

Full Length Research Paper

Aflatoxin B₁ production in chillies (*Capsicum annuum* L.) kept in cold stores

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An attempt has been made to isolate and enumerate the mycoflora invading chillies kept in cold storage since May, 1999. Chilli pods were collected from the cold stores at monthly intervals for a period of one year between December 2002 to November 2003. The incidence of molds on unsterilized as well as surface sterilized samples was recorded. Species of *Aspergillus* found to be dominant on stored chillies were screened for antimicrobial activity. The toxins from *A. flavus* and *A. niger* exhibited antibacterial and antifungal activity. Incidences of *Alternaria alternata*, *Fusarium* spp. and *Mucor* spp. was low on stored chillies when compared to *Aspergillus* spp. Chilli seeds aseptically collected from the pods were also tested for mycoflora. Natural occurrence of aflatoxin B₁ in chilli pods kept in cold storage was tested. Results from HPLC analysis revealed that the samples were contaminated with aflatoxin B₁ to the extent of 5.5 µg Kg⁻¹.

Key words: Chillies, aflatoxin B₁, cold storage.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the important crops of India. Guntur region in Andhra Pradesh is the largest chilli producing area in India. The use of cold stores for chilli storage has now become almost a general practice among farmers. After harvest, the dried chillies were kept in cold stores as the produce fetches premium price due to excellent retention of the colour. During storage, chillies may be infected with molds. Many agricultural commodities such as cereals, oil seeds, spices, dry fruits and feeds have also been reported to be contaminated with the toxins produced by molds (Jelinek et al., 1989, Vasanthi and Bhat, 1998, Reddy et al., 2001). Aflatoxins are toxic metabolites elaborated by *Aspergillus flavus* and *A. parasiticus*. These toxins are highly carcinogenic and elicit a wide spectrum of toxic effects when foods and feeds contaminated with aflatoxins were ingested (Peshta and Bonday, 1990).

As chillies are often preserved in bulk in cold stores, an

attempt was made to determine the incidence of molds and natural contamination of aflatoxin B₁ in chillies kept in cold storage.

MATERIALS AND METHODS

Chilli samples kept in cold storage since May, 1999 were used. Samples were collected at monthly intervals for one year (December 2002 – November 2003). One set of samples was surface sterilized with 4% sodium hypochlorite for 2 min, rinsed thoroughly with sterilized distilled water. These pods were blotted to dry and placed on Czapek- Dox agar plates (surface sterilized samples). Another set of samples was rinsed with sterilized distilled water, blotted to dry and kept on agar plates (unsterilized ones). The samples were incubated for seven days at room temperature and molds on chilli pods were recorded. In order to collect the seed, the pods were carefully dissected under aseptic conditions and the seed samples (unsterilized and surface sterilized) were placed on agar medium to isolate mycoflora.

Dominant fungi isolated from fruits and seeds were screened for toxin production by taking antimicrobial activity as the test criterion. *A. flavus* and *A. niger* were dominant on chilli pods were cultured on yeast extract sucrose broth for 30 days at 28±2°C. The culture filtrates were extracted with chloroform and concentrated extracts were tested for antimicrobial activity against *Bacillus cereus* (MTCC 430), *B. Subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC

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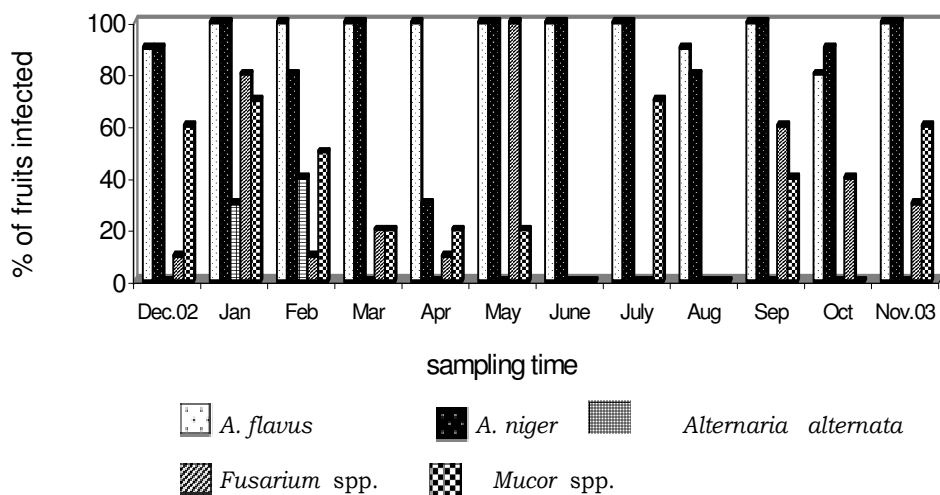


Figure 1. Incidence of molds on chillies (unsterilized) kept in cold storage.

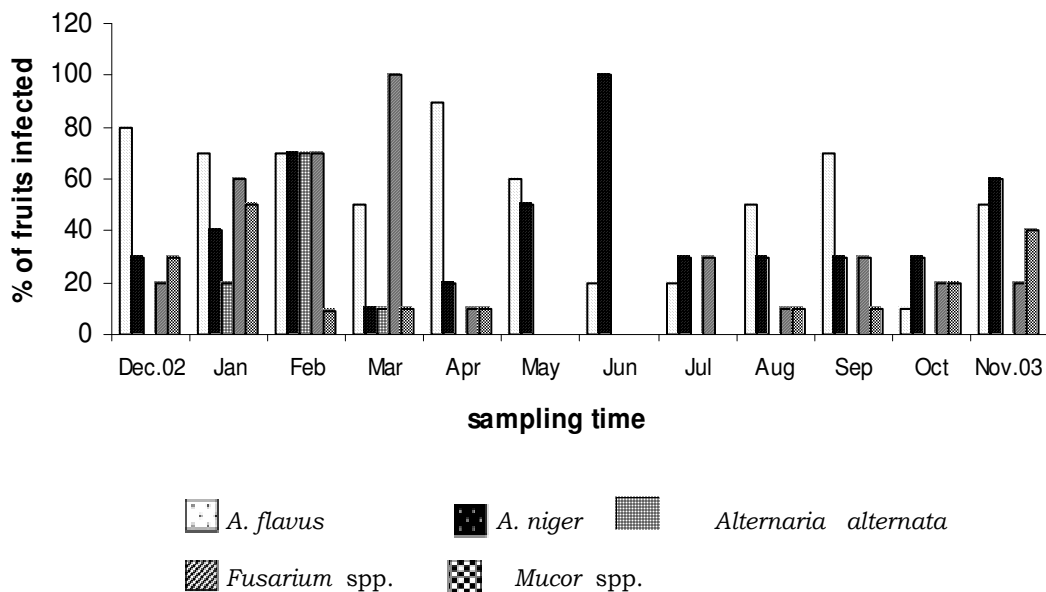


Figure 2. Incidence of molds on chillies (surface sterilized) kept in cold storage.

424), *Escherichia coli* (MTCC 40) and *Candida albicans* (MTCC 183). Chilli samples collected from cold store during Nov. 2003 were screened for the occurrence of aflatoxin B₁. Extraction and purification of toxin was done according to the method given by Madhyastha (1995). The toxin was analysed by HPLC based on the procedure suggested by AOAC (1984).

RESULTS AND DISCUSSION

The data on percent of chilli pods infected with various molds in unsterilized and surface sterilized samples are

presented in Figures 1 and 2, respectively. Mold incidence was high in unsterilized pods as compared to surface disinfected pods. In both the samples, incidence of *Aspergillus* spp. was very high. In many instances almost all chilli fruits placed on Czapek - Dox agar plates were contaminated with *A. flavus* and *A. niger*. Their incidence in both of the samples ranged from 10 to 100%. Fungi other than *Aspergillus* spp. infecting chillies include field fungi such as *Alternaria alternata*, *Fusarium* spp. and *Mucor* spp. Incidence of field fungi was low when compared to storage fungi such as *Aspergillus* spp.

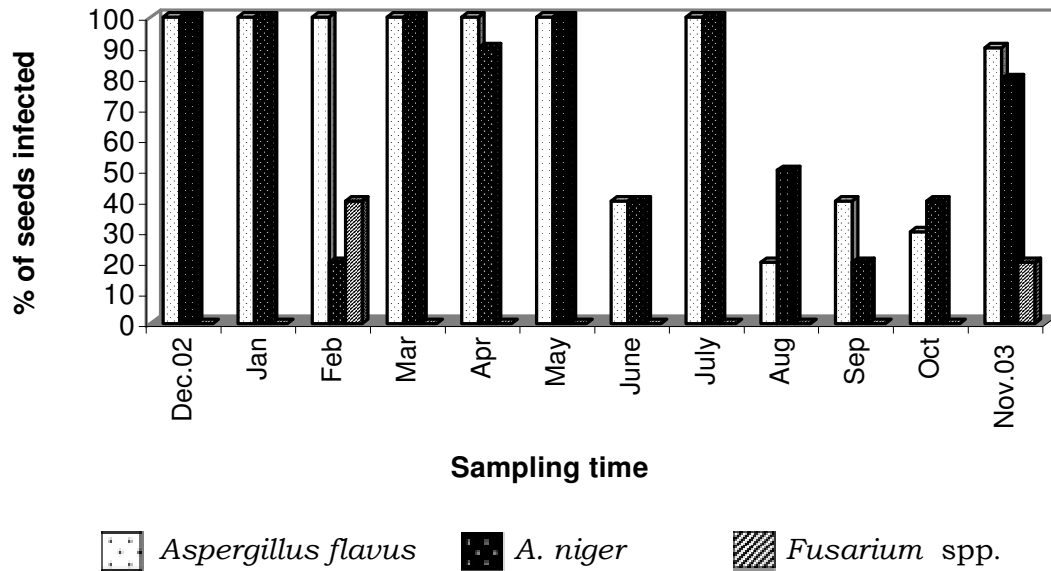


Figure 3. Incidence of molds on chilli seeds (unsterilized).

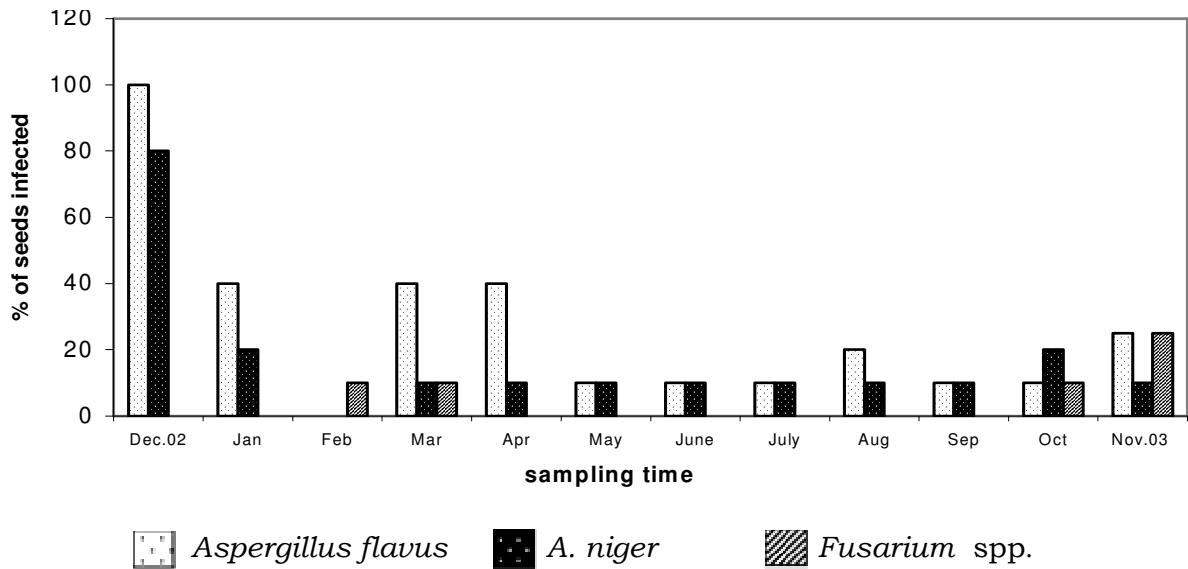


Figure 4. Incidence of molds on chilli seeds (surface sterilized).

The frequency of red pepper infected with *A. alternata* was less. The incidence of *Fusarium* spp. on stored fruits ranged from 10 to 100% on surface sterilized samples as well as unsterilized ones. The amount of fruit infection by *Mucor* spp. in unsterilized samples ranged from 20 to 70% while in surface sterilized ones it was 10 to 50%.

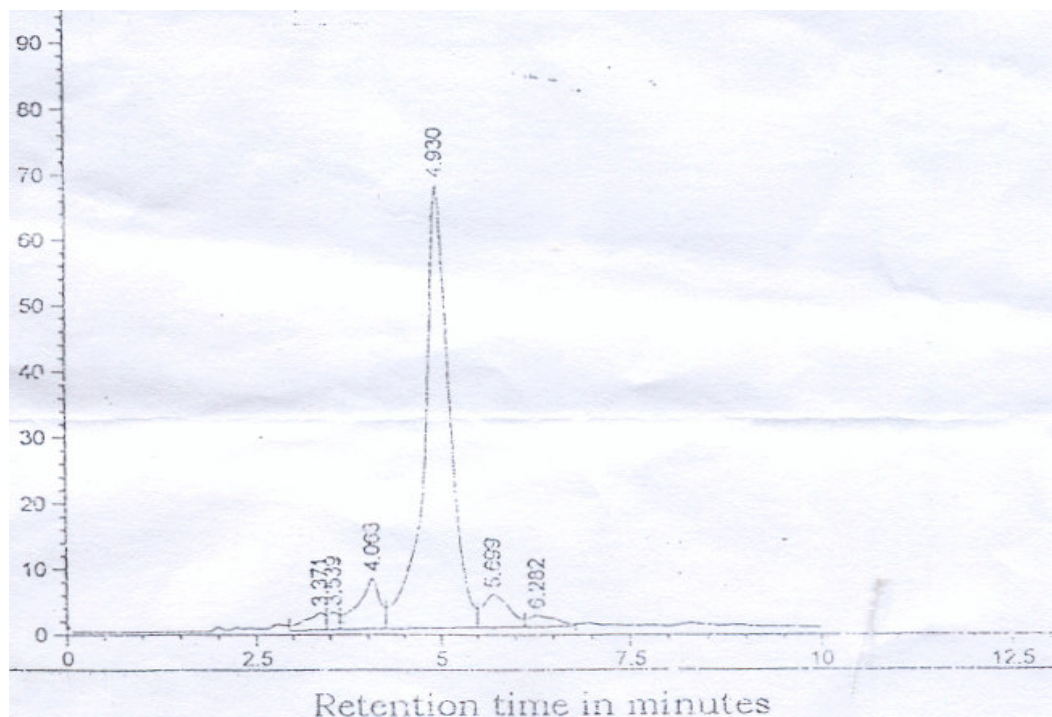
Data on mold incidence on unsterilized and surface sterilized seeds of chilli fruits kept in cold storage are presented in Figures 3 and 4, respectively. *Aspergillus* spp. were the predominant fungi on surface sterilized as

well as on unsterilized chilli seeds. Mold incidence was high on unsterilized seeds as compared to the surface sterilized ones. Incidence of *Fusarium* spp. was low on chilli seeds.

Results on antimicrobial spectrum of *A. flavus* and *A. niger* are presented in Table 1. All the test microorganisms exhibited sensitivity to the toxins elaborated by *A. flavus* and *A. niger*. *C. albicans* exhibited high sensitivity to chloroform extracts of *A. flavus* followed by *B. cereus*, *E. coli*, *B. subtilis* and *P.*

Table 1. Antimicrobial spectrum of Chloroform extracts of *A. flavus* and *A. niger*

Mould	Area of inhibition zone (mm ²)				
	<i>C. albicans</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
<i>A. flavus</i>	360.3	326.5	263.7	234.8	181.3
<i>A. niger</i>	263.4	234.4	207.3	75.3	134.3

**Figure 5.** HPLC chromatogram illustrating the analysis of aflatoxin B₁ in chilli pods kept in cold storage.

aeruginosa Toxins produced by *A. flavus* were relatively more toxic to the test microorganisms as compared to those of *A. niger*.

Chilli pods kept in cold storage since May 1999 were collected and analyzed for aflatoxin B₁ content by HPLC. Aflatoxin B₁ content in red pepper was detected from HPLC chromatogram using aflatoxin B₁ standards. The HPLC chromatogram is presented in Figure 5. The results clearly indicate that concentration of aflatoxin B₁ in chilli pods was 5.5 µg Kg⁻¹.

Storage fungi especially *A. flavus* and *A. niger* were found to be prevalent in stored chillies (Giridhar and Reddy, 1999). Fresh chillies as well as sundried chillies were infected with species of *Fusarium* and *A. alternata* (Adebanjo and Shopeju, 1994). The incidence of field fungi was low on stored products when compared to fresh fruits as suggested by Christensen and Kaufmann (1965). *Fusarium* spp. associated with chilli seeds were found to be toxigenic (Hashmi and Thrane, 1997). Basak et al. (1997) suggested that many of the *Fusarium* spp. associated with chilli seeds could cause fruit rot disease

in chilli. *Bacillus* spp. Have been reported to be highly sensitive to aflatoxins (Madhyastha et al. 1994), Chillies were frequently infected with aflatoxigenic *A. flavus* under storage conditions. Red chillies and chilli powder prepared from dried fruits could be naturally contaminated with aflatoxin B₁ (Reddy et al., 2001). In the present study, mold incidence on chillies kept in cold storage for few years and the extent of aflatoxin B₁ in those samples is reported. Since toxigenic *A. flavus* is found to be associated with chillies kept in cold storage, attempts should be made to prevent the mold attack and elaboration of mycotoxins by the molds. Periodical monitoring of mold incidence and aflatoxin B₁ content are very essential to increase the quality of the red pepper kept in cold stores.

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