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IMPACT OF DISCOLORATION AND PICKING PRACTICES OF RED CHILIES ON AFLATOXIN LEVELS

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Abstract

Red chili is amongst the important market commodities mainly for its pungency, color and their respective therapeutic significances. However, the persistence of aflatoxin contamination in chilies at higher levels is raising the health and economic risks. Post harvest practices may play as crucial role to make the red chilies physically damaged that may lead to increase the levels of aflatoxins. The present study investigated the differences in aflatoxin levels in red chilies with pedicle, without pedicle, normal and discolored pods. The results showed that aflatoxin was higher in discolored pods as well as chilies without pedicle. On an average, chilies with pedicle showed much lower levels of aflatoxins (4.46 ppb) than those without pedicle (9.16 ppb). The discolored chilies contained almost 10 times more (92.13 ppb) aflatoxins than the normal pods. The buildup of aflatoxins was found to be highly influenced ($P<0.001$) by pod discoloration, and absence of pedicle. These results can be beneficial for the understanding of the relation among aflatoxin levels in healthy and discolored chilies as well as chilies with and without pedicles.

Introduction

Capsicum is an economically important genus of *Solanaceae* family. There are approximately 27 species, of which five are cultivated worldwide, i.e., *Capsicum. annuum* L, *Capsicum frutescens*, *Capsicum baccatum* L., *Capsicum chinense* and *Capsicum pubescens* (Csillery, 2006). The amount of chili produced in the world is 24 million tons (Anon., 2005). The main production areas are located in Asia with the annual production of 16 million tons (Anon., 2005). This is among the most valuable cash crop in Pakistan. Pakistan is the sixth largest exporter of chilies in the world (Abrar *et al*., 2009). However, the chili export of Pakistan is badly decline from couple of years back due to the higher contamination levels of aflatoxin. Previous studies have shown that the Pakistani commodities other than chilies like maize have also contained higher level of aflatoxin (Niaz *et al.,* 2012; Khatoon *et al*., 2012).

Aflatoxin is a potent carcinogenic metabolite produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Production of aflatoxin by these fungi can minimize by the antifungal activities of certain materials like propolis, *Ephedra Alata* etc. (Hashem *et al*., 2012; Al-Qarawi *et al.,* 2012). Aflatoxin may alleviate the risk of different diseases including liver cancer, cirrhosis, gastritis etc., especially in African and Asian countries (Atanda *et al*., 2011). According to the European Union (EU), the allowable limit of the total aflatoxin in food for direct human consumption is 4 ppb levels (Herzallah, 2009). Temperature and humidity are the crucial factors that can influence the toxin production before and after harvest of the crop (Cotty, 1991; Russell *et al*., 1976). Improper picking practices and improper post harvest processing of the chilies my lead to increase the aflatoxin production. Aflatoxin infection may also occur due to the mechanical damages, stress conditions or damages by birds, mammal or insects etc (Cotty & Lee, 1990; Dowd, 1998; Guo *et al*., 2003; Odvody *et al*., 1997; Sommer *et al*., 1986). Good quality of red chili may be attributed to the brighter red colored skin with thicker texture and uniformity of pod size. The damages may not only cause the physically deterioration of quality but may also lead to increase the aflatoxin production.

The study describes the impact of discoloration and removal of pedicle from the chilies on the production of aflatoxin. These investigations were also aimed to find out the relationship between picking practices and sorting after drying with the aflatoxin production in chilies.

Materials and Methods

The study was conducted in different chili producing areas of Sindh-Pakistan. The samples of red chili (locally called dandi cut variety) were collected from five chili growing areas in and around Kunri, Mithi, Nagarparkar, Marjhango and Samaro. The collected samples represented the crop year 2008, which was sown in late February to early March, and picked in the second week of September in the same year. The picked chilies were transferred to open-air drying fields, where the harvest was spreaded to single layer on bare sandy soil.

Sampling: To investigate the comparative occurrence of aflatoxin in defective and normal pods, separate samples were collected on completion of sun drying and categorized into following sub-samples.

i. Normal pods
ii. Discolored pods
iii. Pods with pedicle
iv. Pods without pedicle

All the samples were collected in cotton bags and quickly transported to the laboratory and kept in sterile glass jars. The jars were kept in a refrigerator at 4°C. All samples were analyzed within a week for aflatoxin estimation.

Sample preperation for aflatoxin estimation: Each representative sample was converted in to powder form by laboratory grinder (Braun Model # KMS 2). 10 g of sub sample were taken from each sample for aflatoxin analysis. The sub sample was blended with 50 ml of 70% methanol for 2-3 min in an electronic commercial blender (Waring model 51BL31-7011, US). The extract was filtered through Whatman number 1, and 5 ml of the filtrate was used for analysis.

Estimation of total aflatoxins: The quantitative analysis of total aflatoxins was determined through a competitive direct Enzyme Linked Immunosorbent Assay (ELISA) by using a commercially available immunoassay kit Veratox (Neogen Corp., Lansing, MI. USA). The method of analysis was approved by AOAC RI and the USDA-GIPSA (2008-011). The optical density of the analyte was measured through Tecan Sunrise micro plate reader at 650 nm. These OD values were then entered in Log/logit Software (Awareness Technology Inc) in order to plot the standard curves through which the concentrations of aflatoxin (ppb) in samples were obtained.

Calculation of aflatoxin content: The total aflatoxin content (on a dry basis) in each sample was calculated as follows:

$$\text{Aflatoxin content (ppb on dry basis)} = \frac{\text{Aflatoxin value in ppb with moisture}}{\text{Total weight of sample} - \text{moisture content of sample}} \times 100$$

Mycological studies: Association of aflatoxin producing fungi with chilies was also examined by using blotter method (Anon. 1976). Three layers of white blotter were jointly soaked in sterilized water and placed on petri plates. Chili seeds were plated out at different positions in petri dishes. Fungi grown on seeds were identified after 7-8 days reference to Barnett & Hunter (1998); Booth (1971); Domsch *et al*., (1980); Dugan (2006); Gilman (1957); Nelson *et al*., (1983); Raper & Fennel (1965); Raper & Thom (1949) and Thom & Raper (1945).

Statistical analysis: The sample collection and analysis was done in triplicate from each of the test location. A two way ANOVA for a factorial design was applied to determine the sources of variation. Duncan's test was further used to analyze the differences within treatments. All statistical analyses were performed by using SPSS software (SPSS version 17, Inc., USA).

Results and Discussion

Chilies with pedicle vs without pedicle: In order to determine the effect of picking practice on aflatoxin contamination, ripe chilies with and without pedicles were collected after completion of drying. Both types of samples were analyzed for aflatoxin levels in the laboratory. The results indicate that the aflatoxin levels were found to range between 3.0 to 5.92 ppb (dry weight basis) in chilies with pedicle, and 7.82 to 10.79 ppb (dry weight basis) in samples without pedicle (Table 1). Chilies are picked manually often by female field workers when the fruit is mature. However, a proportion of immature fruits are also picked up during the process. Furthermore, as a result of quick plucking action, fruits are often picked in a way that pedicle remains on to the plants. Such fruits as a result of opening up of their viscera are more liable to fungal contamination that may lead to production of aflatoxins. The physical damage to the pods that provides open entry to the fungi, in general, is one of the chief factors to favor the growth of fungi that may lead to the production of aflatoxin (Dowd, 1998; Guo *et al*., 2003; Odvody *et al*., 1997). The deteriorated impacts of this practice can be minimized by optimizing the drying and storage techniques. Because the optimal humidity and temperature that favors fungal multiplication during storage causes severe damages in relation to aflatoxin levels. In general, the suitable ranges for aflatoxin production are 25-33°C (temperature); 97-99% (relative humidity); and 0.95-0.99 (water availability) (Hill *et al.*, 1985; Şimşek *et al.*, 2002, Atalla *et al.*, 2003). The differences between the aflatoxin levels of opened viscera and closed viscera chilies were calculated and expressed in folds (Fig. 1). It was found that the aflatoxin levels in opened viscera chili pods were 1.8 to 2.9 folds (2.1 fold) greater than that of closed viscera chilies.

Table 1. Comparison between the aflatoxins levels in ppb (dry weight basis) in red chilies with and without pedicle.

Study location	Aflatoxins (ppb on dry weight basis)	
	Chilies with pedicle	Chilies without pedicle
Kunri	$5.92 \pm 0.0^{a,A}$	$10.79 \pm 0.10^{b,A}$
Mithi	$5.0^{a,B}$	$10.24 \pm 0.07^{b,A}$
Nagarparkar	$4.39 \pm 0.0^{a,C}$	$7.82 \pm 0.03^{b,B}$
Morjhango	$3.0^{a,B}$	$8.58 \pm 0.05^{b,C}$
Samaro	$4.0^{a,B}$	$8.4 \pm 0.08^{b,B}$
Mean	4.46 ± 1.09	9.16 ± 0.31

Different small letters within same rows are significantly different at $p<0.05$

Different capital letters within same columns are significantly different at $p<0.05$

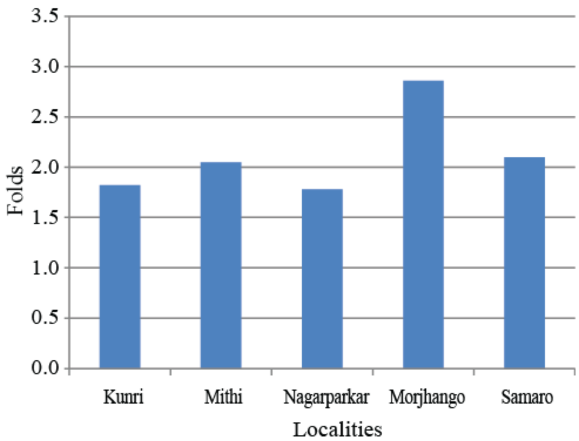

Fig. 1. The differences between aflatoxin levels of with and without pedicle chili pods.

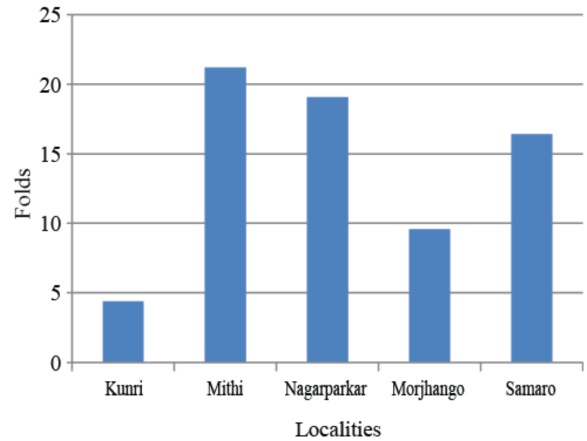

Fig. 2. The differences between aflatoxin levels of normal and discolored chili pods.

Normal vs discolored chilies: The aflatoxins in normal chilies was found to range between 4.1 to 19.54 ppb (X=8.82 ppb-db) and 83.53 to 113.86 ppb on dry weight basis in discolored chilies (Table 2). It is evident from the findings that the discolored chilies are one of the highly significant sources of aflatoxins contamination in chilies. Mycological studies of these samples also shows the presence of fungal proliferation, especially the presence of aflatoxigenic fungi i.e., *Aspergillus Flavus* (X=8.45±1.8) *and Aspergillus parasiticus* (X= 9.45±1.2) in reported results support the aflatoxin synthesis in discolored pods (Table 3). The contamination of this crop with aflatoxin from the growth of *Aspergillus flavus, Aspergillus nominus, A. parasiticus, A. pseudotamarii, A. bombycis,* and others (Paterson, 2007; Samson, 2001; Varga *et al*., 2003) is one of the most serious problem. We could not find any supporting or contradictory findings of previous studies in the literature that relates the discoloration of chili pods with aflatoxin production. This statement may therefore need to further investigate in detail, however opened up new debate for chili researchers in toxicological aspects. In order to quantify the impact of discoloration on aflatoxin levels in chilies, the differences in terms of folds have been estimated and presented (Fig. 2). It is apparent that the aflatoxin levels in discolored pods were found 4.4 to 21.2 folds (14.1fold) higher than normal chili pods. This shows that the presence of such pods (discolored pods) in any consignment is liable to spread the problem on grinding without segregation.

Improper post harvest handling practices at farm level played important role to increase the contamination of aflatoxin in chilies. The aflatoxin in discolored pods was found to be highest amongst other types of pods (Fig. 3). It is therefore suggested to separate the discolored and opened viscera pods from the lot that may decrease the risk of further increase in the level of aflatoxin contamination. This type of practice may help the chili growers to maintain the required standards of quality of their produce and capable to supply aflatoxin free chili to the processors. Consequently, the availability of safe chili for domestic population and export will be a step forward to combat the food safety issues and to support the economical affairs of Pakistan.

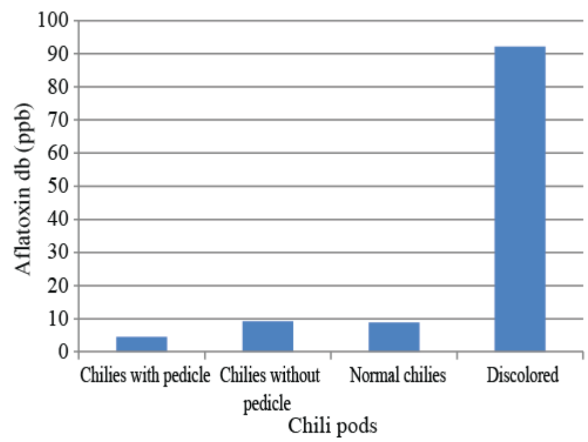

Fig. 3. Mean values of aflatoxin levels in different types of chili pods.

Table 2. Aflatoxins levels in ppb (db) in normal and discolored chilies.

Study Location	Aflatoxins (ppb on dry weight basis)	
	Normal chilies	Discolored
Kunri	19.54 ± 0.40 ^{a,A}	86.14 ± 0.38 ^{b,A}
Mithi	4.10 ± 0.26 ^{a,B}	86.95 ± 0.33 ^{b,B}
Nagarparkar	5.97 ± 0.20 ^{a,C}	113.86 ± 0.50 ^{b,C}
Morjhango	9.42 ± 0.30 ^{a,D}	90.18 ± 0.40 ^{b,D}
Samaro	5.09 ± 0.30 ^{a,E}	83.53 ± 0.18 ^{b,D}
Mean	8.82 ± 5.60	92.13 ± 10.10

Different small letters within same rows are significantly different at p<0.05
Different capital letters within same columns are significantly different at p<0.0

Table 3. Mycological studies of normal and discolored chilies.

Chili pods	Average fungal incidence (%)			
	Aflatoxigenic fungi*		Non aflatoxigenic fungi	
	Aspergillus Flavus	***Aspergillus parasiticus***	***Fusarium oxysporum***	***Alternaria Alternata***
Normal pods	$2.77 \pm 1.01^{a,A}$	$3.12 \pm 0.7^{b,A}$	$8.76 \pm 1.0^{c,A}$	$11.08 \pm 0.5^{d,A}$
Discolored pods	$8.45 \pm 1.8^{a,B}$	$9.45 \pm 1.2^{a,B}$	$12.65 \pm 1.5^{b,B}$	$17.7 \pm 1.9^{c,B}$
Mean	5.61	6.285	10.705	14.39

Different small letters within same rows are significantly different at $p<0.05$
Different capital letters within same columns are significantly different at $p<0.05$

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