J. Univ. Kuwait (Sci.) **8,** 1981

Implication of fungi in the loss of strength of Sudanese cotton fibre

MAHGOUB H. ABDALLA AND NAGAT M. EL-TAYEB

Department of Botany, Faculty of Science, University of Khartoum, Sudan

ABSTRACT

Cellulolytic ability of 25 moulds recovered from Sudanese cotton fibre was explored in relation to its effect on the physical properties of the fibre. Extracellular digestion, by 13 moulds, of carboxymethyl cellulose (CMC), cellobiose, or raw cotton indicated that a *Chaetomium* (out of three *Chaetomium* species), *Aspergillus nidulans* and *A. fumigatus* (arranged in descending order) caused most rapid degradation of pure cotton fibre. The phytopathogens *Cephalosporium* sp. and *Stemphylium* sp. caused least degradation. Moulds that were the best CMC or cellobiose utilisers did not necessarily digest raw cotton substrate at the same rate.

Bundle strength and elongation of fibre, two properties of yarn spinnability, were assessed by immersing raw cotton in metabolites of nine test lint moulds. *Thielavia* greatly weakened bundle strength whilst fibre elongation property was highly reduced by *Cladosporium herbarum*, *Stemphylium* sp., *Aspergillus fumigatus*, *Thielavia* sp. and *Trichoderma* sp., arranged in descending order.

INTRODUCTION

Moulds play an important role in degrading plant products prior to and subsequent to harvest. Special interest has been paid to cellulolytic fungi, which are very effective agents of deterioration, in order to safeguard the quality and quantity of plant products including fibre crops.

Cotton fibre becomes a target for microbial invasion from the very start of its synthesis because of its content of readily utilisable carbon sources (94% cellulose beside non-cellulosic polysaccharides) (Sadov *et al.* 1973). Cotton fibre may thus provide a suitable substratum not only for cellulolytic moulds but also for those that possess low or zero cellulose-decomposing ability such as Mucoraceous moulds. This may be attributed to the availability of soluble sugars during fibre synthesis.

Boll infection results in cellulose degradation in uncollapsed (immature) cotton fibre and this, in turn, affects yarn spinnability (Marsh & Kerr 1961). They reported the induction of a considerable proportion (28–70%) of uncollapsed fibres when cotton, picked from mature bolls, was dusted separately with conidia of *Nigrospora* sp., *Penicillium* sp., *Aspergillus niger*, *A. flavus*, *A. terreus*, *Alternaria* sp. and *Fusarium moniliforme*, but that only 3% fibre damage followed inoculation with *Rhizopus*

stolonifer. A decrease of fibre strength as well as length following fungal infection of lint has been reported by Stands *et al.* (1962). Hence the early presence in the young cotton bolls of cellulolytic micro-organisms may lead to uncollapsed fibres. These uncollapsed fibres exhibit excessive yarn breakage during spinning processes (Bailey 1953) and such weakened fibres fail to fluff (Marsh & Kerr 1961).

Studies of crude mould metabolites indicated the presence of three cellulase components: one is readily adsorbed by cotton causing loss of tensile strength, the other component has a higher activity upon carboxymethyl cellulose whilst the third one possesses a higher affinity towards cellobiose (Selby & Maitland 1965, 1967; Wood 1968, 1969). None of these fractions can separately degrade cotton fibre as effectively as they do when in combination. In the process of cotton digestion by fungal filtrates, it has been suggested that short fibre formation is an intermediate stage of biodegradation of lint (Halliwell 1963; Halliwell & Riaz 1970). Subsequent digestion by the same enzyme complex yields soluble sugars.

The present investigation, the first of its kind in the Sudan, was aimed at assessing the ability of crude metabolites of some local fungi, previously isolated from cotton bolls (Abdalla & El-Tayeb 1981a, b), to degrade the new variety of cotton 'Barakat' and to test their effects on fibre strength.

MATERIALS AND METHODS

The long and extra-long-staple new cotton variety 'Barakat' was utilised. Cotton fibre was picked from sound non-sutured or cracked bolls.

Cellulolysis

An *in vitro* test of cellulolytic ability of fungi recovered from cotton bolls (Abdalla & El-Tayeb 1981a, b) was conducted following the modified technique of Eggin & Pugh (1962). Walseth's (1952) digestion method employing 85% phosphoric acid at 4°C for 4 hr, was preferred to boll-milled cellulose suggested by Eggin & Pugh (1962). Culture plates were inoculated with 5 mm diameter mycelial plugs cut from the periphery of 5–7-day-old cultures and incubated at 32°C for 10 days. The rate of cellulolytic ability of each test mould was visually assessed on the basis of the width of the cellulose-cleared halo around the mycelium.

The following contaminants were chosen because of frequent isolation from lint (Abdalla & El-Tayeb 1981a, b): *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger* (two isolates), *A. terreus*, *Cephalosporium* sp., *Cladosporium herbarum*, *Chaetomium* spp. (three isolates), *Nigrospora oryzae*, *Stemphylium* sp., a sterile orange–yellow mycelium (suspected to be *Epicoccum purpurascens*), *Thielavia* sp. and *Trichoderma* sp. Other less frequent contaminants were also included in this preliminary test.

Assay of crude cellulase enzyme

The fungi listed above were separately grown in shake culture as adopted by Mandels & Reese (1957) for the isolation of cellulase. The fungal culture was grown in 500 ml flasks containing 100 ml medium. Inocula 5 mm diameter were picked from 5–7-day-old cultures. Inoculated flasks were mechanically shaken (60 oscillations/min) for 12 hr per day for 7–10 days at 32°C and subsequently Seitz-filtered.

DIGESTION OF CELLULOSE AND DERIVATIVES BY CRUDE MOULD FILTRATES

Test for carbohydrate yield

(i) Carboxymethyl cellulose assay. A concentration of 0·55% carboxymethyl cellulose (CMC) 50T, dissolved in 0·055 M citrate buffer, pH 5·5, was used as a substrate to confirm the cellulolytic ability of the test fungi. To 9 ml of this substrate 1 ml of the cell-free crude fungal filtrate was added in a test tube. The mixture was incubated at 50°C for 1 hr after which the amount of reducing sugars (mg glucose/ml) was determined by Miller's (1959) method. A Beckman spectrophotometer Model B was employed (Chapman *et al.* 1975).

(ii) Cellobiose assay. An aliquot of 3 ml (0·0667 mg/ml) cellobiose substrate (dissolved in succinate buffer; pH 4·5) was treated with 1 ml crude mould filtrate for 3 hr at 50°C (Selby & Maitland 1967). The reducing sugars yielded were subsequently determined following Miller's (1959) technique.

(iii) Assay of sugar yield in digested cotton lint. Halliwell's (1961) method was adopted whereby about 25 mg cotton fibre (to which 1·3 ml acetate buffer, pH 5·5, were added) were covered with the crude mould metabolic products, using distilled water in the controls. The experiment was incubated at 50°C for 7 days during which time the flasks were periodically hand-shaken for a few minutes to ensure the adsorption of the mould metabolites onto the fibre surface. The soluble sugars were then determined according to Rossness & Wright (1974).

(iv) TLC method. The sugar acetate solutions obtained from cotton fibre previously digested with the crude filtrates of *Aspergillus fumigatus*, *A. nidulans*, *Cephalosporium* sp., *Nigrospora oryzae*, the sterile orange–yellow mycelium and *Trichoderma* sp. were separated on silica gel TLC plates. A standard solution was prepared by dissolving 2% (w/v) cellobiose in chloroform. The chromatograms were developed, using the ascending technique, in 4% methanol in benzene (Tate & Bishop 1962).

Test for fibre strength

Cotton lint (1 g) was immersed in 25 ml acetate buffer together with 25 ml metabolites of each of the aforesaid moulds. The mixture was incubated at 50°C for 7 days. Then the lint was removed, air-dried and tested in the Spinning and Fibre Testing Laboratory of the Gezira Agricultural Research Corporation for loss in fibre strength as compared with the controls which received 25 ml sterile water in place of the fungal metabolic products.

RESULTS

The majority of mycoflora (20 out of 25) previously isolated from Sudanese cotton lint (Abdalla & El-Tayeb 1981a, b) were rated between moderate and fast spreading, 11 colonies being highly cellulolytic, whilst 9 moderately cleared the cellulose of the culture medium (Table 1). These two features were based upon visual assessment. Two isolates of *Aspergillus niger* were noted to differ considerably in both growth rate and

cellulolytic ability. *Arachniotus* sp., *Aspergillus flavus*, *A. fumigatus*, *A. niger* (isolate No. 1), *A. nidulans*, *A. terreus*, *Chaetomium* spp. (isolates Nos. 44 & 45), *Cladosporium herbarum*, *Nigrospora oryzae* and *Trichoderma* sp. were classified as highly cellulolytic (Table 1).

Digestion of carboxymethyl cellulose (CMC) citrate, cellobiose or raw cotton lint with extracellular metabolites of the 13 test moulds resulted in carbohydrate hydrolysis. Data presented in Table 2 demonstrate that *Chaetomium* (isolate No. 44) had the greatest effect in reducing CMC citrate substrate to simpler sugars, followed by *Nigrospora oryzae* and then *Trichoderma* sp. Although *Cladosporium herbarum* was least effective in hydrolysis of CMC, it moderately digested raw cotton fibre. The ability of the other individual moulds to digest the experimental substrates was inconsistent. This may be exemplified by *Cephalosporium* sp. which moderately utilised CMC, but ranked best when the substrate was changed to cellobiose and least when its filtrate was tested on pure cotton fibre. Similarly *Nigrospora oryzae*, the second best CMC digester, was the second least utiliser of cellobiose and moderately degraded raw cotton fibre (Table 2). However, *Chaetomium* (isolate No. 45) was the most efficient biodeteriogen, whilst *Thielavia* and *Stemphylium* were poor cellulose utilisers. This observation matched with the visual observation on cellulose clearance (Table 1) for the first mould but not for the last two. Although *Aspergillus nidulans* and *A. fumigatus* moderately digested CMC, yet they were effective in the degradation of cellulose of raw lint. However, the controls (CMC, cellobiose or raw cotton lint treated with plain water) did not yield reducing sugars or any form of intermediary sugar products.

Further investigation regarding cellulose digestion by some selected cotton fibre moulds indicated that the distance travelled by the spot of digestion products of

Table 1. Visual assessment of cellulose-clearing ability of cotton fibre mycoflora in comparison with the rate of linear expansion on cellulose agar medium

	Degree of cellulolysis			
Growth rate	High	Moderate	Low	Nil
Fast	*Aspergillus fumigatus* *A. niger* (isolate No. 1) *A. terreus* *Chaetomium* sp. (44) *Chaetomium* sp. (45) *Nigrospora oryzae* *Trichoderma* sp.	*Cephalosporium* sp. *Penicillium* sp. *Stemphylium* sp.	*Helminthosporium* sp.	*Alternaria* sp. *Rhizopus stolonifer*
Moderate	*Arachniotus* sp. *Aspergillus flavus* *A. nidulans* *Cladosporium herbarum*	*Chaetomium* sp. (51) *Curvularia* sp. *Eurotium* sp. *Papulospora* sp. Sterile orange–yellow mycelium *Thielavia* sp.	*Aspergillus versicolor*	*Aspergillus niger* (isolate No. 2)

Table 2. Extracellular carbohydrate digestion by cell-free fungal filtrate (of fibre mycoflora) measured in mg reducing sugar/ml substrate (absorbance reading at 575 nm)

Source of metabolic product	Substrate digestion		
	CMC (1 hr)	Cellobiose (3 hr)	Cotton (7 days)
Aspergillus fumigatus	0·58	—	0·21
A. nidulans	0·44	—	0·23
A. terreus	0·68	0·05	—
Cephalosporium sp.	0·68	0·24	0·03
Chaetomium (44)	0·90	0·16	0·06
Chaetomium (45)	0·71	0·04	0·27
Chaetomium (51)	0·72	0·05	0·15
Cladosporium herbarum	0·25	—	0·16
Nigrospora oryzae	0·85	0·03	0·17
Stemphylium sp.	0·64	0·07	0·05
Orange–yellow mycelium	0·56	0·03	—
Thielavia sp.	0·72	0·05	0·08
Trichoderma sp.	0·85	0·06	0·13

—: Not tested.

cellulose by cell-free filtrates from *Aspergillus nidulans* or *Trichoderma* slightly preceded that of the control (pure cellobiose, Rf 0·57). Spots from the control, *Cephalosporium* or *Aspergillus fumigatus* filtrate-digested fibre, had nearly the same Rf values. It was also noticed that the spots developed on TLC plates, including the control, faded away very quickly. Spots derived from cotton fibre digested either by *Nigrospora* or the sterile orange–yellow mycelium did not develop on the TLC plate (Fig. 1). When soaked in mould metabolites, the bundle strength as well as the elongation of fibre were variably lowered as compared with lint soaked in plain water. *Thielavia* filtrate was most effective in reducing bundle strength (Table 3). Metabolic products that had a greater negative effect upon fibre elongation property belonged to *Cladosporium herbarum* (27% reduction as compared to the control), *Stemphylium* (27%), *Aspergillus fumigatus* (20·7%), *Thielavia* (19%) and *Trichoderma* (19%).

DISCUSSION

The association between growth rate, leading to quick substrate colonisation, and the efficiency in cellulose utilisation by lint mycoflora is an indication of possible economic losses. The majority of the test fungi, previously recovered from cotton floral parts including boll locules (Abdalla & El-Tayeb 1981a, b) were highly cellulolytic and fast spreading (Table 1). According to Tansey (1971), thermophiles are two to three times quicker in cellulose digestion than mesophilic species. This observation lends support to the high degree of cellulose decomposition exhibited by *Aspergillus fumigatus* and *A. terreus* cultures (Table 1). The mesophiles *Trichoderma*, *Arachniotus*, *Aspergillus*

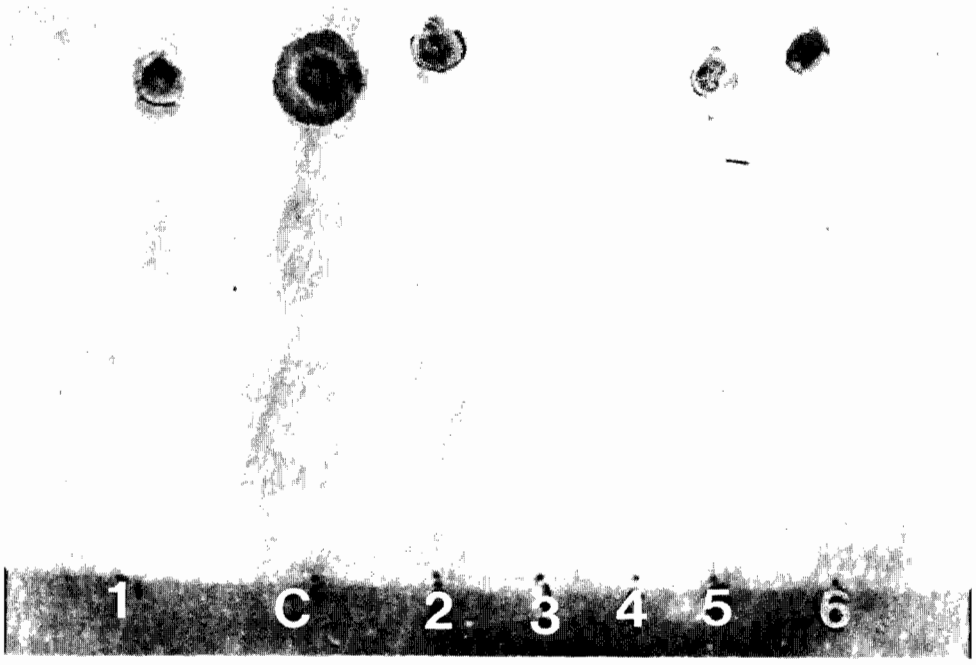

Fig. 1. Thin layer chromatogram showing the separation of sugar acetate solutions yielded by cotton fibre digested by metabolites from: 1, *Aspergillus fumigatus*; 2, *A. nidulans*; 3, *Nigrospora* sp.; 4, Sterile orange-yellow mycelium; 5, *Cephalosporium* sp.; 6, *Trichoderma* sp.; C, Control (cellobiose).

Table 3. Mean fibre strength (stelometer index at 1/8 in., g/tex) and elongation (%) of cotton fibre soaked in cell-free mould metabolic products

Fungal filtrate	Bundle strength (1/8 in., g/tex)	Elongation (%)
Aspergillus fumigatus	21·8	5·0
A. nidulans	20·9	5·5
Chaetomium sp. (44)	24·3	5·6
?*Chaetomium globosum* (45)	22·0	5·5
Cladosporium herbarum	22·9	4·6
Nigrospora oryzae	21·4	5·5
Stemphylium sp.	21·3	4·6
Thielavia sp.	19·5	5·1
Trichoderma sp.	22·4	5·1
Plain water	25·6	6·3

nidulans, *A. niger* and *Chaetomium* spp., contrary to Tansey's (1971) view, may be considered the most efficient cellulose-utilising fungi amongst indigenous isolates (Table 1). Tansey (1971) described *Trichoderma viride* as 'not a quick cellulose decomposer'. It is interesting to note that the two isolates of *Aspergillus niger* recovered from Sudanese cotton lint (Abdulla & El-Tayeb 1981a) behaved differently on cellulose agar plates (Table 1). According to some authors this mould has little or no cellulolytic activity. It is believed that the utilisation of cellulose by *A. niger* must follow the addition of a soluble carbon source to the medium (Simpson & Marsh 1964). *Arachniotus*, *Aspergillus flavus*, *Cladosporium herbarum*, *Curvularia* sp., *Eurotium amstelodami*, *Papulospora* sp., *Penicillium* sp., the sterile fungus and *Thielavia* sp., classified as mesophiles, required a prolonged incubation period in order to degrade cellulose. The hazard arises when cotton storage is prolonged. Tansey's (1971) report on the slow elaboration of cellulose by *Cladosporium herbarum*, and consequently its delayed cellulose utilisation, agrees with the present work regarding our local isolate (Table 2). *Aspergillus versicolor* and *Helminthosporium* sp. exhibited extremely slow cellulose digestibility. Apparently, these moulds may be secondary cellulose utilisers depending either upon a starter of simple sugars, such as glucose, released in the medium (Simpson & Marsh 1964) as a result of the cellulolytic activity of the primary colonising species, or available simple sugars during fibre synthesis. The non-cellulolytic contaminants such as *Rhizopus solonifer*, *Alternaria* sp. and *Aspergillus niger* (isolate No. 2) might have been influenced by the soluble carbohydrates early during fibre formation.

In vitro digestion of CMC, cellobiose or raw cotton fibre with crude mycelial filtrate of selected members of the lint mycoflora yielded free glucose. The cellulase-containing mould metabolites induced brittle lint. Such breakage of lint into short fibre fragments has been explained by Halliwell & Riaz (1970) as the first step in the attack of cellulase on the cellulose structure of cotton fibre. Furthermore the intermediate product (one spot only on TLC plates) of the digested fibre gave an Rf value on TLC plates identical to those of pure cellobiose (Fig. 1). Although the isolation of pure cellulase from the test moulds was not effected, yet the yield of cellobiose and subsequently glucose from crystalline cellulose as well as raw cotton by the fungal filtrate may signify the content of at least the component C_1 of cellulose in the crude mould metabolic products (Halliwell 1975).

The loss of strength and elongation is indicative of early fibre degradation. Cotton fibre exhibited increasing fragility with time of incubation in mould metabolites. *Thielavia* sp., recorded for the first time in the Sudan (Abdalla & El-Tayeb 1981a), greatly affected fibre strength. The stelometer test showed that *Cladosporium herbarum* and *Stemphylium* sp. metabolites effectively lowered fibre elongation followed by *Aspergillus fumigatus*, *Thielavia* and then *Trichoderma* (Table 3). It is interesting to note that *Cladosporium herbarum*, *Stemphylium* sp. and *Thielavia* sp. metabolites lowered yarn spinning efficiency even before appreciably digesting cellulose (Table 2).

ACKNOWLEDGEMENTS

Thanks are due to the Staff of the Spinning and Fibre Testing Laboratory of the Gezira Agricultural Corporation, Sudan.

Mould isolates given numbers in the text were deposited in the Botany Department, Faculty of Science, University of Khartoum.

REFERENCES

Abdalla, M.H. & El-Tayeb, N.M. 1981a. Preliminary survey of cotton flower mycoflora from Sudan. Trans. Br. Mycol. Soc. **76:** 367–70.

Abdalla, M.H. & El-Tayeb, N.M. 1981b. Cotton boll mycoflora from Sudan. Mycopathologia **75:** 39–44.

Bailey, T.L.W. Jr. 1953. Doughnut fibres. Text. Industry **117:** 143–6.

Chapman, E.E., Jacobelli, M.C. & Logan, A.A. 1975. Cellulolytic and amylolytic activity of *Papulospora thermophile*. Mycologia **67:** 608–15.

Eggin, H.O.W. & Pugh, G.J.F. 1962. Isolation of cellulose decomposing fungi from soil. Nature **193:** 94–5.

Halliwell, G. 1961. The action of cellulolytic enzymes from *Myrothecium verrucaria*. Biochem. J. **79:** 185–92.

Halliwell, G. 1963. Advances in enzymatic degradation of cellulose and related materials. Pergamon Press, Oxford.

Halliwell, G. 1975. Hydrolysis of fibrous cotton and reprecipitated cellulose by cellulolytic enzymes from soil microorganisms. Biochem. J. **95:** 270–81.

Halliwell, G. & Riaz, M. 1970. The formation of short fibres from native cellulose by components of *Trichoderma koningii* cellulase. Biochem. J. **116:** 35–42.

Mandels, M. & Reese, E.T. 1957. Induction of cellulase in *Trichoderma viride* as influenced by carbon sources and metals. J. Bacteriol. **73:** 269–78.

Marsh, P.B. & Kerr, T. 1961. Uncollapsed fibres associated with bollrot in cotton. Plant Dis. Res. **45:** 550–1.

Miller, G.L. 1959. Use of denitrosalicylic acid reagent for determination of reducing sugar. Analyt. Chem. **31:** 426–8.

Rossness, P. & Wright, B.A. 1974. *In vitro* changes of cellulose, trehalose and glycogen during differentiation of *Dictyostelium discoideum*. Arch. Biochem. Biophys. **164:** 60–72.

Sadov, F., Korchagin, M. & Matetsky, A. 1973. Chemical technology of fibrous cotton. Mir Publishers, Moscow.

Selby, K. & Maitland, C.C. 1965. The fractionation of *Myrothecium verrucaria* cellulase by gel filtration. Biochem. J. **94:** 578–83.

Selby, K. & Maitland, C.C. 1967. The cellulose of the components involved in the solubilization of cotton. Biochem. J. **104:** 716–24.

Simpson, M.E. & Marsh, P.B. 1964. Cellulose decomposition by the Aspergilli. U.S.D.A. Tech. Bull. 1303, 53 pp.

Stands, I.E., Fiori, L.A. & Groves, N.H. 1962. The utilization of *Diplodia*-damaged bollrot cotton. Part I. Effects on yarn properties and spinning efficiency. Textile Res. J. **32:** 1013–22.

Tansey, R.T. 1971. Agar-diffusion assay and cellulolytic ability of thermophilic fungi. Arch. Mikrobiol. **77:** 1–11.

Tate, M.E. & Bishop, C.T. 1962. Thin layer chromatography of carbohydrate acetates. Can J. Chem. **40:** 1043–8.

Walseth, C.S. 1952. Occurrence of cellulases in enzyme preparations from microorganisms. Tappi **35:** 228–33.

Wood, T.M. 1968. Cellulolytic enzyme system of *Trichoderma koningii*. Separation of components attacking native cotton. Biochem. J. **109:** 217–27.

Wood, T.M. 1969. The cellulase of *Fusarium solani*. Biochem. J. **115:** 457–64.

(Received 6 September 1980)

أثر الفطريات في اضعاف قوة تيلة القطن السوداني

محجوب حسن عبد الله و نجاة مبارك الطيب
قسم النبات بكلية العلوم ، جامعة الخرطوم ، السودان

خلاصة

في هذا البحث تم عزل ٢٥ نوعا من الفطريات من القطن السوداني ، واختبرت قدرتها على تحليل السليولوز وأثر ذلك في الخصائص الفيزيائية للتيلة ، وقد رتبت الفطريات وفقا لنشاطها الانزيمي . كما تم اختيار ٩ أنواع من الفطريات النشطة انزيميا واختبر أثرها في بعض خصائص الغزل مثل قوة التيلة وقابلية الفتلة للاستطالة .