



STUDIES ON PRODUCTION OF AFLATOXIN B1 IN CHILLIES (*CAPSICUM ANNUUM* L.) COLLECTED FROM COLD STORAGE AREA IN WARANGAL DISTRICT OF TELANGANA STATE, INDIA

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Abstract:

Aflatoxins are toxins produced by moulds in food commodities both before and after harvest. After harvest of the produce, if the chilli fruits are not properly dried, it leads to mouldy growth and subsequent aflatoxin production. Chilli pods were collected from the cold stores at monthly intervals for a period of one year between Feb 2019 to Jan 2020. With regard to influence of storage period on aflatoxin three (Teja, Wonder Hot, and U341) cultivars of chilli at Three months interval for a period from Feb 2019 till Jan 2020 varied to an extent of 11.0 g/kg content of Aflatoxin were recorded in Super market of Teja Variety. Among three cultivars, Teja varied in aflatoxin content from three months (2.5g/kg) to 12 months of storage (6.5gm/kg) interval. Similarly, cultivar U341e had the variation of 1.5 gm/Kg (three months) to 4.5 gm/kg (twelve months), at three month interval. Three varieties of Chilli Samples collected from Warangal grain market, Vegetable Market, Kiranam Shop and Super Market of Warangal district of Telangana state were analyzed for aflatoxin B1 content by Potato Dextrose Agar (PDA) method. The average aflatoxin content of samples from Super market had very high quantity of aflatoxin (9.50gm/kg) which was containing less than the permissible limit of 20 µg/kg. Similarly the samples from Warangal grain market had an average aflatoxin content of 11.0 gm/kg, much below the permissible limit.

Key Words: Chillies, Aflatoxin B1, Teja, Wonder Hot, U341, Estimation and Potato Dextrose Agar (PDA)

INTRODUCTION

Mycotoxins are from fungi which contaminate foodstuff (CAST, 2003; Vena^{ncio} & Paterson, in press). Aflatoxins are the most serious and well-known examples: Aflatoxin B1 is the most toxic. *Aspergillus flavus* may be the predominant producer of the B group. Furthermore, it is important to differentiate between natural contamination levels in crops and those which may be present from malicious intent in the current security conscious age (Paterson, in press-a). As per the recently introduced European Union (EU) regulations, only 5 µg/kg of aflatoxin B1 and 10 µg/kg of total aflatoxins are allowed in chillies. India and the United States of America do not have any regulations for this commodity. However, the mycotoxin problem is most acute in developing countries where climate and lack of resources compromise production. The drive for exports to raise revenue and strict regulations in developed countries means that the best quality commodities are sold leaving the remainder, which have higher toxin concentrations as a consequence. Such problems exist for chillies (e.g. Bhat, 1988). Chillies are produced and consumed in developing and developed countries. For example, curries are virtually the national dish of the United Kingdom

Chilli (*Capsicum annum* L.) is one of the important crops of India. Warangal region in Telangana 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 (Peskta and Bonday, 1990).

The aflatoxin adulterated chilli and their product has become a major problem as it causes health hazards to the consumers and it affects the global market as well. After harvesting of the chilli crop, the farmers wait for the good price in the market. The produce undergoes various means of transportation, storage and marketing. During the interval, *Aspergilli* are the predominant contaminant which readily colonize and have potential to produce toxin. The infection occurs on stored fruits and the contamination with aflatoxin deteriorates quality and make the produce unfit for consumption, thereby hitting the export trade in the international market. Post-harvest aflatoxin contamination is most attributable to improper storage. The possible presence of such acutely toxic and carcinogenic substance in foods and in animal feeds had a profound effect on the utilization and trade. Processor and importing countries have placed limits on the levels of aflatoxins permissible in many products. Even when the product reaches to the consumer, the contamination continue to occur as the spore of *A. flavus* common in air and water and the exposed food may be colonized

Materials and methods.

Collection of chilli samples from cold storage.

As chillies are often preserved in bulk in cold storages, an attempt was made to determine natural contamination of aflatoxin B1 in chillies. Chilli samples collected from Warangal Rural Cold Storage Private Limited Unit, (Warangal Grain Market, Vegetable Market, Kiranam Shops and Super market) from Feb 2019 were used for estimation of aflatoxin content in three chilli genotypes namely Teja, Wonder Hot, and U341 were selected for the study. The samples were kept in cold storages after 20 days of harvesting. Samples were then drawn at three month intervals till Jan 2013 as per the standard sampling methods followed by International Seed Testing Association (ISTA). A sample from seed lot was obtained by drawing out small representative portions as primary sample of 1kg at random from different portion of the seed lot measuring from 1000 to 5000 kg. The samples were mixed thoroughly to constitute the composite sample of 500 g. Again submitted sample of 200 g was made and finally reduced to 50 g of working sample for aflatoxin estimation. The next subsequent sampling was drawn from the same varieties and the same lot in next three months interval. The temperature of 4⁰C was maintained during the study. The aflatoxin content was estimated by using indirect competitive Potato Dextrose Agar (PDA) method.

Collection of chilli samples from market three samples of chilli from Warangal Rural Cold storage Units were collected during 2019-20 in the same manner as explained earlier. 500 g of samples were collected and were analysed for aflatoxin contamination. Care was taken in such a way that the samples from same field were not repeated. The chilli samples brought to the market 20 to 30 days after harvesting.

Isolation of Associated Fungi

The Infected samples were the first surface sterilized by washing under running tap water to remove dirt such as sand. A flamed blade was used to cut partly diseased and partly healthy portions of the sample, the cut portions were then surface sterilized using 70% alcohol after which they were rinsed in successive changes of sterile distilled water. They were then inoculated on Potato Dextrose Agar (PDA). This was done for all the samples from the three markets, the plates were incubated at 28⁰C±2. Fungal growth was observed daily. After six days of incubation, a small portion of mycelium from each fungal colony was transferred aseptically into fresh plates containing the medium used. The fungi were purified by repeated sub-culturing

Preparation of Potato Dextrose Agar

1. Suspend 39 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely.
2. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing.
3. In specific work, when pH 3.5 is required, the medium should be acidified with sterile 10% tartaric acid.
4. The amount of acid required for 100 ml. of the sterile, cooling medium is approximately 1ml.
5. Do not heat the medium after addition of the acid Molds will grow as filamentous colonies of various colors.

Identification of isolated organisms

Using sterile inoculating needle, minute portion of each organism was aseptically taken and teased at the center of a clean microscopic slides containing drop of lactophenol cotton-blue stain, covered with cover slips and observed under the microscope. Identification was made with reference to standard textbooks such as Domsch *et.al.*, (1980), Barnett and Hunter (1992).

Results and discussion.

Aflatoxin content was estimated from three cultivars of chilli at three months interval for a period from Feb 2019 till Jan 2020 (Table-1) (Fig-I). Results from Potato Dextrose Agar (PDA) analysis revealed that the samples were contaminated with aflatoxin B1 to the extent of 6.5 gm/kg. Among three cultivars, Teja, Wonder Hot, and U341. Teja varied in aflatoxin content from first month (0.8gm/kg) to 12 months (6.5 gm/kg) of storage (Plate-I, II and III). Similarly cultivar U341 had the variation in aflatoxin contamination of 1.5 gm/kg at three months to 4.5 gm/kg after twelve months of storage. The cultivar wonder Hot showed 2.0 gm/kg after three months of storage. If the farmer does not find a remunerative price in the market he is forced to opt for storing the produce in the cold storage until he gets a good price.

Table.1 Analysis of aflatoxin content from three genotypes of chilli at three months interval from cold storage during Feb 2019 to Jan 2020

Sl.No	Chilli varieties/hybrids	Aflatoxin content (gm/kg)				
		0 Month	3 Months	6. Months	9.Months	12.Months
1	Teja	0.8	2.5	3.5	4.8	6.5
2	Wonder Hot	0.5	2.0	2.4	3.6	4.6
3	U341	0.6	1.5	2.2	3.0	4.5

Table.2 Estimation of aflatoxin content of chili varieties from Warangal after Nine Months Collected from cold storage area in Warangal District in Telangana state

Chilli varieties	Warangal Grain Market		Veg. Market		Kiranam Shop		Super Market	
	A	B	A	B	A	B	A	B
Teja	5.0	10.0	6.0	10.5	6.4	10.8	6.5	11.0
Wonder Hot	4.5	9.0	5.5	9.5	5.7	9.7	5.9	9.9
U341	3.5	7.0	4.0	7.3	4.2	7.6	4.5	7.8
A. Pod infection (Percentage. B Aflatoxin content (gm/kg)								

If the produce is not stored and maintained in proper storage conditions, it might lead to development of moulds particularly *A. flavus* and ultimately aflatoxin contamination. Estimation of toxin at various intervals of storage confirms the presence or absence of toxin till it reaches to the consumer as safe product. The variation in aflatoxin production at different storage interval could be due to the temperature and relative humidity prevailing in the cold storage and the sampling size. The variation between the genotype is due to the resistant mechanism offered by the genotype.

Three samples from Warangal Urban market of Warangal district of Telangana State were analyzed for aflatoxin B1 content by Potato Dextrose Agar (PDA) technique (Table- 2) (Fig-II). The average aflatoxin content of

samples from Warangal Urban market had very less quantity of aflatoxin (10.00 gm/kg) which was less than the permissible limit of (11.0 gm/kg) prescribed by India. Similarly, the samples from Super Market had an average aflatoxin content of 9.50 gm/kg suggesting that the samples are fit for consumption purpose.

After harvesting of the crop, sometimes the farmers sell their produce immediately to get a remunerative price in the market. If the produce is contaminated with the aflatoxin, it then reaches to the traders and then to the consumer. The detection of aflatoxin immediately after the harvest before it reaches to the consumer is very important.



Plate-1 Chillies samples of three varieties (Teja, Wonder Hot, and U341.) Collected by the Corresponding author from Various Places of Warangal geographic region a) Warangal Grain Market b) Kiranam Shop c) Vegetable Market d) Super market place for conducted present Research.



Plate II: Analysis of aflatoxin content from three genotypes of Healthy chilli VS Contaminate Chilli in cold storage during Feb 2019 to Jan 2020



Plate- III. Inoculation of infected surface of Chillies of Three Varieties (Teja, Wonder Hot, and U341) medium after five weeks of culture to identified of Nine fungal species A) inoculation of Infected surface in laminar air flow chamber b) *Aspergillus flavus*, c) *Aspergillus niger*, D) *Mucor* species e) *Penicillium* spp, f) *Aspergillus flavus*, *Aspergillus niger* *Mucor* and *Rhizopus* Sp

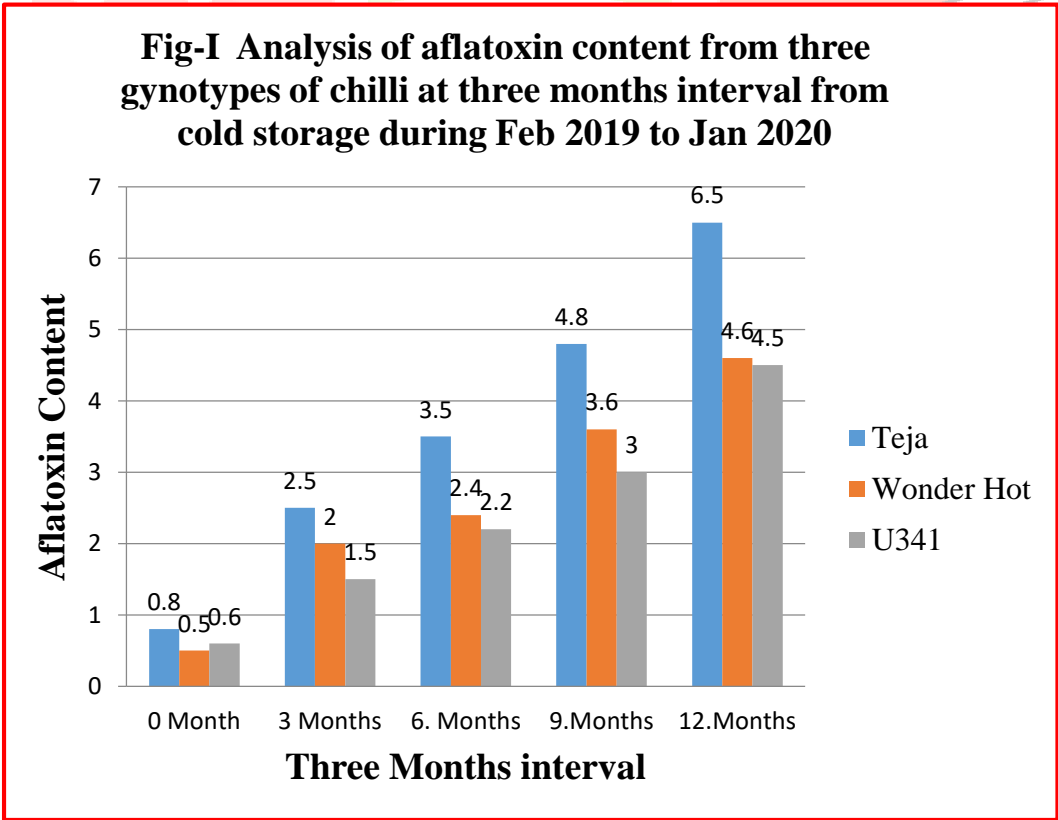
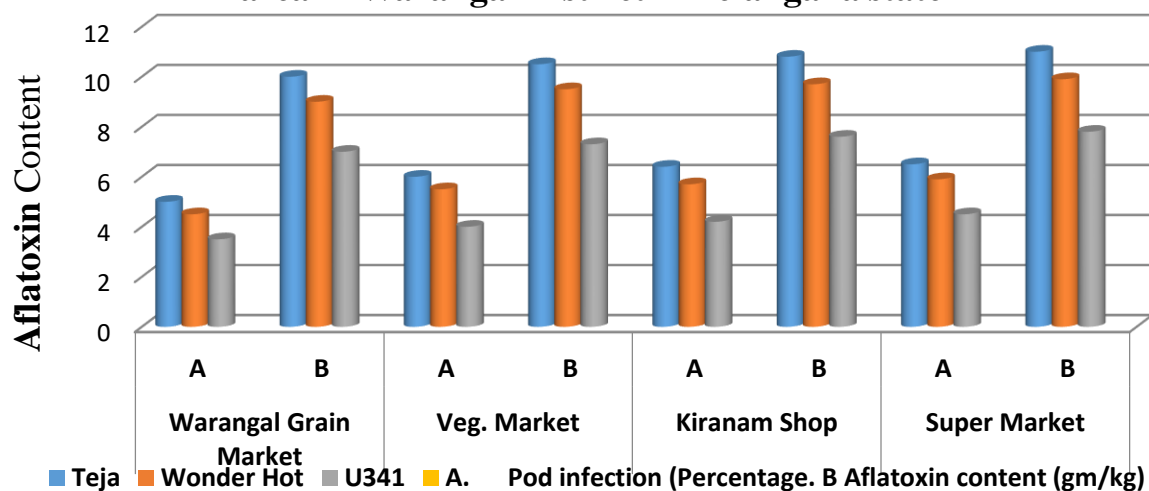


Fig-II Estimation of aflatoxin content of chilli varieties from Warangal after Nine Months Collected from cold storage area in Warangal District in Telangana state



The average aflatoxin content of samples from Warangal was less (9.5 gm/kg) which was remarkably much lower than the permissible limit of 20 gm/kg. Similarly, the samples from Super Market showed an average aflatoxin content of 11.0 gm/kg suggesting that the samples are acceptable for consumption purpose.

The average aflatoxins in 110 samples covering storage length from one year to over 10 year in maize, whole grain rice and brown rice were found to be 0.99, 3.87 and 0.88 mg/kg, respectively at Liaoning Province, China (Liua *et al.*, 2006). An attempt has been made by Ravi Kiran *et al.*, (2005) to estimate aflatoxin content in chillies collected from the cold stores at monthly intervals for a period of one year between December 2002 and November 2003 at Guntur region in Andhra Pradesh.

Natural occurrence of aflatoxin B1 in chilli pods kept in cold storage was contaminated with aflatoxin B1 to the extent of 5.5 mg/kg. One hundred and twenty groundnut samples collected by Chala *et al.*, (2013) from farmers' stores and markets of Ethiopia were used for aflatoxin analysis. Of 120 samples, 93 were positive while the remaining 27 were negative in aflatoxin content. The total aflatoxin levels in the positive samples varied between 15 mg/kg and 11,900 mg/kg. Groundnut samples from 21 selected markets in 10 regions of Ghana yielded high levels of the aflatoxigenic fungus *A. flavus*. A total of 196 nuts and their products marketed in Penang, Malaysia were assessed by Leong *et al.*, (2010) for aflatoxins. Thirty two out of 196 samples (16.30 %) were contaminated with aflatoxins, ranging in levels from 16.60 gm/kg up to 711 µg/kg. The aflatoxin contamination in chilli produce stored in cold storage was high compared to the samples collected from market. This indicates that the samples collected from market were free from aflatoxin contamination at the permissible level. This is safe for consumption. So the consumer and the trader have to take precautions to store the chilli produce in cold storage in order to reduce the risk of aflatoxin contamination.

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