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FUNGAL INCIDENCE AND AFLATOXINS CONTAMINATION IN TWO MAJOR CHILLI VARIETIES OF SINDH, PAKISTAN

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Abstract

Chilli crop is susceptible to microbial attack, particularly toxigenic fungi that produce aflatoxins. The presence of higher levels of aflatoxin in chillies raises health and economic concerns. Post-harvest management may play an important role in causing physical damage to red chillies in presence of moisture, leading to increased aflatoxins levels. A recent study investigated two chilli varieties *viz*., Sanam and Longi collected from various areas of Sindh, Pakistan. The level of aflatoxins in chillies dried on net surfaces was found between the range of 2.3-7.1 µg kg^{-1} whereas samples dried on cemented ground showed aflatoxin contamination from 6.7-14.5 µg kg^{-1}, aflatoxin contamination in most of the samples collected from different areas was less than 20 µg kg^{-1}. The maximum incidence of *Aspergillus flavus* was found in all 81 chilli samples followed by *A. niger, A. parasiticus, A. fumigatus, A. candidus,* and *A. terreus*. These findings indicate that 60-80 percent aflatoxins contamination in chilli samples contained aflatoxins within the acceptable range 20 µg kg^{-1} (ppb) for people utilization as described by the US Food and Drug Administration (FDA).

Key words: Chilli varieties, *Aspergillus flavus,* Aflatoxins contamination.

Introduction

Chilli (*Capsicum annum* L.) is one of the most consumed spices in the world. Mostly cultivated chilli varieties in Sindh are Sanam and Longi. Both varieties are high yielding, profitable in local and international markets and used as a spice in various meals in Sindh (Khokhar, 2013; Sahar *et al*., 2021). With a steady increase in global demand, the production of chilli during the last 10 years has increased on an average of 3.9 percent per year in the world (Asare-Bediako *et al*., 2015; Devi & Thyagarajan, 2020). In Pakistan, the annual production of chilli is 148.1thousand tons with a total cultivated area of 65.1 thousand hectares which is 3.8 percent more than the year (Anon., 2018; Anon., 2019; Channa *et al*., 2020).

The main chilli-producing areas in Pakistan are the lower region of Sindh province including Kunri, Mirpurkhas, Umerkot and other small towns. Kunri is considered the hometown of chillies (Hussain *et al*., 2013) and is internationally known as the largest chilli production centre in Asia (Akhund *et al*., 2017).

It has been assessed that 25% of the world's harvests are affected by molds that are stable natural pollutants of the food chain (Bennett & Klich, 2003; Anon., 2003; Peivasteh, *et al*., 2021). Mycotoxin reduction requires an integrated approach, including government agencies, farmers, food processors, and scientists. This may a significant impact on the costs of food production. An international regulatory standard for mycotoxins in food items specifies the extent of global trade in impure commodities (Bryden, 2007).

Chillies contaminated with aflatoxin and pesticide residues are a major problem in the global market trade. *A. flavus* and *A. parasiticus* strains are the main toxigenic fungi that can produce Aflatoxins (Hua *et al*., 2019). Impurities by aflatoxins can arise in chillies and numerous different kinds of foodstuff, dry fruits, juices, milk, tea, and feed items, particularly in high temperate and moist areas of the globe (Zahra *et al*., 2018; Pallarés *et al*., 2019; Ismail *et al*., 2020; Jiang *et al*., 2021).

The post-harvest approaches focus primarily on aflatoxin elimination. After the contamination of food and feeds with aflatoxin, it is impossible to remove toxins completely. Since aflatoxin impurities has been a major concern in the trading of chillies internationally, recent research was conducted to examine the level of fungal, effect of drying on various surfaces and aflatoxin contamination in chilli varieties, Sanam and Longi produced in various parts of Pakistan for domestic consumption and export.

Material and Methods

Collection of samples: The varieties of dried chillies from the net and cemented ground were collected from lower Sindh. A total 81 dried samples of chilli fruits were collected in autoclave sterilized cotton bags separately from the selected sites and samples were categorized as dried on the net and cemented ground. The samples were brought to the A.G. laboratory of Aerobiology and plant pathology, Department of Botany, Federal Urdu University of Arts, Sciences and Technology, Karachi, Pakistan for analysis.

Isolation of the pathogens: The collected samples were cut into 1.5 to 2 cm pieces and after surface sterilization by dipping in 1% calcium hypochlorite ($Ca(OCl)_2$) for one minute followed by 3 washes with sterilized distilled water. Each sample had three replicates and five small pieces were transferred to Potato Dextrose agar (PDA) medium containing Penicillin and Streptomycin 1 ml per liter. Petri dishes were incubated for seven days at 28±2°C. The percent incidence of *Aspergillus* spp. was calculated by the following formula:

$$\text{Percent incidence} = \frac{\text{Number of samples on which a } \textit{Asprgillus} \text{ sp., appeared}}{\text{Total number of samples analysed}} \times 100$$

Identification of fungi: Isolated fungi were identified using 10X and 40X magnifications under the microscope to examine hyphae, conidiophores, conidia and other morphological characters including colony texture, growth pattern and rate of colony growth on PDA. Guidelines of Ellis (1971; 1976), Barnett & Hunter (1998), Domsch *et al*., (1980), Thom & Raper (1945), Dugan (2006), Raper & Fennel (1965) and Singh (1991) were used for the confirmation of *Aspergillus* species.

Estimation of aflatoxins: The quantitative analysis of aflatoxins was analysed through a competitive direct enzyme linked immunosorbent assay (Sahar *et al*., 2021) by using a commercially available immunoassay Kit Veratox (Neogen Corp., Lansing, MI.).

Results and Discussion

In the present study, 173 isolates of *Aspergillus* spp. were obtained from 41 samples of Sanam variety out of 81 samples of both varieties from different localities of Sindh, Pakistan (Table 1). In Sanam variety, *A. flavus* had the highest frequency (80%) followed by *A. niger* and *A. parasiticus* with 75.61% and 19.51% infection respectively. Filamentous fungi are significant despoiler of foodstuffs during storage and render them unhealthy for human consumption by retarding their nutritional value due to the production of mycotoxins (Costa *et al*., 2019). Chillies are susceptible to infection by aflatoxin-producing fungi and consequent aflatoxins contamination at all stages (Singh & Cotty, 2017). In 40 samples of Longi variety, 132 isolates were recorded. The highly frequent fungi were *A. flavus* with 90.24% infection followed by *A. niger* with 75% and *A. parasiticus* was 20%, respectively. *A. candidus* occurred in low frequency i.e., 2.44% and 5% in Sanam and Longi varieties, respectively. Enamullah *et al*., (2022) evaluated 6 different fungal species and concluded that red chillies were highly infected by *A. flavus* and *A. niger*. Mandeel (2005) reported 17 fungal species associated with red pepper and observed heavy infection by 96 strains of *A. flavus* and 62 strains of *A. niger*. Hussain *et al*., (2013) reported that *A. flavus* and *A. niger* were predominant fungi in Sindh, Pakistan. In comparison to other genera, Bokhari (2007), Singh *et al*., (2008), and Al-Hindi *et al*., (2018) found that *Aspergillus* is one of the most ubiquitous and dominating infection causing genus.

A. flavus and *A. parasiticus* are responsible for the aflatoxins production mainly aflatoxin B_1 in chillies which are extremely toxic and carcinogenic mycotoxins (Su, 2019). Chillies contain aflatoxins, which cause diseases such as liver cancer, gastritis, and cirrhosis, particularly in African and Asian countries, posing a serious hazard to human life and limiting their export (Atanda *et al*., 2011). In the current study, *A. flavus* and *A. parasiticus* were found at 80 and 19.51% in Sanam variety and at 90.24 and 20% in Longi variety, respectively (Table 1). On the opposed, the total aflatoxin level detected in Sanam variety was 19.83 µg kg^{-1} and 44.33 µg kg^{-1} in the Longi variety (Fig. 1), which is higher than the European Union limits 10 µg kg^{-1} (Herzallah, 2009; Iqbal *et al*., 2010).

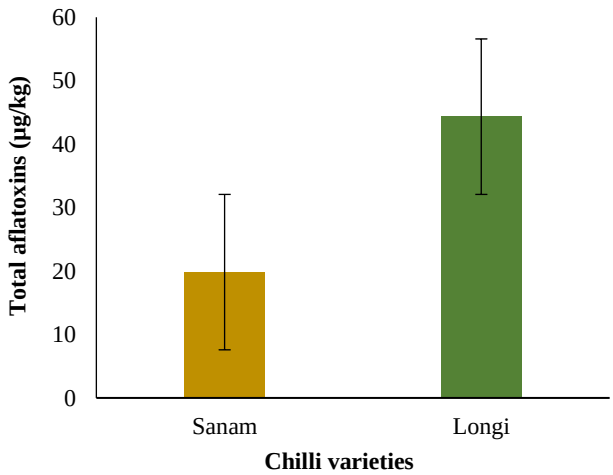

Fig. 1. Total aflatoxins in Sanam and Longi varieties of chilli.

Usually, solar energy is directly used to dried-up harvested produce inthe worldwide (Condori *et al*., 2001). Farmers use to dry their harvested crops mostly on the ground in the open areas which increases the growth of fungi and aflatoxins synthesis (Sahar *et al*., 2017; Marnoto *et al*., 2012). In this study, both varieties (Sanam and Longi) samples dried on the cemented ground were heavily contaminated with *A. niger* (100 & 75%) and *A. flavus* (25 & 58%, respectively). However, Sanam and Longi variety samples on net surfaces showed comparatively low infection with *A. niger* (85 & 73%) and *A. flavus* (5 & 20%). In contrast, *A. flavus* expressed the maximum infection in Longi variety (Fig. 2). When correlating the fungal and aflatoxins in contamination on dried chilli varieties on different surfaces, the current study showed chilli varieties dried on the cemented ground were highly contaminated with aflatoxins ranging from 6.7-11.3 µg kg^{-1} and 9.2-14.5 µg kg^{-1} in Sanam and Longi, respectively. The minimum level of aflatoxins i.e., 2.3-3.4 µg kg^{-1} and 4.8-7.1 µg kg^{-1} was found respectively in Sanam and Longi chilli varieties dried on net (Fig. 3). Sahar *et al*., (2021) also reported that minimum aflatoxin level was obtained in red chillies dried on a lifted net and observed to be significantly different in dried samples from other surfaces.

In this study, the amounts of total aflatoxins in the Sanam variety ranged from 7.08-19.83 µg kg^{-1} and 4.3-44.3 µg kg^{-1} in Longi variety. The highest aflatoxins levels i.e., 44.33 and 19.83 µg kg^{-1} respectively in Longi and Sanam varieties were observed in samples collected from Kunri (Fig.4). Kunri contributes 85% of Pakistan's red chilli production and is known as the largest harvesting hub in Asia (Akhund *et al*., 2017). Samples of Sanam and Longi variety collected from Kunri reported higher aflatoxins contamination, as well as 54 and 90% infection by *A. flavus*, that was more as compared to other areas. Similarly, Hussain *et al*., (2013) also reported that chillies collected from Kunri were highly infected with *A. flavus* followed by *A. niger*. In a recent study, *A. niger* expressed 95% infection in Longi variety from Kunri and 43% in Sanam variety. Hussain *et al*., (2013) and Enamullah *et al*., (2022) also mentioned that *A. flavus* and *A. niger* were the most prevalent fungi isolated from various locations of Sindh, Pakistan (Table 2).

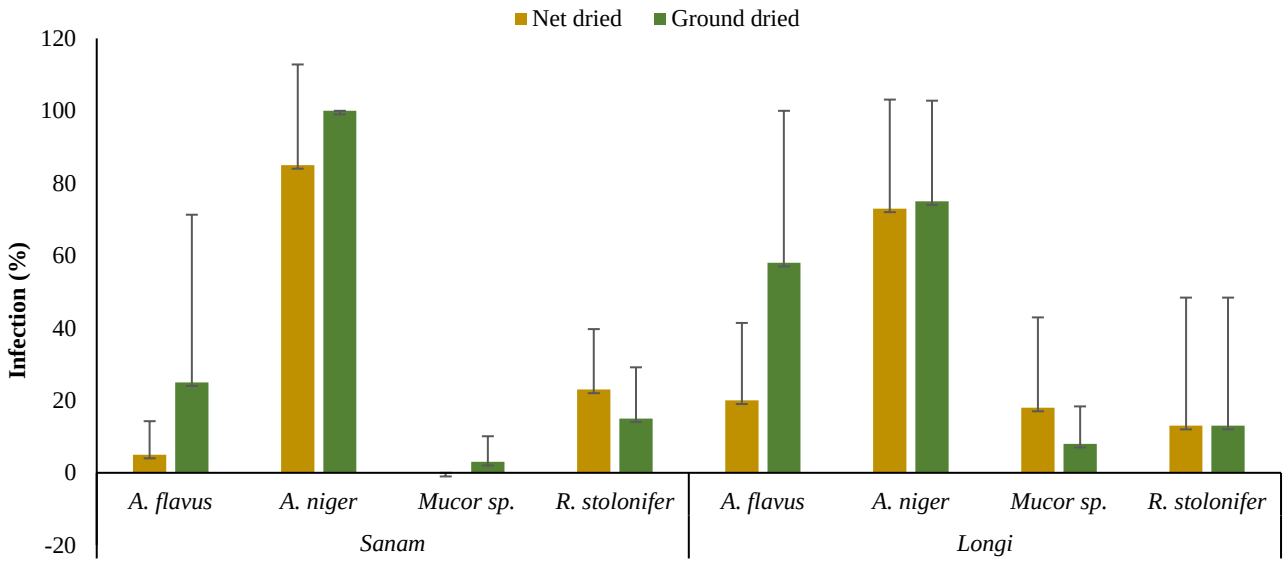

Fig. 2. Infection percentage of fungi in different chilli varieties dried on net and cemented ground.

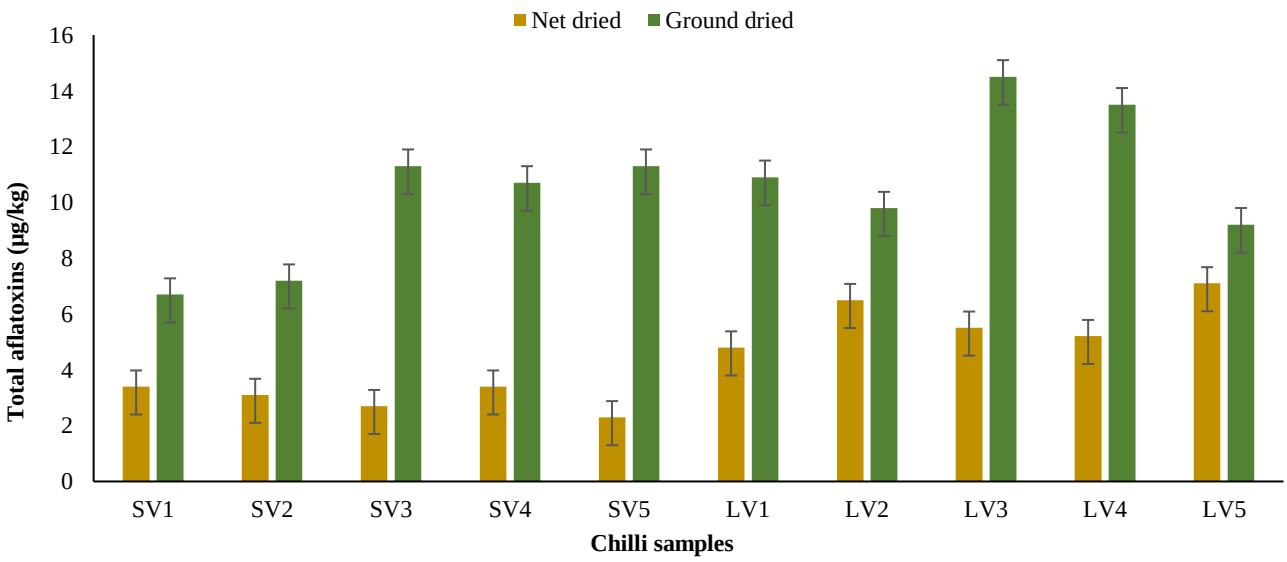

Fig. 3. Total aflatoxins in different chilli varieties dried on the net and cemented ground.
SV1-SV5: Sanam variety, LV1-LV5: Longi variety, V1: Kunri, V2: Umerkot, V3: Mirpurkhas, V4: KGM, V5: Digri

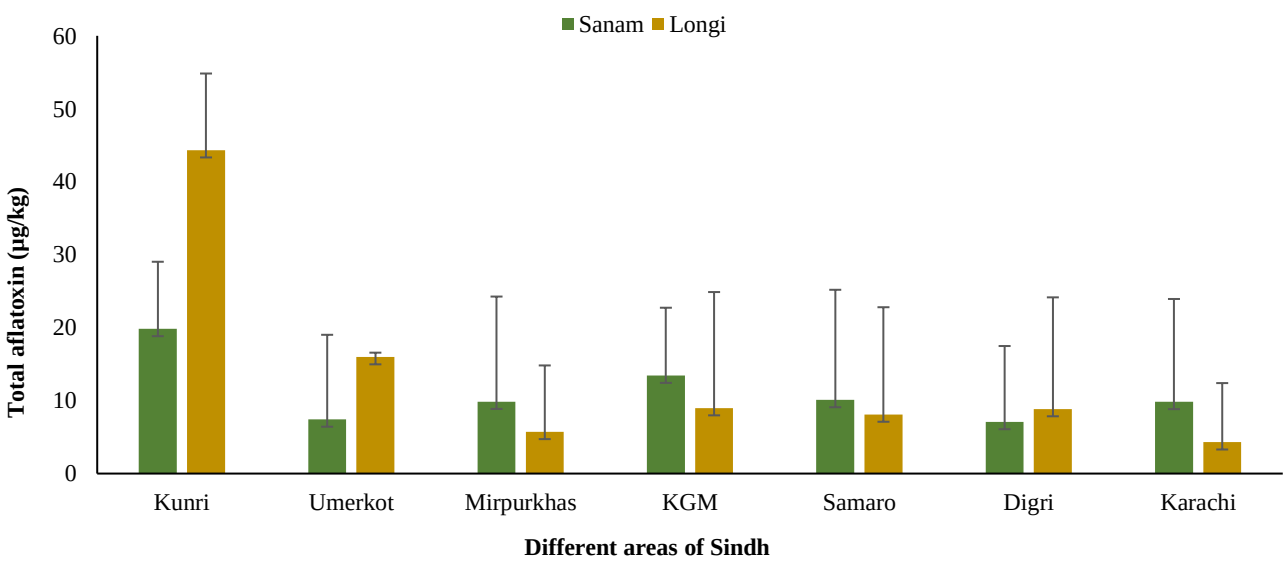

Fig. 4. Total aflatoxins in red chillies collected from different areas of Sindh KGM: Kot Ghulam Muhammad.

Amongst the other areas included in this study, Sanam variety samples from Umerkot showed 15.97 µg $kg^{-1}$and Longi variety from Kot Ghulam Muhammad yielded 13.43 µg kg^{-1} aflatoxins that is considered to be a moderate level of aflatoxins. Whereas, samples of both the varieties from rest of the areas were detected with a negligible level of aflatoxins (Fig. 4) according to Anon., (2014).

The comparative study of red dried chilli varieties collected from different areas of Sindh disclosed that the Longi variety was highly infected with *A. flavus* and *A. niger* from Kunri 90 & 95% followed by Mirpurkhas 88 & 85% and Karachi 89%, respectively. However, Sanam variety from Kunri showed 54 & 43%, Mirpurkhas 49 & 28%, Kot Ghulam Muhammad and Digri 40 & 38% infection with *A. flavus* and *A. niger*, respectively. After *A. flavus* and *A. niger*, *A. parasiticus* was the most isolated fungus and expressed higher infection in Longi variety as compared to Sanam variety (Table 2). The impurities in chilli crops with aflatoxins due to the growth of *A. flavus*, *A. niger*, and *A. parasiticus* is the most serious problems (Sahar *et al*. 2013; Patersona, 2007; Varga *et al*. 2003; Samson, 2001). The results of the current study would suggest that drying chillies on net should be promoted to minimise the aflatoxin contamination that may lead to better quality and increase in export of chillies.

Table 1. Incidence (%) of *Aspergillus* species in chillies varieties from Sindh, Pakistan.

Chilli varieties	Chilli samples	No. of isolates	Species					
			A. flavus	***A. niger***	***A. parasiticus***	***A. fumigatus***	***A. candidus***	***A. terreus***
Sanam	41	173	80	75.61	19.51	2.44	2.44	0
Longi	40	132	90.24	75	20	10	5	7.5

Table 2. Comparative analysis of infection percentage of *Aspergillus* species isolated from Sanam and Longi varieties of chilli samples from different areas of Sindh.

Chilli varieties	Fungi	KUN	UMK	MPK	KGM	SMR	DGR	KHI
Sanam	*A. candidus*	-	2 ± 8.2	-	-	-	-	-
	A. flavus	54 ± 39.3	37 ± 34.2	49 ± 36.7	40 ± 45.7	41 ± 44.3	40 ± 45.7	29 ± 40
	A. fumigatus	0.4 ± 2.9	1 ± 4.1	-	-	-	-	-
	A. niger	43 ± 2.8	17 ± 23.3	28 ± 30.6	38 ± 41.8	8 ± 20.4	38 ± 41.8	18 ± 30.6
	A. parasiticus	1 ± 5.8	2 ± 8.2	1 ± 4.4	3 ± 6.8	2 ± 5.6	3 ± 6.8	-
	A. terreus	-	-		-	-	-	-
Longi	*A. candidus*	8 ± 10.4	-	8 ± 10.4	-	-	-	-
	A. flavus	90 ± 15.1	53 ± 36.9	88 ± 21.2	38 ± 12.8	55 ± 33.4	40 ± 15.1	89 ± 7.1
	A. fumigatus	-	3 ± 7.1	13 ± 14.9	-	3 ± 7.1	8 ± 10.3	-
	A. niger	95 ± 9.3	63 ± 29.2	85 ± 20.7	20 ± 15.1	15 ± 17.7	25 ± 10.3	-
	A. parasiticus	8 ± 10.4	5 ± 9.3	5 ± 9.3	8 ± 10.4	3 ± 7.1	10 ± 15.1	28 ± 14.9
	A. terreus	-	10 ± 15.1	-	10 ± 10.7	-	5 ± 9.3	-

KUN: Kunri, UMK: Umerkot, MPK: Mirpurkhas, KGM: Kot Ghulam Muhammad, SMR: Samaro, DGR: Digri, KHI: Karachi; Mean ± St. deviation

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