

Original Paper



## Fungi associated to lint and cotton seeds with fuzz

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### Abstract

In the industrialization process of cotton lint to produce quality fabrics, the fiber needs to present extrinsic and intrinsic characteristics that enable it to produce high quality yarns. The intrinsic characteristics are genetic attributes while extrinsic define the degree of purity of the product and it is result of the production system, especially the harvest and storage. During this stage, the fiber is exposed to physical and biological agents that cause loss in quality with emphasis on fungi, important contaminants. The contamination can be caused by boll rot symptoms at maturity and induce the degradation of cellulose fiber and change in the color that reduce the quality. The purpose of this research was to identify the fungi associated with cotton lint and seeds with fuzz from growing areas of Brazil and some countries. Samples of lint from the United States, Ivory Coast, Greece, Turkey and Cameroon and cotton seeds from Rio Grande do Norte, Mato Grosso, Bahia, Goiás and Ceará were subjected to humid chambers in Petri dishes during 48 h. In order to identify the fungi associated to samples, it was isolated in culture medium of Malt Extract Agar. The samples from United States and Tukey presented more contamination. The main fungi associated with lint were *Fusarium* spp, *Penicillium* spp, *Phomopsis* spp, *Aspergillus niger* and *Rhizopus* sp. Seeds with fuzz represents an important way of spread of pathogens.

**Key-words:** Pathogen, Fiber, Contamination, Storage

### Introduction

Cotton is one of the vegetable fiber more cultivated and older of the world. The earliest references record their cultivation a few centuries before Christ. In Brazil, little is known about the history of this plant from the malvaceae family. It is known, however, that by the time of the Brazil discovery the natives already cultivated cotton and transformed it into threads and fabrics (Canechio Filho et al., 1972; Galeano, 1999). In the colonial period the colonizers promoted the planting in hereditary captaincies, but with exclusively homemade and subsistence production (Rodrigues, 2015).

As a commodity, cotton is one of the fiber group products of major economic importance, due to the quantity and value of production. Its cultivation is also of great social importance, due to the number of direct and indirect jobs in its chain (Richetti and Melo

Filho, 2001; FAO; ABC, 2017). Almost the entire plant is used economically, especially fiber and seed. Fiber has multiple and varied applications, providing to the fabrics characteristics that are difficult to reproduce by synthetic yarns such as softness, lightness, beauty absorbency and freshness (Gridi-Papp et al., 1992) and is recognized as the most important of textile fibers due to its technological qualities and its wide use. Despite this, its consumption has been falling due to the increase in the consumption of synthetic fibers driven by the drop in the price of oil barrel. Even so, it represents 21% of the world consumption of textile fibers and about 50% of the consumption of these fibers in Brazil (Ruiz, 2017). The world production in 2014 was 26 million tons of lint (FAOSTAT, 2017), while in the 2018 crop it should be around 24 million tons, with China being the largest producer (ICAC, 2017). Brazil is one of the world's largest producers of this

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fiber, with a planted area of about 1.500 million of ha, where about 2.4 million tons of lint are produced (CONAB, 2019).

Among the main industrial applications of cotton fiber we can mention the manufacture of yarns for weaving of several kinds of fabrics, production of hydrophilic cotton for medical and nursing activities, making of felt, blankets and upholstery and obtaining cellulose (Correa, 1989).

For feather industrialization and quality fabrics, the fiber needs to have intrinsic characteristics that enable it to produce high quality yarns and be fully adaptable to the modern spinning mills used in the world's textile industries. Among the intrinsic characteristics of the fiber can be strength, length; uniformity of length, maturity, fineness, strength and elongation (Santana et al., 1999).

Intrinsic traits constitute genetic attributes. Significant changes in its expression result from the influence of adverse environmental factors or inadequate crop management during the season. On the other hand, extrinsic characteristics define the purity of the product. They result from crop management from the opening of the bolls to post harvest and storage procedures. During this period, fiber is exposed to different agents that may determine loss in quality.

Factors that determine loss in fiber extrinsic quality include the lint conditions during processing, short fiber index, neps, stickiness, external contaminants such as chicken feathers, animals hair, synthetic fibers such as polypropylene and polyethylene improperly used to store the product during the hand picking, or natural fibers such as jute and sisal normally used to tie the bags, as well as fungi and other microorganisms that degrade cellulose.

Contamination by microorganisms may occur in the field when many of the fruit rot pathogens migrate to the fiber and may develop in it. Damage can be greater when cotton is not harvested in the right time and is exposed to environmental conditions that are favourable to development of pathogens, especially extended periods of high humidity and rainfall (Hillocks, 1992).

This paper aimed to evaluate the cotton lint from different producing regions of Brazil and the world and seeds with fuzz from the states of Ceará and Rio Grande do Norte, Brazil aiming to identify the

presence of contaminants fungi and seed-borne pathogens.

## Material and methods

The cotton lint samples were obtained from Embrapa Cotton's Fiber Laboratory, located in Campina Grande - PB, and were from the following producing countries: United States, Ivory Coast, Greece, Turkey and Cameroon and from the following Brazilian states: Rio Grande do Norte, Mato Grosso, Bahia, Goiás and Ceará (Table 1)

Five initial samples of 0.1kg were taken from each locality, which were transformed into a composite sample of almost 0.5kg and from it were taken 10 sub-samples of almost 0.01kg which were submitted to the humid chamber in Petri dishes with a diameter of 9cm for 48 hours, aiming to induce favorable conditions for the growth of possible fungi associated with them. After this time, the incubated samples were analyzed under a stereo microscope.

In order to identify fungi associated with the samples, after their presence had been verified, they were isolated directly using a thin-tipped stylet for Malt-Agar Extract culture medium, distributed in 9cm Petri dishes which were incubated at 28° C for 72 hours. From the initial growth of the fungi, subcultures were made in order to isolate the pathogens. After growth, the fungi were examined under a stereo microscope, from which slides were prepared for examination under a 100x magnification binocular microscope and morphological characteristics were compared to those found in the literature.

The incidence of the set of pathogens in the samples was determined as well as the incidence of each pathogen alone in the samples of the different origins. With the objective of verifying if seeds with fuzz obtained from the processing of seed cotton from the states of Rio Grande do Norte and Ceará could become a way of dissemination of important pathogens, the sanitary analysis of seeds from those states was performed. It was decided to analyze only these samples because the other states studied in this research use certified seeds without fuzz which are treated with fungicides in the cotton crop, whereas in the semiarid region of Brazil are used seeds with fuzz and without chemical treatment. Samples from other countries were not considered because they are not used for multiplication in Brazil. The sanitary analysis of the seeds was performed using the Blotter

Test (Neegaard, 1979), using 200 seeds in four replications of 50 seeds, which were distributed at 10 seeds per plastic box. After the incubation period, the seeds were evaluated under a stereo microscope and when pathogens associated with them were observed, slides were made for examination under the optical microscope to identify their genus or species.

## Results and discussion

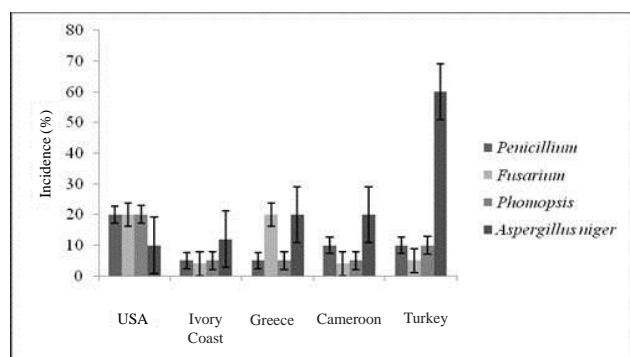
The samples from the United States and Turkey were the ones with the highest incidence of feather-associated pathogens, both with 60% (Table 1).

These incidence level are not associated with the intrinsic quality of cotton produced in the exporting country, but mainly with the conditions of transport and storage. High humidity and higher warehouse temperatures, in addition to lack of appropriate aeration, can induce the growth of lint degrading fungi in these locations.

**Table 1.** Incidence of pathogens in cotton lint from different countries.

Country	Incidence (%)
United States	60,0
Ivory Coast	5,0
Grece	20,0
Cameron	40,0
Turkey	60,0

The main fungi associated to lint in the sample from the United States were *Fusarium* spp, *Penicillium* spp and *Phomopsis* spp. The three genera presented an incidence of 20% in the samples (Figure 1).



**Fig. 1.** Genera of pathogens in cotton lint from diferent countries. \*The bars on the graph represent the standard deviation of the mean

Marsh and Bollenbacher (1949) pointed out that fungi of the genus *Fusarium* are the most common found in association with cotton lint in the United

States. This research confirms these results because the incidence of 20% of samples from that country were contaminated with this fungus. One the other hand, *Penicillium* spp. was found at higher levels in lint samples from that country. This genus is reported by Marsh and Bollenbacher (1949) as one of the most frequent in american cotton samples studied by them.

Samples from Turkey had a 60% of *Aspergillus niger* contamination. This pathogen is one of the most important found associated with lint in the world. According to Lagi re (1974), *A. niger* was one of the most present among 39 species found in lint from different countries in Africa, Latin America and Asia. This specie was also present in samples from Cameroon and Greece, although to a lesser level than those from Turkey showing the cosmopolitan character of the fungus when associated with cotton lint.

In Iran, Mirzaee et al. (2013) found *Fusarium semitectum* and *Nigrospora oryzae* associated with cotton lint. They also identified *Exserohilum rostratum* as a new causal agent for cotton bolls and lint rot. In addition, *A. niger* and *Rhizopus* spp. were often isolated from lint, even before harvesting. A wide variety of fungi can associate with cotton lint and can cause fiber degradation. Lagi re (1974) in a literature review work listed 95 species of cotton lint associated fungi.

The most pathogens that affect cotton bolls can also cause lint damage. If cotton is not harvested at the appropriate time and remains exposed to unfavourable environmental conditions, especially rainy and high relative humidity for extended periods, the growth of pathogens on the lint may be sufficient to cause changes in the color.

Most fungi that occur as lint contaminants produce cellulolytic enzymes (Hillocks, 1992). Under favorable conditions for fungal development for long periods before harvesting, they may induce cellulosic fiber degradation still in the field, which may result in damage to the industrialization process. One of the problems that can worsen with field contamination is the short fiber index. According to Freire (2015), this is related to the number of pre-cleaning performed in the cotton processing. In addition to the dirt inherent in the development of lint fungus, cellulose degradation can increase the number of fiber breaks in the pre-cleaning process prior to cotton processing. According to Hillocks (1992), among the most common fungi found in the lint is *Alternaria* species

such as *Alternaria alternata* and *A. macrospora*, *Nigrospora* sp, *Rhizopus* sp., *Aspergillus* sp and *Penicillium* sp, while Lane and Sewell (2006) pointed out that *Aspergillus niger* was the most common specie identified in association with cotton lint.

Even if there is no degradation of fiber cellulose, its darkening causes loss in the quality, resulting in lower market value for fiber. One of the pathogens that cause greater darkening of cotton fiber is exactly *A. niger*, due to the large amount of spores produced that infiltrate between the fibers in lint bales, inducing its darkening and consequent loss of quality.

Samples from Ivory Coast had low incidence levels of most fungi with *A. niger* as the predominant lint-associated specie. This fungus is often present in association with the lint worldwide. The association of fungi with lint has also been a concern for occupational health. Lane and Sewell (2006) evaluated cotton lint samples from 12 producing regions of the world and identified the same species found in this research indicating that they are prevalent. In addition to finding *Cladosporium* sp. and *Alternaria* sp. observed that *Aspergillus* was the most common genus and *A. niger* the most frequently found species. The authors pointed out that there is growing evidence that inhalation of fungal spores and their fragments and toxins can cause respiratory disease, particularly in indoor and industrial environments.

In the samples from Brazilian producing states, we found the same genera of lint-associated fungi as those found in samples from other producing countries, except *Phomopsis* (Figure 2).

It was found that the samples from the state of Rio Grande do Norte presented 100% of contamination. This means that, they were contaminated by at least one fungus species. In the present case *Aspergillus niger* was the fungus that induced the highest contamination in that state, being found in all samples. Also in Rio Grande do Norte, the fungus *Penicillium* sp. was present in 40% of the samples including in association with *A. niger* increasing the level of fiber extrinsic quality deterioration. *Penicillium* spp. was also the fungus most found in cotton samples from the state of Bahia. In this state, *A. niger* presented lower incidence, and was present in only 20% of the samples against 80% in the first. *A. niger* was the only fungus found associated with cotton from the state of Ceará, being present in 100% of the samples.

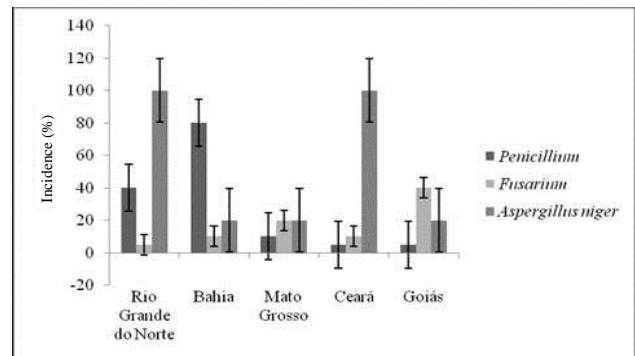


Fig. 2. Incidence of fungi in cotton lint from different producers states of Brazil.

\*The bars on the graph represent the standard deviation of the mean

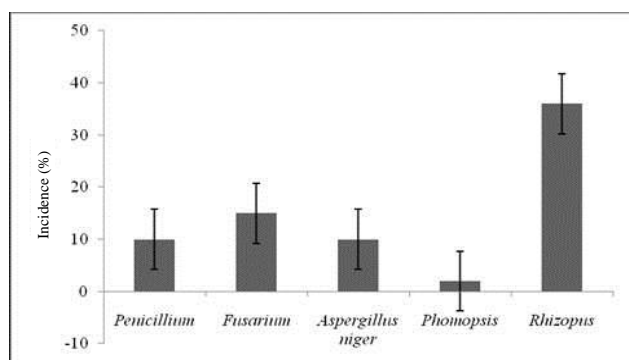
The states of Mato Grosso and Goiás were those with the lowest rates of lint contamination, whose maximum value was obtained by *Fusarium* spp. in Goiás, contaminating 40% of the lint. *Fusarium* spp and *A. niger* were also present in Mato Grosso samples in 20% of the samples.

Three species of *Fusarium* are reported by Marsh and Bollenbacher (1949) associated with cotton lint. They are: *Fusarium moniliform*, *F. oxysporum* and *F. roseum*. In this work, the *Fusarium* species associated with lint were not defined; however an important feature is the frequent presence of this fungus in cotton samples from the Midwest states. This fact is attributed to the endemic presence of *Fusarium* in the region as a causal agent of damping-off. In all states of the region there are broad environmental conditions for the development of this pathogen (Goulart, 2005).

Based on these results, it can be seen that the samples from the Midwest states showed better extrinsic characteristics with respect to fungal contamination than the samples from the Northeast states. This phenomenon may be related to the fact that in the Midwest the technology used in the cotton production system is also applied in postharvest, where the processing and storage of the lint are performed under optimal conditions, thus reducing the possibility of fungal development on the lint. These data confirm the concerns expressed by Beltrão (1999) regarding the extrinsic characteristics of cotton from semiarid region of Brazil. According to the author, the improper storage conditions of the lint or cotton seed contribute decisively to the reduction of its extrinsic characteristics, inducing loss of product quality.

Regarding the sanitary analysis of seeds with fuzz, it was found that the samples from the state of Ceará had 36% incidence of *Rhizopus* spp, fungus more present in the samples. *Fusarium* spp. with 15%, followed by *Aspergillus niger* and *Penicillium* spp., with 10% were the fungi with the highest incidence level in the seeds. *Phomopsis* was present in only 2% of the seeds (Figure 3).

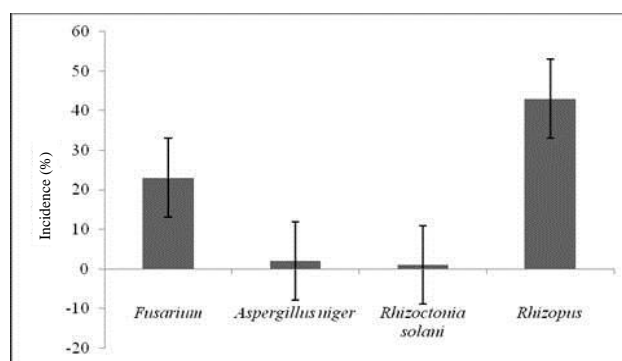
There was prevalence of *Fusarium* spp. in seeds from the semiarid region. This is an important fungus in view of its ability to cause damping-off. In this sense, the use of seeds with high levels of *Fusarium* incidence may mean increased incidence of damping-off for which the treatment of seeds with fungicides is recommended, which in this case would be impaired by the presence of the fuzz. Therefore, the seed with fuzz becomes an important vehicle for the dissemination of *Fusarium* fungi in the field, which may cause damage due to the loss of the initial stand and the need for replanting.



**Fig. 3.** Incidence of fungi in cotton seeds with fuzz from Ceará State Brazil.

\*The bars on the graph represent the standard deviation of the mean

Seed samples from the state of Rio Grande do Norte also showed an incidence of *Fusarium* spp. (23%) in indexes higher than those presented by seeds from the Ceará state. However, the diversity of fungi genera associated with them was smaller compared to the first one. It is important to observe the high incidence of *Rhizopus* spp. (43%). *Rhizoctonia solani* and *A. niger* were also found in low incidence (Figure 4).



**Fig. 4.** Incidence of fungi in cotton seeds with fuzz from Rio Grande do Norte State of Brazil

\*The bars on the graph represent the standard deviation of the mean

Therefore, it was verified that both seeds from the state of Ceará and Rio Grande do Norte, presented incidence of fungi potentially causing damping-off. In the case of Rio Grande do Norte, it is important to point out that, besides the presence of *Fusarium* spp., there was incidence of *R. solani* associated with seeds. Therefore, it is important that preventive measures such as seed treatment are taken, especially in those regions where environmental conditions are favourable to the occurrence of damping-off.

It is important to point out that *R. solani* can survive in soil or in the seeds. The inoculum carried through the seeds may be responsible for pre- or post-emergence damping-off, and may cause root rot. On the other hand, seeds usually deteriorate in the presence of pathogens. Even without inducing damping-off, seed-associated fungi can reduce their physiological quality, which will result in less vigorous plants and possible losses in germination power with consequent reduction of the final crop stand.

In this work it was verified that the seeds with fuzz, although still being used in commercial cotton areas in the semi-arid region of Brazil, may be an important way for the dissemination of inoculum of pathogens that cause damping-off. It is recommended that the cotton grower of the Brazilian semi-arid region use seeds without fuzz, submit the seeds to the sanitary analysis in order to identify possible associated pathogens, in order to prevent their spread and to establish preventive measures such as seed treatment aiming at the control of these pathogens.

## Conclusion

The fungi presented as contaminants of the lint from different producing countries, representing as a

potentially important for its degradation;

The cotton seeds were infected with fungi that can determine the loss of viability during storage and by *Fusarium* species that are potential cause of damping-off and wilt.

It is important to make the seed treatments to avoid initial diseases to reduce stand losses and spread of pathogens potentially important.

**Conflict of interest:** All authors declare no conflict of interest.

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