STUDY OF STICKINESS EFFECT ON FUNGAL DETERIORATION OF COTTON FIBERS

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Abstract: *In the present study, the influence pattern of cotton fiber stickiness on fungal contamination was studied. By means of DNA analyzing for microscopic fungi (Sanger method), the main biodestructors were identified. Thanks to empirical observation on the behavior of contaminated zones using thermo-detection and analysis of microfungi morphology, visible contamination of cotton with microfungi was defined as an alternative indicator of fiber stickiness determination. Based on the results of this study, a novel method facilitating infected cotton classification was developed.*

Keywords: *cotton fibre, stickiness, biodeterioration, fungal contamination, micromycetes, thermodetection, cotton classing, trehalulose.*

1 INTRODUCTION

Natural, artificial and man-made fibers are common used as raw materials for production of a wide range of fabrics and clothing. Global cotton production and consumption is rising annually. The cotton fiber market attracts manufacturers with the ability to create textiles with high hygienic properties, which more often are in great demand with consumers. Uncontaminated cotton fibers could be produced in the traditional way, but such cotton is characterized by high cost. Manufacturers often use low-quality polluted cotton. Growing in the fields, cotton is exposed to external environmental conditions including atmospheric precipitations, insects, and microorganisms. All these factors cause pollution of cotton fibers, which, in turns, has negative influence on yield value, spinning process and quality of cotton fibers.

The most widespread reason of bacterial and fungal infection of cotton is stickiness (the presence of disaccharides and polysaccharides). The stickiness defect is caused by complex factors as insects securing adhesive sweet liquid that pollutes fibers and microorganisms deterioration [1]. By means of high-performance liquid chromatography of aqueous extracts from cotton, nine main sugars have been found and only one of them is able to block spinning equipment and interfere production. It was a trehalulose ($C_{12}H_{22}O_{11}$) - oligosaccharide, sucrose isomer [2]. A key factor that leads to cotton stickiness due to trehalulose has a low melting point, two or three times lower than for the rest of sugars, according to Heket [3] about 48°C.

Trehalulose is originally entomological sugar and injects on fibers exclusively with secretions (“honeydew”) such cotton pests as aphids (Aphis Gossypii) and whiteflies (Bemisia spp.) [4]. The secret of these insects also contains trehalose, but this saccharide has other chemical and physical properties (melting point, functional groups, etc.).

Honeydew on the surface of cotton fiber could be transferred to a metallic surface of the spinning machines or rubber rollers, etc., resulting in lapping. As a result, it may lower the efficiency of the yarn production and yarn quality [5].

This research investigates interrelation of microscopic fungi deterioration of cotton with its stickiness. The main objective was to study the phenomenon of cotton fiber stickiness and determine whether fungal deterioration is responsible for sticky properties or fungal contamination serves as a consequent effect of stickiness.

2 EXPERIMENTAL

2.1 Materials

The objects of this study were two samples of Central Asian cotton fiber, with different selection and grown in different places. The first sample of cotton fiber is referred to the S-4727 selection supplied by the ginning factory №253 (Myrzakent, Kazakhstan). The second one is the AN36 selection supplied by the ginning factory №069 (Akhunbabaev, Uzbekistan). In this work, a multistage sampling was used according to GOST R 53236-2008 [6]. The cotton samples were exposed in conditions of a temperature of 20±2.0°C and a relative humidity of 65±4%.

2.2 Methods

Cotton Classing

The classing method was used for visual assessment of cotton fiber according to GOST R 53234-2008 [7]. This method was used to determine visible microbial contamination, color, class of weediness and type along the length of the staple formed manually. The special ICS-Texicon equipment was used for providing the lighting in UV range.

High Volume Instrument Analysis (HVI)

HVI analysis was used for testing of length, uniformity in length, strength, elongation at break, micronaire (tinting and maturity), and color. The analysis was performed by USTER HVI 900 SA (CIRAD, France) according to ASTM D5867 [8].

Thermodetection

Thermodetection of cotton fiber samples was carried out by means of SCT thermodetector (CIRAD, France) in accordance with BS EN 14278-1 [9]. The temperature was 84±4°C with hot pressure time of 12±2 s. The force applied was 780±50 N for aluminum hot plate and 590±50 N for an upper wooden board.

Benedict test

To determine the stickiness of cotton fiber Benedict test was performed according to GOST R 53030-2008 [10] (alkaline solution). The numerical values of aqueous extract from cotton fiber was obtained by measuring of optical density (590 nm) using a spectrophotometer PE-5300V (Russia).

Detection of sugar by color reaction

The method of stickiness detection by color reaction was carried out according to ISO 12027:2012 [11]. The droplets of honey dew on a cotton were transferred to paper for color reaction by reagent based on paraaminobenzoic acid. The resulting image on the color reaction paper was compared with a series of standard replicas and the grade was assigned.

DNA sequencing analysis for microscopic fungi (Sanger method)

The specific belonging of microscopic fungi under study is determined by Sanger sequencing analysis carried out by HITACHI Biosystems 3130 (All-Russian Research Institute of Agricultural Biotechnologies, RAAS). The purification of amplicon was performed by Omnix columns. The sequences having length of more than 530 peaks were obtained by using capillary electrophoresis. The obtained DNA sequences were compared with NCBI GenBank international basis [12].

3 RESULTS AND DISCUSSION

According to physical-mechanical analysis by HVI test, the samples under investigation have close parameters of quality (Table 1).

Table 1 Characteristics of cotton fibres under investigation

Parameter		Selection	
		S-4727	*AN36*
Type	Classing	5	6
Code		35	33
Grade		II	II
Class		first	first
Length (UMML) [mm]	HVI test	28.0	25.9
Micronaire		4.2	4.3
Strength [g/tex]		25.5	29.1
Elongation [%]		6.0	6.1
Reflectance [%]		74.1	70.8
Yellowness [+b]		9.9	11.3

The micronaire value is 4.2-4.3, which indicates average roughness of the fibers. The S-4727 selection is characterized by long fibers with average strength (25.5 g/tex), AN36 sample has short fiber length with higher values of strength (29.1 g/tex).

Parameters of color grade defined by HVI meet the results of cotton classing. The cotton samples are medium-fibrous with low trash count. Both the cotton samples have color characteristics from matte-white to cream with pale yellow spots.

However, both samples of cotton are characterized by a high microbial deterioration inspected without magnification. During the classing, numerous black-brown spots being similar to mold fungi contamination were observed on the fiber surface. Figure 1 shows a cotton ball locally affected by microfungi, which was detected by classing.

The thermodetection represents the method for the stickiness determination by simulating the interaction of the working surfaces with fibers, recreating loads and temperatures.

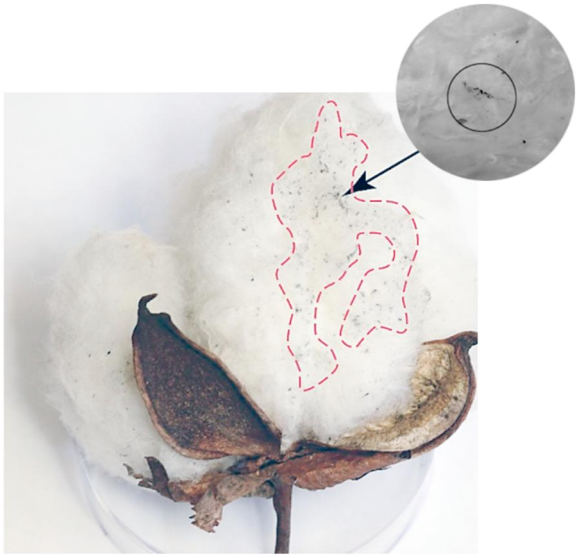

Figure 1 Visible contamination by microscopic fungi easily detectable in cotton fibers at classing (AN36 sample)

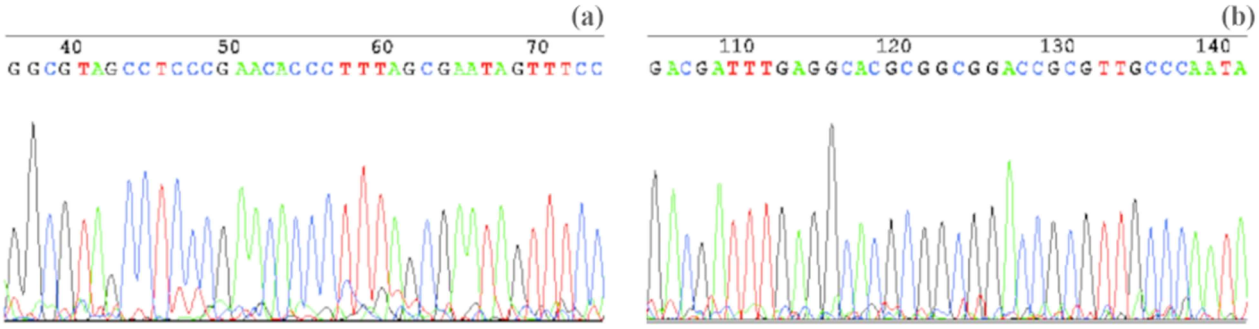

Figure 2 Sequences fragments of the DNA molecules sequencing peaks for the first sample (S-4727 selection)

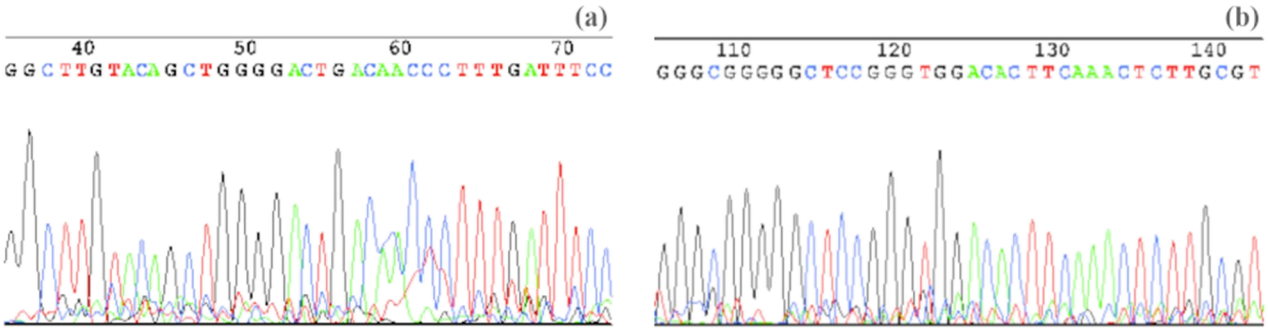

Figure 3 Sequences fragments of the DNA molecules sequencing peaks for the first sample (AN36 selection)

When analysing the stickiness of the cotton samples by the method of thermodetection, the matching of infected zones with the sticky points was fixed. Provided, that exactly these areas were identified as zones of visible contamination by bacterial and fungal infection. In the following, it was necessary to determine the reasons for the concordance of zones affected by microfungi with zones of increased stickiness, and thus to understand whether the fungi are sticky on its own or positioning on the sticky areas defines their presence.

The Sanger analysis results are shown in Figure 2 (S-4727 selection) and Figure 3 (AN36 selection). According to NCBI GenBank database, the fragments sequence of peaks of the both first and second samples are determined as DNA of microfungi *Cladosporium cladosporioides*. Despite the many differences between selected cotton fibre samples (morphology, period of aging, areas of growth), the results of the DNA sequencing method indicate the identity of species of microfungi on both cotton samples.

According to Dugan et al. [13] *Cladosporium* genus includes more than 772 species fouling a variety of crops, including cotton fibers. However, there is no evidence of the ability of *Cladosporium cladosporioides* to release any sticky substances. In case of morphology analysis of *Cladosporium* microfungi, it was stated that the fungal cell wall mainly consists of hexoses (34-47%) and β-1,3-glucan, including a certain amount of galactose and mannose [14]. Galactose and mannose are monosaccharides (hexoses) with a melting point of 167°C and 132°C, respectively. Therefore, such saccharides could not have a significant contribution to stickiness.

Due to the facts that *Cladosporium* does not display the stickiness or release adhesives, the reason of sticky points and visible contamination matching is that fungi use trehalulose as a nutrition source. According to observations of entomologists Rekacha and Dobretsova [15], saprophytic fungi could develop on the cotton aphid excrements, causing further devaluing the fiber quality. As previously noted, the cotton aphid (Aphis Gossypii) and whitefly (Bemisia spp.) emit the “honey dew” containing trehalulose. Consequently, localization of visible fungal contamination could be used for identification of sticky areas and evaluation of infection without using instrumental methods.

Thanks to obtained regularities and the current US classification codes of United State Department of Agriculture (USDA), the system of stickiness rate for infected cotton was developed. The samples of Central Asian cotton fiber (about thirty thousand tons) were assessed by infection intensity. The most typical forms of fungal contamination on fiber revealed were assigned by codes. The photos of typical fungal contamination forms are shown in Figure 4.

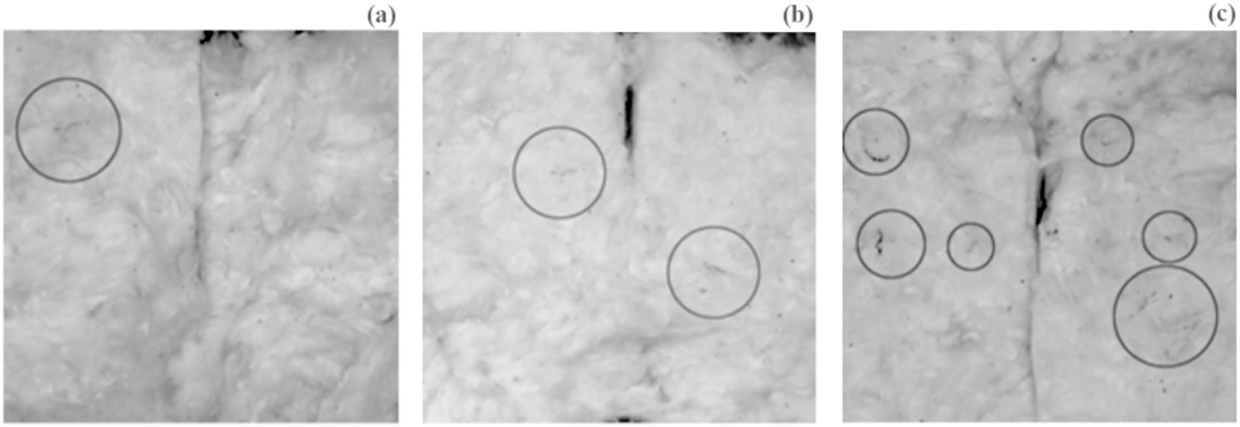

Figure 4 The signs of visible fungal contamination in cotton fibers: low degree – classer code 040 (a), average degree – classer code 041 (b), strong degree – classer code 042 (c)

The developed technique requires using of the visual organoleptic assessment regulations according to GOST R 53234-2008. When cotton sample has separate zones of fungal contamination at least in one of the scanned layers (Figure 4a), it is assigned a code 040. When fungal contamination is observed in two layers of, but not more than two, a cotton fiber sample is assigned a code 041 (Figure 4b), more than two layers - the code 042 (Figure 4c). A code 042 corresponds to strong biofouling that can cause difficulties in processing and be a threat to staff working health (fungal infection). The characteristics of signs according to developed technique of cotton fiber are shown in Table 2. The authors propose to integrate this method of cotton evaluation in GOST R 53224-2008 [16] as an alternative way to identify infected cotton.

Table 2 The assessment system of visible fungal contamination (VFC) degree of cotton fibers

Codes	Brief description	The VFC description
0	None	No signs of VFC
1	Signs of VFC	Barely visible hyphae of microfungi in a separate layer of cotton fiber samples
2	Medium VFC	Noticeable hyphae of microfungi in no more than two layers of cotton fiber samples
3	Strong VFC	Areas of VFC in more than two layers of cotton fiber samples

Classification codes of fungal contamination were convert into numerical values respectively: no code = 0; 040 = 1; 041 = 2; 042 = 3. The results of stickiness index assessing are presented in Table 3.

To compare the accuracy and comparability of stickiness index assessing of cotton fiber using the developed express method and standard techniques, the correlation analysis according to Spearman was performed [17]. Comparative analysis of Spearman rank correlation and statistical reliability of the developed method in comparison with the most-used ones is shown in Table 4.

The data obtained by the developed express method with a high degree of statistical reliability coincide with the data of the advanced thermodetection method, with a correlation of 0.7.

Table 3 Results of the evaluation of cotton fiber stickiness

Method of evaluation		S-4727	AN36
Developed method	code	040 (1)	042 (3)
Thermo-detection (sticky points)	AV	11 (ligth)	37 (strong)
	SD	0.7	0.9
	CV [%]	146.1	14.9
Color reaction (grading)	AV	grade C (medium)	grade C+ (medium+)
	SD	2.3	0.8
	CV [%]	31.2	8.1
Benedict test (solution optical density)	AV	1.05 (strong+)	0.9 (strong+)
	SD	0.4	0.1
	CV [%]	38.1	10.9

Note: *AV* – average value, *SD* – standard deviation, *CV* – coefficient of variation.

Significant differences in results of the developed method and the data obtained by color reaction and Benedict methods are due to the fact that these methods are aimed to determine the total amount of sugars of cotton fiber. However, the total amount of sugars considerably exceeds sugars of entomological origin providing stickiness.

Thus, the developed express method in high degree of reliability characterizes the stickiness of a cotton fiber infected with visible fungal contamination. The developed express method is an alternative, non-labor-intensive way of detecting contaminated cotton when assessing its quality.

Table 4 Comparative analysis of the developed method with the most-used ones

Compared methods	ρ	λ	S' [%]
Developed / thermodetection	0.7	3.2	0.99
Developed / color reaction	0.2	0.7	0.48
Developed / Benedict test	0.2	0.9	0.67

Note: ρ – Spearman rank correlation coefficient, λ – multiplier for statistical reliability, S' – statistical reliability.

4 CONCLUSIONS

The work results prove that microfungi *Cladosporium cladosporioides* found in cotton fibers and identified by DNA sequencing by Sanger are not sticky. The matching of visible microfungi contamination areas with sticky points at thermodetection allows the use of microfungi as an indication of stickiness at the visual cotton classing assessment. The obtained data on cotton classing induces the opportunity to determine the most common forms of fungal contamination and assign. The data obtained according to the developed express method coincides with the data of the most advanced thermodetection method with a high degree of statistical reliability (more than from 99%), with a correlation of 0.7. The proposed methodology allows evaluating the degree of stickiness only for infected cotton.

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