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MOULD CONTAMINATION OF RUBBER

BY

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INTRODUCTION

Fungal contamination of rubber has been recorded as early as 1919. De Vries (1919) observed that the fungi which normally occur on rubber, in the tropics, are various species of *Penicillium* and *Aspergillus.*

Sheet rubber becomes overgrown with fungi mainly during storage, and because of current practice in Ceylon sheet rubber is stored four to five times, at different stages, before it is finally shipped out. Storage over long periods of time at any one place is perhaps the biggest contributory factor to fungal infection of prepared rubber. Contaminated rubber is sold at a considerable discount, causing economic hardship to smallholders, whose rubber, unfortunately, is most prone to fungal attack.

Sheet rubber dried over a slow-burning fire, which produces excess smoke, is resistant to fungal attack, because of the presence of small quantities of creosotes deposited on it during the treatment with smoke. However, this effect does not last for more than five to six days. During this period the fungicidal effects of the smoke constituents wear off and the rubber sheet then becomes a suitable substrate for the growth of fungi.

Fungal growth not only down-grades the rubber by discolouring it; but Heinisch & Khur (1956) have observed that it can lead to loss in weight, which may amount to more than 30%. Weight loss is caused by the decomposition of carbohydrates in the rubber too. The fungi not only spread on the surface of the sheet, but also enter and spread within it.

This paper gives the results of a scientific study of the problem of mould contamination of rubber and the possibilities of checking it by physical means, without the use of chemicals.

MATERIALS AND METHODS

Isolation: Pieces of contaminated sheet rubber were collected at various rubber purchasing depots and from estate stores. Samples were brought into the laboratory in sealed polythene bags. Small pieces of these samples were incubated in humid chambers for periods of one to four weeks, when they were completely over-grown with fungi. Using a sterile needle, samples of various fungi, identified on the basis of colour and colony characteristics, were picked out and plated on malt agar. Typical cultures were despatched to the Commonwealth Mycological Institute for identification. Stock cultures were maintained in the laboratory in malt agar in Mc-Cartney bottles and under oil immersion.

Temperature control: The effect of temperature on spore germination was tested in incubators, using agar-coated glass slides, sprayed with conidial suspensions of the selected fungi, made up in glass distilled water. Inoculated slides were first dried in a desiccator to remove the excess water from the agar surface, and incubated at 100% relative humidity (RH). Spore germination was recorded after 24 hours, taking five readings of 100 conidia each.

Humidity control: Trials were carried out on the selected species maintained at different RHs, at room temperature 28° ± 2°C, by the use of saturated salt solutions. Agar-coated glass slides sprayed with spore suspensions were used as in the experiments on temperature control. In every case at least five counts of 100 conidia were made from each slide after incubation for 24 hr.

RESULTS

Fungal species: Twelve different species of fungi were isolated from contaminated samples of sheet and crepe rubber. The frequency of occurrence of the more common fungal species isolated from stored rubber, expressed as a percentage of 70 samples tested, is as follows:—

(*a*) *Penicillium citrinum*	61%
(*b*) *Aspergillus niger*	57%
(*c*) *A. ochraceus*	44%
(*d*) *P. frequentans*	6%
(*e*) *Paecilomyces varioti*	2%

The first three species *viz. P. citrinum, A. niger* and *A. ochraceus* were often found together on the same sample of contaminated rubber. All mouldy rubber was found to be infected with more than one species of fungi.

Three out of the twelve fungi isolated *viz. A. niger, A. ochraceus* and *P. citrinum,* were selected for further studies. These three species had distinct colour differences during growth and sporulation which made them convenient for study. The growth rates of all three were almost the same, *P. citrinum* showing slightly faster growth and more profuse sporulation. Field investigations showed that these three species are the most frequently occurring contaminants on smoked sheets as well as on crepe.

Storage being considered the most important factor, trials were begun, to determine the best storing conditions under the following lines:—

(1) Adopting different temperatures,

(2) Storage at different RHs,

(3) Checking the factors other than temperature and RH which promote fungal growth.

Effect of temperature: No germination was recorded at 15°C in any species, but almost 100% germination was observed between 20 and 37°C. The upper limit for *A. niger* was not determined as its conidia continued to germinate profusely even at 40°C. Spore germination in *P. citrinum* and *A. ochraceus* ceased at 40°C.

TABLE 1

EFFECT OF TEMPERATURE ON GERMINATION OF CONIDIA OF THREE TYPES OF FUNGI

	Percentage germination at							
Fungus	15°	20°	25°	30°	32°	35°	37°	40°
A. niger	00	98	98	99	89	92	87	80
A. ochraceus	00	63	98	96	99	97	80	00
P. citrinum	00	78	96	—	97	99	92	00

The selected species of fungi reacted differently when incubated at high temperature. Growth rates of the mycelial strands, and the production of conidiophores and conidia changed with the rise in temperature. In the case of *Penicillium* spp. changes took place only upto 38°C, after which germination did not occur. Germ tube lengths were determined at different temperatures from 25°C upwards. Thirty five to fifty measurements were taken at each level, avoiding highly distorted strands as far as possible.

The number of longer germ tubes decreased as the temperature increased, so that germ tubes longer than 350 μ were no longer observed at temperatures higher than 30°C in *Penicillium* spp. (Fig. 1).

Conidiophores produced at temperature above 30°C were abnormal, and often the first germ tube produced by the conidia began to bud off conidia, immediately after emergence from the spore. The conidia thus produced did not resemble the characteristic spore type produced under optimum conditions of incubation. As the fungus does not produce the typical conidiophores at high temperature, the number of conidia produced under such conditions was also significantly less, and this was clearly observed at temperatures higher than 34°C (Figs. 2a and 2b).

Mislivec & Tuite (1969) in their studies on the temperature and RH effects on species of *Penicillium* observed that the latter tend to germinate on materials with low moisture contents at slightly higher temperatures than at their optimum temperatures.

Effect of relative humidity: The effect of RH on spore germination of the selected fungi is shown in Table 2.

TABLE 2

EFFECT OF RH ON SPORE GERMINATION (ON AGAR-COATED GLASS SLIDES)

	Percentage relative humidity									
Fungus	100	96	90	85	80	75	63	52	43	42
A. niger	95	85	0	0	0	0	0	0	0	0
A. ochraceus	81	88	0	0	0	0	0	0	0	0
P. citrinum	90	90	0	0	0	0	0	0	0	0

Table 2 clearly illustrates the importance of RH on the growth of moulds. None of the species tested germinated at 90% RH or below that figure. Therefore, a further series of RH tests were carried out to establish the pattern of growth of moulds on sheet rubber. In these trials 5 cm square pieces of fresh uncontaminated ribbed smoke sheet (RSS) were washed in sterile distilled water, air-dried and tested for growth of five species of fungi, frequently occurring on RSS, by placing a 0·4 cm diameter disc of inoculum cut from a five-day old culture of the fungus, growing on malt agar in the centre of the pieces of RSS and incubating at various RHs. The test fungi included the three species being studied, and two other less common species *Paecilomyces varioti* and *P. frequentans.*

P. varioti grew rapidly on malt agar, but its growth on RSS was insignificant compared to that of the other species. In the latter, growth was observed at 100% and 96% RH within three days of incubation. Growth at 96% RH was apparently better than at 100% RH, mainly because water droplets condensed on the pieces of rubber, as well as on the walls of the desiccator at 100% RH. None of the fungi

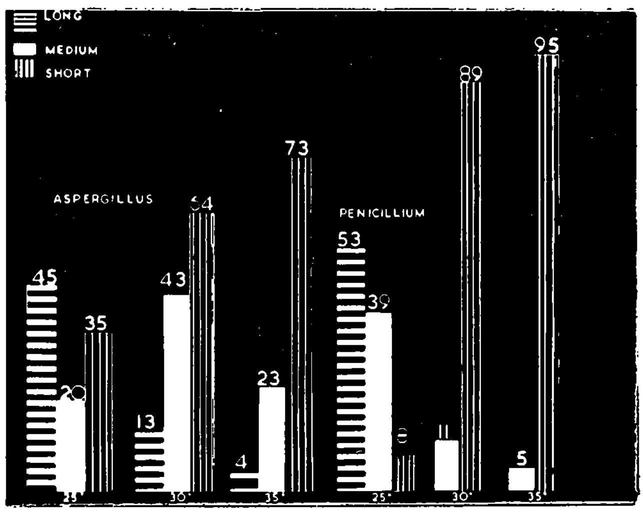

Fig. 1. Lengths of germ tubes at different temperatures

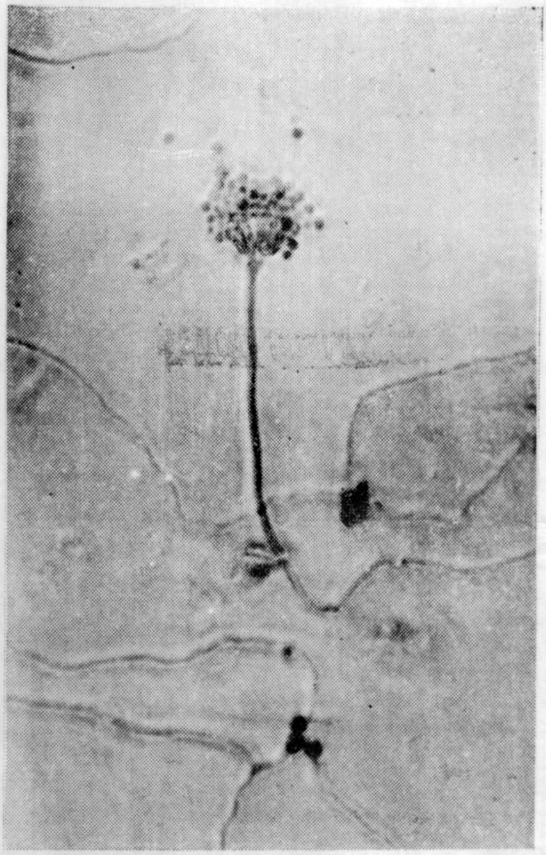

Fig. 2a. Germination and sporulation of *A. niger* at normal room temperature. (Note the complete conidiophore.)

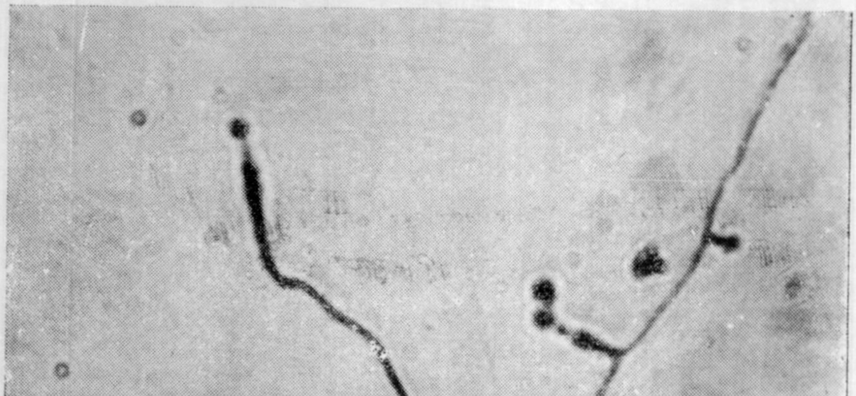

Fig. 2b. *A. niger* producing abnormal conidiophores at higher temperatures

grew in contact with free water, even after eight days whereas they grew profusely in the spaces between the droplets of water. At 96% RH, the fungi completely overran the rubber producing a mycelium and sporulating profusely after seven to eight days. Growth started only after six to seven days at 90%, and the rate of growth was slow, colonies being sparse with little sporulation. *Penicillium* spp. commenced growth after 12—15 days at 85% RH, and covered the sample after two weeks with sparse mycelium. At 80% RH slow growth commenced after 29 days, whereas at 75% these species failed to show any signs of growth upto 50 days. The inoculum introduced got desiccated at RH levels below 80% except for *Penicillium* spp., which dried out at 75% RH at least for 50 to 60 days.

DISCUSSION

These studies have clearly shown that mould growth can be controlled by manipulating temperature and RH. The lower limit of temperature for the growth of the fungi studied was 15°C. Storage of rubber at 15°C (59°F) is not feasible in Ceylon as this would require refrigeration, which would be too expensive. Further, the atmospheric humidity is relatively high in the wet low country districts of Ceylon, where rubber is grown, so that even if rubber is stored at 15°C, moisture condensation would occur with the inrush of fresh air when the door of the store house is opened. The control of fungal growth by storage at high temperature would require the store house to be heated to over 40°C or (104°F). This will involve practical difficulties during the handling of rubber, as labourers would not be able to work continuously, in a store house maintained at over 40°C.

The importance of RH on mould growth has been illustrated here. The prospects of controlling mould growth in sheet rubber, by storage under conditions that would ensure that RH would be kept down as low as possible appear to be bright.

Good quality smoked sheet contains 0·3—0·6% moisture, and will continue to absorb moisture from the atmosphere on exposure, taking upto 1·4—2·8% moisture. Heinisch & Kuhr (1956) found that 0·8% moisture was sufficient to promote mould growth on RSS. However, Galloway (1935) working on textiles has shown that the development of moulds is more closely related to the RH of the atmosphere than to the moisture content of the material. Our studies and observations have confirmed Galloway's findings. It is clear that mould growth can be retarded to a large extent by the proper management of rubber purchasing stores, with a view to reducing RH.

RH of the storing compartments depends on:—

(*a*) The locality of the store houses, whether they are on wet or moist grounds, surrounded by water-soaked earth banks. These tend to keep the storage compartments at high humidities, promoting mould contaminations during wet periods;

(*b*) Ill-ventilated gloomy rooms;

(*c*) Type of rubber stored.

It was often observed during our inspections that rubber dealers large and small, collect wet scrap and partially dried sheets. Store houses of type (*b*) will undoubtedly provide optimum conditions for mould growth (Fig. 3).

Other reasons for mould growth

(1) Dumping of sheet rubber on bare floors, and stacking rubber right up against walls. Due to high humidity water condenses on the floors and walls during wet periods, and mould growth starts on sheets touching the floor and the walls.

(2) The propagation of the fungi is encouraged by the high humidity conditions brought about by storing wet scrap and sheets along with good rubber.

(3) Good quality rubber gets stacked on top of mouldy rubber, with the result that fungal growth continues unabated.

(4) During wet periods these fungi continue to grow on wooden planks too, even in the absence of rubber and retain enough inoculum to contaminate the next batch of smoked sheets to be stacked on them (Fig. 4).

(5) Most depots store old contaminated rubber in the form of cut ends from smoked sheets, remilled rubber, and scrap along with fresh high quality rubber. This is of course suicidal, because the excess inoculum available helps fungal growth even under sub-optimum conditions (Fig. 3).

(6) The dissemination of fungal spores is encouraged at almost all store houses, by workers disturbing sheets and scrap heaps, laden with numerous fungal spores, right inside their main storage compartments. No precautions are taken whatsoever, and we have seen labourers happily dusting out mouldy RSS right over fresh or high grade rubber.

Storing is such a neglected factor that some licence holders collected rubber in small retail shops where other consumable goods were handled. At one such depot a porous container full of common salt was by the side of a heap of recently collected rubber. The floor around the salt container was wet and some of the rubber had got contaminated.

Existing methods of storage are quite unsatisfactory. We are sorry to have to be critical, but we feel that it is the duty of the Department of Commodity Purchase to set the example and take necessary steps to check and correct the situation, in its purchasing depots, as we have often observed that they are as much to blame as the private trader. Permits should only be issued to people who can provide clean and proper store houses, with provision to store rubber on elevated stands, with adequate space between stacks to facilitate the movement of air. Provision must also be made for sufficient space to store good rubber away from scrap and contaminated sheets.

Most dealers can even go to the extent of storing No. 1 RSS in dehumidified rooms. It is abundantly clear that dealers make adequate profits to maintain proper store houses. The Government should look into this problem early and take the necessary steps to stop this wanton neglect, which is costing the country dearly. The Rubber Research Institute has spotlighted this matter time and again without any effect, so that we have now to bring it to the notice of the people concerned.

(7) Another very important factor which contributes to mould contamination of RSS is that producers delay sales of their produce, expecting to obtain better prices later on, in the process, bad storage down-grades the rubber and the producer suffers heavy financial losses.

This could be illustrated with the following incident which took place at Horana in August 1969.

Fig. 3. A rubber dealer's store house where storage conditions leave much to be desired

Fig. 4. Mould infection spreading on to the wooden plank on which the smoked sheets are kept

On the 6th August the price of sheet rubber was Rs. 1.16, on 8th August the price went upto Rs. 1.19¾, then the price dropped to Rs. 1.13 on the 11th August, but went up again to Rs. 1.15¾ on the 13th August, from then onwards the price went down steadily; because of the fluctuation of the prices producers held back their stocks from the 11th August when the price was at Rs. 1.13. Finally the stocks had to be released by the 6th of September, when most of the rubber had got contaminated. Depots down-graded this rubber to No. 3, 4, 5 and 6. Some producers had held on for up to 1½ months expecting a price increase. Most of the stocks held back on the 11th August were already seven to eight days old.

This is a human problem that has no easy solution, but it is of vital importance to the smallholder not to stock his rubber for long periods. With the new barter agreements being planned for rubber (Anon, 1969) we should be extra careful to produce and ship the best quality. Therefore, if some form of price stabilisation can be introduced to encourage small producers to sell their rubber at regular intervals, it will help obviate the necessity for market speculation.

In conclusion we are quite convinced that mould contamination can be reduced to a minimum by observing a few basic factors in storage and inducing smallholders to sell their rubber at regular intervals without speculating on the market.

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QUESTIONS AND ANSWERS

Question: Does mould in any way deteriorate the properties of rubber, or is it only a visual blemish? (Anon).

In what ways do these moulds affect the technological and physical properties of rubber? (Anon).

Answer: A physical change is definitely existing, shown by the loss in weight during prolonged storage — there is no other change except for the visual blemish. (Confirmed by Dr. B. C. Sekhar).

Question: (*a*) Is there a chemical that could be added at the time of coagulation for preventing mould formation?

(*b*) Could evaporating formalin in the store room prevent mould formation? (Anon).

Answer: (*a*) The paper read dealt with physical control as no safe chemical has been found to control mould growth.

(*b*) Formalin is a good fungicide — this helps to prevent fungal contaminations for a short period, until the volatile formaldehyde evaporates.

Question: (*a*) What method would you adopt to re-do mouldy sheet rubber? Please state chemicals that could be used.

(*b*) How would you disinfect (1) smoke house, (2) store room ? (Anon).

Answer: (*a*) A mouldy sheet can be washed in water using a brush to scrub the sheet; an organic fungicide can also be used along with this, and the sheet should be re-smoked well.

(*b*) Smoke houses need not be disinfected using chemicals, if smoking is not completely stopped at any stage. Fungicides like formaldehyde are used by some people, the effect of formaldehyde does not last.

Question: Can you recommend any chemical that could be used in the washing of mouldy RSS to prevent or minimise reinfection? (Anon).

Answer: Mr. M. Nadarajah answering this question said that paranitrophenol can be used to prevent reinfection.

Question: On the question of unsatisfactory storage practices of the small scale rubber dealers, could you lay down the minimum standards necessary for storage by these dealers? (Anon).

Answer: The most important factor is the proper storage — this was dealt with in the paper.

Dry spacious buildings should be used for this purpose. Almost all dealers do not possess such facilities to store good clean rubber away from (*a*) contaminated sheets, (*b*) wet rubber, (*c*) scrap. Smoked sheets should not be stored on bare cement floors, on wooden planks, or against walls. They should be encouraged to use aluminium dexion racks at least one and a half feet from ground level, leaving enough space between racks for free air flow. Rubber should not be stored along with other perishable material like paddy, coffee, coconut scrapings, *etc*.

Storage for long periods should also be avoided, dealers should not hold back stocks, waiting for market fluctuations. They should be advised to sell the rubber within four to six days.

Buying of wet rubber or uncured raw rubber should be discouraged or else they should be advised to send such rubber straight to the drying rooms without leaving them anywhere near the dry sheets.

Question: Will mouldy RSS re-processed into brown crepe, be as good as normal crepe or would it be similar and inferior as scrap crepe ?

Answer: The Head of the Rubber Chemistry Department said that work on this line to determine the better type of rubber had not been carried out.