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STUDIES ON THE DISTRIBUTION, GROWTH, AND CELLULOLYTIC ACTIVITY OF FUNGUS ORGANISMS CAUSING DETERIORATION OF RAW AND FINISHED PRODUCTS

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Introduction

Fungal deterioration of all kinds of raw and finished goods is a severe problem in East Pakistan, because the climatic conditions are such as to furnish nearly optimum temperature and humidity during the six rainy months from May to October.[1] This fungal deterioration is of especial importance in the storage of cotton yarn and jute fibres, and therefore it was considered of importance to undertake a systematic study of fungal deterioration with reference to: (1) the most frequently occurring fungus organisms in mildewed materials, (2) the optimum conditions for their growth, and (3) their cellulolytic activity with reference to cotton and jute fibres. The present communication describes the results obtained in these three directions.

Distribution of Fungus Organisms Isolated from Deteriorated Materials

A systematic isolation of fungus organisms from various mildewed substrata such as cotton fabrics, jute fibres, leather articles, paper, rexin coverings, camera lenses and other industrial finished products was carried out. Altogether a total of 24 organisms were isolated from the above sources.

The medium used for isolation was Waksman's glucose peptone medium (glucose 10 g., peptone, 5 g.; potassium dihydrogen phosphate 1.0 g.; magnesium sulphate 0.5 g.; agar, 20. g.; water 1000 ml.; adjusted to *p*H 3.8-4).

To each sterilised petri dish about 20 ml. of sterilised medium was added. After the medium had solidified the isolation was made in several ways as follows :

(i) Spores were dusted or discharged naturally over the medium.
(ii) Spores were transferred by touching the head of the organisms with a sterile needle.
(iii) Spores were discharged over the medium by scraping the substrata, such as leather goods, rexin coverings etc., by means of a sterile scalpel.
(iv) In case of cotton threads, jute fibres etc., the infected portions were cut into small pieces, transferred to culture dishes by means of sterile forceps and placed on the medium.

The culture dishes were incubated at 30°C. for 3-5 days before isolation could be made. In many cases a second or third isolation had to be made for freeing from bacterial and fungal contaminations. Single hyphal and monosporic cultures were made finally for obtaining pure culture.

For the identification of *Penicillium* and

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Aspergillus species Czapeck's medium (sodium nitrate, 2 g.; potassium hydrogen phosphate, 1.0 g.; potassium chloride 0.5 g.; magnesium sulphate 0.5 g.; ferrous sulphate 0.01 g.; sucrose, 30 g.; agar, 20 g.; water 1000 ml.) was used. Raper and Thom[2] used this medium for specific identification. For other fungi potato dextrose agar medium (peeled potato, 200 g.; glucose, 20 g.; agar, 20 g.; water, 1000 ml.) was used and identification was made from Gilman's [3] "Soil Fungi" and from Smith's [4] "Introduction to Industrial Mycology."

The organisms isolated from different substrata have been listed in Table 1.

It will be seen from the above table that *Penicillium* and *Aspergillus* appeared most widely in most of the mildewed articles and they were represented by 10 and 7 species respectively. *Memnoniella echinata* has been found to cause damage to many items made up of cotton fabrics when exposed to the weather condition in humid tropical areas.[4] Among other organisms *Curvularia lunata* and *Fusarium conglutinans* from Vita glucose tablets, *Paecilomyces* sp. from camera lens, *Chaetomium globosum*, *Trichoderma lignorum* and *Cunninghamella* sp. from jute sackings may be mentioned.

Spore Germination Tests in Relation to Controlled Humidity

The next step in the present study was to determine the influence of relative humidity upon the germination and the elongation of germ tubes of the fungi *Memnoniella echinata*, *Penicillium terlikowskii*, *P. funiculosum* and *Chaetomium globosum* isolated from deteriorated cotton fabrics and jute sackings in the previous experiment. For the case of *Aspergillus niger*, Bonner[5] could find the relative humidity requirement for the germination to be as low as 70%.

A simple method of conducting such test was devised following Clayton's[6] work by suspending cover glasses with spores of the test organisms over different solutions supposed to produce theoretically definite humidity. Salt of oxalic acid of analytical reagent grade, analyzed sucrose, and redistilled water were used in the preparation of the required solutions.

The humidity chambers were set up by a series of air-tight wide-mouthed bottles (250 ml. capacity) having their lids and upper region of the neck portion grounded. The chambers were thoroughly cleaned and washed with redistilled water.

The experiment was carried out with 100, 99.85, 99.04, 99.03, and 96.0% relative humidities. These were respectively made out of redistilled water, 0.10, 0.50, 1.0 molar solutions of sucrose and saturated solutions of oxalic acid.[6]

TABLE 1.—LIST OF FUNGI ISOLATED FROM THE RESPECTIVE SUBSTRATA.

Fungi	Substrata
Aspergillus flavus ..	leather
A. fumigatus	leather, Vita glucose
A. luchuensis	jute, cotton
A. niger	cotton
A. ruber	leather
A. sydowi	leather, condensed milk
A. terreus	cotton
Chaetomium globosum ..	jute
Curvularia lunata ..	Vita glucose
Cunninghamella sp. ..	jute
Fusarium conglutinans ..	Vita glucose
Memnoniella echinata ..	cotton
Paecilomyces sp. ..	lens
Penicillium adametzi ..	silk
P. canescens	paper
P. charlesii	leather
P. citrinum	paper, condensed milk
P. funiculosum ..	jute
P. phoenicium	tobacco
P. restrictum	rexin, Vita glucose
P. terlikowskii ..	cotton, leather, wool
Penicillium sp. ..	cotton
Penicillium sp. ..	paper
Trichoderma lignorum ..	jute

To each of the humidity chambers for 99.85, 99.04, and 98.03 percentages of relative humidity, was added 46 ml. of the respective molar solutions of sucrose. The chambers were then autoclaved at 100°C. for 10 minutes to inactivate enzyme possibly present in sucrose. Later, 10 mg. of potassium dichromate was dissolved in 4 ml. of water and was added to each of the chambers after they had cooled. Potassium dichromate was added to prevent the growth of any organisms in these sucrose solutions. To the remaining two sets of humidity chambers, one received 50 ml. of redistilled water for giving 100% humidity, the other one received 50 ml. of saturated oxalic acid solution and thus used for the purpose of humidity chamber giving 96.0% relative humidity.

Cover glasses used as spore-carriers were thoroughly cleaned in acid and rinsed several times in distilled water and kept for 24 hours in redistilled water and dried with clean cloth. Non-nutritive spore carriers were used because the presence of extraneous material would effect the relative humidity surrounding the spores.[6]

Spores were brushed over the cover glass. Each spore carrier was then attached to one end of a glass rod with paraffin. The paraffin was heated before its use to get rid of any volatile materials. The lower end of the glass rod bearing the spore carrier was embedded on a glass ring which was attached to the glass lid of the humidity chamber with paraffin (Fig. 1). Three such spore-carriers were embedded in each humidity chamber. The chambers were shaken gently at regular intervals of time taking care that no splashing of the solution within them occurred. This was done to minimize stratification of atmosphere or of the solution within the chamber.

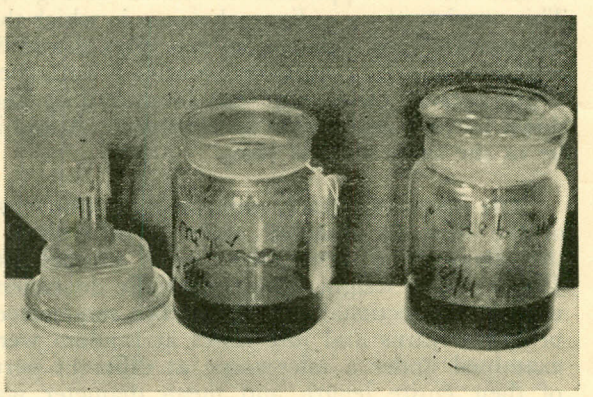

Fig.I.— Spore carrier and humidity chamber.

At the end of each test spores were stained with cotton blue glycerine and observed under microscope for making counts. The germinated spores with their germ tubes this way stained blue and helped easy counting and observation. Spores mounted in this way also could be kept for long time for later observation.

Rectangular cover glasses were used in this experiment for carrying spores of the organisms to be tested. Spores were brushed at one end of the cover glass and this end remained suspended towards the solution inside the chamber and examined after 24 and 48 hours incubation at 30-31°C. Usually ten counts were taken from one end of the cover glass to the other, pushing it horizontally and from ten different microscopical fields. For finding out percentage of germination altogether about 1000 spores were counted for each of the organisms, *M. echinata*, *P. terlikowskii* and *P. funiculosum* while with *Ch. globosum*, a little over 500 ascospores were observed. The length of the germ tubes were measured by means of ocular micrometer and the mean result of 10 measurements are noted in Table 2.

Data presented in Table 2 showed that with all the four organisms tested the higher the relative humidity the greater was the percentage of germination of the spores. The mean percentages of germination of spores of the four test organisms at a relative humidity of 100% after 24 hours were between 70-80% and after 48 hours approximately 10% more spores germinated. At relative humidity of 99.85, 99.04 and 98.03%, the germination rates were progressively lower than those obtained at 100%. The mean germination at a relative humidity of 99.85% after 24 hours was 26-31% and after 48 hours 30-36%. Similarly, at 99.04% the mean germination of spores with the four organisms ranged between 20-25% after 24 hours and 25-30% after 48 hours; at 98.03% the figures were 12-16% after 24 hours and 15-17% after 48 hours, whereas germination was zero to very few at 96%.

As noted above the length of the germ tubes were also found to be strongly dependent upon the percentage of relative humidity, an increase from 98% to 100% producing a two to four fold increase in length of the germ tubes.

Assay of Cellulolytic Activity of the important Fungi attacking Cotton Yarns and Jute Fibres

Three species of fungi, *Memnoniella echinata*, *Penicillium terlikowskii* and *Aspergillus niger*

TABLE 2.—GERMINATION OF SPORES AND LENGTH OF GERM TUBES OF FOUR FUNGI AT DIFFERENT RELATIVE HUMIDITY AFTER 24 AND 48 HOURS EXPOSURES.

Organism	Mean germination of spores at relative humidity							
	100%		99.85%		99.04%		98.03%	
	Exposure		Exposure		Exposure		Exposure	
	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.	24 hrs.	48 hrs.
Memnoniella echinata ..	70	88	30	33	24	25	14	16
Penicillium terlikowskii ..	73	87	31	34	25	26	15	16
P. funiculosum	80	90	30	36	23	30	16	17
Chaetomium globosum ..	70	83	26	30	20	27	12	15
	Mean length (in μ) of the germ tubes							
M. echinata	50	78	30	58	26	46	19	21
P. terlikowskii	50	74	27	54	22	47	18	25
P. funiculosum	46	63	32	46	23	34	17	27
Ch. globosum	47	68	39	60	32	51	24	26

isolated from cotton fabrics and two species, *Chaetomium globosum* and *Penicillium funiculosum* from jute sackings were used in this experiment. All determinations on the rate of cellulose decomposition were made with cotton threads and jute fibres, which were cut into a number of equal pieces for the work. About 30 pieces of the threads and fibres were placed on one microscope slide and tied at both their ends. Two such slides were placed in a single pair of petri dishes equipped with two Whatman No. 4 filter papers. Four dishes thus equipped were autoclaved for 15 minutes at 15 lbs. pressure. After cooling 3 ml. sterilised water was added to each dish. Spores were dusted over the sterilised threads and fibres by the help of camel hair brushes, also sterilised previously by autoclaving. After inoculation the plates were kept in a saturated moist chamber. This was made by filling the lower part of a large sized glass dessicator with redistilled water and placing the dishes on the wire-netting which rested over the false bottom.

After incubation at 30–31 °C. for the requisite number of days the thread and fibres were removed. Harvesting was accomplished by transferring them to 70% ethanol for several minutes and then washing with distilled water. The excess water was drained and the threads and fibres laid on blotting paper to dry. The dried material was conditioned at 55% relative humidity for 24 hours before the breaking strength was measured.

The breaking strength of the fibres was determined with an apparatus as shown in Fig. 2. This consists of an ordinary balance in which the left pan has been replaced by two clips A and B. A is suspended from the left beam while B is attached to the balance pillar and can be moved up and down by rack and pinion arrangement. A and B lie in the same vertical line.

The fibres were rarely uniform in the true sense of the term as observed under a microscope ; the diameter is never the same throughout. The breaking weight being essentially dependent on the cross sectional area of the fibre, some pieces of cotton yarns and jute fibres at random were examined under a microscope at different parts of their entire length and mean diameter was determined. These mean diameters of different

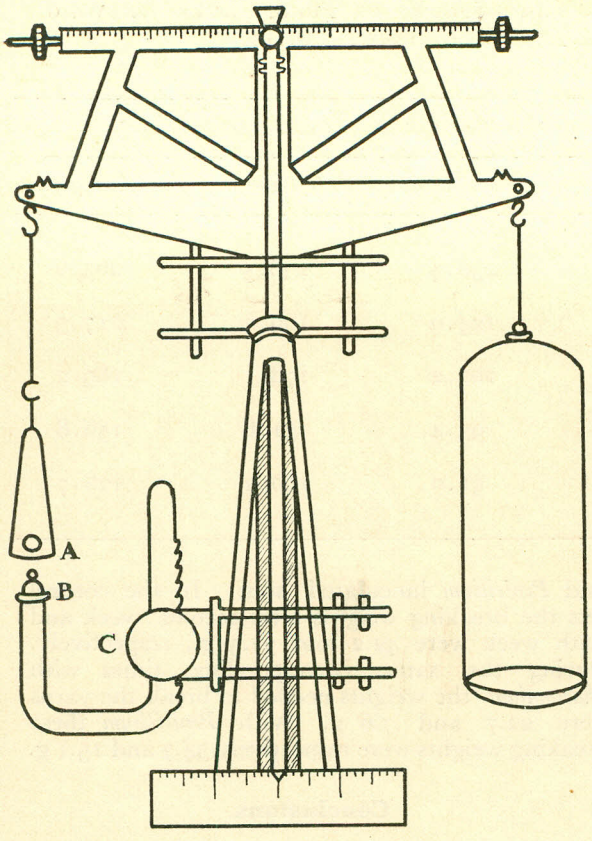

Fig.2.— Showing the parts of the apparatus for determination of breaking strength of fibres.

pieces of cotton yarns and jute fibres though somewhat varied but for the present purpose were considered to be uniform.

After carefully clamping a single fibre at both ends A and B, C was slowly turned to stress the fibre properly so that on releasing the pan the pointer remained at the zero position. The tightening of the fibres by the clamp A and B required the greatest skill ; if it be too tight at either end the fibres break evidently with a lesser weight. When this took place the reading was discarded. The weights were very slowly and gradually increased, for it had been found that a fibre not breaking with a greater weight broke with a smaller one when the latter was placed at once. It was also noticed that at a certain weight the fibre did not break as soon as the beam was raised but only after a few seconds.

The yarn used for the experiments on cotton was of 22 count.* One of the four dishes containing samples, mounted and sterilized as described above, was kept as control and the other three were respectively inoculated with the spores of the test organisms, *M. echinata*, *P. terlikowskii* and *A. niger*, by brushing over the threads. After inoculation the dishes were kept in the moist chamber. Assay of the breaking strength was made after one, two, three, four, and six-weeks' growth of the organisms by taking out each time from each dish about 10 to 12 pieces of cotton yarns, washing, conditioning and then measuring the strength as described above. Fig. 3 shows the mode of growth of *M. echinata* on fibres of cotton yarns.

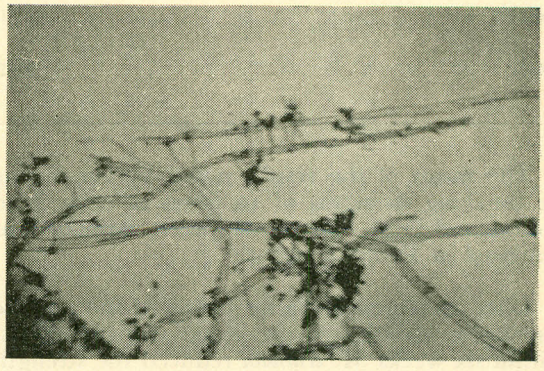

Fig.3.— *Memnoniella echinata* on cotton fibres showingconidiophores and conidia.

With jute fibres similar steps as above were followed. But here the organisms used were *Chaetomium globosum* and *Penicillium funiculosum* and the measurements of the breaking strength started from second week. Figure 4 shows a single perithecium of *Ch. globosum* growing on jute fibres.

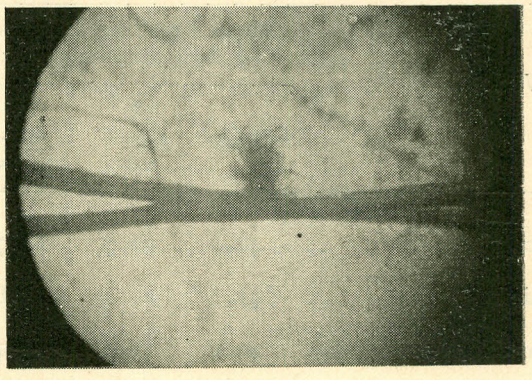

Fig.4.— *Chaetomium globosum* on jute fibres showing a single perithecium

* By courtesy of No. I Dhakeshwari Cotton Mills, Narayanganj, Dacca.

TABLE 3.—COMPARATIVE CELLULOLYTIC ACTIVITY OF THREE SPECIES OF FUNGI AFTER GROWING THEM ON COTTON FIBRES FOR DIFFERENT LENGTHS OF TIME (IN WEEKS).

Week No.	Mean breaking weight of 10 measurements in g.			
	Control	*M. echinata*	*P. terlikowskii*	*A. niger*
First	290.8	230.4	238.5	265.6
Second	282.5	124.0	189.3	217.5
Third	276.8	102.2	178.1	189.2
Fourth	271.0	93.4	131.6	159.8
Sixth	261.8	51.0	100.2	128.7

The mean of the results noted by measuring the breaking strengths of 10 fibres of each kind (cotton and jute) per week per treatment was tabulated in Tables 3 and 4.

Data presented in Table 3 showed the relative destructive abilities of the three test organisms. Within six weeks *M. echinata* reduced the strength of the yarns to one fifth of their original strength and the other two organisms could reduce the strength a little over half with reference to the yarns present in control dishes.

Table 4 showed the relative degradations of jute fibres from week to week with *Chaetomium*

TABLE 4.—COMPARATIVE CELLULOLYTIC ACTIVITY OF TWO SPECIES OF FUNGI ON JUTE FIBRES AT DIFFERENT INTERVALS OF TIME.

Week No.	Mean breaking weight of 10 measurements in g.		
	Control	*Ch. globosum*	*P. funiculosum*
Second	42.2	22.7	34.7
Third	41.1	21.3	29.8
Fourth	40.2	11.9	23.4
Sixth	37.5	7.6	15.1

and *Penicilium* inoculated sets. In the control sets the breaking weights after second week and sixth week were 42.2 and 37.5 g., respectively. During the same corresponding times with *Chaetomium* the weights needed to break the yarns were 22.7 and 7.6 g. With *Penicillium* these breaking weights were respectively 34.7 and 15.1 g.

Conclusions

In this experiment with *Memnoniella echinata* the percentage of loss in breaking strength at 7, 14, 21, 28, and 42 days harvest was approximately 16, 55, 63, 66 and 78, respectively. The breaking strength values for the corresponding harvests were 230.4, 124.0, 102.2, 93.4 and 51.0 g., respectively. The values of the average of controls has been used in all calulations. A small loss of breaking strength was found for the controls during the whole period of the work (Fig. 5), and this may be attributed to stray bacterial contamination occuring when the moist chambers were opened every week for removal of test samples.

The percentage of loss in breaking strength of the cotton yarns with *Penicillium terlikowskii* after 7, 14, 21, 28, and 42 days harvest was approximately 14, 31, 35, 52 and 60, respectively. The breaking strength for corresponding harvests were 238.5, 189.3, 178.1, 131.6 and 100.2 g., respectively. The loss of breaking strength was much less when compared to *Memnoniella echinata*.

With *Aspergillus niger* treated cotton yarns the percentage of loss in breaking strength at the above intervals of time was 4, 21, 32, 42 and

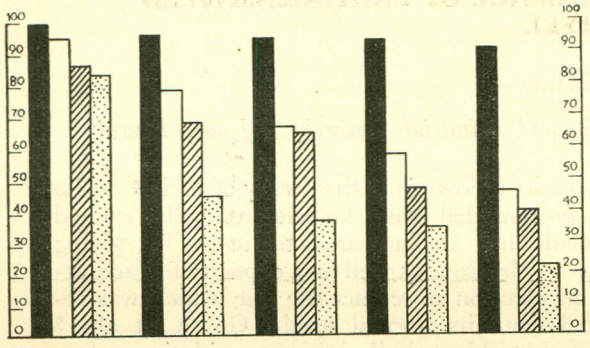

Fig.5.— Percentage of strength of variously treated cotton yarns after five successive periods.

Control yarns.

Yarns on which *Aspergillus niger* had been grown.

Yarns on which *Memnoniella echinata* had been grown.

Yarns on which *Penicillium terlikowskii* had been grown.

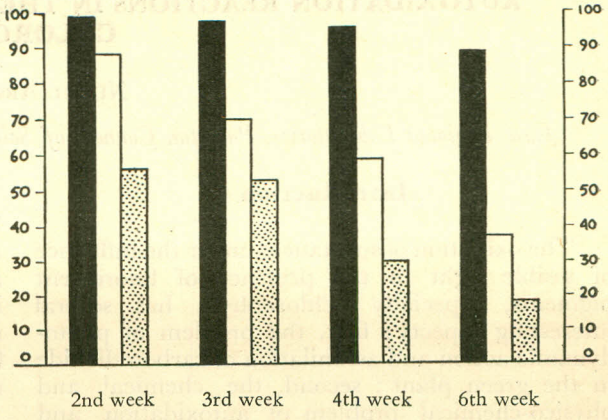

Fig.6.— Percentage of strength of variously treated jute fibres after four successive periods.

Control fibres.

Fibres on which *Penicillium funiculosum* had been grown.

Fibres on which *Chaetomium globosum* had been grown.

53, respectively. Their breaking strengths were respectively 265.6, 217.5, 189.2, 159.8 and 128.7 g.

Among the three fungi tested on cotton yarns *M. echinata* was found to cause greater loss in the breaking strength of the yarns than the other two.

The jute fibres when treated with *Chaetomium globosum* and *Penicillium funiculosum* the percentage of loss in breaking strength with *Chaetomium* was far greater than the *Penicillium*. The percentage of loss in breaking strength after 14, 21, 28 and 42 days harvest was 43, 47, 70 and 81, resepectively, with the *Chaetomium*. In the corresponding intervals of time with *P. funiculosum* the percentage of loss in breaking strength was 11, 30, 41 and 62, respectively (Fig 6).

References

1. Q.A. Ahmed, Pakistan J. Forestry, **2**, 91—115 (1952).
2. K. B. Raper and C. Thom, *A Manual of Penicillia* (The Williams and Wilkins Company, 1949).
3. G.C. Gilman, *A Manual of Soil Fungi*, (Collegiate Press Inc., Ames, Iowa, 1945).
4. G. Smith, *An Introduction to Industrial Mycology* (Edward Arnold Ltd., 1954) fourth edition.
5. J. T. Bonner, Mycologia, **40**, 728 (1948).
6. C. N. Clayton, Phytopath., **32**, 921 (1942).