



Isolation and Screening of Chilli Pepper for Fungal Contamination and Aflatoxin Production

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i930462

Editor(s):

(1) Dr. Hon H. Ho, State University of New York, USA.

Reviewers:

(1) Anjana Pradhan, Govt of Sikkim, India.

(2) Sherifa Mostafa M. Sabra, KSA.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/68528>

Original Research Article

Received 05 March 2021

Accepted 11 May 2021

Published 12 May 2021

ABSTRACT

Improper postharvest management of red chilli pepper results in the invasion of *Aspergillus* spp. It is the most common pathogen that infects chilli resulting in the production of aflatoxin - the most potent biological toxin. The present study was aimed to study the occurrence of naturally occurring fungal pathogens in the commercially available chilli pepper in India. About twenty samples were collected from retail stores and commercial markets of Tamil Nadu, India. Isolation and purification of the naturally occurring fungal pathogens were carried out using a potato dextrose agar medium. Results showed that the samples were contaminated with *Aspergillus flavus*, *A. parasiticus*, and *A. niger*. Scanning electron microscopy (SEM) was done to morphologically confirm the pathogens. SEM analysis also showed the internal structural damage caused by the pathogens. Followed by the isolation, all the samples that contained *A. flavus* were tested for aflatoxin production using the thin layer chromatography (TLC) method. It was found that a total of 16 chilli samples were tested positive for aflatoxin production. It could be seen that the tropical climatic conditions of India increased the probability of aflatoxin production in the chilli pepper.

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Keywords: Chilli pepper; fungal contamination; *Aspergillus spp.*; TLC; aflatoxin.

1. INTRODUCTION

Chilli is one of the most commonly consumed spices in the world, known for its pungency, anti-inflammatory and anti-bacterial properties. India is the leading country in the production of chillies contributing 41.11% of the world's production with around 55.11% of the total vegetable cultivated area. India is also the largest consumer and exporter of dry Chilli. About 20% of produced vegetables are lost due to spoilage by pathogenic contamination [1]. Common pathogens that cause spoilage of harvested chilli are *Aspergillus flavus*, *A. parasiticus* and *A. niger*. Among these mentioned pathogens, *A. flavus* is the commonly occurring pathogen in chilli pepper that has the tendency to produce secondary metabolites called aflatoxins. The aflatoxins are the most potent naturally occurring mycotoxins and are a huge health concern due to their high toxicity as manifested by their lowest tolerable daily intake (TDI) compared with that of other known mycotoxins [2].

Aflatoxin production by *Aspergillus spp.* was highly reported in tropical countries. Pre-harvest stress caused by poor agricultural practices like irregular irrigation/drought conditions, and poor postharvest practices like improper drying, poor transportation and storage of harvested chilli are major causes of *Aspergillus spp.* contamination. Aflatoxins B₁, B₂, G₁, G₂, M₁ and M₂ are different types of aflatoxins with varying degrees of toxicity. Among these, Aflatoxin B₁ is of particular importance, as it is highly carcinogenic [3] and classified by the International Agency for Research on Cancer (IARC) as a class I carcinogen [4]. Hence, it is necessary to screen the harvested chillies for the presence of aflatoxins to avoid health implications and economic loss. Several chromatographic methods are available for the screening and detection of aflatoxins. The traditional thin layer chromatography (TLC) method is considered a powerful screening tool for the presence of aflatoxins. Hence, the present study was conducted to understand the occurrence of naturally contaminated fungal pathogens in the commercially sold chilli samples. The samples were also tested for the presence of aflatoxin production by the pathogens.

2. MATERIALS AND METHODS

2.1 Materials

Red chilli samples were collected from retail and commercial stores of Tamil Nadu and stored in Ziplock bags until further analysis. Potato dextrose agar, TLC plates, aflatoxin B₁ standard, chloroform, acetone, sodium hypochlorite, ethanol and methanol were procured from sigma chemicals for the analysis.

2.2 Screening of Samples for Fungal Contamination

Chilli samples collected from retail stores were sterilized using 1% sodium hypochlorite followed by 80% ethanol. The sterilized samples were pat dried with filter papers. After sterilizing the samples, they were plated in the potato dextrose agar medium and incubated for 48 h (Fig. 1). Agar block containing different species was then aseptically transferred into new plates for purification and screening [5].

2.3 Scanning Electron Microscopy Analysis

A scanning electron microscope was used to study the morphological changes in chilli pepper (Model: Quanta 250, FEI, Asia). The samples were sputter-coated with gold to avoid sample damages during analysis [6].

2.4 Aflatoxin Production

The samples that contained *A. flavus* were tested for the production of aflatoxin using the TLC method [7]. Methanolic extract of chilli pepper was prepared by grinding 10 g of whole dry chilli with 50 ml of sterile water and filtered through two layers of muslin cloth. This extract was used for TLC. Chloroform and acetone (9:1) were used as the developing solvent. The solvent was taken to completely cover the bottom of the chamber to a depth of approximately 0.5 cm. The chamber is covered to prevent evaporation of the solvent. The chilli extract was placed on the TLC plate using a Pasteur pipette against the aflatoxin standard. The loaded TLC plate was placed in the TLC chamber with the sample line towards the bottom and the chamber was covered. When the solvent reached 1/3rd length of the plate, the plate was removed and the chromatogram was viewed under UV light at 365 nm [7].



Fig. 1. Plating of chilli samples in potato dextrose agar medium

3. RESULTS AND DISCUSSION

The number of samples contaminated with the fungal pathogens is presented in table 1. It was found that all the samples tested were contaminated with *A. flavus* and *A. niger*. Almost 6 samples had *A. parasiticus* contaminations in them. Drought stress results in the reduction of the plant's natural defence mechanism, making it prone to *Aspergillus spp.* infection and increases the production of the secondary metabolite aflatoxin by *Aspergillus spp.* [8,9]. Aflatoxin

production by *Aspergillus sp.* was reported in locations with high temperature (25-42°C), dryer climate (with humidity of 60%) to wetter climate (with humidity of 85%) [10,11].

SEM analysis to study the morphology of the *Aspergillus spp.* in the chilli pepper was performed. The morphology of *A. flavus* with the conidia is presented in Fig. 2. The growth of pathogens in the chilli samples resulted in damage of the internal chemical structures of the samples (Fig. 3).

Table 1. Number of samples contaminated with selected fungal pathogens

Pathogen	No. of samples
<i>A. flavus</i>	20
<i>A. parasiticus</i>	6
<i>A. niger</i>	20

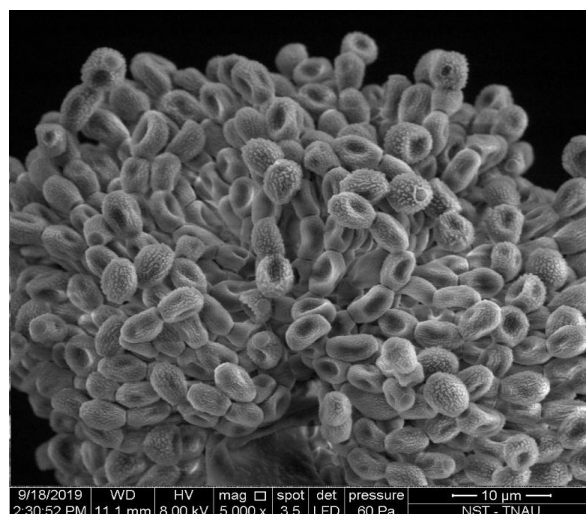


Fig. 2. SEM imaging of *A. flavus* in chilli pepper

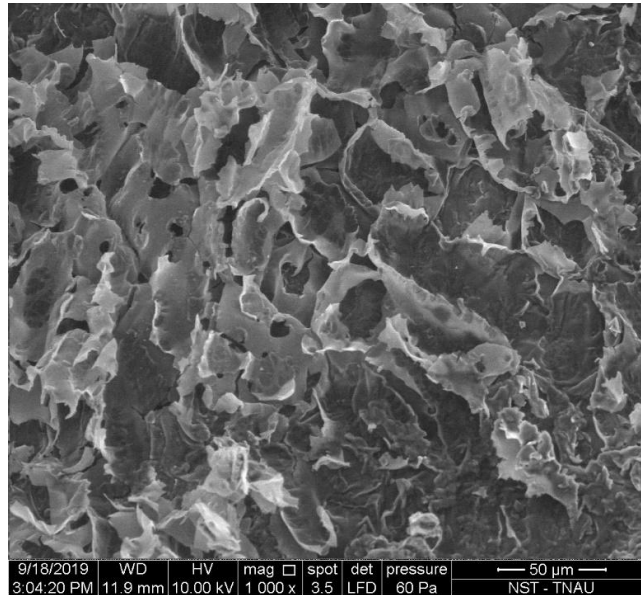


Fig. 3. Structural damage caused by *A. niger* in chilli pepper

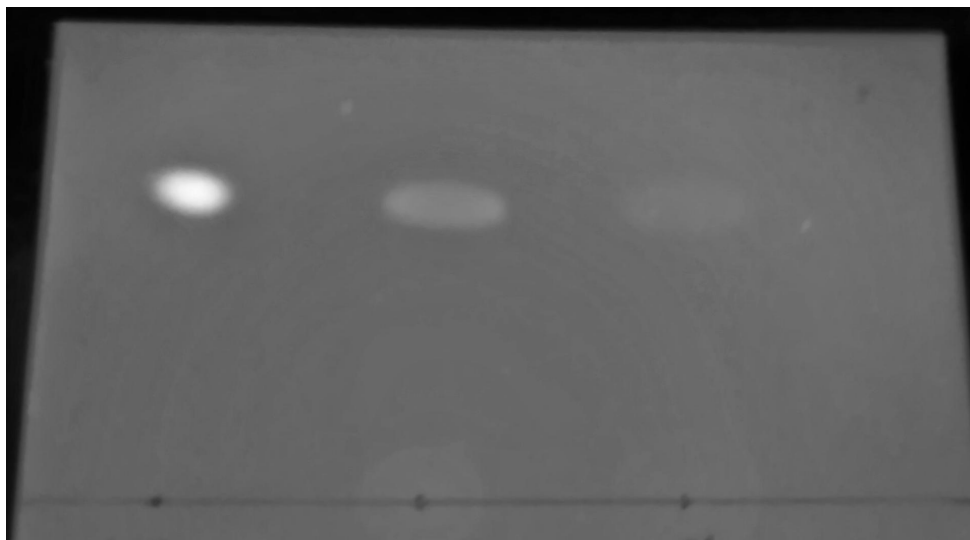


Fig. 4. Aflatoxin B₁ screening using TLC

Under favourable environmental conditions of tropical temperature (24 - 43°C) and relative humidity (60-85%), *A. flavus* produce secondary metabolites called aflatoxins [10]. Aflatoxins are the most toxic and common mycotoxins in food and feed [12]. *A. flavus* produces aflatoxin B₁ and B₂, whereas *A. parasiticus* isolates produce aflatoxin G₁, G₂, M₁, B₁, and B₂. *A. flavus* produces a number of airborne conidia and propagules that infect plants [13]. Changes in environmental temperature influence the expression levels of regulatory genes (aflR and

aflS) and aflatoxin production in *A. flavus* and *A. parasiticus* [14–16]. Hence, India being a tropical country, it is important to understand the ability of the pathogens in chilli samples to produce aflatoxins. Thin layer chromatography (TLC) is among one of the oldest techniques used for aflatoxin detection [17]. The higher sample throughput of TLC suggests that it is one of the quickest, simplest and most effective analytical techniques and is most suitable for the screening routine which is usually important in the duration of the storage and exchange of commodities

such as foodstuffs and feedstuffs etc [18]. In the present study, all the samples tested contained *A. flavus* and hence it was assumed that aflatoxin was produced by *A. flavus*. No tests were performed to identify the fungal source for aflatoxin. TLC was used to screen the presence of aflatoxin irrespective of its production by *A. flavus* or *A. parasiticus*. TLC analysis followed by the pathogen screening showed that 16 out of 20 samples were contaminated with aflatoxin B₁ (Fig. 4). Fast screening of the pathogens using TLC will help in sorting and reducing the aflatoxin development in the produces.

4. CONCLUSION

The results showed the presence of *Aspergillus spp.* in the chilli samples collected from retail stores of Tamil Nadu, India. All the samples tested contained *A. flavus* and *A. niger*. TLC confirmed the production of aflatoxin by the naturally occurring *A. flavus* present in the chilli samples. Sorting and reducing the aflatoxin contaminated samples is highly important to avoid spreading in the value chain. Creating awareness as a public health issue, surveillance, following good agricultural practices and proper postharvest practices will help in aflatoxin mitigation.

ACKNOWLEDGEMENT

This study was funded by the School of Post Graduate Studies, Tamil Nadu Agricultural University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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