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Mycotoxin levels and incidence of mould in Turkish rice

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Abstract One hundred unpackaged rice samples, each weighing 500 g, were randomly collected at retail stores and open markets in the largest rice growing area (Thrace) in Turkey and analysed for mould counts, predominant mould genera, moisture content and mycotoxin levels. Mould counts ranged from 1.0×10^1 to 1.5×10^4 cfu/g in 70 of 100 samples, and the correlation between moisture content and mould count was significant ($p \leq 0.05$). *Aspergillus* spp. and *Penicillium* spp., potential mycotoxin producers, were the dominant moulds. In one area from which samples were collected, the mycotoxin content of rice was found to be positively correlated with moisture content; samples with higher moisture also contained higher numbers of moulds. The levels of total aflatoxins, aflatoxin B1 and ochratoxin A were higher than the maximum tolerable limits (4, 2 and 3 μg/kg, according to the EC Regulation and the Turkish Food Codex) for 32, 14 and 30 of 100 rice samples, respectively. This is the first comprehensive report of ochratoxin A levels in rice grown in Thrace, Turkey.

Keywords Mycotoxins · Mould contamination · ELISA · Public health · Rice · Turkey

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Introduction

Rice (*Oryza sativa* L.) is a very important foodstuff for billions of people. It is the dominant grain for half of the world population and provides 20% of the world's dietary energy supply, with wheat and maize supplying 19% and 5%, respectively (FAO 2004). Rice cultivation is carried out in subtropical environments with sufficient warmth and high humidity.

Mould contamination in cereal grains, which can occur at the farm or at the site of storage, affects the yield, quality and nutritional value of the products (Aran and Eke 1987). Mould growth is possible when the moisture content exceeded 13–15% (Jay 1996). The Food and Agriculture Organization (FAO) estimates that at least 25% of the world cereal production is contaminated with mycotoxins (Dowling 1997). Most of the mycotoxins in rice are removed during the milling process (Takashi et al. 1984).

The toxic effects of a number of mycotoxins on human and animal health have led to an increase in legislative provisions aimed at con-

trolling their presence in food and feed. Mycotoxins potentially present in mouldy rice include aflatoxins, ochratoxin A (OTA) and *Fusarium* toxins (Miraglia and Brera 2000). Aflatoxins are toxic secondary metabolites produced by some species of Aspergilli, especially *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius*. These metabolites are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic agents. The liver is most affected by the carcinogenic and toxic activities (Peraica et al. 1999). Aflatoxin B1 (AFB1) is the most potent hepatocarcinogen known in mammals and is classified by the International Agency of Research on Cancer (IARC) as a Group 1 carcinogen (IARC 1993). The maximum tolerable limits for aflatoxins allowed in cereals by EC Regulations (2006) and the Turkish Food Codex (2008) have been set at 4 μg/kg for total aflatoxin (total AF) and 2 μg/kg for AFB1. Ochratoxin A is a naturally occurring mycotoxin which is produced by several species of the genera *Aspergillus* (e.g., *Aspergillus ochraceus*) and *Penicillum* (e.g., *Penicillum verrucosum*). It has been shown to be hepatotoxic, nephrotoxic, teratogenic and carcinogenic to animals and is classified as a possible human carcinogen (category 2B) by the International Agency for Research on Cancer (IARC 1993). Moreover, OTA is suspected to be the causative agent behind Balkan endemic nephropathy (BEN), a kidney disease in Southeastern Europe (Pfohl-Leszkowicz et al. 2002). The EC Regulations (2006) and Turkish Food Codex (2008) have set a maximum tolerable limit for OTA at 3 μg/kg for all products derived from unprocessed cereals.

Cereals have been grown in Anatolia (Turkey) for thousands of years and are an integral part of life in rural areas. Wheat, barley, rice, maize, oats, rye, millet, spelt, canary grass and mixed grains are the main cereals grown in Turkey. In 2007–2008, rice consumption in Turkey was reported to be 8.7 kg per person/year (Portal of Food and Agriculture in Turkey 2009). Although Turkey is one of the most important producers and exporters of rice in Europe, it ranks relatively low in the world's paddy rice production (Table 1). The Thrace region of Turkey, i.e., the European part of the Marmara region, is the largest rice growing area, producing 488,404 metric tons of rice in 2006. This region is followed by the Black Sea region, which produced 170,539 metric tons of rice in 2006 (Gaytancioglu 2007). The Thrace region in Southeast Europe is a transpass corridor between Europe and Turkey.

Table 1 Rice production of different countries in the world

Country	Paddy production in metric tons (2006)
China	183,276,048
India	139,137,000
Indonesia	54,454,937
Thailand	29,641,871
Brazil	11,526,685
Japan	10,695,000
Italy	1,412,957
Spain	845,900
Turkey	709,800
Greece	167,247
Portugal	120,000

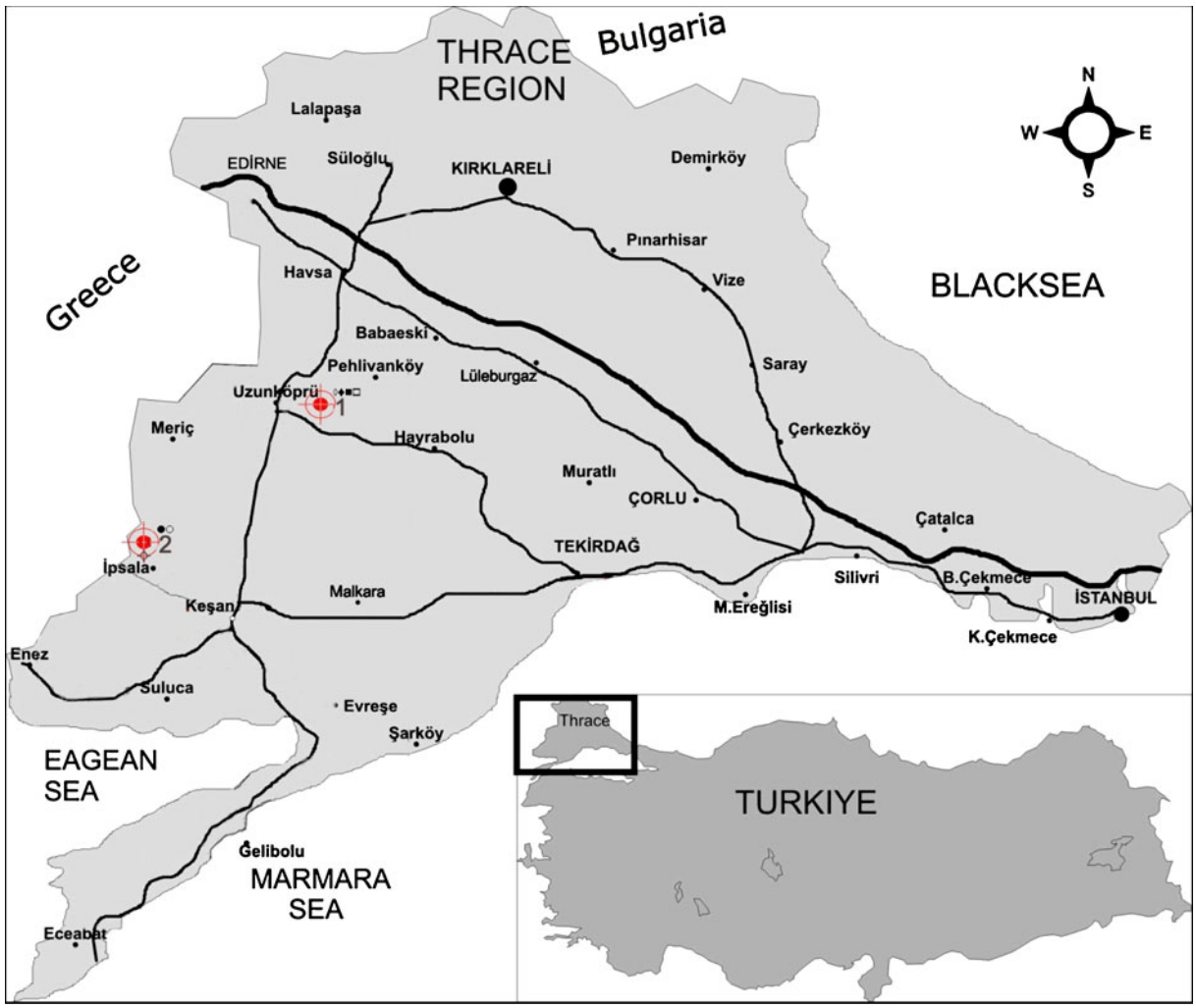
FAOSTAT 2006; Gaytancioglu 2007

The objectives of this study were: (1) to measure moisture content and determine total mould counts in rice produced in the Thrace region, (2) to investigate the incidence of potential mycotoxin-producing moulds in Turkish rice, and (3) to determine if levels of mycotoxins were in accordance with the maximum tolerable limits of the EC Regulation and Turkish Food Codex.

Materials and methods

A total of 100 rice samples from open packages, each weighing 500 g, were obtained from two different rice-growing areas [Uzunkopru (Site 1) and Ipsala (Site 2)] in the Thrace region in the summer of 2006 (Fig. 1). Samples were transported to the laboratory under refrigerated conditions (4–6°C) and analysed within 4–6 h.

For microbiological analyses, a 10-g portion of each rice sample was placed in a sterile stomacher bag and homogenised in 90 ml sterile 0.1% peptone water [Oxoid CM 9 (Basingstoke, UK)] for 2 min using a Stomacher 400 (Seward Medical Ltd, London, UK) as recommended by Hocking et al. (1992) for homogenisation of solid and semi solid samples. Serial dilutions of the homogenate were prepared with the same diluent and samples



A. flavus ■□ **AFB1,** *A. parasiticus* ◆◇ **Total AF,** *P. verrucosum* ●○ **OTA**

Fig. 1 Location of study areas and rice sampling points in Thrace, Turkey (*1* Uzunkopru, *2* Ipsala)

(0.1 ml, in duplicate) were surface plated on Dicloran Rose Bengal Chloramphenicol agar (Oxoid CM 727). This selective agar inhibits the growth of bacteria which may interfere with colony development by moulds in non-sterilised foods (Park et al. 2005). Plates were incubated at 25°C for 5–7 days in the dark. Mould colonies were counted and the number of samples showing growth of a particular genus of mould was determined.

After the enumeration of moulds, colonies were sub-cultured on malt extract agar (MEA) [Merck 1.05398 (Darmstadt, Germany)], which is recommended in the Second International Workshop on Standardization of Methods for the Mycological Examination of Foods (Hocking et al. 1992) as a medium for use in identifying moulds. A portion of each colony was transferred to the surface of MEA. Plates were incubated at 25°C for 5–7 days. Mould genera and species were identified by macroscopic and microscopic characteristics according to taxonomic keys (Klich 2002; Pitt 1979; Samson and Pitt 2000; Samson et al. 2002).

The moisture content of each rice sample was determined as described in ISO 712 (1998).

The Ridascreen® Total AF test [R-biopharm, Art. no, R4701 (Darmstadt, Germany)], a competitive enzyme immunoassay for the quantitative analysis of aflatoxin residues in cereals and feed,

was used as instructed by the manufacturer (Enzyme Immunoassay for the Quantitative Analysis of Aflatoxins 2002). Briefly, a 10-g sample of rice was weighed into a screw-top glass vial; 50 ml of 70% methanol/distilled water (35 ml ethanol, 15 ml distilled water) was added and mixed with a magnetic stirrer (Janke & Kunkel, Germany) for 10 min at 23 ± 1°C. The extract (100 µl) was filtered using filter paper (Whatman no. 1) and diluted by combining with 600 µl of dilution buffer. The diluted filtrate (50 µl) was then transferred to each well in a microtitre assay plate. Duplicate total AF standards and the prepared sample solutions were added to in a microtitre plate wells. The concentrated total AF enzyme conjugate was diluted 1:10 (1 + 9) with the corresponding sample buffer. The detection limit of the total AF test using this procedure 1 is 0.05 µg/kg. The mean recovery rate is 85% and the average coefficient of variation is 15% (Enzyme Immunoassay for the Quantitative Analysis of Aflatoxin Total 2002).

The Ridascreen® AFB1 30/15 test (R-biopharm, Art. no R1211) is a competitive enzyme immunoassay for the quantitative analysis of AFB1 in cereals and feed (Enzyme Immunoassay for the Quantitative Analysis of Aflatoxin B1 2004). The rice sample (10 g) and 50 ml of 70% methanol/distilled water were mixed by stomaching for 3 min. Distilled water (1 ml) was added to 1 ml of extract (filtered by a Whatman no. 1); duplicate 50-µl aliquots of the AFB1 standard solutions and diluted test samples were deposited in separate wells of a microtiter plate. Subsequently, enzyme conjugate (urea peroxide, 50 µl) and anti-aflatoxin antibody solution (tetramethyl-benzidine, 50 µl) were added to each well and the mixture was incubated for 30 min at 23 ± 1°C in the dark. The liquid was then removed from the wells before rinsing (twice) with 250 µl of washing buffer (10 mM PBS-Tween 20 (0.05%) buffer, pH 7.4). The stop reagent (1 N H_2SO_4; 100 µl) was added and the absorbance was measured at 450 nm in an ELISA reader. The lower detection limit of the AFB1 30/15 test is 1.0 µg/kg. The recovery is stated to be 80–100% and the mean coefficient of variation is 8% (Enzyme Immunoassay for the Quantitative Analysis of Aflatoxin B1 2004).

The Ridascreen® OTA test (R-biopharm, Art. no R1301) is a competitive enzyme immunoassay for quantitative analysis of OTA in cereals, feed, beer and pig serum (Enzyme Immunoassay for the Quantitative Analysis of OTA 2003). Each rice sample (2 g) was weighed into a centrifuge vial and mixed with 4 ml of distilled water and 0.2 ml α-amylase solution. The solution was prepared by dissolving 0.5 g of porcinepancrease [1.000.000 U, Sigma A-3176] in 1 ml PBS buffer. The components were mixed by shaking (IKA Labortechnik, Germany) for 20 min at 23 ± 1°C before the addition of 1 ml of 5 N HCl. Following additional shaking for 5 min, 10 ml of dichloromethane (Merck, 1.06049) was added. The vial was shaken vigorously by centrifuging for 15 min (3,500×g, 15°C). The upper aqueous layer was removed and discarded. The dichloromethane layer, filtered (Whatman No 1) into a new screw cap centrifuge vial, was mixed with equivalent volumes of 0.13 M $NaHCO_3$ (pH 8.1) and centrifuged for 15 min (3,500×g, 15°C). The aqueous phase (100 µl) was diluted with 400 µl of 0.13 M $NaHCO_3$ (pH 8.1) buffer; 50 µl was deposited into each well in the microtitre assay plate. Duplicate standard solutions (50 µl; 0, 25, 75, 225, 675 and 2,025 ng/kg OTA in aqueous solution) were deposited in individual wells. The concentrated OTA enzyme conjugate was diluted 1:10 (1 + 9) with the corresponding sample buffer. Diluted enzyme conjugate (50 µl) was added to each well. Solutions were mixed gently by rocking of the plate for 2 h at 23 ± 1°C in the dark. The liquid was poured off wells and the microwell holder was inverted and vigorously tapped against absorbent paper to ensure complete removal of liquid from the wells. The substrate (50 µl of urea peroxide) and 50 µl chromogen (tetramethyl-benzidine) were added to each well, mixed thoroughly, and incubated for 30 min at 23 ± 1°C in the dark. The stop reagent (100 µl) was added to each well and measured at an absorbance of 450 nm. The recovery rate for OTA is 85% and the average coefficient of variation is 14% (Enzyme Immunoassay for the Quantitative Analysis of OTA 2003).

The mean values for absorbance of the standards and the samples were determined according to the Rida® Soft Win program (RI-

DAVIN.EXE) distributed by Ridascreen (R48 Biopharm).

Statistical analysis of mould counts was done based on absolute values. Colony counts were converted into logarithmic values. One-way ANOVA and Duncan's multiple range tests were used to analyse log mould counts. Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS 1997).

Results and discussion

Quantitative and qualitative data on mould contamination in Turkish rice and relation to moisture content

Yeasts and moulds may contaminate cereal grains at populations exceeding 10^4 cfu/g. Growth of moulds is effectively eliminated when grains are properly dried to less than 13–15% moisture (Huang and Hanlin 1975; Jay 1996). Rice, however, is an aquatic plant and is usually harvested at much higher moisture levels (30–50%). Mycotoxin-producing moulds may contaminate grains and produce high quantities of mycotoxins during storage (Park et al. 2005).

The moisture contents and mould counts in rice samples are presented in Table 2. The moisture contents in 20 samples from Site 1 and 16 samples from Site 2 were higher than the legal limits (>15%) stated in the Codex Alimentarius (1995). The highest mould count (1.5×10^4 cfu/g) was detected in rice samples collected at Site 1. In addition, mould counts in rice with different moisture levels from rice from Site 1 were statistically significant from those from Site 2 ($p \leq 0.001$). In a study carried out in the United Arab Emirates, the moisture content of rice samples ($n = 500$) varied between 5.7% and 15.3% (Osman et al. 1999). Trenk and Hartman (1970) reported that *A. flavus* can grow and produce aflatoxin in corn kernels with moisture content above 17.5% at temperatures of 24°C or higher.

Methodology of mycotoxin detection and quantitation

As shown in Table 3, the moulds isolated from rice were broadly represented by four genera. In total, 212 isolates were obtained from 100 samples. *A. ochraceus* was the most frequently detected species, found in 30% of 100 rice samples, followed by *P. verrucosum* (28%), *A. flavus* (24%), *A. parasiticus* (20%) and *A. niger* (18%). Several researchers have reported the frequency of *Aspergillus*, *Penicillium* and *Fusarium* in rice samples (Osman et al. 1999; Park et al. 2005). Makun et al. (2007) found *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Mucor*, *Rhizopus*, *Trichoderma*, *Curvularia*, *Helmenthosporium* and *Cladosporia* in 196 mouldy rice samples in Nigeria. Similarly, the others have reported *Aspergillus* spp. as one of the most predominant moulds in grain from flood-affected paddy fields in India (Begum and Samajpati 2000; Reddy et al. 2009). Reddy et al. (2009) showed *A. flavus* and *A. niger* contamination was dominant in milled rice samples. In

Table 2 Moisture content and mould counts in rice samples

Geographic region	Moisture content		Total mould counts (cfu/g)			
	≤15%	>15%	Contamination levels (cfu/g)			
	(Log mean x±Sx[a] (n))	(Log mean x±Sx (n))	<10	10–<10^3	10^3–<10^4	10^4–<10^5
Uzunkopru (Site 1) ($n = 50$)	1.06 ± 0.18b ($n = 30$)	1.73 ± 0.28a ($n = 20$)	10[b]	29	8	3 (1.5×10^4)
Ipsala (Site 2) ($n = 50$)	0.78 ± 0.17b ($n = 34$)	1.15 ± 0.30ab ($n = 16$)	20	24	4	2 (1.2×10^4)
Total ($n = 100$) (%)	64 (64%)	36 (36%)	30 (30%)	53 (53%)	12 (12%)	5 (5%)

[a]Means in a column and row not followed with the same letter are significantly ($p \leq 0.001$) different
[b]The mould counts which were less than the assay detection limit (10 cfu/g) are calculated as "0" for statistical analysis

Table 3 Identification of mould species in rice samples

Genera	Moulds species	Uzunkopru (positive samples)	Ipsala (positive samples)	Total (%) ($n = 100$)[a]
Aspergillus	*A. ochraceus*	16	14	30 (30%)
	A. flavus	14	10	24 (24%)
	A. parasiticus	12	8	20 (20%)
	A. fumigatus	6	–	6 (6%)
	A. niger	4	14	18 (18%)
	A. terreus	2	–	2 (2%)
	A. versicolor	2	–	2 (2%)
	A. candidus	–	2	2 (2%)
Penicillium	*P. verrucosum*	10	18	28 (28%)
	P. citrinum	2	6	8 (8%)
	P. aurantiogriseum	2	2	4 (4%)
	P. viridicatum	–	2	2 (2%)
	P. islandicum	–	6	6 (6%)
	P. cyclopium	–	4	4 (4%)
	P. digitatum	–	6	6 (6%)
	P. oxalicum	–	2	2 (2%)
Fusarium spp.		8	4	12 (12%)
Mucor spp.		6	4	10 (10%)
Other[b]		14	12	26 (22%)
Total number of mould isolates		98	114	212

[a]Number of total rice samples
[b]Three genera were found at low frequency: *Cladosporium* spp., *Scopulariopsis* spp. and *Geotrichum* spp.

another study, Park et al. (2005) reported that rice was naturally contaminated by *A. ochraceus* spores. Other genera found at low frequency included *Cladosporium* spp., *Scopulariopsis* spp. and *Geothricum* spp.

In a study reported by Ha et al. (1979), the frequency of *Penicillium* spp. in rice increased from 14.5% to 26.9% with increased moisture content during storage. *Penicillium* and *Aspergillus* were the predominant genera in the samples, followed by *Alternaria* spp. Another study *P. citrinum* and *P. islandicum* were reported to be predominant penicillia in milled rice samples produced in Argentina and Paraguay (Tonon et al. 1997). The percentage of rice samples contaminated with potentially toxigenic moulds in our study revealed that the incidence of *Penicillium* species (especially *P. verrucosum*) in the rice samples from Site 2 was higher than that from Site 1. Accordingly, this supports a correlation between the presence of *P. verrucosum* in Turkish rice samples and the presence of OTA in the same samples. Similarly, Park et al. (2005) examined rice in Korea and found higher levels *Penicillium* spp. in the Northern region and *Aspergillus* spp. in Southern region, indicating differences in their ecological positions and preferential geographic regions. Based on results from our study, geographical distribution of toxigenic moulds and mycotoxins detected in rice samples were categorised into either the southern or northern regions in Thrace (Fig. 1). In this way, we examined both the effect of geographic factors such as latitude on mycotoxin incidence as well as the difference in OTA production by separate genera (Park et al. 2005).

Types and concentrations of mycotoxins in Turkish rice

Poor sanitation and handling conditions during the harvest, drying, transport and storage stages of cereal production can result in fungal contamination and subsequent formation of mycotoxins (Aydin et al. 2007). Turkey has encountered aflatoxin contamination in various foods exported and/or consumed in the country (Aydin et al. 2007; Camlibel 1995). The levels of mycotoxin contamination in Turkish rice samples examined

in our study are shown in Table 4. Fifty-six percent of the 100 samples were found to contain total AF levels ranging from 0.05 to 21.4 μg/kg. Most importantly, 32% of the samples exceeded the maximum tolerable limit (>4 μg/kg) for total AF as stated in the EC Regulation (2006) and Turkish Food Codex (2008). The highest levels of total AF in the samples were 21.4 μg/kg (Site 1) and 20.1 μg/kg (Site 2). In a study reported by Bandara et al. (1991), aflatoxin levels in parboiled rice were found to be significantly higher than in raw milled rice, with the highest levels of AFB1 and AFG1 being 185 and 963 μg/kg, respectively.

Levels of AFB1 in 58 (58%) of 100 samples were higher than the detection limit (1 μg/kg) and levels in 14 (14%) rice samples were found to be higher than the legal limits of the EC Regulation (2006) and Turkish Food Codex (2008) (Table 4). In the United Arab Emirates, AFB1 was detected in 160 (64%) long grain rice samples and 81 (32%) short grain rice samples at levels ranging from 1.2 to 16.5 μg/kg (Osman et al. 1999). Sales and Yoshizawa (2005) reported that the incidence of AFB1 in rice ranged from 0.025 to 11.0 μg/kg in the Philippines. In another study, in which AFB1 was estimated for 1,200 samples by ELISA, 67.8% of the samples were positive to AFB1 (Reddy et al. 2009). Toteja et al. (2006) examined parboiled rice collected from India and found 38.5% of the samples to be positive for AFB1. More recently, 9% of rice samples in Ecuador were shown to be contaminated with aflatoxins with a range of 6.8–40 μg/kg (Mühlemann et al. 1997). Bandara et al. (1991) analysed 597 rice samples and AFB1 was detected in 72 (12%). In our study, the percentage of samples positive for AFB1 was similar to that reported by Reddy et al. (2009) and Osman et al. (1999).

Commonly used analytical methods for the determining aflatoxin concentrations in foods include high performance liquid chromatography (HPLC) with fluorescence detection (FLD), thin layer chromatography (TLC) and immunochemical methods such as ELISA (Lin et al. 1998). ELISA is often favoured over conventional HPLC and TLC methods because it has a high throughput and requires low sample volumes, minimal sample extraction and clean-up. This method is rapid, simple, specific, sensitive and portable and can be fully quantitative for the detection of mycotoxins in food and feeds in the field (Trucksess 2001). However, the antibodies produced are often cross-reactive with compounds similar to mycotoxins. An extensive study of the accuracy and precision of the ELISA method over a range of commodities is essential before commercial use (Zeng et al. 2005). The ELISA test kit has been validated for the detection of total AF in grain and grain products (e.g., milled rice, wheat, and corn) by comparison with HPLC (Zeng et al. 2005). It has been shown that the Agraquant® total AF ELISA test kit is effective in measuring total AF for several commodities. Good accuracy and precision for grain and grain products was

Table 4 Mycotoxin levels of rice samples

Geographic region	Mycotoxin levels								
	Total AF levels (μg/kg)			AFB_1 levels (μg/kg)			OTA levels (μg/kg)		
	<0.05[a]	0.05–4.0	>4.0[b]	<1.0[a]	1.0–2.0	>2.0[c]	<0.025[a]	0.025–3.0	>3.0[d]
Uzunkopru (Site 1) ($n = 50$)	18	10	22 (21.4)[e]	16	24	10 (17.2)[e]	14	26	10 (80.7)[e]
Ipsala (Site 2) ($n = 50$)	26	14	10 (20.1)[e]	26	20	4 (14.4)[e]	14	16	20 (5.7)[e]
Total ($n = 100$) (%)	44 (44%)	24 (24%)	32 (32%)	42 (42%)	44 (44%)	14 (14%)	28 (28%)	42 (42%)	30 (30%)

[a]Under the minimum detection limit

[b]Above the maximum tolerable limit for total AF

[c]Above the maximum tolerable limit for AFB_1

[d]Above the maximum tolerable limit for OTA

[e]The highest mycotoxin (total AF, AFB_1 and OTA) level

found for quantitating aflatoxin in the range of 4–40 μg/kg. In another study, competitive direct ELISA was validated against HPLC as a reference and other methods, including a mini column method and the VICAM Aflatest® system for AFB1 in peanuts (Lee et al. 2005). These researchers declared that ELISA was acceptable as an analytical method despite the high expected sampling variation. An acceptable correlation between ELISA and HPLC for AFB1 analysis was obtained when different sample extracts were used. More importantly, the accuracy of ELISA was validated against a reference method applying HPLC/FLD and showed an exceptionally good correlation between ELISA and HPLC when the same sample extracts were used.

Significance for public health

The incidence of AFs in foods and feeds is relatively high in tropical and subtropical regions, where climatic conditions provide an optimal environment for the growth of moulds. Furthermore, a correlation between dietary exposure to AFs and the incidence of human liver cancer in some areas, especially in Africa and Asia, has been shown (Hill et al. 1986). OTA is predominantly found in cereal grains, cereal products, legumes, oilseed, coffee beans and feed (Zinedine et al. 2007). The frequency of contamination of rice analysed for OTA was 72%. In our study, OTA levels were found to be higher than the legal limits of the Turkish Food Codex (2008) and EC Regulation (2006) in 30 (30%) rice samples (Table 4). The highest OTA level reported here was 80.7 μg/kg in a sample from Uzunkopru (Site 1). Rice contaminated with OTA has been reported in several studies. Indeed, Zinedine et al. (2007) reported a high frequency of contaminated rice from Morocco with 15% of total samples above the EU legal limits. Zaied et al. (2009) reported that 28% of 96 rice samples were contaminated with OTA in Tunisia; the highest level of OTA was 150 μg/kg. In Vietnam, OTA in rice was found at concentrations of 21.3–26.2 μg/kg (Trung et al. 2001). In a study performed in the United Kingdom, Scudamore et al. (1997) detected OTA in three out of 40 (7.5%) rice samples, with OTA levels ranging from 1–19 μg/kg. Incidences of OTA in samples of non-organic and organic rice obtained from Spain were 7.8% and 30%, respectively (Gonzalez et al. 2006). In Egypt, Abdelhamid (1990) reported the occurrence of OTA in 33% of rice germs and rice germ cake, with an average value of 577 and 4 μg/kg, respectively. Juan et al. (2008), found that 14 out of 100 rice samples exceeded the maximum level of OTA in cereals allowed by European Commission Regulations (2006). The highest frequency of positive samples (30%) and the most contaminated sample (47 μg/kg) was found in Casablanca City in Morocco (Juan et al. 2008). These results were similar to those of Zaied et al. (2009) (28% positive samples) and our results (30% positive samples). Ochratoxin-producing moulds can clearly contaminate rice and other grains and produce critical levels of OTA during storage. Rice is a good substrate for the characterisation of OTA-producing *A. ochraceus* strains (Juan et al. 2008).

Conclusion

The results of this study confirm that rice, among grains and grain-derived products from Mediterranean countries, can be contaminated with aflatoxins and OTA. The risk of human exposure to mycotoxins in contaminated grains and grain products is an important public health issue. In several areas of Eastern Europe, where chronic exposure to OTA occurs, involvement of this mycotoxin in cancer of the urinary system and in kidney pathologies typical of BEN is suspected. Studies suggesting a correlation between consumption of foods containing OTA and BEN show higher OTA contamination levels in cereals from endemic areas as compared to cereals from non-endemic areas. High levels of OTA may lead to a higher incidence of BEN and urethra, renal, and pelvis tumors in the region. Our study is the first to report on the occurrence of OTA in rice from Thrace region of Turkey. The presence of OTA in 30% of rice produced in this region poses a potential risk to public health.

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