or more, (Sadgopal 1959, Menon 1960). Agaru deposits may have a hollow centre surrounded by a very dark brown or black region which at its outer limits may merge into lighter brown portions before white wood is reached (Plate 1). It is said that traces of aromatic oils can be found in this peripheral white wood as well as in the coloured portions.

There are a number of grades of agaru of which the best and darkest are used in incense mixtures while the lower grades (dhum) are extracted by steam distillation to provide an oil (agar attar) that is used in the perfume industry. Agaru is also said to be used in embalming and to have insect repellent properties. Details of the collection and grading of agaru and its subsequent processing have been given in a number of papers (De 1927, Sadgopal & Varma 1952, Sadgopal 1959, Rao & Kuldip Bhatia 1959, Menon 1960, Dass 1965), and are also reviewed by Burkhill (1966).

Distillation is carried out at three centres in Assam and the Naga Hills (Menon 1960) and on smaller scale it provides an important cottage industry in the Sylhet region of Bangladesh. In recent years, since the partition of India and Pakistan, the latter has suffered a decline because many of the forest areas where *A. agallocha* occurs are in India, while the distilleries are largely in East Pakistan. Further difficulties arose with the liberation of Bangladesh, so that now many of the cottage industries in the Sylhet region have gone out of business while others are threatened with closure.

Authorities differ on their views on how far a tree with valuable agaru deposits can be detected from its external appearance. De (1927) is definite that there is no outward indication that a tree contains agaru and Sadgopal & Varma (1952) appear to take this view but they add that professional agar collectors can detect agar bearing trees successfully. Menon (1960) describes how trees are tested for agaru deposits by borings before they are felled, which also implies that the agaru collector requires considerable experience as well as a non-destructive sampling technique, if he is to set about his work efficiently. However, there are others (Bose 1938, Rao & Kuldip Bhatia 1959, Bhattacharya *et al.* 1952) who claim that trees with agaru deposits are diseased and show signs of ill health, particularly a dieback of the top and outer branches and a yellow tint to the woody tissues. Dass (1963) and Sadgopal (1960) also state that wounds, stem distortions and rotten branches provide evidence of agaru deposits within a tree, regarding these symptoms as the result, rather than preceding conditions, of agaru formation, while Bose (1938) goes so far as to claim that trees with agaru deposits eventually die of the condition.

Whatever the truth may be in this matter, it seems clear that indiscriminate felling of *A. agoalcha* by unskilled collectors has been continuing for many years (Bose, 1938) and that this has been greatly aggravated by the interference with normal supplies of agaru to distillation centres in recent years. The position is now so serious that *A. agallocha* is in grave danger of becoming extinct in parts of Bangladesh and Eastern India. It was because of these circumstances that the present limited investigation was undertaken, in the hope of obtaining a better understanding of the origins of agaru and, through this, contributing to the conservation of *A. agallocha* and the industry that it serves.

Previous Studies on the Origins of Agaru

The belief that the formation of agaru deposits in *A. agallocha* is due to fungal activity goes back at least fifty years and provides the basis of most investigations into their origin. Indeed, of the various authors consulted as part of this investigation, only Sadgopal (1959) suggests that insects, as well as fungi, might be involved in the etiology of the material.

The earliest investigation appears to have been made by Bose (1926) who found dark coloured septate hyphae and signs of delignification of the cell walls in longitudinal microsections of wood

associated with agaru, in a number of independently collected samples. A fungus was isolated consistently from these specimens which corresponded with the hyphae seen in the wood sections, but which was only identified as a member of the Fungi Imperfecti. Later, however, Sadgopal (1959) refers to a *Torula* sp. isolated by Bose, which may be the fungus which was used to inoculate *A. agallocha* for successful production of agaru. A little after the first investigation of Bose, Tunstall (Anon., 1930, 1932) isolated *Aspergillus rubber* Thom & Church (syn. *Aspergillus sejunctus* Bainier & Sartory), a *Penicillium* sp. and a *Fusarium* sp. from wood of *A. agallocha* containing agaru in Assam. All three isolates were inoculated experimentally into healthy *A. agallocha* (presumably free of agaru deposits) by insertion of inoculum into holes bored to various depths horizontally into the stems. Examination of the inoculated trees 3-6 months afterwards revealed signs of agaru formation in the wood and the original fungal inocula were reisolated successfully from these tissues. The experiments were terminated at the end of 1931 when contamination of inoculum points in the test trees by an unidentified basidiomycete occurred (Anon. 1932). The cultures of the fungi used as inoculum are reported as lost during World War II (Sadgopal 1959).

At about the same time Bose appears to have resumed his work as he reports (Bose 1943) on inoculation trials with a *Cladosporium* sp. which had been presumably obtained from wood containing agaru deposits. Inoculations were made in three successive years (1939-1941) and the first of these, when examined at intervals of one and two years, showed formation of agaru gum close to the treated region, with abundant hyphal colonisation. Unfortunately the trees used for all the studies of Bose up to this time were situated at Kohima and Imphal and were lost during the fighting in these regions during World War II. However, by 1950 Bose had re-established his experiments, using his original isolates, but nine years later no results had been reported (Sadgopal, 1959).

The next investigation into the cause of agaru deposits was made by Bhattacharya, Datta & Baruah (1952) who confirmed previous observations that there was a consistent association between wood containing the oleoresin and profuse colonisation of these tissues by dark septate hyphae. *Epicoccum granulatum* Penz. was consistently isolated from the specimens examined and re-inoculation of woody tissues of *A. agallocha* resulted in colonisation very similar to that of wood with naturally occurring agaru, with delignification of the cell walls and formation of resinous cavities bearing drops of oil. It seems possible that this investigation is the same as that described by Sadgopal (1959) and attributed to Baruah alone.

In addition to these three independent studies Sadgopal (1959) refers to some (apparently unpunlished) work by Dr. K. Bagchee at Dehra Dun in 1952 who isolated a *Cylindrocladium* sp., a *Fusarium* sp. and a sterile mycelial culture from samples of wood with agaru. The *Cylimndrocladium* sp. was obtained consistently while the *Fusarium* sp. was found once only from a range of collections. These two isolates with the *Torula* sp. obtained earlier by Bose were used independently and in combination to inoculate trees in the same region of Assam as the previous trials of Bose (1926, 1938, 1943) and Bhattacharya *et al.* (1952). At the same time investigations of the anatomical and entomological aspects of agaru formation were tarted by Dr. K. Choudhury. No accounts of the results of these field inoculation trials or the work of Choudhury have been traced.

No other accounts of investigations into the origins of agaru, published in the last fifteen years, have been traced but the Forest Pathologist for Bangladesh, Mr. Md. Abdur Rahman, isolated a *Sphaeropsis* sp. from wood containing agaru in 1972, which he regards as a possible cause of the condition (personal communication, 1975). In addition, some experiments have been made recently at Lowacherra, near Sylhet, to determine the relation between wounding and agaru deposits (vide Mr. A. W. Khan, Deputy Chief Conservator of Forests) and in a typewritten report by Mr. M. Mosharraf Billah to the Bangladesh Forest Department at Dacca (1975) there is reference to inoculation experiments using agaru as inoculum. The latter are claimed to have been successful.

The Present Investigation

This has been based on four collections of wood containing deposits of agaru which were obtained at intervals during 1975 and 1976 through the kind offices of Mr. A. W. Khan and are described below.

Specimen No. 1

A small sliver of uniformly brown wood, about 3 cm x 1 cm x 0.25 cm, of unknown provenance, obtained during the author's visit to Bangladesh in March 1975.

Specimen No. 2

A roughly cylindrical sample, about 20 cm long and 3 cm in diameter, consisting of white wood with several longitudinal streaks of brown agaru deposit, associated with a small branch scar in at least one instance. Received September 1975.

Specimen No. 3

A much larger sample than No. 2 measuring approximately 15 cm x 8 cm x 8 cm with pronounced agaru deposits along the grain. The most prominent of these were hollow in the centre and, on splitting, were found to contain evidence of old branch scars. While the agaru deposit was fairly sharply delimited, the outer parts where distinctly lighter in colour and showed less uniformity than the darker central region. Received February 1976.

Specimen No. 4

Similar to No. 3, but with pronounced bluestain throughout the greater part of the white wood. Received May 1976.

Specimens 2, 3 and 4 were all collected from Kachargul Village near Juri Forest Office in the Sylhet district.

All four specimens were used for microscopic examination and the last three supplied material for attempts to isolate and identify fungi associated with agaru.

Microscopic Examinations

These were all based on longitudinal sections, about 15-20 micron thick, prepared with a sledge microtome from small pieces excised from dark brown or light brown deposits, or from surrounding white wood 0.5-1.0 cm from the agaru, a few samples included material from brown and white zones. All pieces were boiled in water until waterlogged when they were sectioned, stained in saffranin and picro-aniline blue (Cartwright 1929) and mounted in Gurr's Neutral Mounting Medium. A few sections were also retained to be tested for lignification using 5% alcoholic phloroglucinol and concentrated hydrochloric acid. Thirty-four permanent mounts have been prepared in this way which have been examined and are now stored in the laboratory. The results of these are summarised in Table 1, showing the distribution of oleoresinous deposits and four broad categories of fungus mycelium in relation to the agaru deposits and surrounding wood. A note on the presence of delignification is also included.

The most striking fungal colonisation showed spiral hyphal growth in the tracheid walls (spiral cavitation) typical of invasion of soft rot fungi. This occurred in Specimens 2, 3 and 4 where it was closely associated with oleoresin deposits, but was absent in Specimen 1 (which was uniformly infilitrated with oleoresins).

Relatively large, septate dark hyphae could be found in most sections, often penetrating cell walls by fine boreholes typical of bluestain fungi. Where these were relatively sparse, they were largely confined to ray parenchyma. These hyphae were particularly evident in Specimen 1 and in the bluestained white wood of Specimen 4. In one set of sections mature pycnidia of *Botryodiplodia theobromae* Pat. were found within vessels of the woody tissues. Vissible connections could be traced between these and dark septate hyphae.

Plate 2 shows part of a section with spiral cavitation and colonisation by bluestain hyphae.

Fine hyaline hyphae which were clearly not young stages of bluestain hyphae were evident in most sections to a greater or less extent. It is thought that the greater part of these may have belonged to saprophytes, such as the *Penicillium* and *Aspergillus* spp. which appeared so regularly in later attempts to culture the fungal associates of agaru. On two occasions of a few stunted conidiophores, very similar to those of *Penicillia* were found within vessels of sections which lends some support to this hypothesis. Where they were plentiful, hyphae of this type often formed loose mats in vessels or on the inner surface of the cavities of agaru deposits.

It was also evident from the occasional presence of clamp connections and the association of hyphae with large boreholes, that this group included a proportion of wood destroyeding basidiomycetes. The occurrence of these features is noted in Table 1 but no attempt has been made to provide even an approximate quantitative estimate.

TABLE 1. Summary of Observations Made From Microsections of Wood Containing Deposits of Agaru.

Specimen No.	Colour of Wood	Oleoresin	Spiral Cavitation	Coarse Brown Hyphae	Colourless Hyphae	Evidence of Wood Destroying Basidiomycetes.	Evidence of Delignification	Slide Numbers
1	Dark Brown	xR	—	xx	x	—	—	1
2	Dark Brown	xxR	xxx	x	x	x	—	2
3	Dark Brown	xxR	xx/xxx	—	x/xx	—	—	7, 8, 9, 11, 12,19
4	Dark Brown	xxR	—/xx	—	—/xx	x	—	28, 33
2	Light Brown	xxR	xxx	x	x	—	—	3, 4
3	Light Brown	R/xxR	—/xx	—	—	x	x	5, 6, 10, 13, 14 23, 24, 25, 26,27
4	Light Brown	R/xR	—/xx	—/xx	—/x	x	x	28, 33, 34
3	White	—	—	—/x	xx/xxx	x	x	15, 16, 17, 18, 20, 21, 22
4	White	—	—	xx	x	x	x	29, 30, 31, 32,33

Symbols—, x, xx etc. indicate intensity of fungal colonisation or deposits in the sections, on the following basis :

—Absent, x Occasional, xx Frequent, xxx Very Frequent

R denotes oleoresinous deposits in ray parenchyma and additional x symbols show extent of these in other tissues

Values noted thus —xx/xxx denote variation in frequency or distribution between slides

Small delignified parts of the tracheids and vessels occurred in sections from white wood but these were never associated with oleoresin deposits.

A re-examination of Specimen 3 showed that there were occassional small faint brown flecks and streaks in the white wood at an appreciable distance from agaru deposits, that might represent the earliest visible stage of agaru formation. Sections cut from these areas showed they were colonised by fine hyaline hyphae which in one case showed one or two clamp connections and elsewhere were associated with large boreholes. Where the small flecks were more pronounced in the wood, spiral cavitations could sometimes be found in addition to possible basidiomycete hyphae. It would be speculative, however, to conclude on the basis of this slender evidence that agaru deposits arise where the wood is colonised by basidiomycetes followed by soft-rot fungi.

No evidence was found in any of the sections of structures corresponding to the 'brush-like' fungal clusters described by Bose from his microscopic studies (Bose 1938).

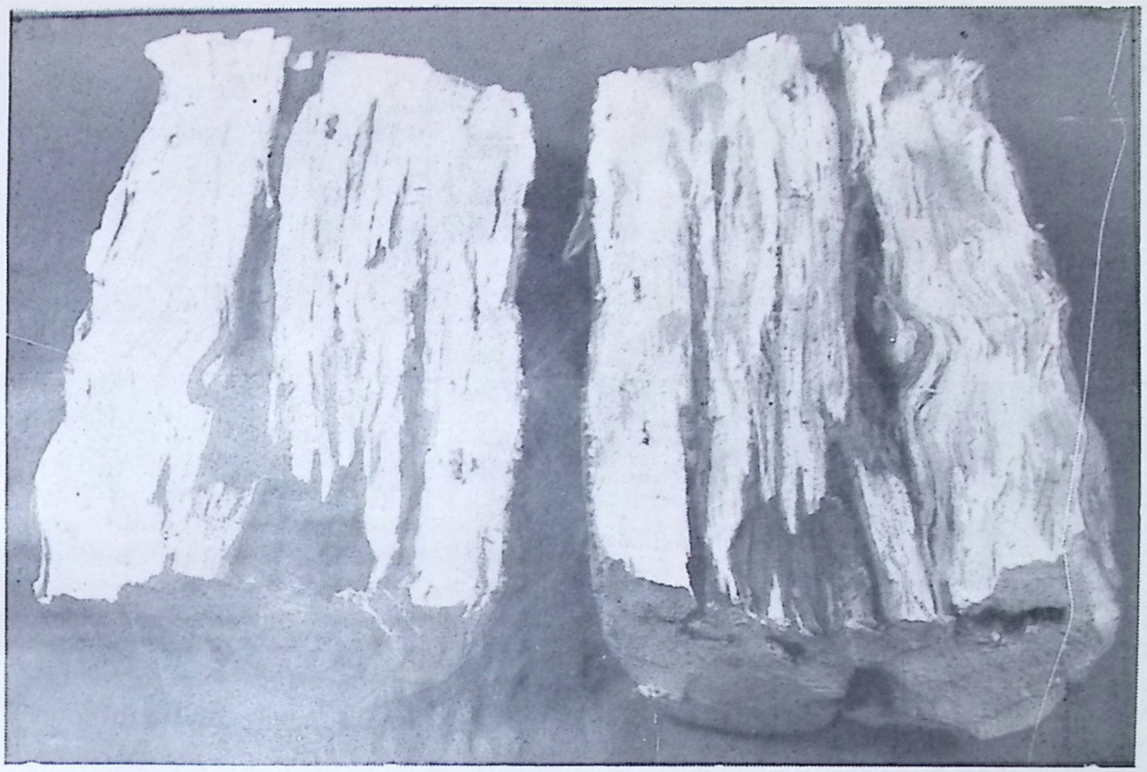
Fungal Isolations

Attempts to identify the fungi associated with agaru deposits and surrounding wood were confined to Specimens 2, 3 and 4 ; Specimen 1 was excluded as it was too old and small to yield useful information.

Apart from some unsuccessful attempts to incubate pieces of wood with agaru in damp chambers for the isolation of fungal associates, these studies were based on the plating out of small splinters, approximately 0.5 cm long, excised aseptically from the surfaces of freshly split specimens. As far as possible, these were taken from regions that were close to those used for microsections showing varying degrees of agaru deposit.

Between three and five splinter samples were transferred per plate and incubated at 25° C for 11-14 days. Plates were inspected at regular intervals over this period and isolates transferred to tubes of 3% malt agar medium for further growth and identification as they appeared.

In all three trials 3% Oxoid malt agar was used for the initial plating, and in isolations from



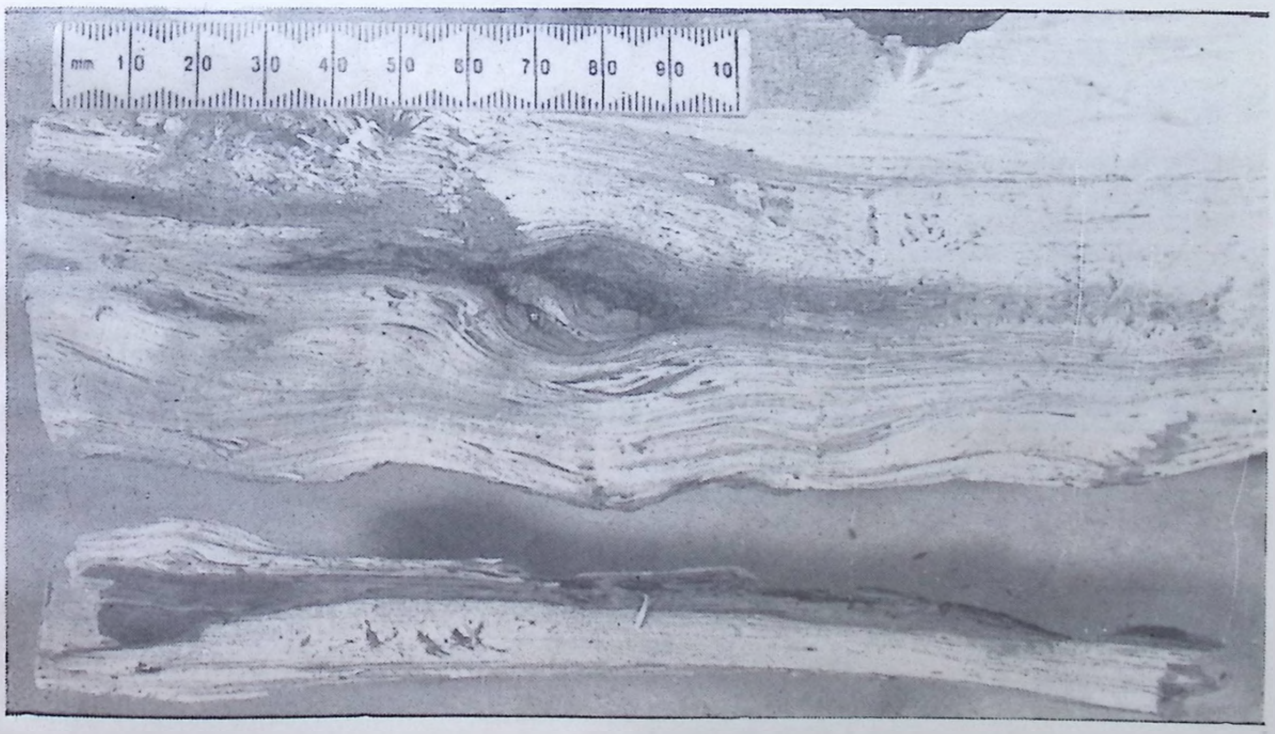


Plate I. Two samples of wood of *A. agallocha*, split to show agaru deposits and inclusion of old branch scars in these regions (Specimens 2 and 3).

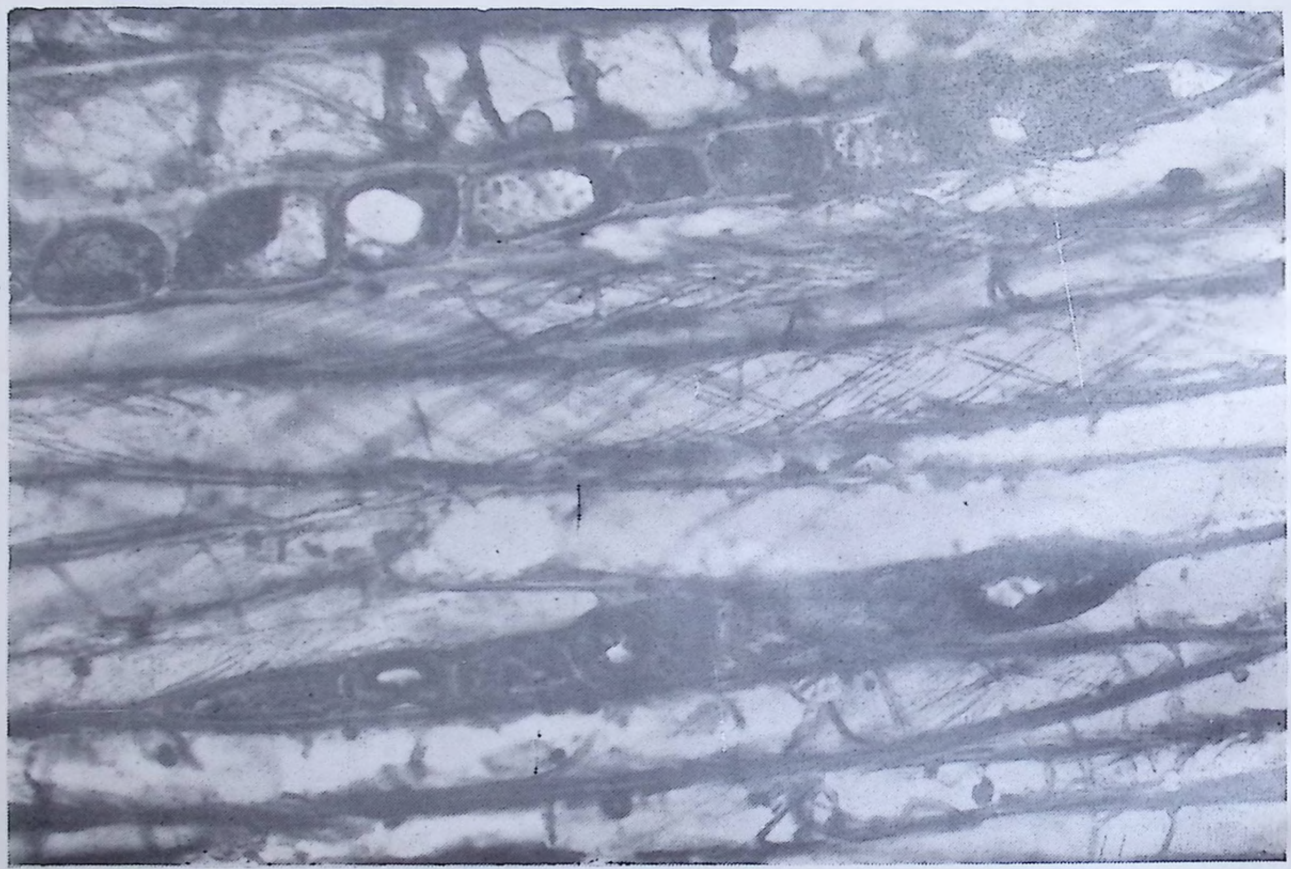


Plate II. Longitudinal section of wood containing agaru to show associated dark brown hyphae and spiral cavitation of tracheid walls.

Specimen 2 this medium with additional crystamycin (an antibiotic preparation of penicillin and streptomycin) was also used as a precaution against possible excessive bacterial contamination. However, this refinement appeared to be unnecessary and was discontinued in later work with Specimen 3, in which 3% malt agar only was used.

TABLE 2. Distribution of Isolates From Specimen No. 2

	DARK BROWN		LIGHT BROWN		WHITE	
	A	D	A	D	A	D
Aspergillus and Penicillium spp.	xxx	xxx	xxx	x	xxx	xxx
Fusarium sonali	x	x	x	—	—	—
Botryodiplodia theobromae	x	x	xx	xx	—	—
Trichoderma viride	—	x	—	—	—	x
Phialophora parasitica	xxx	xxx	xx	xx	—	—

This is a summary of the results of two trials in which the number of samples plated for each treatment combination varied, in total, between 48 and 90, the smallest number being from light brown regions of wood.

Frequency of species isolated are denoted thus :
--Absent

x upto 5% total isolates in treatment
xx between 5-40% total isolates in treatment

xxx more than 40% total isolates in treatment

Medium used : A = 3% malt agar

D = 3% malt agar + crystamycin

This notation is also in Tables 3 and 4.

TABLE 3. Distribution of Isolates From Specimen No. 3

	DARK BROWN	LIGHT BROWN	INNER WHITE	OUTER WHITE
Aspergillus and Penicillium spp.	xxx	xxx	xxx	xxx
Botryodiplodia theobromae	—	—	xxx	xxx
Phomopsis spp.	x	xxx	x	—
Sterile mycelium	x	—	x	—

Medium used 3% malt agar (Oxoid). 40 samples for each treatment combination.

For symbols see Table 2.

TABLE 4. Distribution and Frequency of Isolates From Specimen No. 4

	DARK BROWN			BLUESTAIN TAIN			INNER WHITE			OUTER WHITE		
	A	B	C	A	B	C	A	B	C	A	B	C
Aspergillus and Penicillium spp.	xx	-	-	–	–	–	–	–	–	xxx	–	–
Fusarium solani	xx	–	xx	–	xxx	xx	xxx	–	x	–	–	–
Botryodiplodia theobromae	xxx	–	–	xxx	xxx	x	xx	xxx	xx	–	–	–
Phomopsis spp.	xx	–	–	–	–	–	xx	–	–	–	–	–
Cunninghamella echinulata	xx	xx	–	–	–	–	–	–	–	–	–	–
Trichoderma viride	x	–	x	–	–	–	–	–	–	–	–	–

Media used :

A = 3% malt agar (Oxoid)

B = Selective medium for basidiomycetes (Hunt & Cobb 1971)

C = Selective medium for bluestain fungi ; 3% malt agar with 0.1% Cu.

Note : 20 samples per treatment combination.

For symbols see Table 2.

As the media used for Specimens 2 and 3 favoured fast growing sugar fungi, to the exclusion of slower organisms, the range of media for Specimen 4 included one which was selective for basidiomycetes (Hunt & Cobb 1971) and one of malt agar with additional copper which had been used successfully elsewhere for bluestain fungi. The results obtained from this last trial were somewhat disappointing (Table 4) as, although the selective media supressed the appearance of the fast growing organisms, other slower growing fungi did not replace them.

In all trials by far the most frequently isolated fungi belonged to the genera *Aspergillus* and *Penicillium*, which were present in all specimens and, at one time or another, in dark brown, light brown and white regions of wood. The following species have been identified within this group, which are listed below in order of frequency.

Penicillium citrinum Thom (most frequent)

Aspergillus tamarii Kita

A. sejunctus Bainier & Sartory

(=*A. ruber* Thom & Church) see Tunstall (Anon. 1932).

A. chevalieri (Mangin) Thom & Church
A. *restrictus* G. Smith
A. flavus Link ex Fr.
A. caesilluz Saito

Fusarium spp., all identified as *F. solani* (Mart.) Sacc. were also isolated from Specimens 2 and 4 in all sampling regions, but always at a much lower frequency than the Aspergilli and Penicillia.

Botryodiplodia theobromae Pat. was the most commonly occurring single fungus species in all samples, but predominant in white wood regions. As has been indicated above, this species probably accounts for most of the colonisation by bluestain fungi that was observed in the microsections.

A hitherto underscribed *Phomopsis* sp. was isolated on several independent occasions from Specimens 3 and 4 and will be described in due course by Dr. E. Punithalingam of the Commonwealth Mycological Institute.

Trichoderma viride Person ex S. F. Gray was found surprisingly rarely and *Cunninghamella echinulata* (Thaxter) Thaxter & Blakeslee was only found in Specimen 4 where it was associated with dark brown agaru deposits and was one of the few species to grow freely on the selective medium for basidiomycetes.

The most interesting isolate was *Phialophora parasitica* Ajello, Georg. & Wang, which was only found in appreciable quantities in Specimen 2, where it was closely associated with the heaviest desosits of oleoresin. This fungus has been only recently described from a human skin infection (Ajello *et al.* 1974) and has since been associated with various diseased conditions in plants (Hawksworth *et al.* 1976).

As the genus *Phialophora* includes a number of species known to be soft rot agencies (Seehan *et al.* 1975) a small test was set up to determine whether this fungus and the *Phomopsis* isolate would produce typical spiral cavitation in the tracheid walls of *Aquilaria agallocha*. Small billets of white wood, cut from Specimen 3, were autoclaved (15 lb for 30 minutes) in boiling tubes with 15 ml of water agar. These were then inoculated with the two fungi and incubated in the dark for five days at 25°C, followed by thirty days in a glasshouse at about 20°C. After this period the billets were completely colonised and the fungi were sporulating on the surface when they were prepared for sectioning and microscopic examination. This showed that, while the woody tissues had been colonised throughout by both fungi, only *P. parasitica* had produced spiral cavitation of the tracheid walls. The latter was extensive and closely similar to that found to be associated with the agaru deposits in Specimens 2, 3 and 4.

However, it would be rash to assume from this observation that *P. parasitica* is the sole cause of spiral cavitation in our microsections, as *A. flavus*, *F. solani* and *T. viride* have all been shown to be capable of soft rot activity at times (Seehan *et al.* 1975) and might produce a similar artefact. It is the more regrettable, therefore, that neither facilities nor material were available for the extended testing of all of our isolates that were possible causes of the spiral cavitation observed in our sections.

Sterile mycelial cultures were isolated from a few splinter samples from Specimen 3. These have remained unidentified and their role, if any, in agaru formation is not known.

DISCUSSION

If there is to be any chance of recovery in the agaru industry of Bangladesh, or of the survival of *A. agallocha*, it will be essential to improve our understanding of the nature and origins of the product and develope a non-destructive technique for detecting agaru deposits in native trees.

The discovery, about fifty years ago, that agaru in *A. agallocha* was closely associated with mycelium in the affected tissues (Bose 1926), seems to have led to the tacit but widely held view that this was due to the invasion of the tree by a single specialised fungus.

This is implicit in the later work of Bose (1938, 1943) the researches of Tunstall (Anon. 1931, 1932), Bhattacharya *et al.* (1952) and the investigations described by Sadgopal (1959), in all of which single fungus species found associated with agaru deposits were used in inoculation trials.

However, the number of positive results reported from the use of these varied inocula is probably sufficient evidence alone to suggest that agaru arises

from a much more generalised cause than was envisaged in these researches.

The results of our present limited investigation also support this view, as the range of fungi observed and isolated has varied between specimens and there has been little consistency in their distribution through the host tissues.

Indeed, it seems likely that oleoresinous deposits such as agaru may arise as a direct response of the stem tissues of *A. agallocha* to wounds with subsequent invasion by weak pathogens.

In a recent paper, Shigo (1975) has described the events leading to decay in a tree as falling into the following three broad stages, (1) host response to wounding, (2) invasion by pioneer micro-organisms and (3) the decay of dead cells. The pioneer invaders of stage (2) will elicit a response in the host which will depend on the aggressiveness of the micro-organisms, the kind of wound and the state of health of the host and may result in 'compartmentalisation of the invaders by gum secretions, tylose formation and other forms of barriers.

It is suggested that the oleoresinous deposits of agaru are a protective reaction of this kind and that further experiments to determine the origin of agaru should test this hypothesis.

While it is not appropriate at this juncture to discuss the design of these trials in detail, it seems clear that they should be set up, if possible, on even-aged crops and should compare only a small number of treatments. The latter could comprise one or two types of wounds alone and in combination with a crude form of inoculum (such as finely divided, freshly collected agaru wood) and include a full set of controls.

A large number of replicates will be essential, not only to ensure a reasonable standard of accuracy, but to allow for destructive sampling at intervals which may be necessary over an extended period of years. Although the first object of these experiments is not to seek a single fungus as a primary cause of agaru, samples should be taken to allow records of fungal associations in the trees as well as chemical analysis for oleoresin formation.

While trials of this kind may provide reliable information on the cause of agaru and lead to techniques for the induction of these deposits in *A. agallocha,* it will probably be a matter of years before this stage is reached.

In the meantime, some improvement in non-destructive techniques for the detection of agaru in native *A. agallocha* will be essential if the tree is to survive the haphazard felling that now threatens its survival in economically significant quantities.

This need may be met by an instrument that has been developed in North America for detecting rot, stain and other defects in standing trees (Shigo, 1975 ; Skutt *et al.* 1972 ; Tattar *et al.* 1972). The Shigometer as it is called, detects the presence of defects through variations in the resistance of the wood to a pulsed electric current. The test involves the insertion of a wire probe into the tree but the wounding that this requires is minimal. Some applied research would clearly be necessary to adapt the Shigometer principle for agaru detection but this would be well worth while in view of the considerable improvement in the efficiency that this could provide for agaru collection in the field.

Acknowledgements

I am endebted to the Deputy Chief Conservator of Forests, Bangladesh for the provision of material for this small investigation, to my colleagues at the Commonwealth Mycological Institute for identification of fungi, and Mr. John Savory and Miss Janice Carey of the Princes Risborough Laboratory of the Building Research Establishment for their interest and help. I am grateful to Mr. David Fry for taking the photographs.

The study arose from a short assignment by the author to Bangladesh in March 1975. This visit was arranged through the auspices of the Ministry of Overseas Development as part of the arrangements for technical co-operation between the United Kingdom and Bangladesh.

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