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Occurrences of Mycotoxins Contamination in Crops from Pakistan: A Review

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Abstract: The aim of present review paper is to provide a comprehensive overview on mycotoxins effecting crops in Pakistan. Mycotoxins are toxic secondary metabolites produce by different toxigenic fungi species. The most prevalent mycotoxin in crops is Aflatoxin which causes serious health problems. In Pakistan limited information is available on toxicological data of mycotoxins in crops. Few researches after the year of 2007 highlighted the presence of different mycotoxins in different crops like rice, wheat, chilies and maize. Potential threats to human beings and animals after consumption of contaminated food materials.

Key words: Chilies · Aflatoxin · Maize · Wheat · Fungal contamination

INTRODUCTION

Potential health hazards and risks are caused by toxic and infectious food material that produce by microorganisms and lead to infectious diseases and long term negative health impacts [1, 2].

Naturally present toxins in food are plant secondary metabolites, bacterial toxins, phycotoxins and mycotoxins. Whereas mycotoxins are metabolites produce by fungi which contaminate food like crops, fruits and vegetables [3, 4]. Those fungi species which produce mycotoxins are known as phytopathogenic organisms that infect crops and fruits [5].

The mycotoxin producing fungi species are Aspergillus, Fusarium, Alternaria and Penicillium. There are many types of mycotoxins but the most toxic one is Aflatoxin B1 type of mycotoxin. Fungi *Aspergillus flavus* is predominant producer of the B1 aflatoxin.

Pakistan is basically agricultural based country which generates its revenue from crops. In Pakistan various cases of mycotoxins contamination of agricultural products has been reported, highlighting the facts that major portion of our wheat crops are under stress from fungal contamination [6].

Mycotoxin in Chilies: *Capsicum annuum* L. and *Capsicum frutescens* Mill are two varieties grown in Pakistan and commonly known as Chili belongs to family Solanaceae has significant economic value in Pakistan because it is not only using as spice but also eat as vegetable. It also consider as basic ingredient of everyday food in Pakistan. That's why it uses throughout the year however it grows seasonally, harvesting in mid July up to end of November month. Red chilies usually dried under sun heat just for 4 to 7 days or may be until the chilies have remaining 33% fresh mass. This lead to substantial possibility for moisture accumulation at some storage stages, which is favorable to the aflatoxin producing fungi growth. Even drying on the soil involves the collection of diseased chilies and takes for granted to remove the damaged chilies. End of chilies pod stem has glands which produce capsaicin which is responsible for chilies spiciness and also has significant medicinal value.

Chilies consumed all over the world, like in cooked dishes especially in curries. According to regulations of European Union (EU), in chilies only 5μg per kg B1 aflatoxin and 10μg per kg any other aflatoxin group is allowed. But in Pakistan there has no regulations has been set for chilies [7]. However problem due to mycotoxin in foodstuff require regulating some action to protect production. Pakistan is sixth largest country to produce chilies (around 2 lac tons annually) and marketing both red and green chilies [8]. In Pakistan Chili are grown at 473,000 hectares area in Pakistan. Three province contributing to produce this crop like Sindh 82%, Punjab 10.6% and Balochistan 6.1% of the total production.

Developed countries require good quality in commodities so for generating revenue by exporting products Pakistan needs to control mycotoxins production in chilies and related products [9]. In Pakistan,

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very limited information is present on mycotoxins in chillies. One study conducted in 1985 by Shamshad *et al.* [10] in which it has been reported that *A. flavus* was found in chillies samples taken from Pakistan. But no information found on aflatoxins concentration in Pakistani Chilies. For Pakistan National Master Agricultural Research Plan (1996-2005) recognized that there is requirement for research to conduct in order to increase the quality and yield of chilies crop by controlling fungus attack [11].

In Pakistan, studies since 2007 showed that chilies production may be highly contaminated by aflatoxin. This is not only major threat to population health but also a constraint on development.

One study in 2007 found on constraints facing by chili as a crop, like pests attack and diseases. The aim of this study was to highlight the aflatoxin concentrations in chillies to provide assistance to local farmers in controlling, managing and improve yield and quality. Researchers found aflatoxin B1 in all obtained samples of chilies. Moreover, high concentrations were obtained from all ground samples. Research stated that no relation found on detection of aflatoxin and *Aspergillus flavus*. However a direct relationship was found between aflatoxin B1 and Aflatoxin B2 concentrations [12]. Another study carried out on presence of aflatoxin of B group (AFB1) in Pakistan chilies samples. Total 22 pod samples and 22 powdered samples were selected and analyzed by using HPLC. Results revealed that 16 pod samples and 19 powdered form samples were found contaminated containing 73% and 86.4% AFB1, respectively. Mean concentration in powdered samples was 32.2 µg/kg which was greater than chilies pods that was 24.69 µg/kg. However concentrations found in powdered sample was ranged from 0.00 to 89.56 µg/ kg and 0.00–96.3 µg/kg for chilii pods. These concentrations presented higher than the limit set by the European Union. Study also highlighted the limits of detection and quantification that was 0.05 µg/kg and 0.53 µg/kg for powdered and pods, respectively [13].

Effect of winters in Pakistan on contamination of mycotoxins in chillies was studied in 2011. Researches made comparison of samples (n=43) collected during winter and samples (n=42) collected during summer for aflatoxins contamination in chillies. Samples were analyzed by HPLC along fluorescence detection. In winter season collected sample concentration was ranging from 0.00-52.30 µg/kg for pods and 0.00-74.60 µg/kg for ground samples. Whereas, in summer collected samples, concentration was ranging from 0.00-61.50 µg/kg for pods and 0.00-95.90 µg/kg for ground samples. Results revealed that aflatoxin contamination found in greater concentration in chilies samples collected during summer season and winter season chili samples and hence winter chilies samples were in better quality with respect to contamination [14].

Mycotoxins in Wheat Grains: Wheat is one of major crop in Pakistan, used as staple food in all part of the country [15]. Being major component of food, about 40 to 45% of nutrition is derived from the consumption of wheat, which is considered as an essential part of meal in everyday life of rural and urban population [16]. Pakistan being agricultural country, its agricultural sector is considered as back bone of economy, contributing about 21% to GDP of country [17]. Wheat is one of the major contributors to GDP after cotton, rice, maize and sugarcane [18]. Internationally Pakistan stands 6th in wheat production throughout the world and about 35-40% of national income is obtained through export of this commodity [19]. It has been exporting wheat to different countries (USA, Canada, Australia and France) in past few years [20]. Various constrains have been identified playing vital role in low quality and reduced yield of wheat crop [21, 22]. The quantity and quality of wheat is lost a lot in Pakistan during post and pre harvest operations because of fungal contamination [23, 24]. Due to these prevailing condition, the quality of wheat, unluckily doesn't meet the criteria of international standard, so it is expected to have loss in marketing and can also leads to decline in yields. In order to meet the challenges of competition in international market, this has become crucial for all exporters especially Pakistan to certain quality control system [25]. This can be obtained by adopting post-harvest activities in very scientific way according to preconditioning of ISO [26].

Wheat can be contaminated by variety of fungi at different stages of harvest i.e. inappropriate drying, storage, packaging and transportation [27]. The most important and most prevalent strains of fungi reported from different stored grains of wheat in Pakistan, are various species of *Apergillus*, *Penicillium, Fusarium* [28, 29, 30]. The most widely reported mycotoxins in wheat crop are aflatoxins and zearelenone and some traced concentration of deoxynivalenol [31,32]. In the presence of favorable environmental conditions fungal growth is facilitated that results in the increased rate of mycotoxins production during storage condition [25, 33]. The occurrence of subtropical climate in Pakistan, enhance the risk of contamination due to the presence of desired factors that increase the chances of fungal growth [34].

These factors are; variable temperature regimes, high humidity and nearness to sea. Similarly the season of wheat cultivation is of major concerned in Pakistan, cultivated in November to December while harvested in May to June which greatly increases the chances of fungal contamination at pre harvest stage. Secondly our former sector is not aware of modern technologies, they still use outdate and old traditional technologies like the use of sensitive varieties, lack of crop rotation, reduced or no tillage. These are all factors which double fold the chances of fungal attacks. In Pakistan, rural areas former usually store wheat grains in clay bins which absorb moisture content from the surrounding and therefore increases the risk of mycotoxins production in grains [18]. Mycotoxins are recognized throughout world, as one of major agent of food contamination. The risk of contamination of different agricultural commodities most importantly wheat and rice as staple food is increases and the hazards of using the contaminated products have been highlighted, not only in Pakistan but also throughout the world [35]. These commodities are vulnerable to mycotoxins contamination in the presence of high humidity and high mould contents if not stored appropriately [36]. Once these products got the chance of contamination in the fields, fungal contamination continue through different stages of harvesting such as packaging, storage and various other processing stages, as long as the environmental conditions especially moisture conten and temperature are conductive to fungal development [37]. There are certain other factors called stress factors such as shortage of water and pests and nonresistant quality of seed that can increased the probability of mycotoxins production in wheat crops [38, 39]. Mycotoxins are climate dependent that is considered as key driving force in fungal accumulation and mycotoxins production [40, 41]. In this regard Pakistan is more vulnerable to such climatic events like alteration in temperature, humidity, drought and flooding [42]. Pesticides, heavy metals and soil fertilizers are other potential triggers of concern in developing countries. Changing weather pattern in Pakistan may threaten the availability of food in future, if they affect staple food like wheat.

Wheat is not only used as important part of our meals but also used in the various other products [43]. Extensive studies in Pakistan illustrated the fact, that mycotoxins are also a source of contamination for consumers not only in wheat flour but also in the form of other wheat derived products (Aspagati, Macroni, noodles etc), due to changing life style of people towards eastern food habits [18]. So once the contaminated wheat is entered through our food chain it might poses various significant health hazards in human at different levels from diverse sources [44]. The consequences of these health hazards are reported to be very complex due to carcinogenic, hepatic, teratagenic nature of mycotoxins. Humans in developing countries like Pakistan are exposed to such contamination throughout their life due to the widely consumption of mycotoxins contaminated food (Wheat). The continuous exposure to such sources may lead to sufficient concentration of mycotoxins in the body and can adversely affects animals and human health [45]. However, the risk of contamination is dependent on intake dose, toxin type, mode of action, exposure time period and defense mechanism [46,47,48,49]. In human the major health illness associated with mycotoxins contamination includes Liver cancer, bile duct proliferation, lethargy and edma. Several cases of liver cancer have been reported from Pakistan due to consumption of mycotoxins contaminated cereals [50].

Mycotoxins in Maize: Maize (*Zea mays* L.) is an important crop and it has a significant value in the economy of Pakistan. It is used for multiple purposes and considered as the third essential cereal following wheat and rice [51]. People especially of the mountainous areas consume it as food. Its major uses are for human consumption, used as an animal feed and also for the production of oil etc. The products of maize are widely used such as corn flakes and corn flour etc. 1.02 million hectares of maize was produced in the years of 2006 and 2007 and the overall production was 3.09 million tons [52]. Because of its vast utilization its production was increased in the years of 2008 to 2010. In 2008 the production was 3313 million tons but in 2010 it was 3487 million tons [53]. Punjab and KPK are the main areas for growing maize. And the cultivation of maize is done twice a year, once in autumn and second time in spring.

A wide range of poisonous fungi may attack the maize grain [54, 55]. Maize has been widely studied for the contamination of the mycotoxin [56]. Worldwide surveys showed the common fungi which affect the maize crop are *Aspergillus flavus, A. parasiticus, A. ochraceus* etc [57, 58, 59, 60]. A number of mycotoxins are produced by these fungi and these can damage the products of agriculture. The aflatoxins and ochratoxins are the most toxic among the mycotoxins which damage the maize crop [61, 62]. There are various reports in the literature on the mycotoxins in maize in Pakistan.

As Pakistan is located in the subtropical to the tropical region so the environmental conditions of Pakistan are ideal for growth of maize in some regions. At the same time the facilities for the storage of maize are so poor which enhance the growth of fungus. This as a result leads to the production of mycotoxin. So, the mycotoxin production has become an important issue in Pakistan but very little publications are available about the contamination of maize by the mycotoxins. Also these publications cover only small regions for the description and presence of mycotoxins in maize [32, 63, 64]. There are several reports for the aflatoxins and ochratoxin A in local varieties of maize[65,66,67].

The weather conditions are favorable for maize cultivation but during the stages of growth till harvest certain factors enhance the production of *Aspergillus* species which as a result produce aflatoxins as secondary metabolites. The moisture content greatly affects the maize during the storage conditions and in some of the cases although there is less moisture content available to the maize but that may also cause the mycotoxins production [68]. This shows that there are other factors also which cause the mycotoxin production such as water activity and temperature etc. Some other factors such as the structure of the storage area as well as the physical situation of the grain also cause the growth of fungi in maize [69, 70].

The safe limit according to FDA and WHO for aflatoxin B1 and ochratoxin A in food is 20 µg kg-1and 5 µg/kg, respectively [71]. Maize which contains 20 µg kg^{-1} should not be allowed to sell and it should not be commercially used. Despite the human consumption such kind of maize should not be used as an animal feed also [72].

A survey was done about the maize of South West Pakistan and it was found to be contaminated with aflatoxins. *Aspergillus flavus* was the main fungus present which damaged upto 59% of the kernels of corn [73]. It was found in the most of the studies that *Aspergillus flavus* was the most common specie of mold present in the maize. A study was conducted by Shah *et al* [68]which they found that the *Aspergillus flavus* had the highest incidence in the maize kernels taken from the swat valley. They studied the incidence of mould and also contamination of aflatoxin B1 (AFB1) and ochratoxin A (OTA) contamination also adjacent minerals content of maize kernels from Swat Valley in 2007. A major number of samples contained the AFB1 and OTA above the safe permissible limits which showed that the consumers of that area may be in risk of aflatoxins as well as ochratoxins poisoning. AFB1 content was present from none to 30.92 µg kg^{-1}.Similarly OTA ranged from <0.001 to 7.32 µg kg^{-1}.

The samples of rural areas were more dangerous for the local people as well as for animals. The regular use of such crop cause damage to animal and human health. People should be made aware about such problems in the rural areas such as they should have awareness of the risks associated with health. The samples in any area should be properly detected and measured for mycotoxins concentration before storage and buying. Similarly a study was conducted by Saleemi et al. in which they studied the toxigenic fungi in maize as well as in the maize gluten meal by isolation and identification method. Samples were collected from the Faisalabad district for two years. The numbers of samples collected were 82 of maize and 8 of maize gluten meal. The contamination of fungi was 56% and 75% in the maize as well as maize gluten meal respectively [74]. There is a requirement to establish as well as to enforce maize quality standards and regulations related to mycotoxins in any area of Pakistan.

Animals in domestic farming majorly depend on the grazing while they need concentrate in very small portion. Animals like cows, buffaloes, goats feed on the maize crop especially in the northern areas of Pakistan. While on the commercial level, dairy feed contributes up to 70% to meet the needs of animals. This may increase the risk of different types of mycotoxin production in the dairy feed [75]. Compound feed provided to the animals mostly include dairy feed, silage and mixed rations. Silage is used as an animal feed from the past few years by ensiling of crops such as alfalfa, sorghum and corn. During this process, several factors such as moisture content, temperature, insufficient drying, condensation, insects and some other conditions could produce or stimulate aerobic growth of fungi. Such undesirable growth of toxic substances leads to the nutritional loss and its economic value [76, 77].

Mycotoxin in Rice: ice (*Oryza sativa* L.) is one of the most important dietary staple foods and cereal crop almost all around the world and most especially in Asia [78]. Rice provides the bulk of daily calories to humans and other animals. Its unique taste makes it easy to combine with any other food to enjoy better taste and nutritional balance [79, 80].

Studies have been carried out in various parts of the world regarding the fungal attacks and mycotoxins infestation in rice and its products. Contamination of agricultural commodities especially of rice by mycotoxins

is a serious issue for human health in the tropical and sub-tropical regions, as in these regions, the climatic conditions, agricultural and storage practices contribute to fungal growth and toxin production [81, 82]. Shotwell *et al.* [83]evaluated different agricultural commodities such as, rice, wheat, corn, soybeans and sorghum etc. for mycotoxin invasion. They found that rice served as the best substrate for the production of aflatoxins.

Pakistan is one of the major basmati rice producing country. Rice is cultivated in wet season (kharif). Rice is cultivated on about 10% of the total arable land in Pakistan that accounts up to about 2.963 million hectares. In 2008–2009, the rice production was 6.952 million tonnes that contributed 5.5% of the total agriculture produce in that period and made a 1.6% share in the country's GDP [84]. Internationally, Pakistan is the 14th major rice producing country and stands 6th largest exporter of rice, contributes 6% in the world's rice export. The contribution of rice industries in the country's economy is about 21.75% [85]. The basmati rice cultivated in Pakistan has been a favorite food among the global community. The major yield is obtained from Punjab and Sindh provinces. Pakistan exports basmati rice, mainly to: Saudi Arabia, Qatar, U.A.E, Bahrain, UK, Malaysia, Yemen, USA and Kuwait. Rice is frequently examined for Aflatoxin contamination as per mandatory requirement of phytosanitary certificate based on its wide consumption within the country and exportation to many other countries. Asghar *et al.* [86] conducted a study in Pakistan, from 2006 to 2011. They analyzed a total of 2047 samples of basmati rice for the presence of AF-B1, AF-B2, AF-G1 and AF-G2. They found that, 73.3% samples were found to have an average concentration of 1.15µg/kg of F-B1, which was lower than European Union's Maximum Tolerated Level (MTL of 2µg/kg), whereas in 26.7% samples, AF-B1 was not found within detectable limit (>1µg/kg). In the remaining samples, some concentrations of Aflatoxins were found that were within the acceptable limits set by EU and USA. However, only 0.44% of the samples, the concentration of AF-B1 ranged between 4.07–6.91µg/kg, which exceeded the tolerable limit set by EU. Although it was observed that none of the samples contained aflatoxins that could exceed the tolerable limits set by USA (FAO and FDA) and Pakistan of 20µg/kg as assigned by food authorities of USA (FDA and FAO) and Pakistan Standard & Quality Control Authority (PSQCA) [87]. It was also reported that after 2007, Pakistan was hit by unscheduled rain and drastic floods that significantly affected the rice crops. The major impacts were observed up till 2011, as serious losses were observed by the country in quality as well as quantity of the rice products [86].

Hussain *et al.* [88]conducted a survey in Pakistan and reported that 70% of the rice samples were found contaminated with Aflatoxins with an average concentration of AF-B1 and other Aflatoxins to be about 3.7µg/kg and 4.9µg/kg, respectively. Another study was carried out in Pakistan, in which a total of 599 samples of rice were collected and analyzed for aflatoxin contamination. The average concentrations of AF-B1 and AF-B2 in brown, white and sella rice were found to be 0.56-0.73µg/kg and 0.02-0.03µg/kg, respectively, while in August (2010), the levels for AF-B1 and AF-B2 were found 16.65µg/kg and 2.64µg/kg, respectively. It was reported that warm weather and climate provides favourable environmental conditions for fungal growth led by mycotoxins production [89]. All these researchers concluded that Pakistani rice products, most especially, the basmati rice was found to be contaminated with some levels of Aflatoxins but these levels do not concurrently present a potential risk to human health. Although there is a need for further investigations in this field that requires regular monitoring and performing strict routine analysis as per food quality control measures. The concentrations of mycotoxins can be further reduced by good and advanced processing and storage practices. Along with these, other measures such as the use of botanicals, microbiologicals and cooking methods, regarding the reduction of mycotoxins contamination in rice, can also serve the best in managing these issues [90].

CONCLUSION

Keeping in mind the significant role of fungi in mycotoxins production in crops, it is strongly recommended that seed lots should be regularly monitored through certain modern technologies, in order to determine the health status of seeds prior to be exported. The seed contaminated with fungal spores should be treated at pre harvest level as a control measure of spread of fungal diseases. The review also suggests that fungal growth need to be controlled both in field and storage prior to consumption for saving the humans and animals health from deleterious impact of contamination. The chances of mycotoxins contamination in cereal grains like wheat, rice and maize were increased due to high moisture content and inappropriate storage temperature. Therefore all seed grains must be maintained at proper storage conditions to stop fungal proliferation.

Post harvest practices also need to properly improve with use of modern technologies in drying, storage and transportations. Decontamination,, detoxification and cleaning are some other remedial measure that can be adopted in order to cease or reduce contamination to some extent. To ensure the health safety of consumers, the concerned regulatory authorities are suggested to take into account this serious issue of food contamination due to fungal growth and controlling strategies should be adopted and quality control system of food should be practice and improved.

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