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Electron Microscopic Study of Fibre Surface After Endotoxin Removal Treatments on Raw Cotton

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Abstract: A respiratory disease common in textile workers is observed to be caused by endotoxins (Lipopolysaccharides) attached to the raw cotton being processed in the mills. Different remedial measures were performed and their effect on the cotton fibre surface was studied under electron microscope.

Key words: Fibre Surface, Endotoxin Removal Treatment

Introduction

Cotton is the most popular textile fibre in the service of mankind as a variety of wear and upholsters. Besides its versatility, there is usually a disease causing toxic agent 'Endotoxin' in the raw cotton. 'Brown lungs' disease "Byssinosis" is observed to be caused by inhaling the said agent being air born when the cotton is ginned and opened in the mills for processing. Basically, It is Lipopolysaccharide produced by gram negative bacteria which colonize cotton plant in the field and during harvesting, the endotoxin are carried to the ginning factories and mills. Victim of this toxic agent is commonly the poor textile worker and for the sake of his health, their removal from the raw cotton by different methods has been tried, along with microscopic studies and spinning performance of fibers before and after various treatments in this regard. The treatments mainly included mechanical cleaning by Shirley Analyzer, washing, steaming (auloclaving) and flash heating the lint.

Natural surface coating of the fibre is removed or redistributed during washing; whereas, the fibre surface structure may remarkably be affected by the flash heating and steaming processes, however, no pronounced fibre change occurs in the case of mechanical removal through Shirley Analyser.

Researches by Potter and Corbman (1966) reported that under the microscope, cotton resembles a collapsed, spirally twisted, flat and ribbon like structures with a rough surface, containing a wide inner canal (lumin) and a granular effect. de-Gruy *et al* (1973) studied in detail the fibre surface by using SEM (Scanning Electron Microscope) and reported that overheating produces permanent effects on certain properties of cotton. Similarly, Krakhmalev *et al* (1979) examined the fibre wall deformation by heating under electron microscope; whereas, Wilton *et al* (1980) gave frequent washing treatments to raw cotton fibre and reported a remarkable reduction of loose particles from the fibre surface along with removal of fibre surface coating, illustrating some deposits on some places of the fibre. The present study was suggested to examine the effect of endotoxin removal techniques on the cotton fibre surface under related latest electron microscope.

Materials and Methods

Present study was conducted in the departments of Fibre Technology, Microbiology and High Tech. laboratory of the Univ. of Agriculture, Faisalabad. Following are the details of the methods applied to record the data:

Cotton samples of varieties V_1= SLH-1, V_2= CIM-1100, V_3= CIM-448 Cotton V_4=FH-634 were collected from Ayub Agricultural Research Institute, Faisalabad (L_1) and its substations at Sargodha (L_2) and Jhang (L_3).

Mechanical Cleaning Treatment (T_1): In this treatment cotton samples were mechanically cleaned with the help of " Shirley Analyzer MK-2" according to the instructions given in its operational manual.

Steaming or autoclave treatment (T_2) was done at 121°C and at pressure 18 pounds for half-an-hour, Nasir (1998).

Flash heating treatment(T_3) was given to the samples in an electric furnace at 250°C for 25-30 seconds, Russelle and Domelsmith (1993).

Water Wash treatment (T_4) was performed as under:

Cotton samples were washed with water at 60-65°c keeping water : cotton ratio as 50:1, using wetting agent Kieralon-CD, Anonymous (1998) and then washed samples were air-dried.

Endotoxin Content: All the glassware was depyrogenated by heating it at 180°C overnight. A weighed sample of 2 grams was extracted with 200ml Pyrogen-free water by shaking in a glass stoppered flash. Finally, the endotoxin content was determined by the method suggested by Broglen and Reber (1978), Akhtar (1995) and Russelle *et al* (1996).

Electron Microscopic study was performed to examine the change in fibre surface after each treatment with the help of SEM model S-2380-N Hitachi Japan. The process adapted was as for its operational manual, Anonymous (1997).

Results and Discussion

The results obtained from the set of experiments for endotoxin level and electron microscopic examination of the treated cotton varieties are presented and discussed here under:

Endotoxin Level: The analysis of data in this respect presented in Table(a) shows highly significant results for treatments, varieties, location as well as their first and second order interactions.

Duncan's Multiple Range test for the comparison of individual mean values indicates highly significant elimination of endotoxin under all the treatments except the mechanical treatment (T_1). In Autoclave treatment (T_2) a libration of 17.42 ppm down to 6.98 ppm, recorded a redaction of 60%. Present results are found almost at par with the findings of Nasir (1998) who noted 68 to 75% reduction of endotoxin under this treatment. Similarly, wade (1983) submitted that vapour phase treatment (Steaming) was effective in reducing LPS content.

Heating treatment (T_3) caused remarkable reduction level from 17.42 ppm to 4.91 ppm. i.e. a reduction of 72 % in toxin content. The present result correlates upto to a certain extent with findings of Russelle and Domelsmith (1993), they recorded a reduction in endotoxin as 90% at 250 to 255°C for 15-20 seconds flash heating. The difference in results might be due to the fact that they employed gas fired pilot plant dryer for this purpose while under present Investigations electric furnace was used.

The highest endotoxin extractoin of 83% is estimated for cotton samples processed under water wash treatment (T_4) by reducing ppm level from 17.42 to 3.03. Earlier, Sassar (1980) reported that cotton dust containing endotoxin causing pulmonary disfunction was water extractable. Similarly, Russelle & Domelsmith (1988) stated that water washing was the first treatment proposed for reducing the byssinogenic potential of cotton. Present results get confirmation by the findings of Nasir (1998) who reported reduction of 78 to 84% of endotoxin through water wash treatment.

Variety wise comparison of endotoxin level ranked the four commercial varieties as V_1, V_3, V_4 and V_2, with their level recorded as 9.16, 8.44, 7.77 and 6.98 ppm. respectively. The present variations might be due to the environment and location change.

The non-significant change for the mean values of endotoxin for stations L_1 and L_3 are 9.17 and 8.93 ppm. respectively; which differ significantly from L_2 (6.16 ppm). The findings of Olenchock *et al* (1983) confirm the present results, he observed variation in endotoxin contamination in the cotton bales from different countries. Location wise maximum removal of LPS is observed in the samples from Sargodha station.

Electron Microscopic study reveals the effect of different techniques on fibre surface as under:

Mechanical treatment shows no effect on fibre surface and the electron microscopic view is found just like that of the natural non-treated cotton lint-fibre. Therefore, there is considered no need for its EM photographs.

Autoclave treatment illustrates the fibre surface changing [Fig.b at magnification x3000] into a comparatively smooth, cylindrical, slightly swoollen structure, possibly due to the pressure and steam effect of the autoclave upon cellulosic wall texture. The cylindrical structure is not just like that of alkali treated cotton fibre, where as, alkali treated structure of fibre is more cylindrical & lustrous ; Moreover, the lumen of the fibre not found clear in this case, no cracking, no shrinakage and no rupture or peeling of surface cellulose is observed. The present results are confirmed by the findings of Cannizzaro *et al* (1970) who reported the morphological change in fibre surface, particularly after treatment of vapour phase or steaming. He studied the fibre surface by Light and Electron microscope.

From the EM photographs after flash heating treatment [Fig.c at magnification x3000], the fibre surface is examined as distorted, burnt to yellowish brown but at certain portions of fibre, convolutions are found still present. The surface after treatment seemed to be rough or course with signs of strength and elongoation loss ultimately. The present findings in this case are at par with the research report of Krakhmalev *et al.* (1979) that the primary causes of fibre weakening are observed during heat treatments along with fibre wall deformation, seen under electron microscope. Similar views were given by de-Gruy *et al.* (1973) that over heating produced permanent effect on such properties of cotton as elongation at break, degree of polymerization and breaking length due to the degradation of cellulose.

Table-1: Analysis of Variance for Endotoxin Level

S.O.V	D.F	S.S	M.S	F-VALUE
Treatment (T)	3	4462.62	1487.54	3231.055**
Variety (V)	3	94.03	31.34	68.080**
VxT	9	47.99	5.33	11.582**
Location (L)	2	268.46	134.23	291.558**
Tx L	6	150.75	25.12	54.574**
VxL	6	37.77	6.29	13.676**
TxVxL	18	42.01	2.33	5.070**
Error	96	44.19	0.46	
Total	143			

** = Highly significant

Individual Comparison of Means by DMR test

Treat-ment	Endotoxin level	Varieties	Endotoxin level	Loca-tions	Endot oxin level
T_1	17.42a	V_1	9.16 a	L_1	9.17 a
T_2	6.98b	V_3	8.44 b	L_2	8.93 a
T_3	4.90c	V_4	7.77 c	L_3	6.16 b
T_4	3.03d	V_2	6.98 d		

Any two means not sharing a letter in common differ significantly at 0.05% level of probability.

Note: a,b, c and d are used separately for each column.

Fig. a. Longitudinal view of raw cotton fibres (X 3000)

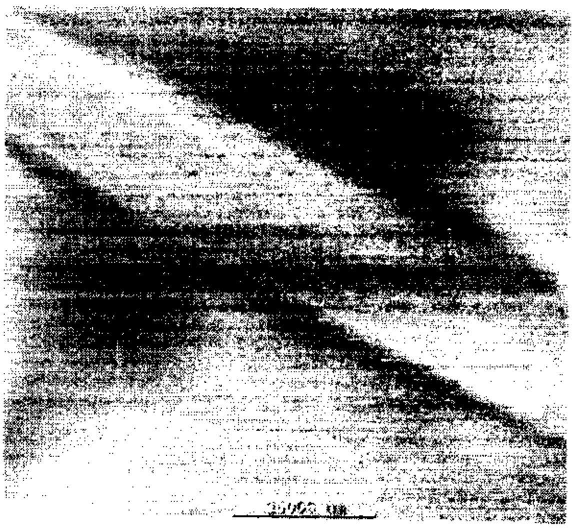

Fig. b. Longitudinal view of autoclaved cotton fibres (X 3500)

Fig. c. Longitudinal view of flash heated cotton fibres (X 3000)

Fig. d. Longitudinal view of washed cotton fibres (X 3500)

Washing treatment effect [Fig.d at magnification x3500] has been noted remarkably drastic.
Fibre surface study under EM confirmed the removal of waxes, fats, pectins and other primary wall impurities along with maximum LPS reduction. As a result, the surface looks somewhat shrinked, swoollen with heavier deposits at different places, along with fibers entanglement the longitudinal structure of surface is found quite uneven due to the removal of natural coatings to a great extent. Present results are found in line with the findings of Wilton *et al.* (1984) who performed the microcopical examination of cotton fibre samples washed under different conditions to remove byssinogenic agent 'endotoxin' from the raw cotton. They found that both transmission electron microscopy and scanning electron microscopy of the treated cotton fibre surfaces showed a reduction in the number of loose surface particles. Changes were observed also in the nature of coatings of the fibre surface just like that of in the present study.

Conclusions

- Endotoxin removal percentage ranked maximum in case of washing treatment followed by Flash heating and Autoclave treatments. While, mechanical treatment howed almost zero effect on fibre surface structure.
- Washing and flash heating caused structural deterioration of fibre, so they are not feasible commercially.
- Autoclave proved the most suitable treatment.
- Location wise, Sargodha station and variety wise CIM-1100 operated as the best one in this regard.

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