MICROSCOPICAL STUDY ON THE DEGRADATION OF COTTON FIBRES BY FUNGI

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The fungal degradation of Indian cotton fibres was studied using microscopical methods. A correlation was found between alkali swelling properties and congo red absorption property of the degraded cotton fibres. The cellulolytic activity of 33 cotton attacking fungi was determined.

When fungi degrade cotton fibres, not only the tensile strength decreases at a rapid rate but certain microscopical characteristics of fibres also change[1]. The microscopical studies including the presence of fungi within the lumen of the infected fibres, the congo red absorption by degraded fibres and alkali swelling properties of tendered fibres have been frequently used to evaluate the extent of fungal deterioration of different cellulosic fibres[2-5]. No such work particularly on Indian cotton fibres has been published so far and so a detailed investigation has been undertaken to study the microscopical aspects of fungal degradation of cotton fibres. The cellulolytic index of the fungi attacking cotton fibres has also been determined.

MATERIAL AND METHOD

Thirty-three species of fungi (Table 1), isolated from outdoor weathered and soil buried cotton fabrics, cordages and yarns were taken for the present study. The cellulolytic activities of these fungi were determined by the fabric test method adopted by Srivastava & Nigam[6].

0.1 g cotton fibre and 100 ml Greathouse mineral salt liquid medium[6] were taken in each 250 ml conical flask and sterilized at 1.1 kg per sq cm steam pressure for 15 minutes. The spore suspension of different fungi were made by adding 10 ml sterilized distilled water to 15 days old cultures. In case of sterile fungus, hyphal suspension was used. One ml suspension was asceptically inoculated into the flasks. The flasks were incubated for 10 days at $30 \pm 2°C$ after which the fibres were washed with distilled water and subjected to following tests.

Microscopic examination—The fibres were examined under high power microscope for surface and lumen growth of fungi after proper staining as per method of Basu and Ghose[1].

Alkali swelling test—The test described by Coward & Spencer[2] was used. The fibres were treated with 18 per cent *NaOH* for 15 minutes on glass slides at room temperature. The width of swollen fibres was measured at five different places. The average width represented alkali swelling value.

Congo red test—The test described by Bright[3] was used. The fibres were treated with 25 ml of 11 per cent *NaOH* for 30 seconds and then quickly washed with water. The fibres were placed in saturated solution of congo red for 5-6 minutes, shaken continuously. The fibres were again washed with distilled water until the washings became colourless. The fibres were examined microscopically in 18 per cent *NaOH*. The damaged fibres characterized by deep staining or uneven staining were counted and this represented congo red value.

TABLE 1

CELLULOLYTIC INDICES OF FUNGI AND THEIR ATTACK ON COTTON FIBRES

Sr. No.	Fungi	Cellulolytic* activity	Microscopical studies**			
			Surface growth	Lumen growth	Swelling value (μ)	Congored value (%)
1.	*Rhizopus nigricans*	0.3	P	N	16.41	8.75
2.	*Cunninghamella echinulata*	0.0	M	N	27.36	25.0
3.	*Chaetomium globosum*	29.0	P	P	38.91	81.25
4.	*Phoma sp.*	42.5	M	N	41.95	71.25
5.	*Trichoderma viride*	47.0	M	N	34.05	70.00
6.	*Aspergillus flavus*	2.2	P	N	17.02	11.25
7.	*A. fumigatus*	40.3	P	N	38.30	80.00
8.	*A. nidulans*	5.3	R	N	19.45	12.50
9.	*A. niger*	0.3	P	N	15.80	7.50
10.	*A. oryzae*	51.5	M	N	41.95	93.75
11.	*A. sparsus*	4.7	M	N	18.85	15.00
12.	*A. sydowi*	0.9	P	N	17.63	11.25
13.	*A. terreus*	42.0	P	N	41.95	78.75
14.	*A. terricola*	0.6	P	N	17.63	7.50
15.	*Penicillium thomii*	74.5	R	M	41.95	81.25
16.	*Penicillium sp.*	1.2	R	N	16.41	7.50
17.	*Penicillium sp.*	1.2	P	N	15.80	8.75
18.	*Peacilomyces varioti*	2.8	M	N	15.80	10.00
19.	*Botrytis cinerea*	28.7	M	N	38.30	82.50
20.	*Pullularia pullulans*	0.0	R	N	16.41	6.25
21.	*Stachybotrys atra*	38.1	M	R	38.91	81.25
22.	*Humicola sp.*	32.1	P	R	34.05	81.25
23.	*Memnoniella echinata*	30.6	M	R	32.22	75.00
24.	*Cladosporium herbarum*	28.1	R	N	26.75	53.75
25.	*Curvularia lunata*	75.1	M	R	41.34	92.50
26.	*C. pallescens*	65.0	M	N	40.13	87.50
27.	*Helminthosporium sp.*	33.6	P	N	37.09	86.25
28.	*Alternaria tenuis*	28.7	M	M	23.10	41.25
29.	*Fusarium chlamydosporum*	34.7	R	N	27.97	50.00
30.	*F. moniliforme*	47.0	P	N	31.61	61.25
31.	*F. roseum*	31.9	P	N	29.79	42.50
32.	*Myrothecium verrucaria*	100	P	M	43.19	90.00
33.	Grey sterile fungus	1.5	R	N	16.40	12.50
34.	Control	0.0	—	—	15.80	6.25
	C. D. at 0.05 P.	—	—	—	2.146	6.224

*expressed as percentage loss in breaking strength of cambric cloth to control.

**P—Profuse, M—Moderate, R—Poor, N—Nil.

RESULTS

Cellulolytic activity—The cellulolytic activities of fungi, isolated from cotton materials, in terms of percentage loss in breaking strength (B. S.) of cotton cambric fabric to control has been shown in Table 1. Five categories of fungi were recognised :

(*i*) Highly cellulolytic (causing B. S. loss of 75% or more)—*Myrothecium verrucaria, Curvularia lunata* and *Penicillium thomii.*

(*ii*) Fairly cellulolytic (causing B. S. loss between 51-75%)—*C. Pallescens, Aspergillus oryzae, Trichoderma viride.*

(*iii*) Moderately cellulolytic (causing B. S. loss between 26-50%)—*Chaetomium globosum, Phoma* sp., *Aspergillus fumigatus, A. terreus, Botrytis cinerea, Stachybotrys atra, Humicola* sp., *Memnoniella echinata, Cladosporium herbarum, Helminthosporium* sp., *Alternaria tenuis, Fusarium chlamydosporum, F. moniliforme* and *F. roseum.*

(*iv*) Poorly cellulolytic (causing B. S. loss between 6-25%)—*Aspergillus nidulans.*

(*v*) Non cellulolytic (causing B. S. loss between 0.0-5%)—12 species (Table 1).

Fungal attack on cotton fibre—Undegraded cotton fibre showed prominent spiral windings with one or two spiral lines (fissures) appearing faintly. As the degradation increased the fissures became deeper and enlarged. In highly degraded fibres the fissures extended to the lumen and the fibres on swelling opened into a ribbon (after treating in alkali and congo red). The fissures extended the entire length of nearly all the fibres examined. The surface of the fibres was found to be rough. In some cases, the fungus hyphae were observed in the lumen of the cotton fibres, while in others fibres were found surrounded by fungal hyphae without being penetrated. It was also observed that the penetration of fungal hyphae was through the cracks in the cotton fibre wall and also from the ends. After penetrating into the lumen of the fibre the hyphae were found to grow from inside to outward direction.

Degradation of cotton fibre—The degradation in cotton fibres was qualitatively estimated in terms of surface and lumen growth of fungi in the fibres (Table 1). Three categories of fungi were recognised :

(*i*) Fungi showing profuse or moderate growth both on the surface as well as in the lumen of the fibres (Sr. No. 3, 28 and 32 in Table 1).

(*ii*) Fungi showing profuse growth on the surface of the fibre but exhibiting poor or nil growth in the lumen of the fibres (Sr. No. 1, 6, 7, 9, 12-14, 17, 22, 27, 30 and 31 in Table 1).

(*iii*) Fungi showing poor growth on the surface but moderate growth in the lumen of the fibres e.g. *Penicillium thomii.*

The degradation of cotton fibres was studied semiquantitatively in terms of alkali swelling and congo red values (Table 1). Majority of fungi exhibited comparatively higher alkali swelling value than control. This indicated that these fungi caused comparatively higher degree of fibre deterioration. However, in *Aspergillus niger, Penicillium* sp. and *Paecilomyces varioti* the alkali swelling values were equal to control.

Congo red value in most of the fungi exhibited the trend similar to alkali swelling value. The fungi showing higher alkali swelling value also showed higher congo red value. In *Rhizopus nigricans, A. flavus, A. nidulans, A. sydowi, Penicillium* sp., *Paecilomyces varioti, Pullularia pullulans* and sterile species, the congo red values were nearer to control (Table 1). Few fungi showing moderate alkali swelling value showed poor or nil congo red value and vice versa (Table 1). *Pullularia pullulans* showed poor alkali swelling value but nil congo red values whereas *A. niger, Penicillium* sp. and *P. varioti* showed poor congo red values but nil alkali swelling values.

DISCUSSION

To evaluate the extent of deterioration in cotton fibres it is frequently desirable to know whether a particular fungus is potentially capable of decomposing cotton cellulose or not. The mere growth of a fungus on a tendered sample does not prove that the fungus is cellulolytic. With this view, the cellulolytic activity of different fungi has been determined. The results of cellulolytic activity in

the present investigation substantiated the results obtained by earlier workers [7-9]. But there are many disagreements in the results as to the relative cellulolytic activity of different fungi. *A. niger* reported to be moderately cellulolytic[10] has been found non cellulolytic. *A. oryzae* reported to be non cellulolytic[11] has been found to be fairly cellulolytic. *A. sparsus* not tested earlier has been found non cellulolytic. *Penicillium thomii* and *Botrytis cinerea* recorded first time from cotton textiles showed definite cellulolytic activity. Considerable variations in cellulolytic activity has been found among species of the same genus.

Regarding fungal attack on cotton fibre it will be seen that the first effect of fungal attack is the digestion of the outermost cuticular layer of the fibre. This observation is in agreement with the earlier work[12-13]. It is further confirmed by swelling characteristics of the fibres. Increased swelling observed in the fibre is characteristic of degree of damage to the fibre since the increased swelling has not been observed in undegraded fibres (Table 1). Bright[3] has explained that swelling of undamaged fibre in alkali is limited by a restrictive effect of its outer wall and this limitation is removed to some degree whenever the outer wall is weakened or broken by a deteriorative agency. The degradation of outer wall of the fibre is also manifested by increased absorption of congo red. The fungal hyphae grow inside the lumen of the cotton fibre due to presence of nitrogenous substances.

Several contradictory statements have been made regarding penetration and growth of fungi in the lumen of cotton fibre Siu[14] and Siu & Reese[15] noted that the hyphae of attacking fungus entered the lumen of the fibre by direct penetration of the primary and secondary walls. Bright[3], however found distinct tendency of a fungus to grow into the lumen through the cracks and broken wall and not by penetrating the wall. In present case the fungal hyphae have been found penetrating through the cracks in the wall of the fibre. There is general agreement among the investigators that the fungi while entering into the cotton fibre grow profusely in the lumen and the fungal growth proliferate from inside to outward direction[14 & 16]. This has been confirmed by the present work. The fungi sporulate on the surface of the fibre.

The high cellulolytic activity of cotton attacking fungi seems to be directly related to the ability of these fungi to grow on the surface as well as in the lumen of the cotton fibres. A high degree of correlation has been found between degree of growth in lumen and degree of fibre deterioration. However, few highly cellulolytic fungi grow only on the surface of the cotton fibres. It is to be expected on theoretical ground that a fungus growing on the surface of the fibre should cause less damage than the one growing internally. The loss in tensile strength of a fibre due to profuse surface growth of highly cellulolytic fungi may be due to removal of successive layers of the fibrils by fungi from the surface of the fibres[17].

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