

Mycotoxins Analysis in Fresh and Dry Fruits from Pakistan - A Review

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Abstract: In Pakistan consumption of fruits has dramatically augmented about 20% and each year fruits production is affected due to microbial spoilage. The contamination of fruits with mycotoxins is investigated to find out how they affect human health and animal food chain. Mycotoxins are metabolites of fungi and comprises of fumonisins, aflatoxins, ochratoxins, deoxynivalenol (DON), trichothecenes, zearalenone and patulin. Mycotoxins are reported to be carcinogenic, tremorogenic, haemorrhagic, teratogenic and dermatitis to a wide range of organisms and cause toxic responses under naturally occurring condition. In this review, the major focus is on identification and detection of different mycotoxins in fruits and to study their impacts on human health among Pakistani population. A variety of fruits such as apple, citrus and mangoes and dry fruits are produced and exported to other parts of the world but dilemma of mycotoxins contamination is affecting export of fruits from Pakistan. The Food and Agriculture Organization (FAO) estimated that about 25% of the world's agricultural production is contaminated with mycotoxins which cause huge losses for farmers in Pakistan. These losses resultant in reduced crop yields, lower quality, reduced animal performance and reproductive capabilities and increased disease incidence.

Key words: Mycotoxins • Aflatoxins • Trichothecenes • Patulinhaemorrhagic • Teratogenic • Dermatitis • FAO

INTRODUCTION

Mycotoxins in fruits and fruit-processed products [1] revealed that the commonest mycotoxin contaminants of fruits worldwide are Patulin, Ochratoxin and Aflatoxins and Alternaria [2] contamination has been reported in oranges, apple and apple juices, dried apricots, dates, prunes, musts, dried figs and raisins [3]. Aflatoxins are most frequently reported in dried figs and raisins worldwide at significant levels of up to 550 and 63 µg/kg, respectively [4].

The presence of mycotoxins results in depreciation in quality of fresh fruits during harvesting and storage [5]. In Pakistan the nutritive value of different fruits such as apple, grapes, mango etc are assessed along with the microbial contaminations [6, 7]. It is suggested that international trade in fresh fruits influence significantly due to plant pathogens which infect fruits either prior to or after harvest during transit and storage conditions [8]. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens which influence the fruits economic value and human health [9, 10]. For example,

Patulin, is produced by *Penicillium* species is a known hazard in the apple juice industry where its levels are under regulatory control (50 ppb in apple juice) [11] while Orange juice and fermented fruit beverages are also susceptible to mycotoxin contamination from *Fusarium* and *Aspergillus* species [12].

According to the EU limits for different samples of fruits such as apples, citrus and mangoes were investigated by Parc laboratory in Karachi but currently Pakistan faces export restrictions by Japan (mangoes), Philippines (citrus fruit) and Italy (apple) due to pesticide, insecticide contamination and quarantine issues. The International Agency for Research on Cancer (IARC) concluded that no evaluation was made respecting the carcinogenicity of patulin to humans [13].

In one of the report Dr. Mubarak Ahmed (Director General of Parc), said:

“Food contamination was a major issue in Pakistan that had not only endangered public health but also badly affected the country's exports”.

It is estimated that fresh fruits contain high levels of sugars (86%) and nutrients (97.55%) with low pH which is responsible for fungal decay of fruits [14]. Pathogenic organisms can enter fruits through damaged surfaces, such as punctures, wounds, cuts and splits [15]. Fungal fruits infection may occur during growing, harvesting, handling, transport, post-harvest storage and marketing and after purchasing [16] conditions which make them prone to fungal decayed [14].

Fungal spoilage of fruits at low temperature [17] much attention in recent times owing to its potential health hazards [18]. The most common species of mycotoxins on fruit were *Botrytis*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Mucor* and *Penicillium* species [19]. Among microbes, *Penicillium expansum*, is a major causative agent of post harvest decay in fruits known to cause harmful effects in humans [20]. With the passage of time it is important to identify and detect fungal contaminants in fresh fruits because some moulds can grow and produce mycotoxins on these commodities and can cause allergies [21]. Contaminations of fruits with Aflatoxin have been associated with occurrence of liver cancer among population of Karachi [22].

Contamination of Tomato Fruit with Mycotoxins: In 2008, G. Dal Bello reported that postharvest tomato fruit rot caused by *Trichothecium roseum* [23] is evident for the first time in Argentina. The fungus was consistently isolated from diseased fruits and shown to be the causal agent according to Koch's Postulates [24]. Although the economic impact of the disease is minimal, *T. roseum* is known as a producer of mycotoxins [25]. Therefore, its presence on fruits decreases the quality of those food products and causes a risk to the consumers [27]. Similarly, Wilson and Payne [28] revealed that the presence of Aflatoxins in fresh tomatoes, cucumber and peach grown in Pakistan at level ranging from 31.21 to 268 µg/kg. This suggests that the contamination of fruits with mycotoxins increases the urge of research in this area [29].

Impact of Different Mycotoxins in Apple and Apple Juices: Pakistan is producing approximately 35, 1900 tonnes of apples since 2007 [30]. But usually its harvesting and cultivation is confined to the northern areas, Balochistan, hilly areas of Khyber Pakhtunkhwa and Punjab [31]. Different varieties of apples produce in Pakistan are Kala Kulu, Amri, Mashhadi, golden delicious and red delicious etc. [32]. It is one of highly nutritious and healthy fruits which contain variety of macro and micronutrients i.e. carbohydrates, proteins, fats and water

[31]. In February 1866, *Pembrokeshire* quoted in a magazines, which depicts the importance of apple in daily life :

"Eat an apple on going to bed and you'll keep the doctor from earning his bread"

According to Mehmood *et al.* [33] apple is one of the most important fruit species existing around the world and is being cultivated throughout the world. Approximately 111600 hectares area is required for its cultivation, leads to the production of about 3666300 tons per year. These facts are also claimed by Hasan [34]. *Aspergillus flavus*, *A. niger*, *Penicillium expansum* and *Rhizopus stolonifer* were the most often isolated fungi, attack on fresh apple fruits. *Alternaria alternata* was found in rotten apple fruits, followed by *A. niger*, *A. flavus*, *P. expansum* and *R. stolonifer* which is further supported by Karaibrahimoglu *et al.* [35].

Karaibrahimoglu *et al.* [36] determined the pH values of apple juice varying with the variety of apples such as Red delicious and Golden delicious. The pH of red apple along with the peels lies within range of 4.1-4.9.

Mushtaq *et al.* [37] also screened mycotoxins in fruits grown in Sindh, Pakistan. It was evaluated that most of the fruits (apple, mango and peach) were contaminated by deoxynivalenol (DON) which were below the detection limit [38]. Samples of fruits were analyzed by Competitive Direct Enzyme Linked Immunosorbent Assay (CD-ELISA) and thin layer chromatography (TLC) [39]. In latest survey, different species of apples were selected from three different survey points of Rawalpindi and Islamabad by Abbas *et al.* [40]. Sample areas were Peer Wadai and Margalla hills. In order to check injury sites on apple fruits, a conidial suspension of 100 µL was prepared and applied on fruits for almost 8 days [41]. Results confirmed that Polygonium (50 µL/wound) in methanol extract can be used for antifungal properties and were very effective in mycotoxins growth on apples [42].

Facts about Mycotoxin in Apples: Mukhtar *et al.* [6] studied nutritional and microbiological aspects of apples of common variety available in local market and household consumption. Peeling reduced both volume and pH of apple juice extracted [43]. The energy, carbohydrates, fat, protein and fiber contents were high in Golden delicious variety while moisture in Kala kulu and the ash contents were high in Red delicious [44]. Bacteriological analysis showed that gram positive bacteria were the dominant strains associated with apple surface. The most common genus

of fungi grown on the surface was *Aspergillus* and *Penicillium*. It was observed that washing with cold running tap water reduced the microbial contamination on apple surface [20].

Measures to Overcome Mycotoxicity: Fruits preservation by sun drying process is a common practice in Pakistan [45]. Both fruits dried for preservation purposes such as dried apricots, plums, dates and dried figs and dried nuts are more vulnerable to fungal infections and mycotoxins [44]. The preserved fruits are exposed to mycotoxins during drying process in trays [46] while dried nuts are usually exposed to fungal infections during drying periods on trees [47]. Various studies have been conducted to reveal the presence of mycotoxins in dried fruits. Aflatoxins occurrence in dried dates, figs and raisins has been studied by Zohri and Abdel-Gawad [48]. Apricots and plums exposed to ochratoxins have also been identified [49]. Dried figs exposed to fumonisin [20] and dates contaminated with zearalenone have been reported in different studies conducted worldwide [43].

Mycotoxicity Analysis of Mangoes: Mango (*Mangifera indica* L.) is one of the most important fruit of the world and it is known as “The king of fruits” [50]. It is generally cultivated in tropical and subtropical parts of the world. Due to its luscious taste, aroma and striking color has achieved the recognition in the world. The main cultivar of mango is Asia with 76.9% of the total world production and amongst countries Pakistan is at 5th number with a part of 7.6% in the world market of mango production [51]. It is extremely a delicate fruit and does not survive in cold environment so it is a short seasoned fruit [52] and also susceptible to attack by pathogenic fungi, which in addition to causing rots may also make them unfit for consumption by producing mycotoxins [53]. A lot of predicament associated to postharvest life of mango are linked with microbial and fungal decline of fruit. These problems established an immense deal of consideration during the most recent decades. The fungal species infect mango fruits at various points of farm chain like preharvest, transportation, storage or processing [54]. Some species of fungi (Aflatoxins) are the most potent natural carcinogens, mutagens and teratogens [55]. Lot of research is made on to evaluate the presence and the effect of mycotoxins on the [56].

Nazim *et al.* [57] reported the presence of mycoflora in fruit juices (mango juice). They also tested the pH of juice and concluded that pH of the mango juice is 5.75. *Aspergillus niger* was found to be prevailing fungus in mango juice sample followed by *A. clavatus*. Recently,

incidence of the quick decline of mango trees found to be 20% and more than 60% in Punjab and Sindh provinces of Pakistan, respectively [58].

The majority of the researchers reported that *Botryodiplodia theobromae* causes the decline in the variety of mango in Pakistan [59]. Mahmood *et al.* [60] studied the rapid decline of mango plants due to the fungal growth. They take the samples of contaminated parts from mycotoxins such as (root, bark and seeds of mango) from Punjab. The study reveals that *Fusarium oxysporum* and *F. solani* are present in root samples of plants whereas *Botryodiplodia theobromae* is present in bark samples. Inoculations with pure culture of *B. theobromae* to the mango seedlings caused 86.7% mortality, which confirmed the cause of quick decline.

Khanzada *et al.* [61] also reported the mango plantations in different areas of Sindh found to endure from a decline disease and in severe conditions, the disease results in death of the plant. They concluded that this is due to association of *Lasiodiplodia theobromae* mycotoxins in different varieties of mango fruits. Iqbal *et al.* [62] designed a study to evaluate the occurrence and intensity of different decline disorders existing in mango mounting areas of the Punjab, Pakistan. Four disorders twig blight, tip dieback, gummosis and bark cracking is prominent with 55.0, 50.0, 25.0 and 25.0 % occurrence while intensity ranged from 16.0- 50.0 % [63].

Methods for Mycotoxin Detection in Mangoes: Chatha *et al.* [64] studied effect of two methods (gamma irradiation along with UV-C and hot water) on the production of mycotoxin. The results indicated that the non-thermal techniques (gamma irradiation and UV-C) are relatively better over the conventional hot water treatment in production of aflatoxins in mango so, gamma irradiation and UV-C attain the promising method to replace conventional method [32].

Iqbal *et al.* [65] studied the association of fungus with the mango malformation (MM) disease. In this study they examine the malformed parts of mango species of the Punjab province of Pakistan and discover the connection of four fungi such as *F. mangiferae*, *F. pallidorozeum*, *F. equiseti* and *Alternaria alternata*, while *F. mangiferae* confirm to be the major infecting fungus of the Mango disease.

Malik *et al.* [66] indicated that about different mango diseases which are becoming serious threat to economy of Pakistan. The evidence is presented that pathogen *Ceratocystis fimbriata* causes sudden death of mangoes in Pakistan possibly in concert with *Botryosphaeriaceae* fungi like *Lasiodiplodia*

theobromae or *Nattrassia mangiferae*. Results of the study explain that *Lasiodiplodia theobromae* and *Nattrassia mangiferae* are weak pathogens as compared to *Ceratocystis fimbriata*.

Measures to Overcome Mycotoxins Contamination in Mangoes:

Most of the workers studied the use of different techniques in order to reduce these diseases which cause the quick decline of the production of fruits (mango) like Sahi *et al.* [67] evaluate the effect of fungicides in order to reduce the impacts of mycotoxin (*Botryodiplodia theobromae*), the causal organism of quick decline of mango (*Mangifera indica* L.), in the production of mango in Pakistan. The result findings divulge that these fungicides are extremely efficient even at a very low concentration against *B. theobromae* and the effectiveness of the fungicides increased with increased dosage rate.

Amin *et al.* [68] also done their research on the effects of fungicides and plant activators restrain the disease growth in fruits and vegetables. This study is targeted to estimate the prospective of pre and postharvest fungicidal applications. *Alternaria alternate*, *Phomopsis mangifera* and *Botryodiplodia* species is found to be allied with mango fruit stem end rot (SER) of Punjab, Pakistan, while *Penicillium* species. And *Aspergillus sp.* is recognized to be connected with secondary infections in the diseased mango fruit tissues. The results reveal that the postharvest fungicidal management provides significantly better disease control as compared to preharvest applications and also better colour development was observed in the fruit subjected to postharvest fungicidal treatments as compared to preharvest applications.

Results after Analysis: Shahbaz *et al.* [69] planned a study to characterize the isolates of the fungus *Lasiodiplodia theobromae*, test their virulence and evaluate different fungicides to find out effective ones for field application. The results of the study reveals that ten isolates were identified from 10 mango growing districts of the Punjab province of Pakistan and resulted in 66.66% mortality and 3.0, 4.9 and 4.5 cm² pathogenicity lesion, respectively. The results of fungicide applications showed 100% decrease in fungus growth.

Analysis of Mycotoxins in Dry Fruits in Different Regions of Pakistan

Methods for the Detection of Mycotoxins in Dry Fruits:

In Pakistan there is variety of mycotoxins on dried fruits which varies from a few hundred per gram of fruits to

thousands [70]. Presence of spores of bacteria and molds are likely to be responsible for degradation of quality of fruits by mycotoxins before or after drying processes [71]. Spoilage of most dry fruits usually occurs during storage, handling and transport.

Methods for the Detoxification of Mycotoxins:

Along with identification of mycotoxins there is also a need to investigate the methods for detoxification of mycotoxins in dried fruits. Mobeen *et al.* [72] evaluated the microwave heating method for investigation of detoxification level during heating process. Study investigated the presence of aflatoxin B1 in the range 5-183 µg/kg and aflatoxin B2 in the range 7-46 µg/kg in peanuts and peanut products such as roasted, salted peanuts, peanut butters. For detoxification the products were heated to 92°C using microwave which indicated the 50-60% reduction in toxicity level up to non-detectable limits.

Results of Samples of Dried Fruits Contaminated with Mycotoxins:

In Pakistan 180 samples of dried fruits and nuts taken from different localities of Northern areas and KPK were analyzed for presence of aflatoxins in peanuts, pistachio, figs apricots and walnuts. The results indicated the presence of aflatoxins in 16 samples above the EU regulation limits i.e. 4mg/kg. The highest contaminations were found in pistachio and peanut samples i.e. 14mg/kg and 14.5mg/kg, respectively. Percentage of total aflatoxins contamination in dried fruit samples were dates (10%), raisins (20%), peanuts without shell (50%), peanut with shell (40%), pistachios without shell (50%), pistachios with shell (20%), walnuts with shell (40%), walnuts without shell (70%), dried apricot (20%), apricot kernels (26%), dried mulberries (26%), dried figs (50%), almonds without shell (30%) and pine nuts with shell (20%) [73].

Peanut, the main crop of Pothohar region was investigated for presence of aflatoxin in 72 samples of salty roasted and unripe peanuts. The results indicated the detection of aflatoxin in 82% samples, with concentration 14.25µg/kg to 98.80µg/kg. The results indicated the fungal contamination in fields as well as in storage sites [40]. Mushtaq *et al.* [37] studied the 125 processed food samples including processed peanuts for aflatoxin detection. The results indicated the presence of aflatoxin in 60% processed peanut samples in concentration ranging from 0.27-2.08µg/kg.

Saleemullah *et al.* [74] reported the presence of mycotoxins in nut fruits i.e. walnut, peanut and almonds at different storage time. The results showed increasing contamination with increased storage time i.e. 7.5-19.7µg/kg (2-3 months) and 11.9-30 µg/kg (12-18months).

CONCLUSION

It can be concluded that inconsistencies in reports and lack of survey data on the prevalence and level of mycotoxins in fruit products makes the risk assessment of mycotoxins in fruit products challenging in Pakistan. The contamination of mycotoxins in the food chain is an inevitable and poses serious threat to food safety.

To overcome these problems public awareness among different stake holders like farmers, processors, exporters and consumers may be helpful to combat the situation has to be created along with the adoption of proper pre-harvest and post-harvest technologies can play an imperative role.

Food hygiene trainings and implementation of strict rules and regulations with changing food safety perspectives might be helpful to curtail the contamination of mycotoxins in Pakistani foods. Intensive screening of microbes may lead to detection of microorganisms. Mycotoxin contamination in our local foods is posing a serious threat to Pakistani population resulting in increase of several chronic and acute diseases. The situation is alarming for policy makers as well as masses.

With the application of molecular biology techniques, the potential mycotoxin degrading microbial strains can be engineered to significantly improve the quality and safety of foods from mycotoxins contamination to protect consumer's health. Finally a most useful practical technology should be developed from economical point of view. It is proposed from different studied that it is not the responsibility of mycotoxicologist to overcome mycotoxin contamination in different agricultural commodities but participation of chemist, botanist, biotechnologist and GIS expertise is equally encouraged.

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