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Aflatoxins in Dried Figs in Turkey: A Comparative Survey on the Exported and Locally Consumed Dried Figs for Assessment of Exposure

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ABSTRACT

Dried figs collected from various exporting companies (2,461 samples) and local stores (219 samples) were analyzed using reverse-phase high-performance liquid chromatography (RP-HPLC) to determine and compare the incidence of aflatoxins, the effectiveness of the regulation limits and monitoring system to select non-compliant samples and the mean daily aflatoxin exposures. The incidence rates of aflatoxin contamination were higher in the domestic samples (47.5%) than in the samples intended for export (23.6%). According to the European regulation limits (2 ng g^{-1} for aflatoxin B1 and 4 ng g^{-1} for total aflatoxin; sum of B1+B2+G1+G2), non-compliant samples were 6 and 24.2% of the dried figs for export and domestic market, respectively. The respective rates of 2.2 and 16.4% were obtained with the national limit. The dietary intakes of aflatoxins through the consumption of dried figs were calculated as 1.27 and 0.2 ng kg^{-1} body weight×day for domestic market and export, respectively. Dried figs contaminated with high levels of aflatoxins can lead to acute and chronic human toxicities. Reducing contamination and exposure to an acceptable level by the implementation of strict periodic monitoring and application of effective new prevention measures might also help to decrease the significant health and economic risks in exported and domestic commodities.

Keywords: Aflatoxins, Daily exposure, Dried figs, Regulatory limits.

INTRODUCTION

During the pre- and postharvest period, naturally occurring mycotoxins contaminate a wide variety of food and feed matrices for human and animal consumption. Aflatoxins are the most abundant mycotoxins due to the widespread occurrence of the toxigenic strains of the Aspergillus molds; Aspergillus flavus, Aspergillus parasiticus, and Aspergillus nomius. These strains exhibit great variability in different geographical areas (Pildain *et al.,* 2004). The interaction of environmental factors such as temperature and moisture, the prevalence of aflatoxin producing strains in the fungal flora of the crop and their relative toxigenicity, influence the total amount and relative proportion of individual aflatoxin production and accumulation. Therefore, their levels are unpredictable and widely vary around the world generating difficulty for the Codex Alimentarius Commission to develop a uniform prevention method.

Aflatoxins are considered to be a significant threat to human and animal health due to their highly toxic, carcinogenic, tetratogenic, hepatotoxic and mutagenic characteristics depending on the duration and level of exposure (Pariza, 1996; Chu, 1997). Therefore, although varying

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from country to country, the European Union (EU) has imposed vigorous regulations to limit the presence of aflatoxin B1 (2 ng g^{-1}) and total aflatoxins (4 ng g^{-1} for the sum of B1+B2+G1+G2) in food products (EC 2003). Exported Turkish dried figs to EU have been rejected in recent years due to high levels of aflatoxin contaminations and caused significant economic loss. Turkey is a candidate country for EU membership and therefore most of the rules and regulations of the Turkish Food Codex and the Turkish Standards are supposed to be harmonized with the EU norms and directives. Nevertheless, recent amendments in the national standards allowed higher aflatoxin contents (10 ng g^{-1} in total aflatoxins) in dried figs for the domestic market in order to minimize the effects on trade (TGK, 2009).

Aflatoxin B1 is considered as the most toxic among the known four major naturally occurring aflatoxins (B1, B2, G1 and G2) and has been classified as Group I human carcinogen by the International Agency for Research on Cancer (IARC) in 1993 (IARC, 1993). The occurrence of aflatoxins has been reported in many staple foods and ingestion of contaminated food is the primary source of exposure. They cause a variety of adverse health effects in humans and are considered as the main reason for the development of hepatocellular carcinoma which is the fifth most common cancer in the world and the third cause of cancer-related deaths (Sun and Chen, 2003; Del Pozo and Lopez, 2007; Shephard, 2008). Consumption of highly contaminated food items can have serious exposure consequences and result in acute aflatoxicosis. No animal species and human beings can develop immunity to the acute toxic effects of aflatoxins. Therefore, estimating the daily intake of aflatoxin by determining the contamination level and consumption rates will be very informative and play an important role in guiding food safety authorities.

Colonization of toxigenic strains of Aspergillus and subsequent aflatoxin production were observed in figs particularly during ripening and reached the highest level at the shrivelled-ripe stage (Buchanan *et al*., 1975; Boyacioglu and Gonul, 1990; Boudra *et al.*, 1994; Senyuva *et al.,* 2007; Iamanaka *et al.,* 2007). Buchanan *et al.* (1975) also mentioned that sun-drying on the tree or in the field favored the fungal infection and aflatoxin accumulation until the moisture loss had progressed to the point that fungal growth ceased (Buchanan *et al.,* 1975). Moreover, fig fruits contain high levels of sugars such as glucose and fructose, amino acids like proline and asparagine and minerals like zinc. Nutritionally rich chemical compositions of fig fruits could support and stimulate the production of aflatoxins (Reddy *et al.,* 1971; Payne and Hagler, 1983; Luchese and Harrigan, 1993).

Aydin Province of Turkey has favorable climatic conditions to grow figs. Although the recent drought reduced the production of dried figs production from 66,500 in 2006-2007 to 42,500 tons in 2008-2009 season, Turkey is still the number one producer and exports approximately 90% of its production mostly to the EU (Aegean Exporters, 2009). Despite the decrease in the production rate, the number of alert notifications and rejection of imported Turkish dried figs due to aflatoxin contamination have increased during the five year period (2003-2008) from 27 to 95 and the amount of rejected lots has reached 800 tons (RASFF, 2009).

Aflatoxins in dried figs not only cause significant health concerns but also economic loss in exported and domestic commodities. The objectives of this study were to compare and present the differences in contamination levels and product quality in dried figs for export and for domestic market, to determine the effectiveness of the regulation limits and monitoring system to select non-compliant samples and to calculate the mean daily aflatoxin exposure of an individual from eating dried figs utilizing the mean levels of aflatoxins, consumption per day and the average body weight.

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MATERIALS AND METHODS

Sampling

Dried fig samples for export (2,461 samples) and domestic market (219 samples) weighing 30 and 3 kg, respectively were collected during the 2009 crop year from different local stores and exporting companies located in Aydin Province of Turkey. All sample selections were conducted according to the European Union Commission Directive (EC, 2006). In order to prevent or minimize the effects of sample selection from the big bulk samples and uneven distributions of the aflatoxins on variability, 100 small incremental sub-samples, each weighing 300 g, were randomly selected from different locations throughout the lot. Then, these individual parts were gathered, mixed thoroughly and divided into three 10 kg parts and blended with a high speed blender (Robot Coupe, R23; Robot Coupe USA, Inc., Jackson, MS, USA) to homogenize without water addition. Random selection and mixing were also carefully conducted during the collection of samples from the local stores. A total of 50 g of homogenized samples was taken and analyzed in triplicates.

Sample Extraction for Aflatoxins

Aflatoxins were extracted from 50 g samples using a method developed by the immunoaffinity column provider (Aflaprep, R-Biopharm Rhone Ltd, Glasgow, UK) based on methanol extraction. 50 g samples were mixed with 100 ml of pure water, 150 ml methanol (Merck, Darnstadt, Germany) and 4 g of sodium chloride and blended (Waring 8011S, Torrington, CT) at high speed for 3 min to obtain a homogeneous sample mix. After mixing, the slurry was filtered through filter paper (Whatman # 4) and diluted with phosphate buffered saline (PBS) solution. This diluted solution was passed through an immunoaffinity column (Aflaprep, R-Biopharm Rhone Ltd, Glasgow, UK). Aflatoxins were eluted from the column by passing 2 ml of HPLC grade methanol (Merck, Darnstadt, Germany) and then 2 ml of HPLC grade water and using gravity to collect the eluate into a glass vial at a flow rate of around 5 ml min^{-1}. Twenty micro liters of the eluate was injected into the HPLC.

HPLC Conditions

Utilizing RP-HPLC (Shimadzu, Tokyo, Japan), aflatoxins were separated with a column (C-18, 25 cm, 4.6 mm, 5 µl) and quantified using post-column derivatization (Kobra Cell, 100 µA) (R-Biopharm Rhone Ltd., Glasgow, UK) performed by a reactive form of bromine. The bromine reacts with aflatoxins (B1 and G1) to form more fluorescent compounds to ease the detection of these toxins by fluorescence detector (excitation 362 nm, emission 425 nm). An isocratic pump was used to adjust the flow rate of the mobile phase (water/acetonitrile/methanol (6:2:3, v/v/v)) to 1 ml min^{-1}. The column temperature was 26 o C. The Limit of Detection (LOD) was determined to be 0.2 ng g^{-1} by establishing the minimum concentration at which a known concentration of analyte was reliably detected after injecting the same amount ten times. The correlation coefficient (r) calculated from the six point calibration curve was 0.999.

Validation of the Method

The current method was validated for dried figs in order to verify its effectiveness and to produce accurate results. For this purpose, blank samples of dried figs were spiked with low (2), medium (4) and high (8) ng g^{-1} levels of total aflatoxins containing equal amounts of each aflatoxin (B1+B2+G1+G2) to determine the mean recovery rates by repeating the procedure six

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Table 1: Recovery rates of aflatoxins spiked into dried figs.

	Recovery rates[a] %		
Materials	2 ng g^{-1} (Total)[b]	4 ng g^{-1} (Total)	8 ng g^{-1} (Total)
Dried figs	91.79±4.45	89.33±4.73	93.2±5.74
RSD[c] %	4.85	5.29	6.16

[a] Values are the means of six analyses, [b] Mean value of aflatoxins (B1+B2+G1+G2), [c] Relative standard deviation .

times (Table 1). Moreover, one blank sample of each and samples with known amounts of aflatoxins (FAPAS, Central Science Laboratory, Sand Hutton, UK) were used to check analytical quality assurance and the technical performance of the analysis. These procedures were repeated every 50 sample readings.

Estimation of Daily Aflatoxin Exposure

The mean daily aflatoxin exposure (AE) of an individual from eating dried figs was estimated separately for export and domestic market using the positive sample mean levels of total aflatoxins and aflatoxin B1, seperately (ng g^{-1}), consumption per day (g figs day^{-1}) and average body weight of an individual (60 kg).

RESULTS AND DISCUSSION

Spiking the blank samples of dried figs with low (2 ng g^{-1}), medium (4 ng g^{-1}) and high (8 ng g^{-1}) levels of total aflatoxin containing equal amounts of each toxin produced acceptable rates of recovery (> 85%) and the relative standard deviation (RSD) values ranging from 89.3±4.7 to 93.2±5.7% and 4.8 to 6.2%, respectively (Table 1). The mean recovery rates were determined by repeating the procedure six times. Adjustment of the reported results based on the recovery experiment was performed on all samples.

The comparison of the number of contaminated dried fig samples and their total concentration of aflatoxins exceeding EU and national regulations for export and for domestic market is presented in Table 2. More

$$\mathrm{AE}\left(\mathrm{ng/kg\ bodyweight}\times\mathrm{day}\right)=\frac{[\text{mean concentration of in dried figs}\left(\mathrm{ng/g}\right)]\times[\text{consumption per day (g figs/day)}]}{[\text{average body weight}\left(60\mathrm{kg}\right)]} \quad (1)$$

Table 2. Comparison of the number of contaminated dried fig samples and their aflatoxin concentrations exceeding EU and national regulations for export and for domestic consumptions.

Samples	No. of analyses	No. of contaminated samples	Total range (ng g^{-1})	Mean levels of aflatoxin B_1 (ng g^{-1})	Mean levels of total aflatoxins (ng g^{-1})	No. of samples above EU limits[a]	No. of samples above national limits[b]
Dried figs for export	2461	580 (23.6 %)	ND[c]-278.04	3.49	5.56	147 (6.0 %)	55 (2.2 %)
Dried figs for domestic consumption	219	104 (47.5 %)	ND-267.48	21.06	34.54	53 (24.2 %)	36 (16.4 %)

[a] EU regulations >2 in B1 and >4 ng g-1 in total (B1+B2+G1+G2); [b] National regulation> 10 ng g-1 in total (B1+B2+G1+G2), [c] Not detected.

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than twice the incidence rate was found (47.5%) in the samples for domestic market than the samples intended for export (23.6%), although the maximum contamination levels did not differ greatly being 267.48 and 278.04 ng g^{-1}, respectively. Overall mean levels of contamination for total aflatoxins and for aflatoxin B1 were found to be significantly higher in dried figs for domestic market (34.54 and 21.06 ng g^{-1}) than for export (5.56 and 3.49 ng g^{-1}) (Table 2). The observed differences in dried fig samples were expected due to the fact that exporting companies give utmost importance to choose the least contaminated ones. As a result, highly contaminated figs were mostly encountered in domestic market samples.

Moreover, the low aflatoxin levels in dried figs samples intended for export compared to domestic samples point towards the efficiency of monitoring in reducing aflatoxins. In Turkey, all dried figs intended for export and most of the dried figs for domestic market are separately screened under UV light to remove any showing blue or green fluorescence, which are considered contaminated. Despite the rigorous UV screening before export, 6% of the samples in this study exceeded the EU standards and they will be rejected when tested upon entry into member countries. Due to the internal cavity of the figs, the ostiole, figs are very vulnerable to fungal and insect infestations not only externally but also internally, which significantly decreases the effectiveness of the UV screening process (Stenier *et al.,* 1988; Doster *et al.,* 1996).

Effectiveness of the Regulation Limits to Select Non-compliant Samples

Six percent of the dried fig samples for export were found non-compliant and considered as unfit for human consumption when EU limits (2 ng g^{-1} for aflatoxin B1 and 4 ng g^{-1} for total aflatoxins) were applied. However, this rate decreased to 2.2 % with the implementation of 10 ng g^{-1} national total aflatoxin limit (Table 2). Higher national regulatory limit also caused a considerable decrease in the non-compliant sample rate from 24.2 to 16.4% for the samples sold in domestic market.

In order to determine the effect of the implementation of a separate low level of aflatoxin B1 limit, the number of dried fig samples contaminated with aflatoxin B1 and total aflatoxins, their concentrations and the number of samples exceeding the EU and national limits for export and domestic market are presented in Table 3.

While 5% (123) of the export samples

Table 3. Aflatoxin B_1 and total aflatoxin concentrations and the number of samples exceeding the EU and national limits in dried figs for export and domestic consumptions.

Contamination level (ng g^{-1})	Dried figs for export		Dried figs for domestic consumption	
	No. of cont. samples with aflatoxin B_1	No. of cont. samples with total aflatoxin	No. of cont. samples with B_1	No. of cont. samples with Total aflatoxin
2<	433	369	51	44
2-4	70	88	8	11
4-10	41	68	12	12
10-50	29	46	21	15
50-100	5	4	3	10
> 100	2	5	9	11
No. of Samples Exceeding EU regulations	147 (6.0 %)	123 (5.0 %)	53 (24.2 %)	49 (22. 4 %)
No. of Samples Exceeding National regulations	36 (1.5 %)	55 (2.2 %)	34 (15.5 %)	36 (16.4 %)

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failed to meet the total aflatoxin limit of EU, the implementation of aflatoxin B1 limit increased the non-compliant samples to 6% (147) (Table 3). If EU regulations were also implemented for domestic market, 22.4% (49) of the samples would be considered as non-compliant samples due to total aflatoxin. Although Senyuva *et al.* (2007) reported otherwise, 2 ng g^{-1} limit for aflatoxin B1 was found to be a more stringent test of compliance and can cause more rejections of the exported dried figs than the 4 ng g^{-1} limit for total aflatoxins in this study (Senyuva et al. 2007). However, total aflatoxin limit became the determining factor instead of lower separate aflatoxin B1 limit when the higher 10 ng g^{-1} limit was applied (Table 3).

Turkey has recently set the allowable maximum limit only for total aflatoxins (10 ng g^{-1}) for dried fruits and lifted the separate lower aflatoxin B1 limit (5 ng g^{-1}). Although low aflatoxin limits in dried figs were tried to be avoided due to their importance in trade, the high contamination rates encountered in dried figs for the domestic market in this study has revealed the need to set a separate lower aflatoxin B1 limit by the national regulatory authorities. In order to reduce aflatoxin exposure to the lowest level achievable, monitoring and strict application of the regulatory limits will be very effective tools to prevent human beings from the deleterious effects of this toxin and offer an extra assurance to public safety. Increasing the permissible levels of toxins can only accelerate daily dietary aflatoxin exposures which will eventually have a negative impact on public health.

Levels of Contaminations

A vast majority of the contaminated dried figs for export (75%) and for the domestic market (50%) contained only less than 2 ng g^{-1} aflatoxins (Table 3). Although aflatoxin concentrations of these samples were considered acceptable for export during the testing period, these compliant consignments are sometimes rejected based on the assessment made by the health authority of the importing countries.

Difficulties in accurate determination of aflatoxin concentrations in dried fig samples taken from large consignments are caused by considerable variability observed in sampling, subsampling and in each step of analytical test procedures (Senyuva *et al.,* 2007; Bircan, 2009). The presence of individual figs with extremely high levels of aflatoxins and uneven distribution of this toxin throughout the lot can also cause large sampling errors and variation among replicated subsample test results as also observed in nuts (Doster *et al.,* 1996; Mahoney and Rodriguez, 1996; Fooladi and Farahnaky, 2003). Using the proper homogenization technique which sufficiently minced the dried figs to prevent the remaining big particles, mostly eliminates the variability caused by the improper sampling (Senyuva *et al.,* 2007; Bircan, 2009).

Furthermore, if the fig fruits were not sufficiently dried to prevent the colonization of aflatoxins producing fungi, they might continue to grow and elevate the toxin level of a sample indicated as acceptable during the transport to the EU.

Besides the high incidence rate, frequent occurrence of very high levels of aflatoxins ($\geq$ 50 ng g^{-1}) in domestic fig samples (9%) were observed compared to export samples (0.3 %) (Table 3).

Despite the fact that dried figs have been heavily scrutinized before export by the Turkish food safety authorities, sampled according to European Commission Directives, analyzed by private or governmental laboratories and then permitted to export, increasing number of notifications for consignments of dried frigs from Turkey containing too high levels of aflatoxins have been reported by the Rapid Alert System for Food and Feed (RASFF, 2009).

Highly contaminated non-compliant domestic samples are sometimes either fermented to produce alcohol or used as

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animal feed which put both animals and humans at risk of acute and chronic aflatoxin toxicity. Aflatoxin B1 in feed is metabolized to aflatoxin M1 in animals by enzymes found primarily in liver, which is considered as toxic as aflatoxin B1 and is excreted in milk (Lafont *et al.,* 1989; Van Egmond, 1989; Galvano *et al.,* 1996; Castegnaro and McGregor, 1998; Coffey *et al.,* 2009). Occurrence of aflatoxin in milk and consequently in dairy products is of major concern due to their being one of the most important foods for human diet especially for babies and other risk groups. Therefore, highly contaminated dried figs must be collected, labeled and destroyed due to the fact that they cause significant health problems and economic loss in exported and domestic commodities. In order to prevent the utilization of the highly contaminated dried figs for animal feed or human food, educational programs must also be organized by national authorities with other related groups to inform growers, producers and manufacturers regarding the hazard associated with aflatoxin contamination besides imposing strict rules and regulations.

Estimation of Aflatoxin Exposure

Although the production and export rates change according to weather and economical conditions, Turkey is still the number one producer of dried figs, with Aydin Province as a major contributor. Approximately 50% of a world dried figs production is made in Turkey meeting 75% of the world demand through exports. However, although the domestic consumption rate reaches 800 g in Aegean region where the figs are mostly produced, the average whole country consumption is 135 g per year (Gunal, 2008). Considering the production and export rate, consumption levels are very low and higher levels are encouraged.

Utilizing the positive sample mean levels of total aflatoxins (34.54 ng g^{-1} for domestic market and 5.5 ng g^{-1} for export), consumption per day for Aegean region (2.2 g) and average body weight (60 kg), the intakes of aflatoxins through the consumption of dried figs were calculated as 1.27 and 0.2 ng kg^{-1} body weight*day for domestic market and export samples, respectively. Aflatoxin intake decreased to 0.78 and 0.13 ng kg^{-1} body weight*day if only mean contamination rates of aflatoxin B1 (21.06 ng g^{-1} for domestic market and 3.49 ng g^{-1} for export) were used. The risk of exposure increases in children when the contaminated products are consumed. Comparing the results of the exposure rates and considering the rapid excretion and partial detoxification of ingested aflatoxins (Williams *et al.,* 2004), although the statistical results showed that there is not a significant toxicological risk for Turkish consumer, it is important to indicate that very low consumption of dried figs ameliorates the serious exposure consequences.

The presence of individual dried figs contaminated with extremely high levels of toxins can lead to high risk of exposure and cause acute human toxicities. The maximum levels were found 267.48 ng g^{-1} for domestic market and 278.04 ng g^{-1} for export samples in this study. Consumption of these levels increased the exposure rate to 9.79 and 10.20 ng kg^{-1} body weight×day for domestic market and export samples, respectively. Despite the lower mean levels of contamination which might be considered as acceptable by the standards, consumption of highly contaminated dried figs can have serious exposure consequences. Shephard (2008) also emphasized the importance and the effect of the consumption of highly contaminated food on the daily exposure rate in his study (Shephard, 2008).

The recent increase in the national regulation limit caused 8 % decrease in the non-compliant sample rates as compared to EU limits. This could also increase the probable daily exposure to aflatoxins and the population health risk.

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CONCLUSIONS

Food insecurity emerged from the risk of human and animal exposure to aflatoxin contamination, determination of tolerable exposure level and the economical loss caused by changing the regulations are the dilemmas facing regulatory agencies to cope with. Aflatoxins are considered as unavoidable contaminants in foods and therefore human exposure cannot be completely prevented and therefore their levels should be kept as low as reasonably achievable. Reducing the contamination and the exposure level to an acceptable level by the implementation of strict periodic monitoring and application of effective new prevention measures (in accordance with the principals of good manufacturing practice, good handling practice and hazard analysis critical control points) based on the knowledge of the conditions that favor aflatoxins production and accumulation in dried fruits and other susceptible commodities might prevent the significant health and economic loss in exported and domestic commodities. However, although setting the legislative levels or preparation of guidelines to reduce the risk of aflatoxins exposure can be very feasible and applicable for large-scale industrial processors and exporting companies, enforcement of regulations becomes impractical for small-scale industries (Williams *et al.,* 2004; Shephard, 2008). Therefore, authorities should also continue to develop monitoring programs to take into account difficulties encountered whilst implementing the Directives.

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آفلاتوکسین در انجیر خشک ترکیه: بررسی مقایسه‌ای انجیر صادراتی و انجیر مصرف داخلی به منظور ارزیابی میزان در معرض قرار گرفتن

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چکیده

انجیرهای جمع آوری شده از شرکت های مختلف صادرکننده (۲۴۶۱ نمونه) و فروشگاههای محلی (۲۱۹ نمونه) با استفاده از کروماتوگرافی مایع با عملکرد بالای فاز معکوس (RP-HPLC)به منظور تعیین و مقایسه وجود آفلاتوکسین، اثربخشی حدود قانونی و سیستم پایش بر انتخاب نمونه‌های ناسازگار و متوسط روزانه قرار گرفتن در معرض آفلاتوکسین، مورد تجزیه و تحلیل قرار گرفتند. میزان شیوع آلودگی آفلاتوکسین در نمونه‌های داخلی (۴۷.۵٪) بالاتر از مقدار آن در نمونه‌های در نظر گرفته شده برای صادرات (۲۳.۶٪) بود. با توجه به محدودیت‌های قانونی و مقررات اروپایی (۲ نانوگرم / گرم آفلاتوکسین B1 و ۴ نانوگرم / گرم برای آفلاتوکسین کل مجموع(B1 + B2 + G1 + G2) نمونه‌های ناسازگار به ترتیب ۶ و٪ ۲۴.۲ برای انجیر خشک صادراتی و بازار داخلی بودند. این مقادیر با استفاده از حد ملی به میزان ۲.۲ و ۱۶.۴٪ به دست آمدند. جذب غذایی آفلاتوکسین از طریق مصرف انجیر خشک ۱.۲۷ و ۰.۲ نانوگرم / گرم به ازای هر کیلوگرم وزن بدن در روز به ترتیب برای بازار داخلی و صادرات محاسبه شد. انجیر آلوده با سطح بالای آفلاتوکسین می‌تواند منجر به سمیت حاد و مزمن در انسان شود. کاهش آلودگی و قرار گرفتن در معرض سطح قابل قبول با اجرای نظارت دقیق دوره‌ای و استفاده از اقدامات پیشگیری کننده موثر نیز می‌تواند کمک قابل توجه ای به کاهش خطرات اقتصادی و بهداشتی کالاهای صادراتی و داخلی نماید.

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