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# Potential of Biological Control of Aflatoxins Causing Organisms in Rice and Maize Value Chains in Pakistan

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**Abstract**

Mycotoxins contamination of cropsposes significant economic losses to both crop producers and traders who give market discounts for the contaminated products.Aflatoxin, being one of the most abundant mycotoxins in everyday food commodities such as rice and maize is of utmost importance due to its hepatotoxic, mutagenic and carcinogenic causing characteristics along with the extensive economic losses caused by aflatoxin contamination.Aflatoxins refer to a group of four mycotoxins (B1, B2, G1 and G2) produced primarily by two interrelated fungi, Aspergillus flavus and Aspergillus parasiticus. Rice and maize are prone to aflatoxin contamination during the growing, harvesting, storage, transporting and processing stages. Therefore, there is a need to determine the present status of aflatoxin levels in supply chains of rice and maize in Pakistan for making a comparison with the permissible levels set by the food regulatory authorities in a pursuit to ensure safe food supply. Among aflatoxin management methods,biological control appears to be the most promising and sustainable approach(because the non-toxic strains of aflatoxin-causing fungi occupy the same ecological niche as the toxic strains) for control of aflatoxins in both pre- and post- harvest stages of the respective supply chains.Baseline studies need to be conducted on aflatoxins in target agro-climatic regions to improve the supply chain of cereal grains in Pakistan.

**Key words:** aflatoxins, biological control, production, processing, supply chain.

## 1. Background

Mycotoxins contamination of commodities is a worldwide problem. Mycotoxins are amongst the major health threatsto humans and animals that cause significant economic losses in both developed and developing countries. Mycotoxins contamination of cropsposes significant economic losses to both crop producers and traders who give market discounts for the contaminated products. Among various mycotoxins, aflatoxins, ochratoxin A, trichothecenes (deoxynivalenoland T-2 toxin), zearalenone, and fumonisinshave received much attention due to thehighoccurrence and severe health effects in humans and animals (Bhat, Rai, & Karim, 2010). Aflatoxins, deoxynivalenol, and ergot alkaloids are usually produced at pre-harvest stages, while fumonisins and ochratoxin A are mainly produced during postharvest stagesof food handling and storage (Bhat et al., 2010).Per an estimate by FAO, 25% of the world's crop is yearly affected by mycotoxins (Smith, Madec, Coton, & Hymery, 2016), mainly through aflatoxin contamination. Aflatoxin, being one of the most abundant mycotoxins in everyday food commodities is of utmost importance due to its hepatotoxic, mutagenic and carcinogenic causing characteristics (Bennett & Klich, 2003) along with the extensive economic losses caused by aflatoxin contamination. Aflatoxins refer to a group of 4 mycotoxins (B1, B2, G1 and G2) produced primarily by two closely related fungi, Aspergillus flavus and Aspergillus parasiticus. Strains of A. flavus show great variation in their ability to produce aflatoxins.

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Toxigenic strains of A. flavusproduce only two aflatoxins, B1 and B2, but most strains of A. parasiticus could produce all the four toxins. Both fungi are capable of invading various food and feed crops and contaminating them with hepatotoxic and carcinogenic aflatoxins. Since aflatoxins are potential carcinogens, their quantity in food and feed is closely monitored and regulated in most countries. Aflatoxins are the major cause of liver cancer. Poisoning resulting from ingestion of aflatoxins known as aflatoxicosishas been reported both in humans and animals in many Asian countries (Dhanasekaran, Panneerselvam, Thajuddin, & Shanmugapriya, 2011). Aflatoxins have been reported in cereals, cereal products, fresh produce(Fernández-Cruz, Mansilla, & Tadeo, 2010), groundnuts and beans (Piotrowska, Śliżewska, & Biernasiak, 2013). Aflatoxin production depends on several preharvest factors such as water stress, high-temperature (> 32°C) stress, insect damage to the host plant, susceptible crop growth stages, poor soil fertility, high crop density, and weed competition (Bruns, 2003). Predisposing factors for post- harvest contamination of cereal grains include high moisture contents, longer storage duration, and improper storage conditions(Magan & Aldred, 2007).The climatic conditions in Asian countries are very favorable for aflatoxin producing fungi (Ali, Sardjono, Yamashita, & Yoshizawa, 1998; Wang et al., 1995), both at pre- and post- harvest stages of food supply chains.Abiotic stresses such as drought, temperature, and moisture content invariably lead to aflatoxin contamination of cereal crops during pre- harvest stages(Scully et al., 2009). Sun drying of cereal grains is the usual method employed by most farmers before storage of the food and feed commodities. However, the sun drying method may not be the best-suited drying method in Asian countries as the environmental conditionsarenot under full control.If the cereal grains, especially rice and maize areinsufficiently driedbefore storage, the grains can be highly susceptible to aflatoxin contamination (Choudhary & Kumari, 2010).Preharvest fungal infection is frequently found in semi-arid places whereas thepostharvestincidence of fungus is commonly reported in warm and humid areas (Kumar & Kalita, 2017). Aflatoxin content is a major factor which affects the export of cereal grains (e.g. wheat, rice, and maize), and certain measures are taken to avoid export rejections (Reddy, 2009).

## 2. Rationale

Pakistan is the world's fourth largest producer of rice. Rice is an important cash crop of the country and to the overall national economy as it has been a major source of foreign exchange earnings in recent years. Rice accounts for 2.7% of the value added in agriculture and 0.6% of GDP. Per capita, annual consumption of milled rice was 17kg in 2009(G. o. Pakistan, 2015). Rice is grown on an area of more than 2,571 thousand hectares in Pakistan with an annual production of about 9,194 thousand tons(STAT, 2011). Punjab contributes 67% of the total area under rice and 55% of the total production.While, Sindh contributes 24% to the total area and 34% of the production while only 9% of the total area and only 10% of the total production is contributed by both KPK and Balochistan (R. E. A. o. Pakistan, 2009). A survey conducted in India during 2010 to assess the incidence of aflatoxin contamination in stored paddy samples of a high yielding rice variety reported samples to be below theprescribed Indian regulatory limits of 30 μg/kg (Siruguri et al., 2012).Aflatoxin in rice has long been a problem in Thailand due to (as it is in a tropical zone) much rain and high humidity. Due to thehigh incidence of aflatoxin in Thailand rice, its export potential has suffered thus resulting in huge economic losses (Wright, 1992). The economic losses caused by aflatoxin contamination is also prevalent in rice industry of Pakistan. A recent study was conducted by Mukhtar, Farooq, and Manzoor (2016)to determine aflatoxins in super kernel rice type consumed in different regions of Punjab. Out of 58% contaminated rice samples with aflatoxin B1,35.7% samples were contaminated by aflatoxin B1 above the European Unionmaximum aflatoxin tolerable limit of 5μg/kg(Commision, 2016).Climatic conditions of Pakistan are highly favorable for aflatoxin contamination on rice. Nonetheless, the intensity of infection varies with the place of origin of rice. In a study conducted by Asghar, Iqbal, Ahmed, and Khan (2014) to determine the occurrence of aflatoxin contamination in brown rice in Pakistan, 70% of samples exhibited a mean concentration of 5 μg/kg equals to permissible limit recommended by European Union. Firdous, Ejaz, Aman, and Khan (2012)analyzed 599 samples of white, brown and sella rice from different vendors in Pakistan and reported 50% of samples to be positive for aflatoxin contamination. Maize is one of the highest yielding cereal crops in the world and has a significant importance for Pakistan (Fao, 2014),where therapidly increasing population has increased the demand for already scarce available food supplies. Maize contributes 2.2 % to the value-added agriculture and 0.4% to the GDP.

The cultivated area for maize stood at 1,142 thousand hectares with a peak production of 4,920 thousand tons(G. o. Pakistan, 2015).Punjab contributes 39% to the total area under maize, and 30% ofthe total production; KPK contributes 56% to the total area and 63% of the production while Sindh and Balochistan contribute 5% of the total area and3% of the total production(G. o. Pakistan, 2015).

Maize, in general, is prone to mycotoxin contamination during the growing, harvesting, storage, transporting and processing stages (Bradburn, Blunden, Coker, & Jewers, 1993). Ahsan, Bhatti, Asi, Bhatti, and Sheikh (2010) reported high levels of aflatoxins in maize grains in central areas of Punjab, Pakistan with an average mean value of 30.3 μg/kg. The minimum (10.4 μg/kg) and maximum (49.35 μg/kg) values of aflatoxin contamination with B1 and sum of B1, B2, G1, and G2 were above maximum levelsof 5.0 and 10.0 μg/kg, respectively (Commision, 2016).The incidence of high rates of aflatoxin contamination affects the export market. The main fungal species, which infect the maize grains,are A.flavus, A. parasiticus, and A. nomius. A study on climate change influencing mycotoxins in pre-harvest maize in Italy determined that these fungi species grow well in the range of 19-35°C and produce maximum aflatoxins at 28°C (Sanchis & Magan, 2004). A. flavus invades and infects developing seed of maize in the field before harvest, and mature seeds during harvest and in storage. Preharvest contamination with aflatoxins is aggravated by drought stress and elevated temperature during seed maturation. K Hell, Cardwell, Setamou, and Schulthess (2000) conducted a study to determine storage practices of maize by farmers in Benin, West Africa and reported maize free of insect damage had no aflatoxin contamination, but maize with 70% of the cobs damaged by insects had 30.3% of the cobs contaminated with aflatoxins.

Around 25-40% of cereal grains are contaminated with mycotoxins produced by storage fungi (Dubey, Singh, Prakash, & Mishra, 2012). Many farmers in Pakistan are still using traditional methods of cultivation and harvesting which leads to lower production and higher postharvest losses of about 20-30%(Khan & Khan, 2010). Some of the general supply chain issues facing bothby rice and maize industries in Pakistan are incorrect timing of harvesting and harvesting strategies, improper drying, lack of storage capacities and poor infrastructure, prolonged storage of cereal grains, improper stacking of grain bags, traditional systems of drying grains on open spaces and weather variability(Memon, Zakria, Mari, Nawaz, & Khan, 2011). These conditions potentially lead to aflatoxin contamination. Harvesting mature paddy with high moisture content has a higher respiration rate and is susceptible to mycotoxin attacks. Similarly, maize drying is crucial in reducing themoisture content to prevent aflatoxin growth. Farmers tend to use uncalibrated machines to shell the cobs resulting in broken grains which are a major source of aflatoxin contamination. During harvesting of maize cobs, farmers tend to throw cobs on the ground for drying. This practice leads to an increased risk of aflatoxin contamination in later stages of maize processing. Long term storage of rice grains with higher than 18% moisture due to delayed drying results in microbial growth. Overstacking due to lack of storage facilities with no grain aeration are common practices by local farmers is one of the main cause for postharvest aflatoxin contamination losses in Pakistan. Warm and humid climatic conditions observed in Pakistan coupled with improper harvesting strategies provide afavorable environment for the growth of molds and production of toxins in both field and storage conditions. The first step to improving the supply chain of cereal grains / (selected food commodities) is to conduct baseline studies on aflatoxins in our target agro-climatic regions. The first stepshould be followed by developing efficient pre-harvest strategies and educating farmers in implementing postharvest strategies.

**3. Management of aflatoxins**

Many strategies including control of insect pests(Kerstin Hell & Mutegi, 2011), development of resistant cultivars(Cary et al., 2011),chemical control(Goldblatt, 2012), physical control (solvent extraction, adsorption, heat treatment and irradiation) (Rustom, 1997), and biological control(Cotty, 1994; Dorner, 2004; Pitt & Hocking, 2006)have been investigated to manage aflatoxins in crops. Among these, thebiological control appears to be the most promising and sustainable approach (because the non-toxic strains of aflatoxin-causing fungi occupy the same ecological niche as the toxic strains) for control of aflatoxins in both pre- and post-harvest stages of the respective supply chains. Different organisms, including bacteria(Palumbo, Baker, & Mahoney, 2006)and yeasts(Masoud & Kaltoft, 2006)have been tested for their ability to the control of aflatoxin contamination. Biocontrol of maize crop in Nigeria has been triedby establishing a non-aflatoxigenic strain of A. flavus or A. parasiticus in the soil of a developing crop(Wu & Khlangwiset, 2010), which then displaces or excludes toxigenic strains during crop infection and colonization(Dorner, 2009).

Successful demonstration of the biocontrol principle of competitive exclusion of toxigenic strains of A. flavus by non-toxigenic strains to reduce aflatoxin contamination has been seen in cotton (Cotty, 1994), peanut (Dorner, Cole, & Blankenship, 1998) and maize (Abbas, Zablotowicz, Bruns, & Abel, 2006).

Rice and maize are grown throughout Pakistan. Rice is grown in many areas of Punjab such as Sialkot, Wazirabad, Gujranwala, Sheikhupura, and Faisalabad whereas Jacobabad and Larkanaare known rice growing areas in Sindh. On the other hand, maize is predominantly grown in areas of Faisalabad, Okara, Sahiwal, Multan, and Lahore in Punjab and Peshawar in KPK. For 'through the supply chain' evaluation of aflatoxin in target chains, a sampling strategy for whole crop cycle in selected geographical areas should be developed followed by quantification of the baseline status of aflatoxin, if present. The following strategy should be adopted to build the national capacity for aflatoxin control and management in rice and maize;

### 4. Sample collection

Samples should be randomly collected from various sites of production and processing/storage facilities throughout the year. Analysis of detection of aflatoxin should be conducted at a food science laboratory. Aflatoxin should be analyzed by using a suitable separation technique such as high-performance liquid chromatography (HPLC). A year-round profile of the status of selected supply chain should be developed.

### 5. Laboratory culture for biological control of aflatoxin-causing organisms

Indigenous non-toxigenic strains of potential candidates should be identified, and their cultures should be established at a biological control laboratory. The strategy should base on the application of non-toxigenic strains to competitively exclude naturally toxigenic strains in the same niche and compete for crop substrates. Thus, for competitive exclusion to be effective, the biocontrol non-toxigenic strains must be predominant in the agricultural environments when the crops are susceptible to be infected by the toxigenic strains. In this context, aprotocol for laboratory culture of non-toxigenic strains successful in thecontrol of aflatoxin-causing organisms shouldbe developed.

### 6. Conclusion and Future Outlook

Keeping in view the importance of high-value food commodities of rice and maize in Pakistan, there exists a clear gap on theapplication of biocontrol of aflatoxin in these food supply chains.There is a need to determine the present status of aflatoxin levels in supply chains of staple commodities in Pakistan for making a comparison with the permissible levels set by the food regulatory authorities in a pursuit to ensure safe food supply. Furthermore, the strategies implemented for biological control of aflatoxin contamination, if successful, will go a long way in reaching a higher potential export of cereal grains.The overall goal willbe to increase trade in target commodities thereby increasing incomes earned by farmers. The outcome will also reduce public health risks associated with the consumption of commodities contaminated with aflatoxins. Appropriate technologies that found effective in the control of aflatoxins (along with the commodity value chain) canbe introduced and promoted.

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