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# Biostability of cotton fibers with different natural colors and selection

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**Abstract.** Biodamageability is one of the weak sides of the cotton-plant fiber. Economic loss from the cotton damage caused by microorganisms is significant. Toxic chemical compounds are used to preserve the cotton fiber. This has a negative impact on environment and cotton consumers. The degree of the cotton biodamage depends on selection variety, types of cultivation, storage conditions and other factors. One of the directions in cotton planting is selection of the boll-stained cotton. It was established that naturally colored cotton of different varieties has high biostability. It allows to product hypoallergenic, eco-friendly textile and reduces impact on the environment. The purpose of the work is stability evaluation for microbiological damage of different cotton varieties including those naturally colored during long-term storage in different temperature and humidity conditions. The research lasted for 10 years. The species composition of the cotton microflora was determined, the impact of fiber on microorganisms and dynamics of samples biodamage was studied in the work. The results obtained show preservation of microflora viability on cotton fiber when storing it under normal conditions for a long-term period. With an increase in temperature and humidity necessary for microorganisms' growth, the fiber destruction processes are amplified. It was established that cotton damage degree depends on the regimes and terms of its storage as well as the color of the fiber. Nature-colored cotton is more resistant for microorganisms, some varieties inhibit the growth of mold fungi.

**Key words:** biostability, cotton fibers.

## INTRODUCTION

One of the directions in the field of cotton breeding is the creation of boll-stained cotton selections. The research of nature-colored cotton was subject of a number of works (Elesini et al., 2002; Grigoryev & Illarionova, 2009; Pekhtasheva et al., 2012). The usage of nature-colored cotton is capable of producing ecologically clean textiles and eases-off the environment thanks to refusal to use synthetic dyestuff.

The problem of lint is its biological damageability. Economic damage from cotton damage with microorganisms reaches sizeable amounts (Yermilova, 1991). To save lint, toxic chemical mixtures are often applied. It is negative for the environment and consumers of cotton goods. The grade of cotton biological damage depends on elite selection, ways of cultivation, storage conditions and other factors.

Many authors mentioned in their works higher biostability of nature-colored cotton in separate grades compared to white cotton (Grigoryev & Illarionova, 2009; Pekhtasheva et al., 2012).

The purpose of the work was stability assessment of cotton fibers of different grades with white and nature-colored fiber to microbiological damages on long storage in various conditions of temperature and humidity. The species composition of cotton flora, dynamics of biological damage of samples was defined, and influence of fibers on microorganisms was studied in the work.

## MATERIALS AND METHODS

As objects of the research, samples of cotton fibers with green, beige, brown nature-color were chosen. Also a sample of white cotton from selection 175-F was investigated.

For a research of questions of fibers and microorganisms' interaction bacterium and the microfunguses were used, as they had an ability to damage cotton fibers and their goods. These are bacterium – Bacillus subtilis, Basillus pumilus, Pseudomonas fluorescens, Erwinia herbicola, Bacillus sp. and microfunguses – Aspergillus niger, Aspergillus terreus, Penicillium variabili, Penicillium cyclopium, Penicillium chrysogenum, Chaetomium globosum.

The effect of fibers on microorganisms was assessed with a method of agar plates in a direct impact of fibers on live cells of bacterium and mushrooms growing on the growing medium (meat-peptonic agar for bacterium and a wort agar for mushrooms). For this purpose, 10 mg of a fiber with a mass rolled into a ball were put into the center of a Petri dish on a growing medium, inoculated in a definite form of a microorganism. To control it, the cups sown with bacterium or mushrooms but without a fiber pattern were used. The inoculation of equal number of microbe cells was provided in each dish. This was made with the help of prior preparation of clean culture of microorganism dilutions in sterile physiological solution. This dilution contained 1 billion microbe cells per 1 mL of suspension (10 Turbidity units) which was controlled according to turbidity standards. Then 1 mL of the received suspension was put into a Petri dish and spread evenly on the agar plate surface. Thus, a solidly even growth of a microbe colony was obtained in all dishes. Crops were thermostated at a temperature of 30 °C within one day for bacterium and 7 days at a temperature of 26 °C for mushrooms. The degree of microorganisms' sensitivity to fibers was determined by the presence/absense of a sterile zone around the species and by the solidity change in the microbic colonies growth in the dishes with fibres as compared with test ones. The tests were carried out for each type of cotton fiber and each microbic culture in five replications.

Production of microorganisms from samples of cotton fibers and determination of their quantity were carried out by a seeding technique on dense growing mediums of washouts from fibers (Kotomenkova & Vinogradova, 2007). Identification of microorganisms was carried out by standard techniques using determinants of bacterium and microfunguses.

The research of samples biostability has been conducted during 10 years. The biostability degree of cotton fibers in relation to microscopic flora which is spontaneously found on them during the plant growth from the environment (the so-called 'spontaneous microscopic flora') was evaluated.

The first stage was carried out in 2006. After the initial damage of cotton fibres was determined, they were placed for storage in various conditions.

The first species series was kept in a climatic chamber during 30 days, under the conditions which were most favorable for the microorganisms' growth (air temperature of 26–30 °C, relative humidity was 90–100%).

The second species series was put for long-term storage under normal conditions (air temperature 18–22 °C, the relative humidity was 60–65%) for 10 years. After the 10-year storage, the species were returned to the conditions which were favorable for microorganisms' development.

To evaluate the biostability of cotton fibers, their structure condition was determined at the beginning of the experiment and after each storage stage.

The degree of fibers damage was determined by the optical microscopy method, with application of MIKMED-5 microscope (LOMO, Russia).

For biostability research samples of cotton fibers were cultivated in a climatic chamber for 30 days under normal conditions of storage (air temperature was 18–22 °C, relative air humidity was 65%) and under optimal conditions for growth and reproduction of microorganisms (air temperature was 26–30 °C, relative air humidity was 90–100%).

Damage degree of fibers was determined by a light-microscopical method. At the same time, types of changes of fiber composition were revealed with the subsequent measure calculation of biological destruction according to the following formula (1) (Yermilova, 1991):

$$k = 0.002x_1 + 0.025x_2 + 0.255x_3 \quad (1)$$

where k – is an indicator of biological destruction of a fiber; $x_1$, $x_2$, $x_3$ – is a quantity of damage by classes A, B and C respectively; $k_1$, $k_2$, $k_3$ – are coefficients of weightiness of damages by classes A, B and C.

Damages of Class A include the first changes in fibers surface: fouling by microorganisms and their metabolites, surface unevenness and small cracks.

Class B unites stronger destruction manifestations: deep cracks, swelling, thinning out, side damages.

Class C includes fibers exfoliation, deep local side damage and fibers degradation.

The value of a destruction indicator $K \leq 0.3$ corresponds to the initial changes of a fiber surface which do not affect its internal composition; in the range of $0.3 < K \leq 3.55$; not only a surface is destructed, but internal sites of fibers, which is followed by initial changes of its composition; in the range of $3.55 < K$ is a deep biological destruction of fibers at all levels (Yermilova, 1991).

The fibers structure changes under the influence of microorganisms were studied with the use of electronic microscopy (PEM) and infra-red spectroscopy (FTIR).

For the study of the thin structure of cotton fibers surface an electronic microscope JSM-T200 (JEOL, Japan) was used. The material was assembled on the table and sputtered with gold in vacuum. Each preparation was examined in 20–30 fields of vision; the most characteristic changes were photoed.

Infra-red fibers spectra were taken on a double-beam spectrometer UR-10 (Carlzeiss Jena, Germany) with retrievable automatic prisms. The spectra were taken in a wave number range from 400 to 4,000 $cm^{-1}$. The samples were prepared according to a moulding technique. Potassium bromide was applied as a moulding matrix. The fiber weight quantity constituted 5 mg.

Structure changes of damaged fibers were evaluated by comparing the IR-spectra parameters (the changes in the width, shape and size of the absorption band) before and after they have been influenced by microorganisms. The IR-spectra decoding was exercised on the basis of research works in the given sphere.

Processing of results was carried out by means of methods of mathematical statistics.

## RESULTS AND DISCUSSION

When fibers and microorganisms cooperate the influence is of a bilateral character. Its results largely determine the textile fiber biostability in the process of its storage and operation.

In this respect, the research results should be divided into three parts:

### The microorganisms' reactions to the fibers' impact

The results of impact assessment of the studied samples of cotton fibers on bacterium and microfunguses are presented in Table 1. All the considered microorganisms are the disruptors of cotton.

**Table 1.** The effect of fiber on bacterium and microfunguses

| Microorganisms | Diameter of a growth inhibition zone, mm the color of cotton fiber | | | |
|---|---|---|---|---|
| | green | brown | beige | white |
| 1. Bacterium | | | | |
| *Bacillus subtilis* | 0 | 0 | 0 | 0 |
| *Basillus pumilus* | 0 | 0 | 0 | 0 |
| *Pseudomonas fluorescens* | 0 | 0 | 0 | 0 |
| *Erwinia Herbicola* | 0 | 0 | 0 | 0 |
| *Basillus sp.* | 0 | 0 | 0 | 0 |
| 2. Microfunguses | | | | |
| *Aspergillus niger* | 0 | 0 | 0 | 0 |
| *Aspergillus terreus* | growth intensity decrease* | growth intensity decrease* | growth intensity decrease* | growth intensity decrease* |
| *Penicillium variabili* | 0 | 0 | 0 | 0 |
| *Penicillium cyclopium* | 0 | 0 | growth intensity decrease* | 0 |
| *Penicillium chrysogenum* | 0 | 0 | growth intensity decrease* | 0 |
| *Chaetomium globosum* | 0 | 0 | growth intensity decrease* | 0 |

Note: Decrease of growth intensity as compared to growth in a test group.

The analysis of results shows that self-colored cotton does not possess an antibacterial action.

At the same time, it is established that a beige fiber suppresses growth of microfunguses of *Penicillium cyclopium, Penicillium chrysogenum, Chaetomium globosum* a little. All studied cotton fibers suppress growth of Aspergillus terreus.

White fiber of the cultivated types and the majority of modern cotton grades are the result of a longtime selection.

Fiber of wild species of a cotton is beige or pinkish to bronze (Gossypium anomalum Wawra et Peyr.), yellowish (Gossypium areysianum Defl.), light brown (Gossypium harknessii Brandg.), brown (Gossypium triphyllum Hohr.), dark brown (Gossypium armourianum Kearney.), brownish (Gossypium somalense Hutch.), brownish green (Gossypium klotzschianum Andress.), green (Gossypium sturtianum var. nandawarense (Der.) Fryx.), dark green (Gossypium davidsonii Kell.) tones. As selection practice shows, the sign of a fiber color is connected with his chemical composition, which, in turn, determines durability, and fineness. So, white fiber contains no more than 0.7–0.8% the adipoceratous substances whereas green one contains to 17.0% (Popova & Khafizov, 1985). Color is given to fiber by catechines (aromatic substances from group of flavonoids which oxidation products form the flobafenas, which cause brown, yellow-brown and blackish color and is antiseptic (Grigoryev & Illarionova, 2009).

Hence, the revealed decrease of growth intensity of microfunguses, as compared to the test group, can be a consequence of the fibers' chemical composition peculiarities or of the antagonistic action of the epiphytic bacterial flora contained on fiber. As it is known, the bacteria of the *Bacillus subtilis* group, which are a part of it, produce biologically active connections of antifungal action (Loeffler et al., 1990).

It should be noted that after cultivating the fibers of natural green color on a growing medium with microorganisms the intensity and brightness of green color increased.

The reason of this effect was not studied in the process of the given research. However, the authors consider this effect to be interesting for researchers studying the color of self- colored cotton and its changes.

**The study of microorganisms' quantity and species composition on the fibers**

The study of microorganisms' quantity and species composition on the cotton fibers was exercised with initial samples, before their biostability test.

The quantity of microorganisms separated from the studied samples of cotton fibers was not equal (Table 2). Most microbial cages are separated from a green cotton fiber, least are separated from with white cotton fiber.

**Table 2.** The number of live bacterial cells separated from cotton fibers samples

| Cotton fiber | Total microbial number, kl $g^{-1}$ of fiber | Cotton fiber | Total microbial number, kl $g^{-1}$ of fiber |
|---|---|---|---|
| green | $3.17 \cdot 10^5$ | beige | $2.00 \cdot 10^5$ |
| brown | $1.30 \cdot 10^5$ | white | $0.77 \cdot 10^5$ |

But the number of microorganisms on the fiber is not a direct reason of its biostability (Kremer, 1987).

Among the microorganisms-destructors which are a part of flora separated from samples of cotton fibers epiphytic sporous bacterium of the *Bacillus subtilis* group prevail. The composition of microfunguses is small and quite conformed (Table 3).

**Table 3.** Mushroom micromycetes separated from control samples of cotton fibers

| Cotton fibers | Mushroom micromycetes |
|---|---|
| green | *Aspergillus niger, Penicillium chrysogenum* |
| brown | *Aspergillus niger* |
| beige | *Aspergillus niger, Penicillium chrysogenum, Paecilomyces variotii* |
| white | *Aspergillus niger* |

**The study of cotton fibers' biostability, of various colors and selections**

The results of the biostability research of cotton fibers upon storage in various temperature-humidity modes are presented in Table 4. Fig. 2 presents a diagram of the destruction indicator changes, which demonstrates the speed of cotton fiber destruction in various storage conditions.

**Table 4.** Biological damageability of cotton fibers under various storage conditions

| Self-color of cotton fibers | Exposing period and conditions | | | | | |
|---|---|---|---|---|---|---|
| | 2006, 30 days, temperature 26–30 °C humidity 90–100% | | 2006–2016, 10 years, temperature 18–22 °C humidity 60–65% | | 2016, 30 days, temperature 26–30 °C humidity 90–100% | |
| | Fiber destruction indicator, K | | Fiber destruction indicator, K | | Fiber destruction indicator, K | |
| | Initial fiber, $K_0$ | Exposed fiber $K_n$ | Initial fiber, $K_0$ | Exposed fiber $K_n$ | Initial fiber, $K_0$ | Exposed fiber $K_n$ |
| green | 0.23 | 3.11 | 0.23 | 0.68 | 0.68 | 3.58 |
| brown | 0.22 | 1.59 | 0.22 | 0.57 | 0.57 | 2.24 |
| beige | 0.18 | 0.42 | 0.18 | 0.30 | 0.30 | 1.66 |
| white | 0.14 | 0.47 | 0.14 | 0.41 | 0.41 | 2.33 |

The composition analysis of initial the self-colored fibers (2006) reveals the presence of overgrowing by microorganisms on all models and (in certain cases) an insignificant streakiness (cracks, deepened surface). Value of a destruction indicator for these fibers is $K \leq 0.3$ that shows initial changes of a fiber surface which do not affect its internal composition. After exposing the cotton fibers, an increased total number of damages and the destruction indicator are revealed at all studied fibers. The green fiber was most severely damaged ($k = 3.11$).

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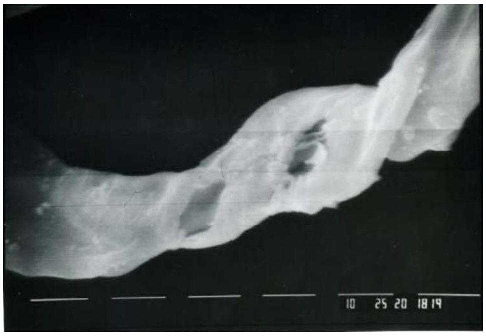
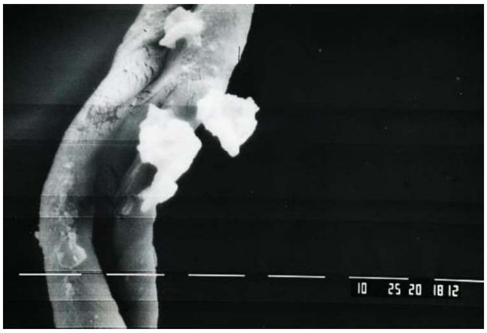
After exposing the cotton fibers in conditions, favorable for microorganisms' development, during 30 days, an increased total number of damages and the destruction indicator are revealed at all studied fibers. The green fiber was most severely damaged ($k = 3.11$). The values of the destruction indicator of this fiber are close to the limit, characterizing the transition to the third qualitative gradation characterizing deep biological destruction at all compositional fiber levels (Fig. 1).

Microscopic examination of samples shows damage, such as destruction of the wall; almost all the fibers have overgrowing. The brown fiber (k = 1.59) has wall damages. Beige and white fibers have high biostability under these conditions: the values of the destruction indicator do not exceed 0.47 (7 times weaker than the green fiber).




a) overgrowing, blotch, deep cracks

b) damage of the wall




c) overgrowing and blotch

d) cracks and wall damage

**Figure 1.** Damages of cotton fibers with microorganisms.

Judging by biological destruction the self-colored cotton fibers stored in conditions, optimum for development of microfunguses and bacterium it is possible to arrange the following row: green > brown > white > beige. In comparison with the initial fiber, the green fiber is damaged 13.5 times stronger, and the beige one is damaged only by 2.3 times.

The research of the nature and the degree of damages of the cotton samples stored in normal conditions (air temperature is 18–22 °C, relative humidity of air is 65%) within 10 years (2006–2016) allows to establish the following. The green (k = 0.68) and the brown (k = 0.57) fibers were most severely damaged, there are damages like swelling, stratifications; the beige fiber had only overgrowing and a weak streakiness; the biological destruction indicator for it has remained at the level of boundary values of the beginning of damage (k = 0.3).

Judging by cotton fiber destruction stored in normal conditions within 10 years, it is possible to arrange the following row: green > brown > white > beige, green and white fibers are damaged 3 times more severely, and beige one only by 1.7 times.

With the increase of relative humidity of air up to 90–100% and temperature up to 26–30 °C, the damage rate of fibers suddenly increased: the white one increased almost by 7 times, the beige and green ones increased more than by 5 times, and the brown one increased almost by 4 times. The white cotton has all types of damages: severe streakiness, swellings, the damaged walls, stratifications. The beige and green fibers demonstrate very strong overgrowing and clump of biomass, strong stratification. Brown fiber has only punctate overgrowing, separate swellings and wall damages.

The results of the analysis of infrared ranges of absorption of the studied fibers will be coordinated with the data of the microscopic analysis. The beige fiber has the amorphisation of composition connected with increased hydrophilicity of a sample (area 1,630 of $cm^{-1}$) and increased proteinous weight due to the growth of microorganisms (the presence of peptide groups in the field of 1,640–1,660 $cm^{-1}$ and 1,540–1,560 $cm^{-1}$). The brown and white fibers are characterized by deep destruction: a large number of ruptures of molecular chains (1,720–2,900 $cm^{1}$) and proteinous weight (increased absorption in the field of 1,540–1,560 $cm^{-1}$). The green fiber has the stronger compositional changes: increased content of carboxyl groups in comparison with the initial one. It demonstrates oxidizing destruction of cellulose, deep amorphisation of fiber as a result of a rupture of the main macromolecules.

The results of the research of dynamics of change of the biological destruction indicator are coordinated with the general damage tendency of cotton fibers by microorganisms (Fig. 2).

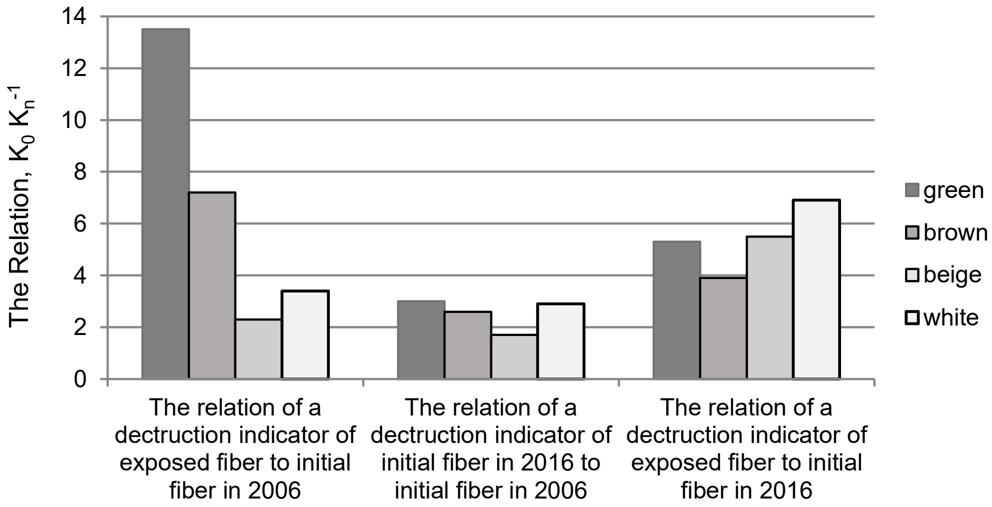



**Figure 2.** Changes in the cotton fibers' destruction indicator in various storage conditions; $K_0$ – the initial fiber destruction indicator (start of storage period); $K_n$ – the exposed fiber destruction indicator.

The analysis of the data of Fig. 2 shows that at exposition of fibers in 2006 green fiber has the largest damage speed by microorganisms, brown one is a little smaller. In beige and white fiber, the rate of change in biodegradation speed is the lowest for the present period.

The change in the structure is due to the presence of amorphous sites in cotton fiber, where the thinnest capillary is the penetration channel of microbial enzymes deep into the cellulose composition (Kotomenkova, 2012).

However, at repeated exposition after 10 years in 2016 the damage intensity of all fibers increases approximately equally highly in connection with reclamation of new surfaces by microorganisms, and deepening the biological destruction processes (transition of damages by classes A and B into damages by classes B and C respectively).

## CONCLUSIONS

The obtained results lead to the conclusion that self-colored cotton doesn't possess anti-microbe qualities in relation to disrupting microorganisms. However, cotton with self-colored beige fiber cause the decrease of growth intensiveness for particular microfunguses, such as: Aspergillus terreus, Penicillium variabili, Penicillium cyclopium, Penicillium chrysogenum, Chaetomium globosum. The similar activity in relation to Aspergillus terreus is determined for all cotton fibers studied in the paper. The discovered fungistatic activity of beige cotton fiber agrees with the results of its biostability evaluation. This kind of cotton demonstrated the greatest stability against the activity of destructing microorganisms among the studied samples.

Microorganisms included into microflora composition of the studied cotton fibers do not show any species diversity; they are known as disrupting microorganisms for cellulosic materials. The micromycetes isolated from the samples are included into a standard set of species which are used in tests for funginertness.

From cotton fibers of various colors a different number of microbe cells was isolated. Their biggest number was stated in cotton of a natural green color. Green cotton fiber has also the greatest biodamage degree among all studied cotton samples according to the results of biostability testing.

The received results demonstrate preservation of flora viability on cotton fiber during keeping it for a long time in normal conditionns without additional power supplies. Biological destruction of cotton fibers takes place under the normal storage conditions too. Increased temperature and humidity of air necessary for the growth of microorganisms lead to increased processes of fiber destruction. It is established that the damage degree of cotton depends on the modes and the periods of storage and on color of fiber.

The received results can be used in selection of nature-colored cotton, decreasing losses in the process of storage of cotton fiber, improving properties of the produced textiles when modeling processes of biological damageability.

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