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Chemical Structure, Occurrence and Health Hazard Status of Ochratoxin A (OTA) in Cereal Food and Feeds of Pakistan: A Review

[1]Abdul Kabir Khan Achakzai*, [2]Samiullah, [2]Mohammad Yaqoob, [3]Mujeeb-Ur-Rahman and [1]Mohammad Younas Khan Barozai

[1]*Botany Department, University of Balochistan, Quetta Pakistan.*

[2]*Chemistry Department, University of Balochistan, Quetta Pakistan.*

[3]*PCSIR Laboratories, P.O. Box, 387 Mastung Road, Quetta, Balochistan, Pakistan.*

profakk@yahoo.com*

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Summary: Ochratoxin A (OTA) is the second most important and naturally occurring mycotoxin after aflatoxin produced as a secondary metabolite by 8 kinds of *Aspergillus* and 6 species of *Penicillium,* which can contaminate a wide variety of food and feedstuffs. But it is mainly produced in cereals by moulds *viz., Aspergillus ochraceus, A. carbonarius* and *Penicillium verrucosum.* There are more than 19 natural and synthetic ochratoxins, the most common and naturally occurring one is OTA. It could be determined by various analytical techniques. However, biochemical method by using aptamers (synthetic bioreceptors) has gained more attention in affinity based assays. OTA being nephrotoxic, carcinogenic (Group 2B), tetratogenic, hepatotoxic and immuno-suppressive imparts several human and animal health problems. It causes a disease known as ochratoxicosis. The main controlling factors which affect the distribution and growth of OTA producing fungi during farming, harvesting, and storage (conditions and periods) of cereal food and feeds are moisture, temperature, medium composition, and the time a product kept under adverse climatic conditions. Results based on the status of OTA contamination in cereals in general and corn and rice food and feed in particular is alarming in Pakistan. Most of the analyzed cereal food in general and feed samples in particular are exceeding the acceptable or permissible limit (5ppb) as proposed by European Union (EU) regulatory commission, and other renowned food and feed monitoring agencies in the world. In the light of published reports, it is suggested that proper harvesting, storage of feed be done and unhygienic method of processing and production be avoided, and there must be also a regular monitoring system to check the health hazard status of OTA of agricultural commodities in general, and cereal food and feeds in particular.

Key words: Mycotoxins, Ochratoxin A (OTA), Ochratoxicosis, Cereals, Food, Feed.

Introduction

Cereals are ubiquitously consumed by human and animals all over the world, and provide them an important source of nutritional energy. Beside the high contents of polysaccharides (e.g. starch) as source of energy, cereals can also provide a balanced diet containing dietary fibre, nutritional protein, and lipids rich in essential fatty acids (i.e., α-linolenic acid, and linoleic acid [1]. According to an estimate, the globally consumed cereals can directly provide about 50% protein and energy necessary for the human diet. While an additional 25% of the same is provided indirectly through livestock [2]. According to FAO the world cereal production in year 2017 is projected at 2597 million tones, only 0.3% (i.e. 9.0 million tonnes) below the year 2016 record, but still above the five-year average. This reduction is mainly due to fall in the wheat output to 740 million tones, representing a 2.7% (i.e. 20.3 million tonnes) decline from year 2016 [3]. Pakistan is ranked at number 3 in Asia and number 8 in the world for major cereal i.e. wheat production by producing an average of 24.3 million tons wheat in the year 2012-2014 [4].

Ochratoxins (OTs) are the hazardous group of mycotoxins produced as secondary metabolite by several moulds. While ochratoxin A (OTA) is the second most important and naturally occurring mycotoxin after aflatoxin. It is receiving great attention through out the globe, because of the hazard it poses both to human and animal health. The contamination of cereal crops with ochratoxigenic fungi and ochratoxins is responsible for heavy losses in cereal crop. OTA and the fungus that produces it do not cause any visible damage to grain and can not be detected through visual inspection, and grading [5].

Types and Chemical Properties of Ochratoxins (OTs)

OTs is a second major mycotoxin or fungal metabolites which are toxic to both human and animals. There are more than 18 types of OTs. Of which 15 are natural and remaining 3 are synthetic. However, there are occurred 5 common OTAs *viz.,* ochratoxin A (OTA); ochratoxin B (OTB); ochratoxin C (OTC); Ochratoxin α and Ochratoxin β.

*To whom all correspondence should be addressed.

They all possess some chemical differences. However, such differences may have obvious influences as toxic potential. OTA is 7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3*R*-methylisocoumarin bonded to L-*β*-phenyalanine through 7-carboxy by amide linkage. The variation in the structure of ochratoxin is illustrated in Table-1 and Fig. 1a. Two enantiomers of OTA i.e. 4*R*-OH-OTA and 4*S*-OH-OTA are also identified. Furthermore, OTA and OTB also exist as methyl and ethyl esters (Table-1 and Fig. 1b). The amino acid (phenylalanine) moiety in OTA is replaced by other amino acids such as tyrosine, valine and serine to produce other analogues of OTA [6]. The synthetic analogues proved to be more toxic (Fig. 1c). However, later on it was reported that the serine, hydroxyproline and lysine also occur naturally [7]. The tyrosine analogue of OTA is also produced by the hydroxylation of OTA by an enzyme phenylalanine hydroxylase [8]. The two acidic functionalities (carboxyl group of phenylalanine and the hydroxyl group at position 8) of OTA make it easily ionizable in physiological systems. The pKa values of OTA, OTB and OTC are 7.05, 8.0 and 7.10 respectively [9]. The change in pKa values is depicted by the variation in the structure of ochratoxins.

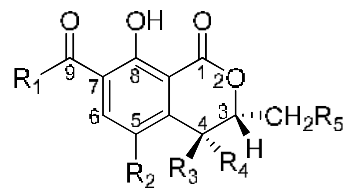
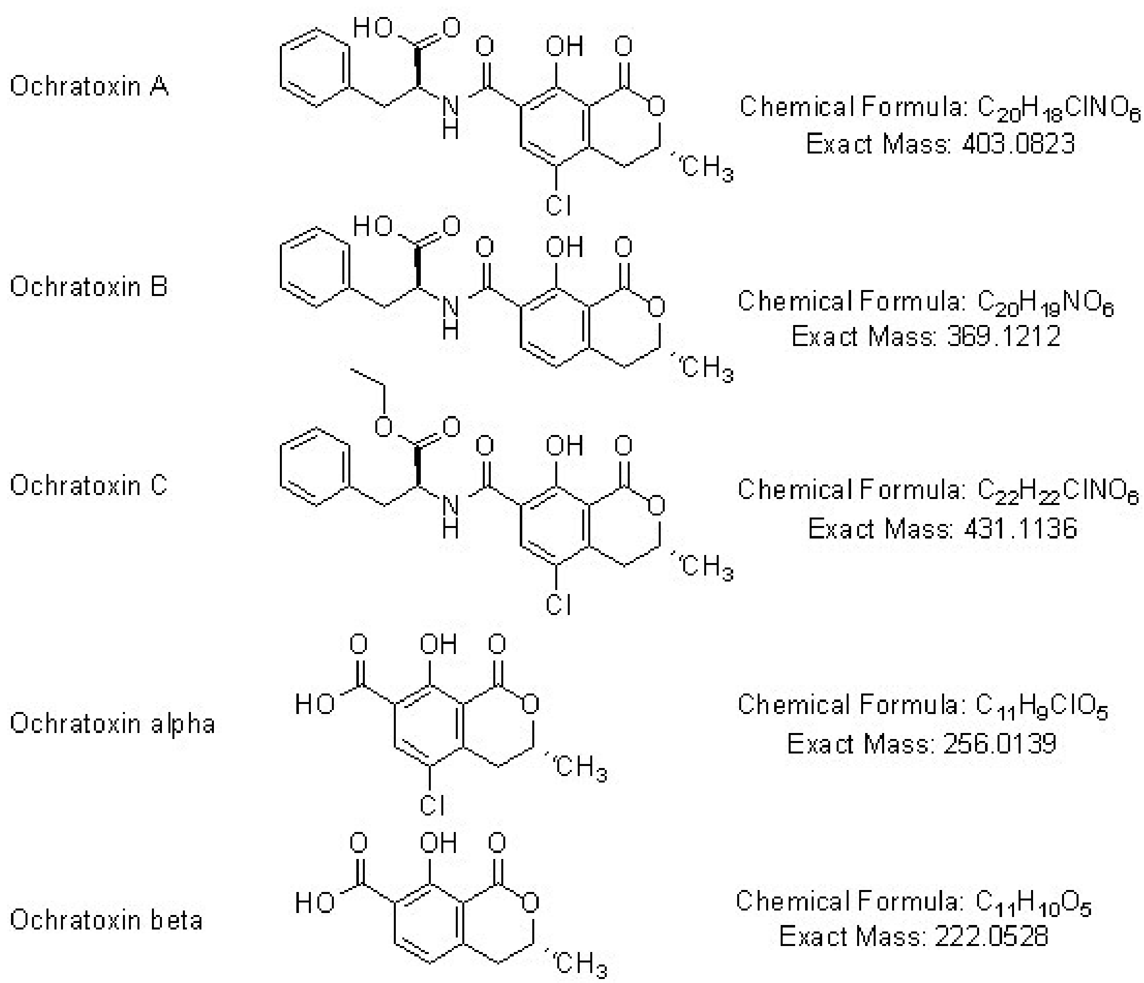
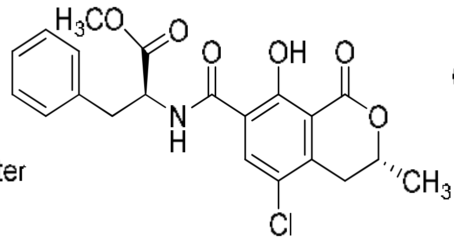


Fig. 1a: General chemical structure for Ochratoxins (See Table-1 for the detailed structure of different R groups).

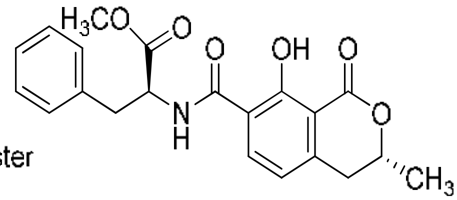


Ochratoxin A methyl ester



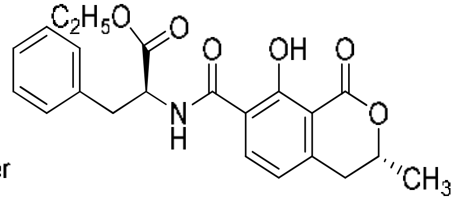
Chemical Formula: $C_{21}H_{20}ClNO_6$
Exact Mass: 417.0979

Ochratoxin B methyl ester



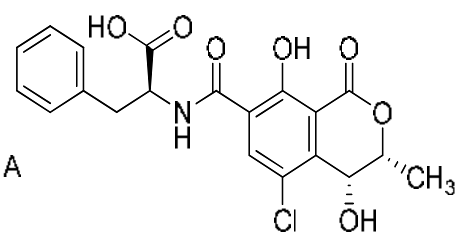
Chemical Formula: $C_{21}H_{21}NO_6$
Exact Mass: 383.1369

Ochratoxin B ethyl ester



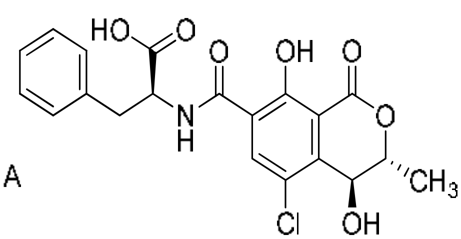
Chemical Formula: $C_{22}H_{23}NO_6$
Exact Mass: 397.1525

4R-hydroxyOchratoxin A



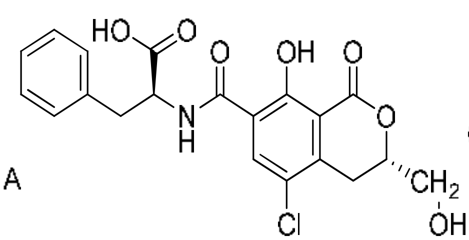
Chemical Formula: $C_{20}H_{18}ClNO_7$
Exact Mass: 419.0772

4S-hydroxyOchratoxin A



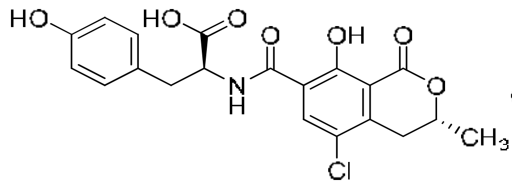
Chemical Formula: $C_{20}H_{18}ClNO_7$
Exact Mass: 419.0772

10-hydroxyOchratoxin A



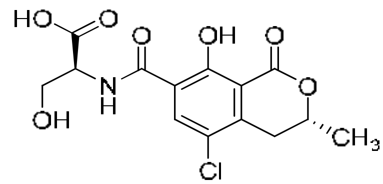
Chemical Formula: $C_{20}H_{18}ClNO_7$
Exact Mass: 419.0772

Ochratoxin A tyrosin analogue



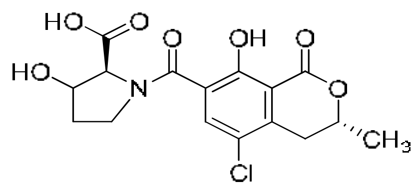
Chemical Formula: $C_{20}H_{18}ClNO_7$
Exact Mass: 419.0772

Ochratoxin A serine analogue



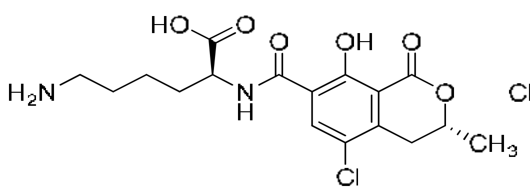
Chemical Formula: $C_{14}H_{14}ClNO_7$
Exact Mass: 343.0459

Ochratoxin A hydroxy proline alogue



Chemical Formula: $C_{16}H_{16}ClNO_7$
Exact Mass: 369.0615

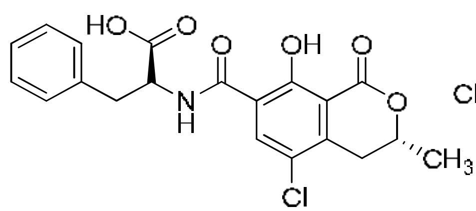
Ochratoxin A lysine analogue



Chemical Formula: $C_{17}H_{21}ClN_2O_6$
Exact Mass: 384.1088

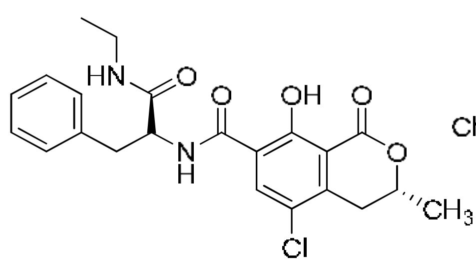
Fig. 1: b. Chemical structure and formulae of 15 different natural ochratoxins (OTs) found in cereal food and feeds.

d-Ochratoxin A



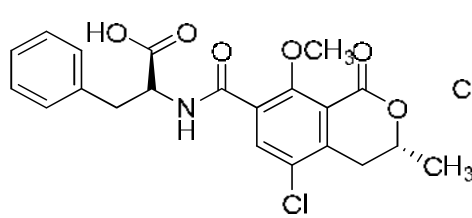
Chemical Formula: $C_{20}H_{18}ClNO_6$
Exact Mass: 403.0823

Ochratoxin A ethyl amide



Chemical Formula: $C_{22}H_{23}ClN_2O_5$
Exact Mass: 430.1295

O-methyl Ochratoxin A



Chemical Formula: $C_{21}H_{20}ClNO_6$
Exact Mass: 417.0979

Fig. 1: c. Chemical structure and formulae of synthetic ochratoxins (OTs).

Table-1: Chemical and physical characteristics of ochratoxins A (OTA) and related metabolites found in cereal food and feeds.

Common Name of Ochratoxins (OTs)	Abbreviations of Ochratoxins (OTs)	Molecular Weight (g/mol)	Molecular Formula	*R[1]	R[2]	R[3]	R[4]	R[5]	State	Melting Point (^0C)	Appearance and Fluorescence	Solubility and UV/Vis	Vapor Pressure (mm Hg at 25^0C)
Ochratoxin A	OTA	403.8	$C_{20}H_{18}ClNO_6$	Phenylalanyl	C1	H	H	H	Solid	90 °C when crystallized from benzene as a solvate and169 °C as non-solvated crystals obtained from xylene	Colorless, Crystals from xylene and xhibits green fluorescence	Soluble in organic solvent (like chloroform and methanol) and Λmax = 213, 331nm	$3.11x10^{-14}$
Ochratoxin B	OTB	369.4	$C_{20}H_{19}NO_6$	Phenylalanyl	H	H	H	H	Solid	221^0C	Crystals from methanol and exhibits green fluorescence	Soluble in organic solvents (like etanol, DMSO, and dimethyl formaldehyde) and Λmax = 218, 319nm	$6.39x10^{-15}$
Ochratoxin C	OTC	431.87	$C_{22}H_{22}ClNO_6$	Phenylalanyl, ethyl ester	C1	H	H	H	Solid	The benzene solvate melting point of $94\text{-}96^0C$, xylene in the crytaline melting point 196^0C	Amorphous white powder	In water 0.2734 mg/L at 25 ^0C and absorbs UV light at wavelengths 333nm	$1.81x10^{-15}$
Ochratoxin A methyl ester	OTAmethest	417.09	$C_{21}H_{20}ClNO_6$	Phenylalanyl, methyl ester	C1	H	H	H					
Ochratoxin B methyl ester	OTBmethest	383.13	$C_{21}H_{21}NO_6$	Phenylalanyl, methyl ester	H	H	H	H					
Ochratoxin B ethyl ester	OTBethest	397.15	$C_{22}H_{23}NO_6$	Phenylalanyl, ethyl ester	H	H	H	H					
Ochratoxin α	OTα	256.01	$C_{11}H_9ClO_5$	OH	C1	H	H	H					
Ochratoxin β	OTβ	222.05	$C_{11}H_{10}O_5$	OH	H	H	H	H					
4R.Hydroxyochratoxin A	OH.OTA	419.07	$C_{20}H_{18}ClNO_7$	Phenylalanyl	C1	H	OH	H					
4S.Hydroxyochratoxin A	OH.OTA	419.07	$C_{20}H_{18}ClNO_7$	Phenylalanyl	C1	OH	H	H					
10.Hydroxyochratoxin A	OH.OTA	419.07	$C_{20}H_{18}ClNO_7$	Phenylalanyl	C1	H	H	OH					
Ochratoxin A, tyrosine analog	OTAtyr	419.07	$C_{20}H_{18}ClNO_7$	Tyrosine	C1	H	H	H					
Ochratoxin A, serine analog	OTAser	343.04	$C_{14}H_{14}ClNO_7$	Serine	C1	H	H	H					
Ochratoxin A, hydroxyproline analog	OTAhydpro	369.06	$C_{16}H_{16}ClNO_7$	Hydroxyproline	C1	H	H	H					
Ochratoxin A, lysine analog	OTAlys	384.10	$C_{17}H_{21}ClN_2O_6$	Lysine	C1	H	H	H					
Synthetic ochratoxins													
d-Oochratoxin A	Dota	403.08	$C_{20}H_{18}ClNO_6$	d-phenylalanyl	Cl	H	H	H					
Ochratoxin A Ethyl amid	OTAethamid	430.12	$C_{22}H_{23}ClN_2O_5$	Phenylalanyl ethyl amid	Cl	H	H	H					
O-methyl Ochratoxin A	O-Ch3.OTA	417.09	$C_{21}H_{20}ClNO_6$	Phenylalanyl, OCH_3 on C-8	Cl	H	H	H					

R1 through 5 refers to that group bound to carbons 9, 5, 4, 4, and the number 3 methyl carbon of ochratoxin, respectively.
*Radicals in OTs metabolites *(Phenylalanine; Tyrosine; Serine; Hydroxyproline; Lysine) [76]

Among all these ochratoxins, OTA ($C_{20}H_{18}ClNO_6$) is the most common and naturally occurring toxic member of OTs group, and contains an isocoumarin moiety linked by a peptide to phenylalanine (Table-1 and Fig. 1b). Its molecular weight is 403.82. OTA is the most prominent member of OTs family, followed by OTB, which differs only by substitution of the chloride by hydrogen at C_5 of the isocoumarin moiety. While OTB ($C_{20}H_{19}NO_6$) and OTC ($C_{22}H_{22}ClNO_6$) are less important and less common, and are being the ethyl and methyl ester of OTA, and their molecular weights are 369.4 and 431.87, respectively. The melting points OTA, OTB and OTC are about 90, 221 and 94-96 ^{0}C respectively, when crystallized from benzene as a solvate. Their vapor pressures are 3.11 x 10^{-14}; 6.39 x 10^{-15} and 1.81 x 10^{-15} mm Hg at 25^{0}C respectively. The maximum absorption peak of OTA and OTB are also noted as 331 and 319nm, respectively [10, 11].

OTA is a weak organic acid, crystalline solid, colorless or white in color and optically active. In acidic and neutral pH, OTA is soluble in polar organic solvent i.e. alcohols, chloroform and ketones, but slightly soluble in H_2O, and insoluble in petroleum ethers and saturated hydrocarbons. While in alkaline medium, it is soluble in aqueous $NaHCO_3$ solution in particular and in all alkaline solutions in general [12]. It poses an intense green fluorescence under UV light in acidic and blue fluorescence in alkaline medium [13]. This molecule is highly stable and possesses a resistance to both acidity and high temperature. Thus when once cereal foodstuffs is contaminated, it is very difficult to totally remove this toxic chemical substance. [14] Stated that the OTA is only partially degraded at normal conditions of cooking. Other researchers also said that this toxic substance can resist 3 hours of high pressure steam sterilization of 121 ^{0}C [15], and even at 250 ^{0}C its destruction is incomplete [16]. However, degradation is observed at low moisture contents when OTA has been treated with an excess of NaOCl [17]. Among above mentioned types of OTs, some are highly toxic, whereas others are low toxic. OTA has been detected in foods, feeds, animal tissues and human blood. OTA is hydrolyzed to its non-toxic α-form (OTα) by microorganisms in the rumen, cecum and large intestine [9]. OTA has often been reported to be more toxic than OTB [18]. The difference in structure of OTs is assumed to be responsible for differences in their toxicities [19]. Most of the OTs is stable chemical compounds, which can not be destroyed by processing and heat treatment of feed and foodstuffs. The more detail about physicochemical properties and structures of remaining natural and synthetic OTs are given in Table-1 and Fig. 1bandc.

Methods for Detection of Ochratoxin A in Food and Feed Stuffs

The presence of OTA in food commodities at very low level may induce toxic effects, therefore selective and sensitive detection of OTA is a prerequisite in order to ensure food and feed safety and to minimize the potential risk to human and environmental health. The current methods used for OTA detection in cereal food and feedstuffs include thin layer chromatography (TLC), ELISA methods, liquid chromatography coupled or not with mass spectrometry (MS), and immunochemical methods [20]. Each and every method having their own merits and demerits depends upon its capability to separate impurities from the analytes, the time of sample preparation and economic aspects. However, for routine testing of OTA a reverse-phase high-performance liquid chromatography (HPLC) seems to be quick and simple methods for the detection of OTA [21]. This method is suitable for quantification of OTA contents in the toxicologically significant range corresponding to 1.5-30ppb. Owing to its fluorescent nature, OTA is generally determined by chromatographic techniques [22]. Despite of its accuracy, these analytical methods are expansive, cumbersome, time consuming and also required qualified technicians. Alternatively, biochemical methods have received considerable attention owing to its low cost, simplicity, rapidity, and high sensitivity. These methods are only based on the interaction between a recognition element and its target inducing a mechanism for molecular recognition. This interaction is then translated into a measurable signal by using a suitable transducer [23]. However, aptamers (synthetic bioreceptors) have gained much popularity in affinity-based assays because of its high affinity, specificity, stability, and its easy chemical synthesis [24].

Ochratoxin A (OTA) Producing Fungi

OTA is a naturally occurring food borne mycotoxin found in a wide variety of agricultural commodities throughout the globe, ranging from cereal grains, dried fruits to wine and coffee. It has been found that there are 9 kinds of Aspergillus (*Aspergillus ochraceus; A. carbonarius; A. niger; A. sclerotiorum; A. alliaceus; A. albertensis; A. lanosus, A. alutaceus and A. sulphureus*) and 6 species of Penicillium (*P. verrucosum; P. nordicum; P. viridicatum*, *P. chrysogenum and P. aurantiogriseum, P. cyclopium* or *P. polonicum*) which can produce OTA [25, 26]. These fungi vary in their optimal growing temperature and water activity, and contaminate various agricultural commodities like cereals, legumes, chillies, dried fruits, and nuts. However, cereals are mainly contaminated by *Aspergillus ochraceus; A. carbonarius,* and *Penicillium verrucosum* [27, 28]. The contamination of mentioned agricultural commodities is generally occurring as a result of suboptimal agricultural practices and poor storage of commodities particularly during the drying process of foods [29]. The kinds and distribution frequency of fungi is depend upon their existing climatic, edaphic and topographic conditions. Major OTA producing fungus in northern Europe is *Penicillium verrucosum,* and more important in warmer climatic zone is *Aspergillus ochraceus* [30].

Among cereals, maize is most susceptible to be attacked by OTA producing fungi. Natural occurrence of OTA contamination in maize and maize based products is a worldwide problem [31]. *Aspergillus niger* is commonly isolated from maize (*Zea mays* L.), and a high incidence of *Aspergillus carbonarius* has also been reported [32]. Both fungal species are the main source of OTs production in maize and other foodstuffs in both sub-tropical and tropical zones of the globe. Studies also revealed that there is a highest contamination of OTA in cereal grains, and to a lesser extent in grape, wine, dried vine fruits, and grape juice [33].

Factors Affecting Concentration of Ochratoxin A (OTA)

There are various internal and external factors which can contribute a significant role in increase of OTA concentrations. But the main controlling factors which affect the distribution and growth of OTA producing fungi during farming, harvesting, and storage (conditions and periods) of cereal food and feeds are moisture, temperature, medium composition, and the time a product kept under adverse climatic conditions. Other factors include the presence of fungal spores, host mechanical, insect, storm and rain damages, moisture stress, mineral nutrients availability, pH, O_2 and CO_2 concentration, and chemical and physical treatments [34-41]. The contamination of agricultural commodities is generally occurring as a result of suboptimal agricultural practices and poor storage of commodities particularly during the drying process of foods [29]. Once in the industrial chain, further contamination may occur from other raw materials, dust adhered, loading and unloading, and residues in the warehouses. In particular, in cereal food manufacturing includes drying, processing, and storage [42-44]. OTA production does not only depend on the genotype of a certain strain of fungi, but also on a wide range of environmental factors such as temperature, activity water (aw), low consuming elements, processing error, insect damage, un-seasonal rains during harvest, and also flash floods, which have a significant influence on fungal metabolism and its growth [45, 46]. Research studies revealed that conditions for producing OTA by *A. carbonarious* is 15-20 ^{0}C, and 0.95-0.98 aw [47]; for *A. ochraceus* is 25-30 ^{0}C, and 0.98 aw [48], and for *P. viridicatum* is 24 ^{0}C, and 0.95-0.99 aw [49]. However, researchers also noted that optimal temperature for OTA synthesis by *A. ochraceus* is 28^{0}C. This synthesis is very low at 15^{0}C or 37^{0}C. On the contrary, *Penicillium viridicatum* develops and can produce OTA at a range of temperature between 4 and 30 ^{0}C. In colder regions, OTA is produced mainly by *Penicillium,* while in the warmer regions it is mainly produced by strains of *Aspergillus* genus [50, 51]. Studies also enumerated that *Penicillium verrucosum* or *P. nordicum* are the fungi mainly responsible for the production of OTA in the cool and temperate regions. While *P. verrucosum* contaminates most of the cereal crops. However, *Aspergillus allutaceus* followed by *A. niger* are mainly responsible for the production of OTA of the tropical and semi-tropical regions of the globe [52].

Diseases Caused by Ochratoxin A (OTA)

Ochratoxins are considered to be the most powerful carcinogens, teratogens, nephrotoxins, and immune-toxins particularly in rats, humans and likely in poultry [53-55]. OTA is a proven carcinogen both in animal and human, and is classified as a group 2B, carcinogen by the International Agency for Research and Cancer (IARC). Kidney is the major organ to be targeted by OTA, but liver could also be a target organ [56]. OTA is also a potential carcinogen in various animal species [34]. OTA has been involved in various human nephropathies since the 1990s *viz.,* Balken Endemic Nephropathy (BEN) [57], and chronic interstitial nephropathy (CIN) [58]. Other adverse effects of OTA include immunotoxicity [59], increased lipid-peroxidation and inhibition of respiration taking place in mitochondria [9].

Status of Ochratoxin A (OTA) in Cereal Food and Feeds of Pakistan

Mycotoxin contamination in general and afflatoxins (AFs) and ochratoxins (OTs) in particular is a burning issue in Pakistan. The farming community and agrarian society in the country have serious concerns about this important issue. In Pakistan, very limited published information is available about the fungal mycobiota and status of their mycotoxin in agricultural products, particularly those used as ingredients in poultry, and animal feeds. Only few reports covering short periods, and smaller regions have described the presence of some toxigenic fungi or mycotoxins in agricultural products [60, 61], and animal feed stuffs [62-64]. There is inadequate research on OTs in the country, but a few groups have recently started research work in this field of study.

Results obtained by [65] indicated that production of OTA by two species of *Aspergillus* i.e., *A. ochraceus and A. sulpureus* (Table-2) depends upon the temperature and substrate, and the production in cereal (corn, rice and wheat) was significantly higher than the maximum permitted level (5 ng/g) as described by EU regulatory commission, and other agencies in the world (Table-3). Results obtained by [28] reflected that out of 275 retail cereal samples (collected from 3 districts of Punjab province) about 50% rice, 26.67% corn, and 25.49% corn products were found to be contaminated by an average value of 12.94; 5.29, and 3.69 µg/kg OTA, respectively. Therefore, rice and corn samples (except corn products) have been found to above the recommended level of OTA (5 µg/kg) by EU and other international agencies. [66] Stated that out of 237 breakfasts cereal samples, about 48% analyzed samples were found contaminated by OTA, and 30% samples were found to be above the permissible limit of EU. While semolina was recorded as the highest (3.90 µg/kg) contaminated breakfast cereal for OTA. The commercial samples of corn and wheat products of Faisalabad, Pakistan collected by [67] exhibited the high level of OTA contamination. In wheat samples highest amount of OTA is recorded in wheat flour (25.90 ng/g), and bread (4.66 ng/g) has lowest level of OTA contamination. While in case of corn samples corn flour showed the highest level of contamination (11.12 ng/g). The level of OTA contamination was found higher than the recommended limits of EU.

In Pakistan different feed ingredients are used in poultry feeds are likely to be contaminated by OTA producing molds. Because most commercial feed mills in Pakistan provide suitable substratum for fungal growth provoked by improper harvesting and storage, poor methodology of consumption and utilization. Therefore, regular monitoring of OTA in poultry is an important pre-requisite to check toxins build-up in poultry feeds. The prevalence study on OTA in feeds is regularly and consecutively practiced in many countries of the world *viz.,* Brazil [68]; Kuwait [69]; Nigeria [70]; Iran [71], and Malaysia [72]. Whereas, in Pakistan there is no as such regular monitoring system for checking both food and feed contamination, which is vital for ensuring the safety of human, animal or poultry feeds. However, in Pakistan the national standardization body i.e. Pakistan Standard and Quality Control Authority (PSQCA), with its Food and Agriculture Division (FAD) deals with the development of standards for foodstuff and its implementation. Presently, PSQCA has set standards only for aflatoxins (among mycotoxins) in certain commodities, but there are no any set standards for other mycotoxins particularly for ochratoxins. That is why the reviewer of the present paper relies on foreign standards of OTs.

Table-2: Average mean values of ochratoxin A (OTA) in cereal food and feed samples reported from Pakistan.

Cereal Samples	Ochratoxin mean values (ng/g or µg/g or µg/kg)	Citation Number
Barley (Bread)	1.50 µg/kg	[66]
Barley ingredient	26.00 µg/kg	[73]
Corn products	3.69 µg/kg	[77]
Corn Flour	11.12 ng/g	[67]
Corn	1.87 µg/g	[65]
Corn	5.29 µg/kg	[77]
Corn (Bread)	1.60 µg/kg	[66]
Cornflakes	2.23 µg/kg	[66]
Corn ingredients	60 µg/kg	[75]
Corn gluten meal (30%)	32 µg/kg	[75]
Corn gluten meal (60%)	70.70 µg/kg	[75]
Maize ingredient	104.4 µg/kg	[73]
Poridge	3.71 µg/kg	[66]
Rice	1.55 µg/g	[65]
Rice	12.94 µg/kg	[77]
Rice ingredient	20.00 µg/kg	[73]
Rice broken	8.8 µg/kg	[75]
Rice polish	5.9 µg/kg	[75]
Wheat Flour	25.90 ng/g	[67]
Wheat	1.3 µg/kg	[78]
Wheat	1.38 µg/g	[65]
Wheat (Bread)	2.13 µg/kg	[66]
Wheat (Bread)	4.66 ng/g	[67]
Wheat ingredient	60.75 µg/kg	[73]
Millet	5.00 µg/kg	[74]
Millet ingredients	2.5 µg/kg	[73]
Rusk	0.98 µg/kg	[66]
Seviyan (Vermicelli)	3.50 µg/kg	[66]
Sorghum ingredient	34.00 µg/kg	[73]
Suji (Semolina)	3.90 µg/kg	[66]

EU = European Union; FDA = US Food Drug Administration

Table-3: The permissible limits of Ochratoxin A (OTA) for cereal and cereal products of various countries.

Country Name	Cereal Products	Ochratoxin A (OTA) limit	Citation Number
1. China	Cereals	5 ppb	[79]
2. EC (European Union)	Unprocessed cereals	5 ppb	[80]
	All products made from unprocessed cereals, intended for direct human consumption	3 ppb	[80]
3. Iran	Wheat	5 ppb	[81]
4. Israel	Cereals, cereal products	50 ppb	[81]
5. Switzerland	All foodstuffs	5 ppb	[81]
6. Turkey	Raw grain	5 ppb	[81]
	Food made from grain	3 ppb	[81]
7. USA	FDA has not yet set any advisory limits or action levels for OTA in any cereal commodity		

Few studies have also been conducted upon the OTA contamination in agricultural products and poultry feeds in the country [60, 73-75]. [73] Studied to assess the OTA content in different cereal feed ingredients. Out of 72 samples analyzed 20 were found contaminated with OTA. The highest content (84.4 µg/kg) was recorded in maize, while millet contained the minimum content (5.00 µg/kg). Researchers also observed that out of 56, OTs were either not detected in most of the analyzed wheat samples, or if detected in very few samples then their values were recorded below the safe limits of human consumption and health. [75] analyzed about 186 samples of poultry feed ingredients and finished poultry feeds. The OTA was determined by them in 63.15 and 29.17% feed ingredients and finished feed samples, respectively with an overall incidence of 50%. Maximum level of OTA contamination (100%) was recorded in corn gluten meal. However, they noted that no feed contained OTA above the acceptable limit as set the criteria by EU on OTA contamination in poultry finished feed.

Conclusion

On the basis of available literature, it can be concluded that the high occurrence of cereals contamination in general and of rice and corn by OTA from Pakistan in particular could be due to its climatic conditions. Most of the analyzed cereal food in general and of feed samples in particular are exceeding the acceptable or permissible limit (5ppb) as proposed by European Union (EU) regulatory commission, and other food and feed monitoring agencies in the world. Both rice and corn are usually grown during hot weather, and harvested during summer in the country. These climatic conditions assist the attack of concerned moulds with maximum production of OTA. The high level found in raw cereals may also be due to the improper storage place and storage periods. Because in our rural areas, people usually store their cereals mainly in mud made bins which can absorb aerial moisture during rainy days. Therefore, it is suggested that proper harvesting, storage of food/feed be done and unhygienic method of processing and production be avoided, and there must be a regular monitoring system to check the health hazard status of OTA of agricultural commodities in general, and of cereal food and feeds in particular.

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